Relativity of Limiting and Optimum Inoculum Loads, Wetting Durations, and Temperatures for Infection by Phytophthora infestans

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ABSTRACT

The infection of potato leaves by *Phytophthora infestans* was investigated under controlled conditions, in a 6 × 5 × 4 factorial experiment with 120 combinations of temperature, inoculum concentration, and wetting duration. The minimum, maximum, and optimum levels of each factor were found to depend on the other two factors, in some cases primarily on one of them and in other cases on a balance of both. For each factor, both the limiting and the optimum range of levels were broad for favorable combinations of the other two factors, and narrow for unfavorable combinations. High spore load had an inhibiting effect when the wetting duration was 6 hours, and long wetting duration had an inhibiting effect when the temperature was 28 C. These findings imply that statements concerning the effect of any one factor on infectivity are relative rather than absolute, and are valid only under certain combinations of other factors which have to be explicitly specified. Phytopathology 61:275-278.

Additional key words: epidemiology, ecology, host-parasite relationship.

As observed in the field, the extent to which climatic factors affect development of *Phytophthora infestans* on potatoes depends on the spore load of the pathogen (9). But little systematic effort has been made to find out how the effect of any given factor is modified by other factors. For example, optimum temp may depend on spore load and on the duration of wetness. This problem has been mentioned in passing in studies with some plant pathogens, but in most cases it seems to be essentially ignored. Such omissions may lead to mistakes in the evaluation of individual factor effects, and may well be responsible for the widely divergent data found in the literature (11).

Published studies of the effect of various factors on plant diseases are usually concerned with one factor at a time. Thus, inoculum load has been found to affect infection in rusts, smuts, early blight of tomatoes, and late blight of potatoes (2, 4, 5, 7, 8). In the case of *Peronospora tabacina* on tobacco, the host-pathogen reaction was found to be affected by inoculum concn, wetting duration, and temp (3), but the joint effect of all three factors was not studied.

In this paper, we present the results of a controlled study of the joint effect of three factors which affect the penetration of *Phytophthora infestans* d By., race 0, into leaves of potato (*Solanum tuberosum* L. 'Up to Date'). The factors investigated were temp, the number of sporangia applied to the leaf, and the duration of the wet period.

MATERIALS AND METHODS.—The experimental treatments were all the 120 combinations of five spore concn, four wetting durations, and six temp. The spore concn were 15, 45, 135, 400, and 1,200 sporangia/cm^2 of leaf (s.p.c.); the most concd suspension was prepared by Schein's method (10), and the other suspensions were obtained from it by progressive threefold dilutions. The wetting durations were 3, 6, 12, and 24 hr. The temp were 5, 10, 15, 20, 25, and 28 C, all ±0.25 C. Three replicate plants with about 10 leaflets each, of which the six oldest ones were inoculated, were used for each factor combination.

The 360 plants were grown for 5 weeks in an air-conditioned greenhouse at 25 ± 2 C, in 0.3-kg pots filled with a sterilized 2:2:1:1 mixture of sandy loam: peat:sand:vermiculite. To make sure the leaves were at the assigned temp at time of inoculation, the experimental plants were placed in their growth chambers for a few hr before inoculation. The leaf temp were measured with thermocouples and recorded on a multipoint recorder. The plants were then withdrawn from their growth chambers and inoculated with the assigned amount of sporangia. The inoculum had been obtained from previously infected potato plants kept at 20 C. Inoculation was performed with Schein's inoculator (10) on a 4-cm^2 target on the underside of each leaf. The accuracy in the number of sporangia per target was within 15% of the desired amount. The inoculated plants were then covered with moist plastic bags, returned to the growth chambers, and kept in darkness for the assigned period of wetness. After the wetting period was over, the plants were removed from their chambers, kept for 15 min opposite a fan for the water drops to evaporate, and incubated in a common growth chamber maintained at 20 ± 1 C, 50-70% relative humidity, and a 12-hr photoperiod.

The efficiency of infection was assessed visually on the fourth day and graded according to the extent of the lesions: 0 and 1 = no infection and traces of infection, respectively; 2, 3, and 4 = lesions covering up to one-third, two-thirds, and the whole of the target area, respectively; and 5 = lesions extending beyond the inoculation site.

Conventional statistical methods were not used for analyzing the results, as they are not appropriate for this type of study. A formal test for interaction would only establish that the effects of the three factors are not additive on the rather arbitrary scale described above; this is fairly obvious (as can be seen from a

cursory scrutiny of the data), and tells us little about the specific type of interaction (i.e., nonadditivity) involved. Methods involving transformations of the data, or fitting polynomial regressions to them, might be helpful if theoretical or empirical information were available which would provide us with a reasonable basis for choosing the appropriate type of transformation or regression; in the apparent absence of any such information, the interpretation and validity of the formal results yielded by these methods would be dubious at best.

The approach adopted in this paper is both more direct and more detailed. The effect of each factor is described in general terms, then investigated in terms of (i) the limiting range and (ii) the optimum range of factor levels.

RESULTS.—The average infection grades obtained with all the combinations of temp (T), inoculum concn (D), and wetting duration (W) are presented in Fig. 1. The corresponding standard errors (shown in the upper right part of the figure) indicate that the variability of the results (i.e., the differences between the three replicate plants) was relatively large at medium infection levels, and relatively small at both low and high infection levels.

The general patterns seen in Fig. 1 can be summarized as follows. For a given combination of T and W, the infection level increased with D; for a given combination of T and D, it increased with W. In both cases several exceptions were found, in which the highest concn (1,200 s.p.c.), or the longest wetting duration (24 hr), resulted in a lower infection than the second-highest concn (400 s.p.c.) and second-highest duration (12 hr), respectively. For a given combination of D and W, the infection level increased with T up to a certain temp (usually 15 C), and decreased for higher temp.

Figure 2 presents a more detailed description of the data. The three subtables in the upper part of Fig. 2 show the limiting ranges of the factors. For example, the notation $\geqslant 135$ in Fig. 2-D(a), inside the region consisting of the three combinations (W = 3 hr, T = 15 C), (W = 3 hr, T = 20 C), and (W = 6 hr, T = 25 C), indicates that, for each of these three combinations of wetting duration and temp, some infection appeared with all inoculum concn from 135 s.p.c. up.

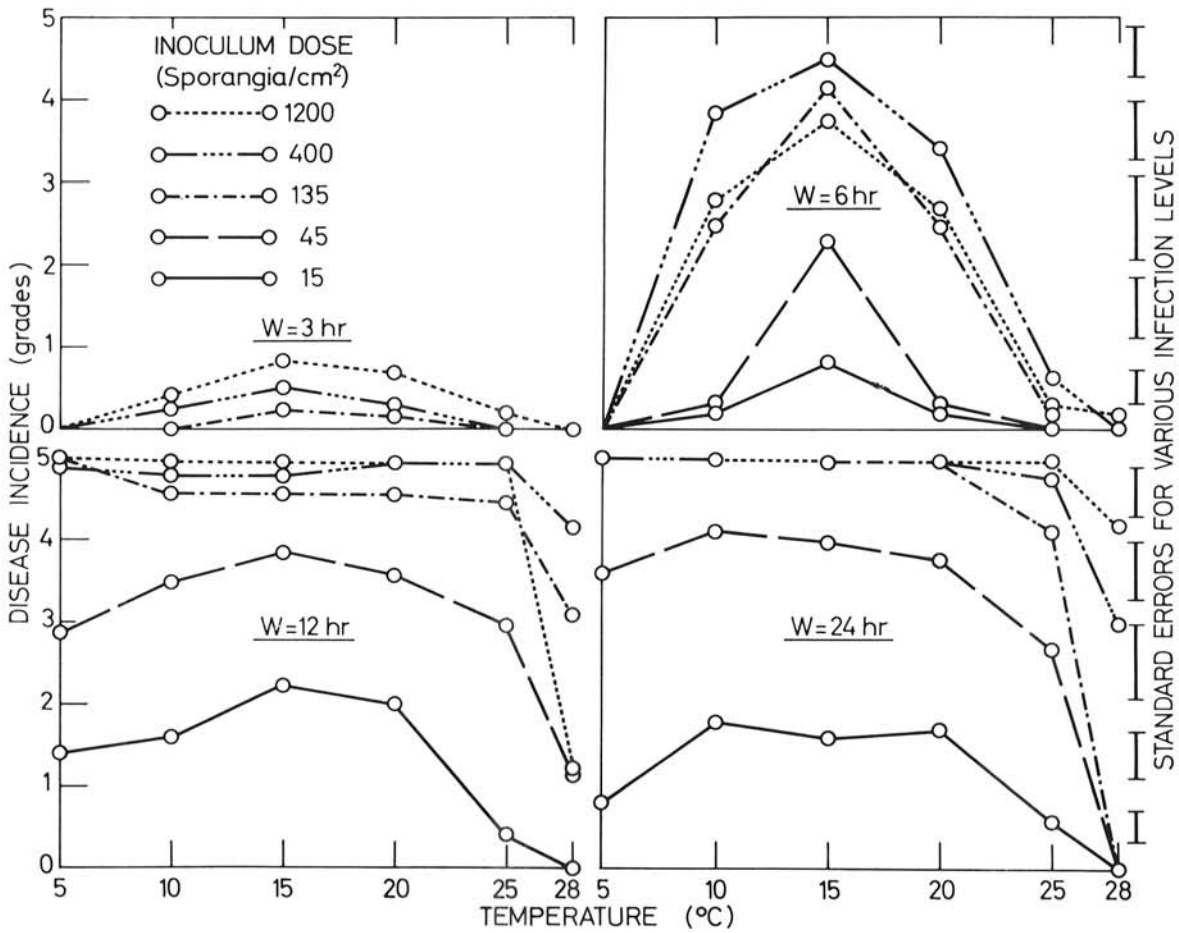

Fig. 1. The effect of temperature (T, in C), inoculum dose (D, in sporangia/cm²), and duration of wet period (W, in hr) on infection of potato leaves by *Phytophthora infestans:* infection levels obtained for all treatment combinations (averages of three replicate plants).

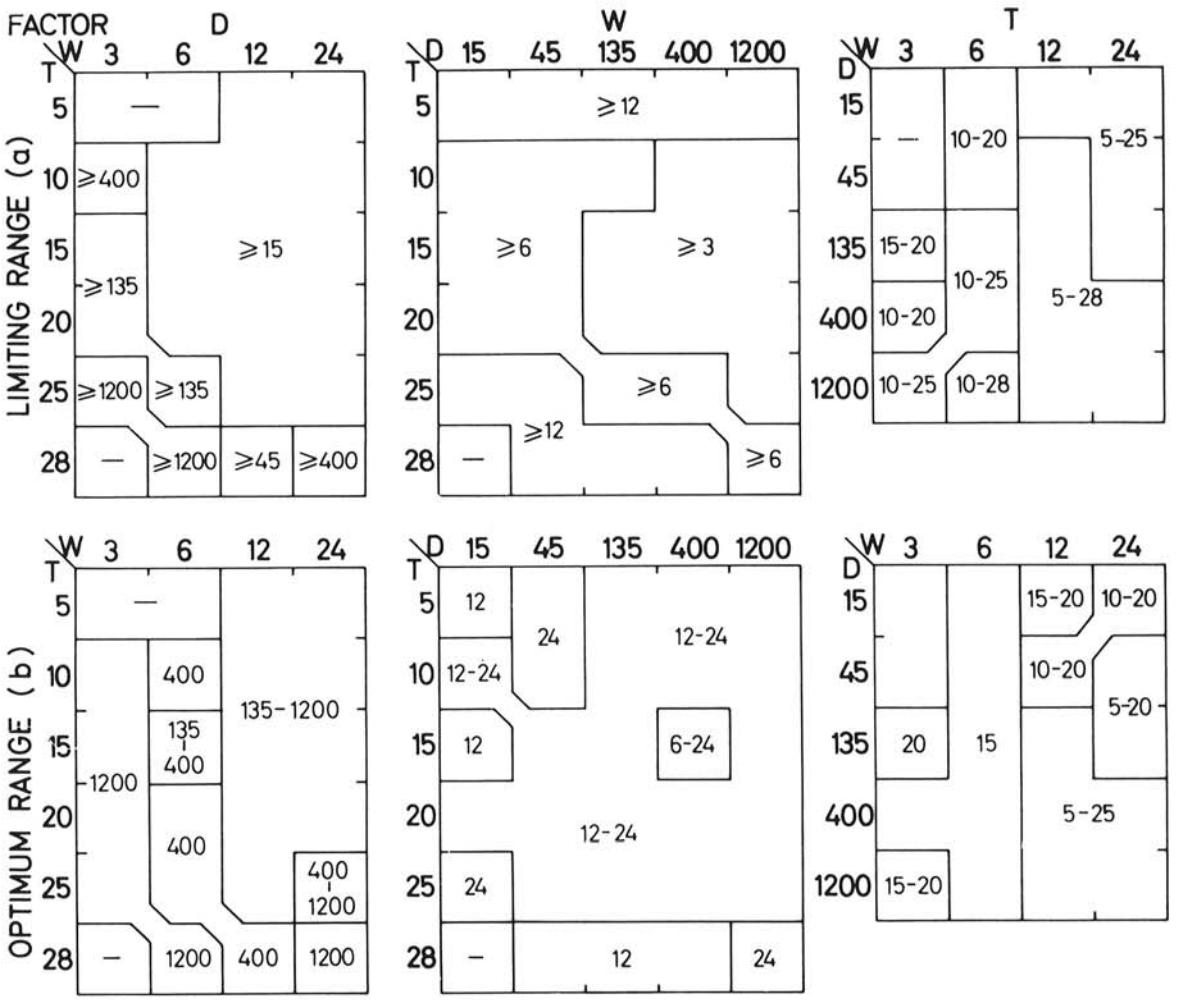

Fig. 2. The effect of temperature (T, in C), inoculum dose (D, in sporangia/cm^2), and duration of wet period (W, in hr) on infection of potato leaves by *Phytophthora infestans:* (a) the limiting range (experimental levels with which any infection at all was observed); and (b) optimum range (experimental levels with which infection was near-maximized) of each factor, for each combination of the other two factors.

The three subtables in the lower part of Fig. 2 show the opt range of each factor; this was defined as that range of the experimental factor levels for which the infection grade was within 15% of the highest level obtained with the same combination of the other two factors.

The min inoculum concn required for infection was found to depend on a balance of both temp and wetting duration (Fig. 2-D, a). Within the range of concn tested, no min was observed when both the temp and wetting duration levels were favorable, or when one of these levels was favorable and the other near-favorable. When either temp or wetting duration got further away from the favorable levels, progressively higher concn minima were observed.

The range of opt inoculum concn was found to depend primarily on wetting duration (Fig. 2-D, b). A broad opt range was observed after wetting periods of 24 or 12 hr; a narrow opt at 400 s.p.c. was found after 6 hr; and only the highest concn was opt after 3 hr. A secondary, and rather irregular, effect of temp was observed mainly at 28 C.

The minimum wetting duration required for infection was found to depend primarily on temp (Fig. 2-W, a). In general, when the temp was either too low or too high, no infection appeared after a wetting period of either 3 or 6 hr, regardless of inoculum concn. For medium temp, the secondary factor of concn came into play; viz, the min wetting period of 6 hr was observed with lower but not with higher concn.

The range of opt wetting durations was 12-24 hr in most cases (Fig. 2-W, b). The most consistent exceptions to this rule occurred at the highest temp (28 C), which invariably resulted in a narrower opt, usually at 12 hr. Similar effects occurred occasionally with the lowest concn and/or at the lowest temp. On the other hand, an unusually broad opt was observed with the combination of 15 C and 400 s.p.c.

The minimum temp required for infection was found to depend essentially on the wetting duration (Fig.

2-T, a). Within the range of temp tested, no min was observed after a wetting period of either 12 or 24 hr, and a min of 10 C was found after 6 hr, regardless of inoculum concn. After a wetting period of 3 hr, the infection, if any, was limited to traces for all levels of temp and concn (Fig. 1).

The maximum temp compatible with infection was found to depend on a balance of both inoculum concn (D) and wetting duration (W) (Fig. 2-T, a). When both the D-level and the W-level were high, or one of these levels was high and the other not too low, infection appeared even at the highest temperature tested. When either D or W decreased, progressively lower temp maxima were observed.

The opt range of temp was found to depend primarily on wetting duration (Fig. 2-T, b). After short wetting periods, a narrow opt (usually at 15 C) was observed. After long wetting periods, broad opt ranges were found, especially with high concn.

DISCUSSION.—The results of this study indicate clearly that the standard concepts of min, max, and opt levels of a factor affecting infection are relative rather than absolute, and may depend on other factors. In our particular case (*Phytophthora infestans* on potato leaves), this relativity was especially striking for the effect of temp. Depending on the levels of spore load and wetting duration, the minimum temp varied from below 5 C to above 10 C, the maximum temperature varied from below 25 C to above 28 C, and the opt temp range varied from a narrow span around 15 C to a broad span covering all the temp between 5 and 25 C.

The common feature of all the relativity effects was that both the limiting and the opt ranges of any given factor were broad for favorable combinations of the other two factors, and narrow for unfavorable combinations. This means that, while all three factors affect the initiation and intensity of infection, a favorable level of one factor or a favorable combination of two factors may compensate for a certain deficiency in the third factor. The detailed nature of the relativity effects varies from one case to another. Thus, the opt spore loads and temp, and the minimum temp, were found to depend primarily on wetting duration, while the min wetting duration was affected primarily by temp. The min spore load depended on a balance of temp and wetting duration, and the maximum temp depended on a balance of spore load and wetting duration.

Another interesting feature is that neither the highest spore load (1,200 s.p.c.) nor the longest wetting duration (24 hr) was always optimum. We are not aware of any theoretical explanation for this phenomenon, and can only speculate about possible reasons. The cases in which the highest spore load inhibited infection reflect either a direct effect of excess spore load or an inhibitory mechanism due to other factors which act only when the spore concn is high enough. Such a mechanism has been described in many fungi such as *Puccinia graminis* f. sp. *tritici* (1). The fact that in our experiments the inhibitory phenomenon was associated mainly with the wetting duration of 6 hr suggests that the mechanism involved operates only within a limited time span. The cases in which the longest wetting period inhibited infection may be due to improper gas exchange over plants enclosed in small plastic bags for a comparatively long time, and/or to the development of a microbial flora adverse to the fungus; the latter phenomenon was described by Leben (6). A similar inhibition of *Peronospora tabacina* on tobacco by long wetting has been observed elsewhere (Rotem & Cohen, *unpublished data*). Our finding that the inhibition by long wetting was associated mainly with the high temp of 28 C may be helpful in future attempts to elucidate the cause of this phenomenon.

The two inhibition phenomena discussed in the preceding paragraph are also relative; i.e., inhibition by one factor depends on the level of another factor. This gives additional weight to the main conclusion of our study, that the effect of any one factor on fungal infection depends on other factors. A finding such as the min, max, or opt level of a factor, an inhibitory effect due to too much of a good factor, etc., is likely to be valid only within a limited range of combinations of other factors, and is therefore fully meaningful only if the conditions of the experiment are explicitly stated. On the other hand, if it is found that a certain factor effect appears under some conditions and does not appear under other conditions, this may provide a clue to the interpretation of the effect. These considerations stress the importance of factorial studies in which the joint effect of several factors on fungal (or other) infection is studied in a systematic layout.

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