Rate of Lesion Development in Relation to Sporulating potential of Pseudoperonospora cubensis in cucumber

Y. Cohen and J. Rotem

The Volcani Institute of Agricultural Research, Bet Dagan, Israel.

Contribution from the Division of Plant Pathology, The Volcani Institute of Agricultural Research, 1970 Series, No. 1736-E; and Department of Life Sciences, Bar-Ilan University, Ramat Gan, Israel. Supported by USDA Grant No. FG-IS-231.

Accepted for publication 9 September 1970.

ABSTRACT

Increased rate of development of lesions caused by *Pseudoperonospora cubensis* in cucumbers was associated with a low sporulating potential of the pathogen. In plants incubated at different temperatures shortly after inoculation, the quickest necrotization occurred at 25 C. In chlorotic lesions already formed, however, the conditions enhancing quickest necrotization were 40 C and darkness. Sporulating potential was highest when plants were incubated at 15 continuously or at 20:15 C day:night thermoperiods, i.e., at temperatures favorable for development of chlorotic lesions. Low temperature regimes delayed appearance and necrotization of lesions, but extended the period of sporangium productivity and increased total sporangium production. Increases in light intensities and in the length of daily photoperiods were associated with an increase in the sporulating potential.

In relation to epidemiology, a distinction is suggested between direct field damage caused by a pathogen and the danger of its spread to neighboring areas. Potential for spread reaches a peak before maximum crop damage occurs. Phytopathology 61:265-268.

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

Decreased sporulation of *Pseudoperonospora cubensis* is associated with necrotization of host tissue in cucumbers (1), but little is known regarding factors affecting the rate of necrotization. It is possible that environmental conditions may exert different effects on lesion formation, and on the rate and duration of spore productivity. In semiarid climates where spells of dry weather may prevent sporulation during a number of sequential nights, the ability of lesions to remain potentially "sporulative" until the weather returns to "normal" may be critical to continuation of the epidemic. Consequently, the influence of lesion condition on the sporulating potential of *Pseudoperonospora cubensis* (Berk. & Curt.) Rost. on cucumbers (*Cucumis sativus* L. 'Bet-Alpha') was studied.

MATERIALS AND METHODS.—Cucumbers were grown in an air-conditioned greenhouse in 0.3-kg pots filled with a 2:2:1:1 sterilized mixture of sandy loam, peat, sand, and vermiculite. Three-week-old plants were inoculated with a sporangial suspension by a Schein inoculator (7), on a 4-cm^2 target, on the lower leaf side with 600 + 15% sporangia/target, and one target/leaf. All plants were kept for 6 hr in a moist chamber at 20 C, then transferred to growth chambers. These methods provided infection of all the inoculated plants.

Temperatures were measured by thermocouples clipped to the leaves, and deviated from the averages in the growth chambers by 0.25 and 0.5 C in the 12-hr dark and light photoperiods, respectively. For illumination, fluorescent VHO, G.E., Gro-Lux (Sylvania), and incandescent lamps were used. Intensities were measured by an Apply perheliometer and expressed in μW/cm^2. In experiments in which light was not a variable, a standard light intensity of 3,069 μw/cm^2 was used. Disease incidence was assessed visually according to the following considerations. The area of *P. cubensis* lesions extends only slightly beyond the inoculated target, but often includes uninfected green patches which usually disappear with further development of the disease. The color of the infected sites changes gradually from green to yellowish-green, then to chlorotic-yellow, and finally to necrotic-brown. These steps in discoloration, as well as the proportion of the discolored area inside the inoculated target, were graded on a 0-5 scale. The yellowish-green lesions were graded from 0.1 to 1.5; the max grade of chlorotic-yellow lesions was 2.5; and of the necrotic lesions, 5.0. The sporulating potential of the pathogen was determined as follows: after a light period of exactly 8 hr, plants were placed for 24 hr in a dark, 20-C moist chamber. The sporangia produced on the infected area were removed by cutting up the infected tissue and shaking it for 10 min in 2 cc FAA (formalin:acetic acid:alcohol, 5:5:90, v/v). The sporangia were then counted using a cytometer.

Variations of the above methods were used in one experiment and are described in the RESULTS section.

In all experiments, the average disease or sporulation value from two leaves of one plant was considered as one replication. Results were analyzed with the aid of a C.D.C. 3600 computer, using either the one-way multiple range test, or the two-way analysis of variance for factorial design (4). In all cases, significant differences in results are presented at the 95% level.

RESULTS.—The most rapid lesion development at constant temp occurred at 25 C for all incubation periods. After incubation of 3 days, it differed significantly from the 20-C treatment; after 5 days, from the 10, 15 and 20 C; after 8 days, from the 10, 15 and 30 C; and after 10 days, from the 10 and 15 C treatments (Fig. 1-A). Lengthening the incubation period reduced the temp level most favorable for sporulation from 25-30 C to 15 C. After incubation of 8 days, only the values from the 15-C treatment differed significantly from those of the other treatments (Fig. 1-B).

Similar phenomena were then studied under varying

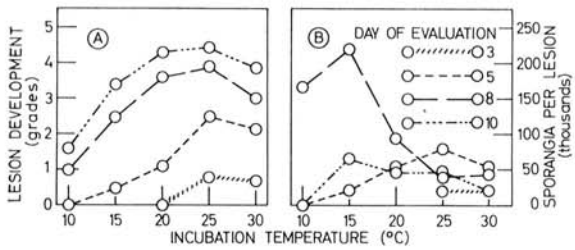

Fig. 1. Effect of constant temp during incubation on development of lesions (A) and on sporulating potential (B) of *Pseudoperonospora cubensis* in cucumbers. (Avg. from 15 replicates)

night:day temp. At 10-C night temp, max lesion development and sporulating potential occurred at the 30-C day treatment after incubation of 3 days, and at the 25-C day temp after longer periods of incubation. For lesion development, 25-C data differed significantly from the 15-, 30-, and 35-C treatments, and for sporulation, data differed from all other treatments (Fig. 2-A). Under incubation at 15 and 20 C by night alternating with various day temp, peaks of lesion development occurred at 25-C day temp; this was significant only for incubation of 4 days at 15-C night temp. Under the same conditions, 20-C coincided with the peaks of sporulation significant for all but the 20-C night:25 C-day treatment incubated for 4 days (Fig. 2-C, D).

The effect of light intensity was more pronounced on sporulating potential than on lesion development. At the highest light intensity, it differed significantly from all other treatments except sporulation at the second-highest light intensity (Fig. 3-A). From Fig. 3-B, it is evident that varying both light and temp affected the tested phenomena, but sporulating potential responded more to light intensity than to temp. At 3,069 $\mu W/cm^2$, the sporulating potential followed a curve sharply contrasting with that of lesion development, being significantly highest at 15 C and lowest at 25 C. Data in Fig. 3-C show that the most rapid development of lesions and the lowest sporulating potential were associated with a photoperiod of 4 hr, while max sporulation and intermediate lesion development occurred with a photoperiod of 20 hr. These values differed significantly from others.

The role of light and darkness in the process of necrosis was tested after infected plants were kept for 5 days at 20 C, until yellow lesions without any trace of necrosis had developed. Eight plants/treatment were then exposed for 24 hr to different temp under continuous light or in darkness. The percentage of lesion area which became necrotic, in plants exposed to light at 10, 20, 30 and 40 C, was 11, 8, 6, and 37%, respectively. Necrosis on plants exposed to the same temp, but in darkness, was much higher: 17, 20, 27, and 67%, respectively. Only data obtained at 40 C (in both light and dark) were significantly different from the other temp, with the dark treatment also significantly higher than the light one. In this test, sporulation was not tested.

Sporulating potential and the number of days spo-

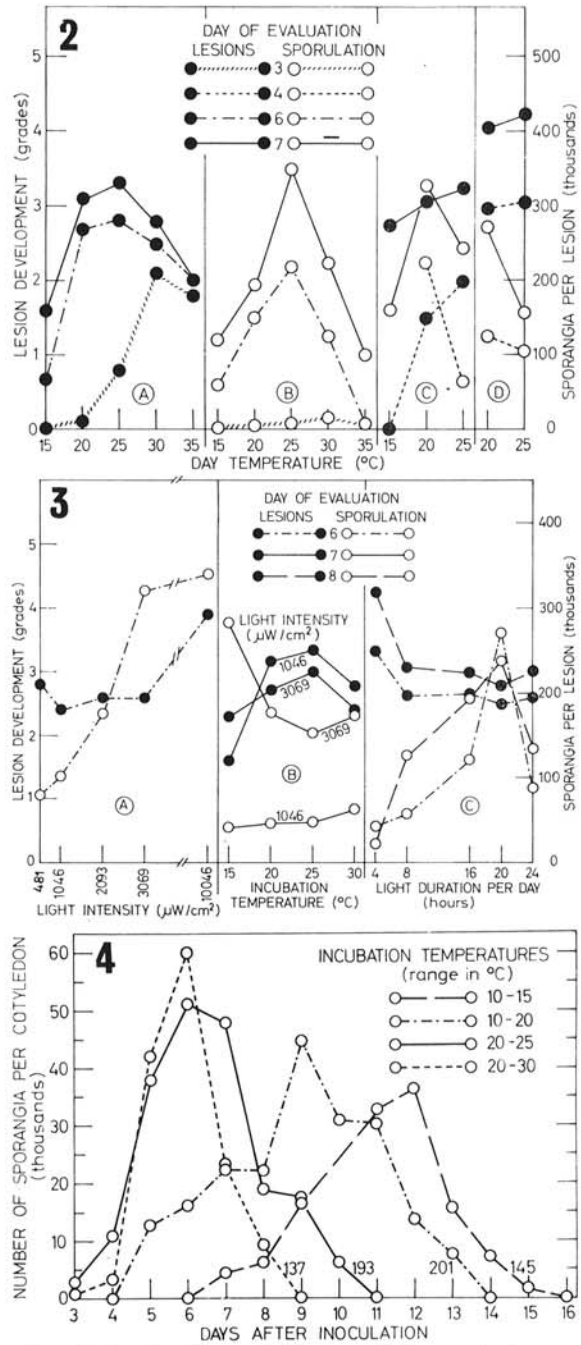

Fig. 2-4. **2)** Effect of thermoperiods on development of lesions (A, C, D) and on sporulating potential (B, C, D) of *Pseudoperonospora cubensis* in cucumbers. Day temp are noted in the abscissa. Night temp were 10 C (A & B), 15 C (C), and 20 C (D). (Avg from eight replicates) **3)** Effects of various light intensities at 20 C (A); two light intensities at various temp (B); and one light intensity at 20 C but for different light periods (C) on development of lesions and sporulating potential of *Pseudoperonospora cubensis* in cucumbers. (Avg for A, B & C are eight, five, and six replicates, respectively) **4)** Sporangial productivity of *Pseudoperonospora cubensis* in cucumbers as a function of incubation temp and time. Figures at the low point of each curve indicate the total number of sporangia (in thousands) produced in each treatment.

rangia are produced over leaves incubated at various temp were determined on plants in the cotyledon stage. The cotyledons were inoculated with 6-mm diam discs of filter paper soaked in a suspension with 110 sporangia/cc and placed on the lower side of each leaf. After a 6-hr moist period, the inoculated plants were placed in growth chambers working on cycles of min: max temp of 10:15, 10:20, 20:25, and 20:30 C, respectively. During the 12-hr period, relative humidity in all chambers was maintained at 55-65%. At the beginning of every night period, the plants were atomized with water and enclosed in moist plastic bags for sporulation. Removal of these bags in the morning was followed by dispersal of some of the produced sporangia in the growth chamber. Evaluation of production was performed before this dispersal started and after it terminated, as follows: Each morning and evening 20 cotyledons were detached from the infected plants and checked for sporangia. The number of sporangia found each morning reflected the production during the moist night-time, but included also sporangia which remained attached to the leaves after previous daytime dispersal. The difference between the number of sporangia found in the morning and the number found on the previous evening reflected the actual productivity of the pathogen at the given conditions of incubation and leaf age. In this experiment, disease incidence of the cotyledons was not assessed.

Figure 4 illustrates the sporulating potential of *P. cubensis* on plants incubated under various regimes of temp. High temp, with min:max of 20:25 C and 20:30 C, were associated with early beginning but also with early termination of sporangial production. The start of sporangia production was comparatively delayed, but lasted for a longer period when the infected plants were incubated at lower temp, with min:max of 10:15 and 10:20 C. In spite of the prolonged period of productivity, the total number of sporangia produced at a min:max of 10:15 C was comparatively small. The highest total number of sporangia per leaf was produced during a period of 10 days under incubation at min:max of 10:20 C.

The relation between necrotization and sporulation in this experiment was similar to that in previous tests. High temp regimes with min:max of 20:25 and 20:30 C accelerated both the first appearance and the final necrotization of lesions. The occurrence of both phenomena was delayed at low temp regimes.

Discussion.—We assume that the development of lesions depends in the beginning on the development of the fungal body inside the infected tissue; viz, colonization. We do not know the optimal temp for colonization but, following the case of *Peronospora tabacina* on tobacco (6), we assume that it is closer to the opt for sporulation than to that of lesion development, i.e., a temp of 15-20 C (1). Necrosis which terminates lesion development develops best at extreme conditions such as 40 C, low light intensity, or even darkness; i.e., conditions not likely to promote the development of mycelium. The fact that lesion development is also advanced by a high intensity of light (Fig. 3) may possibly be explained by the specific conditions in the growth chambers where high light intensities are characteristically associated with the appearance of various symptoms (3). In relation to continuous incubation at one temp, the opt effects on lesion development exerted by 25 C (Fig. 1) represent, in all likelihood, a "compromise" between the slightly lower temp favoring colonization and the slightly higher ones favoring symptom expression. A similar phenomenon was described in the case of *Peronospora tabacina* on tobacco (6).

The sporulating potential is also obviously related to a sufficient degree of previous colonization. Consequently, temp which are too low for colonization also reduce the sporulating potential. This was seen in the experiment in which the greatest number of sporangia was produced at a temp regime in the range of 10-20 C and not at 10-15 C, which, although delaying necrotization of lesions, was too low for a good colonization of the pathogen (Fig. 4).

The positive response of the sporulating potential to increased light intensities and periods is explained rather by the prerequisite effect of light on sporulation (2) than by the effect of light on lesion development.

The almost inverse relationship between the sporulating potential of the pathogen and the development of lesions may be explained by some or all of the following considerations: (i) A high temp favorable for the final development of lesions is less favorable for the growth of the fungus; (ii) necrotic lesions fail to supply the amount of food required to support sporulation; (iii) materials toxic to the pathogen, such as ammonia (5), may develop in necrotic tissues.

The practical implications of the phenomena described in this paper are as follows: (i) In phytopathological research, development of symptoms has often been used as an indicator of pathogen development; the results of some of the studies based on such criteria may have to be reconsidered; (ii) under field conditions, a distinction should be made between the amount of damage some diseases are causing to the crop at a given time and the potential for subsequent spread of the disease. While high temp may accentuate damage to already infected tissues, they should reduce, if only temporarily, the danger of infection to healthy plants. On the other hand, fields in which most of the infected tissue has not yet turned necrotic may be regarded as the most highly infectious and a source of the greatest danger to neighboring healthy fields. This thesis is supported by observations of the epidemiological patterns of late blight in potatoes (*unpublished data*), and by patterns of spore dispersal of *P. cubensis* over cucumber fields (*unpublished data*).

LITERATURE CITED

1. Cohen, Y., & J. Rotem. 1969. The effects of lesion development, air temperature and duration of moist periods on sporulation of Pseudoperonospora cubensis in cucumbers. Israel J. Bot. 18:135-140.
2. Cohen, Y., & J. Rotem. 1970. The relationship of sporulation to photosynthesis in some obligatory and facultative parasites. Phytopathology 60:1600-1604.
3. Dimock, A. W. 1967. Controlled environment in relation to plant disease research. Annu. Rev. Phytopathol. 5:265-284.

4. DIXON, W. J. [ed.]. 1967. Biomedical computer programs. Univ. Calif. Press., [2nd ed.] 600 p.

5. LOVREKOVICH, L., H. LOVREKOVICH, & R. N. GOODMAN. 1969. The role of ammonia in wildfire disease of tobacco caused by Pseudomonas tabaci. Phytopathology 59:1713-1716.

6. ROTEM, J., & Y. COHEN. 1970. The effect of temperature on the pathogen and on the development of blue mold disease in tobacco inoculated with Peronospora tabacina. Phytopathology 60:54-57.

7. SCHEIN, R. D. 1964. Design, performance, and use of a quantitative inoculator. Phytopathology 54:509-513.