Sporulation of Stemphylium botryosum f. sp. lycopersici in Tomatoes and of Alternaria porri f. sp. solani in Potatoes Under Alternating Wet-Dry Regimes

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ABSTRACT

Long periods of wetness are required for sporulation of *Stemphylium botryosum* f. sp. *lycopersici* in tomatoes and of *Alternaria porri* f. sp. *solani* in potatoes. Several short wet periods interrupted by dry ones were able to replace long wet periods. Under these conditions, sporulation of *S. botryosum* f. sp. *lycopersici* was somewhat lower, and of *A. porri* f. sp. *solani* much higher, than under continuous periods of equivalent wetting duration. Both pathogens produced more spores when the wet period was extended from 8 to 16 hours per night and the night/day temperature was elevated from 10/20 to 15/25 or to 20/30 C. In *A. porri* f. sp. *solani* only some of the spores formed each night dispersed during the following day. The number of spores produced each night increased during a week-long period.

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Pathogens which need long periods of wetness for infection may cause diseases after several short, moist incubation periods interrupted by dry ones (1). The effect of the latter phenomenon upon sporulation has not been tested. We found that *Stemphylium botryosum* f. sp. *lycopersici* R. C. & W. in tomatoes, and *Alternaria porri* (Ell.) Neerg. f. sp. *solani* Ell. & Martin in potatoes sporulate in the laboratory after approximately 16 hours of wetness; in the field they sporulate under nightly wettings as short as 8 hours each (7, and Bashi and Rotem, *unpublished*). The purpose of this work was to check under controlled conditions whether a long continuous wet period (CWP) needed for sporulation of both pathogens in the laboratory can be replaced by several short interrupted wet periods (IWP), and how sporulation under these wetting regimes is affected by some temperature regimes.

MATERIALS AND METHODS.—Potato plants (*Solanum tuberosum* L. 'Up-to-Date'), two months old, were inoculated with *A. porri* f. sp. *solani* on the underside of fully grown leaves. Inoculation was made with a Schein inoculator (9), with approximately 700 spores on a target area of 4 cm^2. The inoculated plants were kept for 24 hours in a moist chamber, and then for 8 days in a growth chamber, both at 25 C. In these and other experiments the actual temperatures differed from the set ones by a maximum of 0.5-1.0 C.

Tomatoes (*Lycopersicon esculentum* L. 'Campbell 1327') were sprayed with a spore suspension of *S. botryosum* f. sp. *lycopersici* and kept for 2-3 days in a moist chamber, and then for 8-10 days in a growth chamber, both at 25 C.

Sporulation tests with both pathogens were done with necrotic leaves possessing the highest sporulating

potential (3). Detached leaves were placed between two 30 × 30 cm squares of plastic screening (with 4 × 8 mm holes) held together with paper clips. In the CWP treatments, screens were sprayed with water and placed in an upright position (for drainage of excess water) in moist chambers. These were exposed to given temperature conditions in darkness. In experiments with *A. porri* f. sp. *solani* leaves to be exposed to CWP were first kept in moist chambers at 28 C for 24 hours in darkness for production of conidiophores without spores; then exposed to dryness and irradiated for 8 hours with fluorescent light of 16,786 lux for stimulation of sporulation (Bashi, *unpublished*).

In the IWP treatments, the screens were subjected to night-day variations of wetting, light, and temperature. The night conditions were as described for CWP treatments; i.e., dark and wet. The night temperatures were 10 C lower than the day ones. For the day periods the screens were removed from moist chambers and exposed to 50-60% relative humidity and 16,786 lux of light. Drying and wetting of screens at the beginning of every day and night, respectively, lasted for six night-day periods. In one series of experiments the screens were exposed for the day-time to windless conditions and were rewetted with fine particles of water to avoid detachment of spores. The purpose of this test was to determine the daily addition of spores without losing those spores which had been formed previously. Spore formation on leaves free of previously formed spores was the purpose of another test done with *A. porri* f. sp. *solani* only. The screens were exposed to a relatively strong wind during the daytime and were roughly sprayed with big drops of water for the wetting at night. In the "windy" test screens for evaluation were taken after each night (total present)

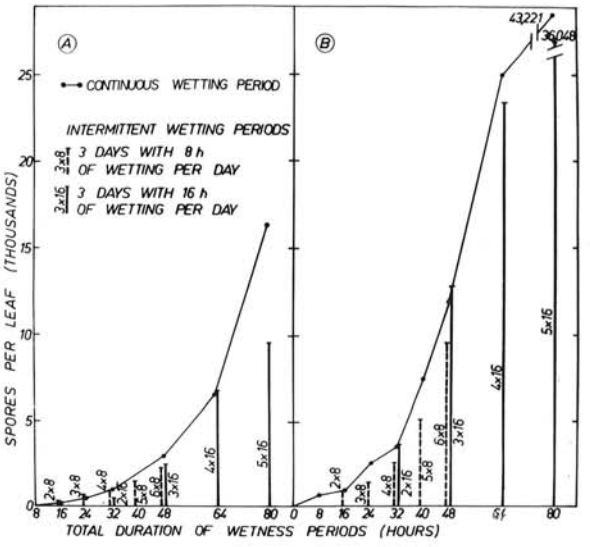

Fig. 1-(A,B). The effect of CWP (continuous wet periods, lines) and IWP (interrupted wet periods, bars) on sporulation of *Stemphylium botryosum* f. sp. *lycopersici* in tomatoes, under conditions unfavorable for spore dispersal. The figures on the abscissa are the total numbers of wet hours, either continuous or interrupted by dry periods. In the IWP treatments, the number of wet periods (2 to 5) and the duration of each (8 or 16 hours) is written near each bar. The duration of each dry period was that required to complete a 24-hour cycle. Night-day temperatures were of **A)** 10 to 20 C, and **B)** 20 to 30 C. Standard errors in various treatments ranged from 11 to 18% of the averages. The figures on the right top in Fig. 1-B indicate the number of spores which exceeded the scale.

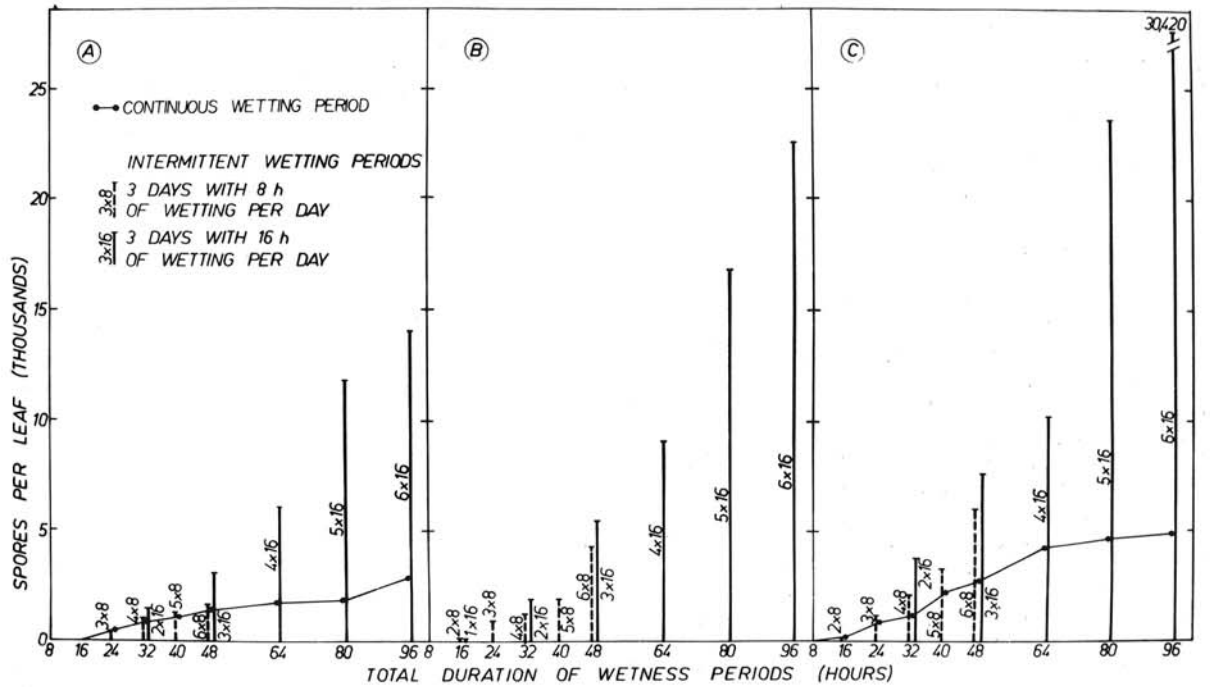

Fig. 2-(A to C). The effect of continuous (CWP, lines) and interrupted (IWP, bars) wet periods on sporulation of *Alternaria porri* f. sp. *solani* under conditions unfavorable for spore dispersal, under night-day temperatures of **A)** 10 to 20 C, **B)** 15 to 25 C (IWP only), and **C)** 20 to 30 C. Standard errors ranged from 9 to 17% of the averages.

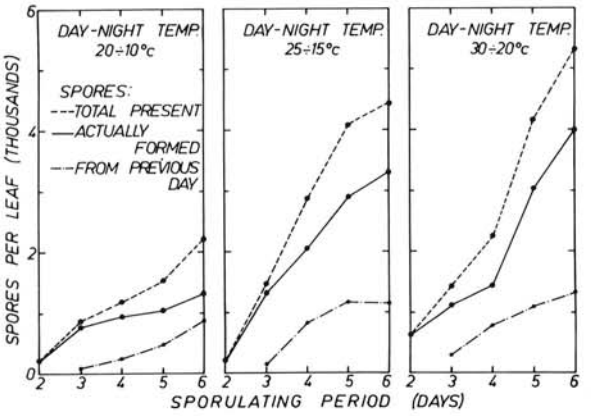

Fig. 3. The effect of IWP on spore production by and dispersal of *Alternaria porri* f. sp. *solani* during a 6-day period. The nights (wet) and days (dry and windy) were 12 hours long. Standard errors ranged from 8 to 19% of the averages.

and day (spores which failed to disperse). The number of spores produced was estimated by subtracting the number which remained on the leaf surface after the day period of dispersal from the number present after the following night period.

In the various tests, the night periods were 8, 12, and 16 hours. The night/day temperature regimes were 10/20, 15/25, and 20/30 C for night/day temperatures, respectively. All tests were made with five replicates of eight leaves each, kept under the same regime.

Separation and counting of spores were done by the filtration method (3). Leaves were first shaken for 1 hour in formalin, acetic acid, alcohol (5:5:90, v:v:v) to detach the spores. It was found that this treatment did not detach the spores used for inoculation. A known volume of the spore suspension was filtered onto a 8-μm Millipore filter which was then examined microscopically. This method was found easier and more accurate than counting the spores with the aid of a cytometer.

RESULTS.—*Sporulation without daily dispersal.*—At 10 C, a continuous wetting period of a minimum of 16 hours was needed for some sporulation of *S. botryosum* f. sp. *lycopersici* to occur. The number of spores produced increased with prolongation of the CWP. In the IWP treatments, two, three, and four wet periods at 10 C and dark, separated by 16-hour dry periods at 20 C and light, provided for similar amounts of spores produced under CWP = 16, 24, and 32 hours, respectively (Fig. 1-A). The IWP treatments of 5×8 and 6×8 (five or six wet periods of 8 hours each, separated by dry periods of 16 hours each), resulted in less sporulation than the equivalent CWP treatments of 40 and 48 hours, respectively. Prolongation of the wet period at night (10 C) to 16 hours, and shortening of the dry period at day (20 C) to 8 hours, provided for better sporulation than equivalent treatments with a short night and a long day. The number of spores formed under long night and short day conditions in the IWP treatments was similar to, or lower than, in the CWP treatments with equivalent wetting duration. The greatest difference occurred between CWP = 80 hours and IWP = 5×16 hours (Fig. 1-A).

Sporulation was affected similarly by night/day temperatures of 20/30 C (night either 8 or 16 hours long). The quantities of spores produced under these conditions exceeded greatly those formed under a night/day temperature regime of 10/20 C (Fig. 1-B).

Because of a 24-hour prewetting of leaves with *A. porri* f. sp. *solani,* the CWP were actually longer than noted in Fig. 2 and the text. Nevertheless, sporulation of *A. porri* f. sp. *solani* was always higher under IWP than CWP treatments of equivalent wetting duration (Fig. 2-A, B, C). The difference in sporulation between equivalent CWP and IWP treatments increased with an increase in the total wetting duration. For instance, at night/day temperatures of 20/30 C, similar amounts of spores were produced at CWP = 16 hours and at IWP = 2×8 hours. However, IWP = 6×8 hours resulted in almost twice as much sporulation as CWP = 48 hours (Fig. 2-A); Likewise IWP of 2×16 hours more than doubled the amount of spores produced under CWP = 32 hours, but IWP of 5×16 hours resulted in approximately five times as many spores as produced under CWP = 96 hours (Fig. 2-C).

Sporulation with daily dispersal.—Despite efforts made to promote detachment of spores formed during each preceding night, many of the spores remained attached to leaves at the end of each windy and dry day. No spores appeared after the first night. The patterns of further sporulation, under three temperature regimes, are presented in Fig. 3. The lower curves in each figure show the number of spores which failed to disperse during each daytime period. The upper curves represent the number of spores present at the termination of each wet period; i.e., those formed during the last night and those which failed to disperse during the previous day or days. The middle curve represents the number of spores actually formed each night; i.e., the total number represented by the upper curve, minus the number of undispersed spores.

As in the former tests, the lowest and highest sporulation was at night/day temperature regimes of 10/20 and 20/30 C, respectively. In each temperature regime, the number of spores actually formed each night (middle curves) increased from the first to the sixth night. The lack of a tendency toward a decrease in sporulation, indicated that the sporulating potential exceeded the six-night experimental period (Fig. 3).

DISCUSSION.—The data presented show that sporulation of *S. botryosum* f. sp. *lycopersici* and *A. porri* f. sp. *solani* in vivo requires a longer wetting period than that provided during one dewy night. Sporulation is then facilitated by IWP regimes for several (at least two) nights. The reaction of the two pathogens to IWP differs. Sporulation of *A. porri* f. sp. *solani* was much higher under IWP regimes than under continuous wetting during which the infected leaves were kept in dark. On the other hand, *S. botryosum* f. sp. *lycopersici* produces somewhat less spores under IWP. This means that this pathogen can tolerate, but not benefit from, IWP. It can be explained by the fact that sporulation of *S. botryosum* f. sp. *lycopersici* in vivo does not require stimulation by daytime conditions, whereas that of *A. porri* f. sp. *solani* does (2).

The number of *A. porri* f. sp. *solani* spores present after each dark and wet period (night) increased from the first until the sixth night. In addition to the actual sporulation,

this was due in part to accumulation of previously produced spores which did not disperse during the previous dry day. A similar phenomenon was deduced from patterns of spore dispersal in the field (6). We lack data for how many additional nights sporulation would proceed on any single leaf, but field observations indicated a period longer than a fortnight (6).

Most studies of the environmental effects on sporulation were done in vitro. Sporulation patterns in vivo may be different, as was shown for *Helminthosporium maydis* in corn (11). Plant pathogens of the *Peronosporales,* like *Pseudoperonospora cubensis* in cucumbers, *Peronospora tabacina* in tobacco, *Plasmopara viticola* in grapes, and *Phytophthora infestans* in potatoes, terminate spore production within one night (4, 5, 12, and Bashi and Rotem, *unpublished*). Some facultative parasites; e.g. *Rhynchosporium secalis* and *Septoria nodorum,* sporulate in vivo under alternating wetting and drying applied in a cycle of a few days (8, 10). This indicated that a long intermittent drying did not interfere with production of additional spores after the initially formed spores were dispersed, but did not prove that sporulation of these pathogens occurs after several short wettings.

Despite lack of data, we suppose that sporulation under IWP regimes is common to many pathogens. Determination of the interrupted wet periods enabling sporulation of various fungi under various temperature regimes is essential for a better understanding of epidemiology, especially in areas where moisture periods are short. Such studies may be valuable for improving simulation of epidemics.

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