

Adaptation of Four Pathogens to Semi-Arid Habitats as Conditioned by Penetration Rate and Germinating Spore Survival

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ABSTRACT

The ability of some plant pathogens to cause disease epidemics in semi-arid habitats with short wet periods was studied. *Stemphylium botryosum* f. sp. *lycopersici* in tomatoes infected equally well after either a long wetting period or after several short, moist periods interrupted by dry ones; its germ tubes survived the interval between moisture periods. In *Phytophthora infestans*, rapid penetration of potato leaves compensated for sporangial sensitivity to

desiccation. Conidia of *Alternaria porri* f. sp. *solani* penetrated potato leaves rapidly and were drought resistant, although infection was higher after a continuous wet period than a few short ones. *Uromyces phaseoli* in beans develops in semi-arid habitats only in humid seasons because of its comparatively slow infection rate and low survivability between short wet periods.

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Wetting periods during the rainless season in Israel consist of ca. 6-8 h of dew and ca. 2-4 h of overhead irrigation applied once in 1-2 wk. Foliar disease epidemics under these conditions are caused by pathogens able to complete the processes of spore germination and infection within the available short moist periods. However, *Stemphylium botryosum* f. sp. *lycopersici* (R., C. & W.) needs 24-48 h of continuous moisture in the laboratory to cause severe infection in tomatoes (*Lycopersicon esculentum* L.) (1). This suggests that interrupted wet periods during spore germination and

infection may be sufficient, as reported for Sigatoka of bananas and purple leafspot of orchard grass (2, 4, 5), and not confirmed in cucumber anthracnose (6).

The purpose of this work was to elucidate mechanisms enabling utilization of short moist periods for infection by (a) *S. botryosum* f. sp. *lycopersici* in tomatoes, (b) *Alternaria porri* f. sp. *solani* Neerg. in potatoes (*Solanum tuberosum* L.), (c) *Phytophthora infestans* (Mont.) De Bary in potatoes, and (d) *Uromyces phaseoli* (Reb.) Wint., in beans (*Phaseolus vulgaris* L.).

MATERIALS AND METHODS.—Plants were

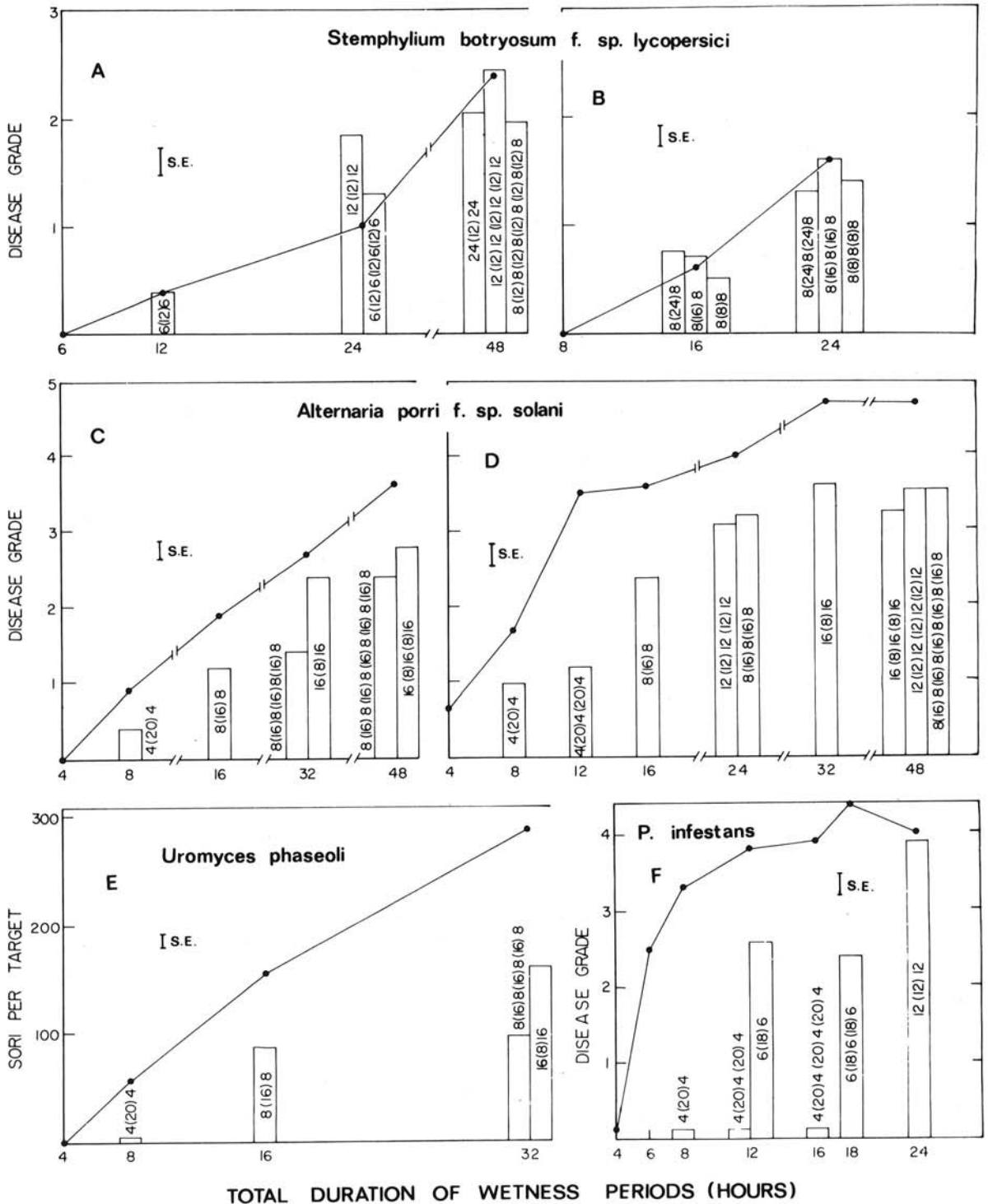


Fig. 1-(A to F). The effect of Continuous Wetting Periods (solid lines) and Interrupted Wetting Periods (bars) on infection of various hosts by their respective pathogens. The time of each wet and dry fraction in the IWP treatments is indicated within or alongside the respective bar, e.g., 6(12)6 means that 2 wet periods of 6 h each were interrupted by one dry period of 12 h. Average standard errors (S.E.) for all treatments are shown. **A and B**) Infection of tomatoes by *Stemphylium botryosum* f. sp. *lycopersici*. **C and D**) Infection of potatoes by *Alternaria porri* f. sp. *solani* at 15 C (C) and 25 C (D). **E**) Infection of beans by *Uromyces phaseoli*. **F**) Infection of potatoes by *Phytophthora infestans*.

grown in a greenhouse in 500-cc pots. Tomatoes (cv. Campbell 1327), potatoes (cv. Up-to-Date) and beans (cv. Bulgarian) were inoculated with their respective pathogens at the age of 10, 10, and 2 wk, respectively. Conidia of *S. botryosum* f. sp. *lycopersici* and *A. porri* f. sp. *solani* were obtained from culture on V-8 agar; sporangia and uredospores of *P. infestans* (race 0) and *U. phaseoli*, respectively, were obtained from previously infected plants. The test plants were inoculated on the underside of each leaf, using Schein's inoculator (10), applying in the case of *Stemphylium*, *Alternaria*, *Phytophthora*, and *Uromyces* spp. ca. 2,500, 1,200, 1,200, and 1,500 spores, respectively, on a target area of 4 cm². The inoculated plants were placed in humidity chambers (8) working on a pre-set program of continuous wetting periods (CWP) or interrupted wetting periods (IWP) as detailed in Results. Relative humidity (RH) in the dry periods of the IWP treatments was 40-45%. After exposure to a given treatment in the humidity chambers, the test plants were transferred to growth chambers (RH 50-60%) for the rest of the 8-day incubation period. The temp in the humidity and growth chambers was 20 ± 1 C except with *A. porri* f. sp. *solani*, which was tested at 25 and 15 ± 1 C. The photoperiod was 12 h and light intensity was ca. 12,482 lx (1,560 ft-c) in the growth chambers and ca. 12,374 lx (1,150 ft-c) in the humidity chambers. Diseases caused by *S. botryosum* f. sp. *lycopersici*, *A. porri* f. sp. *solani* and *P. infestans* were evaluated on a 0-5 scale of grades (0-no symptoms; 5-completely wilted leaves) (1, 9). Bean rust incidence was evaluated by counting the sori. All experiments were carried out with four replicate plants per treatment.

The effects of continuous and interrupted wetting periods on germination of *S. botryosum* f. sp. *lycopersici* and *A. porri* f. sp. *solani* in vitro was tested by placing conidia on cellophane over a moist filter paper in petri dishes at 20 ± 1 C. Unlike the time needed for infection, the time required for germination is short and the wetting periods were 2, 4, and 6 h as CWP; or two or three 2-h moist periods interrupted by 22-h dry intervals in the IWP treatments. During these intervals, the cellophane was dried in jars with P₂O₅ at various temp. The same method was unsuccessfully tried with *U. phaseoli* whose uredospores did not germinate. Sporangia of *P. infestans* were placed in drops of distilled water for either 2, 4, or 6 h (CWP), or for two or three 2-h periods interrupted by 22 h of exposure to 50-60% RH (IWP), all at 15 C. In the conidial species, results were evaluated by the percentage of germinating spores, and the length and number of germ-tubes. In *P. infestans*, the percentage of empty sporangia (zoospores released) was determined.

RESULTS.—Infection trials.—In general, similar infection levels by *S. botryosum* f. sp. *lycopersici* were induced by the IWP and CWP regimes, with equivalent total wetting durations (Fig. 1). In some experiments, however, IWP treatments resulted in either markedly lower or higher infection levels than comparable CWP treatments.

In the first experiment, CWP treatments of 6, 12, 24, and 48 h were compared with IWP treatments in which the total wetting duration of 12-48 h was interrupted by varying dry periods. Infection levels increased from zero at CWP of 6 h to a maximum of ca. 2.5 at CWP of 48 h.

With the IWP regime, two wet periods of 6 h each, interrupted by a 12-h dry period resulted in the same infection level as that obtained with a 12-h CWP. The 24-h CWP resulted in a lower infection level than its two IWP equivalents, whereas the 48-h CWP resulted in an infection level which was similar to, or somewhat higher than, those obtained with its three IWP equivalents (Fig. 1-A).

In the other experiment, two or three 8-h wetting periods, separated by dry intervals of 8, 16, or 24 h induced only slightly different levels of infection from those induced by the CWP (Fig. 1-B).

To test whether the action of IWP treatments on infection is exerted via their direct effect on conidia on the leaf surface, attempts were made to kill the conidia with a fungicide (Maneb 0.1%) after exposure of the test plants to the first wet period of IWP. It was found that the second wetting period of IWP did not increase disease incidence over the level induced by the first wet period.

With *A. porri* f. sp. *solani*, considerable infection resulted from a continuous wetting period as short as 8 h at 15 C and 4 h at 25 C. At 15 C, any increase in the CWP resulted in an increase in infection level. At 25 C increase in the infection level was not observed after the CWP extended beyond 12 h.

Infection levels induced by IWP treatments were lower than those induced by equivalent CWP treatments regardless of incubation temp and total duration of wetness. Each additional wetting period in the IWP treatments resulted in a small, but approximately constant, increase in infection level (Fig. 1-C, D).

The shortest CWP which resulted in bean infection by *U. phaseoli* was 8 h. IWP treatments with shorter wetting periods resulted in negligible infection in comparison with the equivalent CWP treatments (Fig. 1-E).

In the case of *P. infestans*, IWP regimes were ineffectual; whatever the number of separate wetting periods, the resulting effect was similar to that induced by the first such wetting period alone (Fig. 1-F). In another trial, the wet and dry fractions of the IWP treatments were 3-h-long. CWP treatment of 3 h, as well as IWP treatments composed of two and three 3-h wet periods resulted in traces of infection. CWP treatments of 6 and 9 h resulted in appreciable infection of grades 1.4 and 2.7, respectively.

Germination of spores in vitro.—In *S. botryosum* f. sp. *lycopersici* and *A. porri* f. sp. *solani* 96-98% of the conidia germinated after only 2 h exposure to wetness. In *S. botryosum* f. sp. *lycopersici*, the number of germ tubes/conidium (averaged over all treatments) after the first, second, and third period of wetness amounted to 2.1, 3.4, and 3.8, respectively; the corresponding figures for *A. porri* f. sp. *solani* were 1.6, 2.8, and 3.1, respectively.

Germ-tube elongation in the interrupted wetting treatments depended on the temp of the dry intervals. It was greatest following exposure to dryness at 20 C (the lowest temp tested), less at 50 C, and zero at 80 C. Germ-tube development in *S. botryosum* f. sp. *lycopersici* exposed to the CWP treatments was about equal to that obtained with the IWP treatment exposed during the dry intervals to 20 C. Germ-tube development in *A. porri* f. sp. *solani* was better in the CWP than in IWP treatments (Fig. 2-A, B).

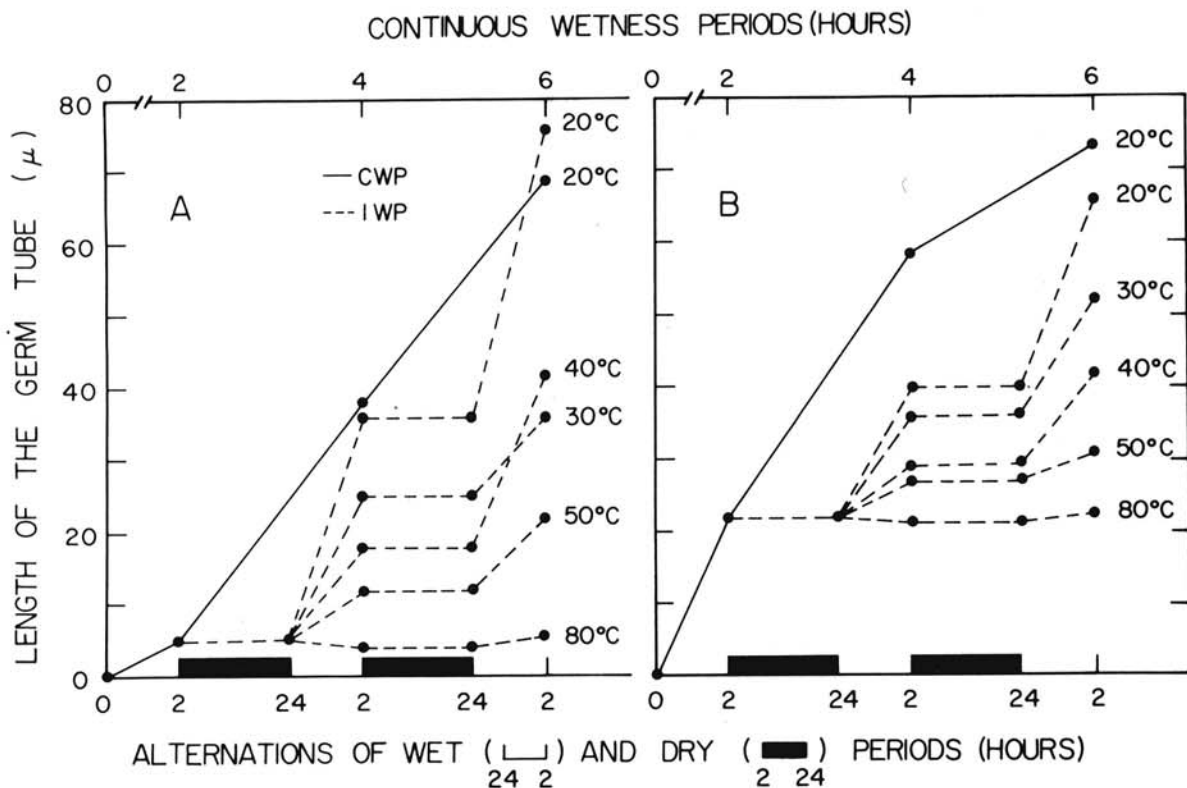


Fig. 2-(A to B). The effect of Continuous Wetting Periods (CWP) and of Interrupted Wetting Periods (IWP) on elongation of germ tubes in A) *Stemphylium botryosum* f. sp. *lycopersici* and B) *Alternaria porri* f. sp. *solani*. CWP treatments were carried out at 20°C. IWP treatments consisted of three wet periods of 2 h each at 20°C alternating with two dry intervals of 22 h each at 20-80°C. Averages of 60 and 30 conidia for *A. porri* f. sp. *solani* and *S. botryosum* f. sp. *lycopersici*, respectively.

Sporangia of *P. infestans* exposed to dry air after the first wetting period failed to release zoospores when returned to moist conditions. Test plant inoculations showed that these sporangia had lost their infectivity.

DISCUSSION.—In semi-arid climates, short wetting periods occur in the rainless season. Under these conditions successful infection by fungal pathogens requires either rapid germination and penetration, or the ability of the germinating spores to survive dry periods and resume growth when rewet. This survivability of germinating spores has been investigated in vitro for a number of species (3), but their ability to cause infection after interrupted wetting periods was not tested. In contrast, in vivo studies of infection following alternate wetting and drying (2, 4, 5) were not accompanied by tests of spore survivability. In our experiments, germination and penetration rates and the use of interrupted wetting periods were investigated in vitro and in vivo.

As in other multi-factor phenomena (1, 9), a favorable level of one factor (e.g., penetration rate) can compensate for deficiencies in the other (e.g., survivability in dryness). *S. botryosum* f. sp. *lycopersici* is a pathogen which requires long wetting periods to penetrate, but succeeds in semi-arid climates because its germinating spores survive dry periods. *P. infestans* sporangia have poor tolerance to drying but the pathogen succeeds in semi-arid climates because of its high germination and penetration rate.

With some pathogens (e.g., *A. porri* f. sp. *solani*) both abilities may be present simultaneously. *U. phaseoli* pathogenesis was not initiated when wetting periods were interrupted; this explains why under Israeli semi-arid conditions bean rust epidemics are restricted to late fall and winter when dew periods are long or are supplemented by rain.

We were not able to evaluate in vitro survivability of germinating spores of *U. phaseoli*. Its dormant spores remain viable for a long time (11) but survivability of dormant and germinating spores may not be correlated.

Both *S. botryosum* f. sp. *lycopersici* and *A. porri* f. sp. *solani* could maintain infectivity during interrupted wetting periods. With the former, infection proceeded equally well during continuous wet conditions, or with an equivalent duration of wet periods interrupted by dry ones. *A. porri* f. sp. *solani* infections were less severe when the germinating spores were dried, even though the spores survived well [Fig. 2 and (7)] and the moisture periods were equivalent to those in the constantly wet treatments. This suggests that the use of interrupted wet periods is dependent also on factors other than survivability. These may be defense reactions of the host initiated during the first wet period which interfere with penetration of germ tubes during the following wet periods.

We suppose that the phenomena described here are common. Organisms like *Cercospora beticola* which

require long wetting periods for infection (12) probably must survive intermittent drying. Determination of the interrupted wetting periods enabling infection by pathogens may improve forecasting and simulation of epidemics, especially in areas where moisture periods are short.

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