Effects of Relative Humidity on Bacterial Scab Caused by *Xanthomonas campestris* pv. *vesicatoria* on Pepper

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ABSTRACT

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Differences in relative humidity (RH) significantly affected bacterial scab (leaf spot) development in pepper plants. High RH with free moisture on the leaves for long periods favored infection. However, *Xanthomonas campestris* pv. *vesicatoria* had modest requirements for high RH in order to cause disease. When inoculated plants were exposed to high RH (>85%) for a few hours during 1 or 2 days, the pathogen could cause disease symptoms. Short periods of unfavorably low RH after inoculation with the pathogen temporarily prevented disease development, but it continued later when high RH conditions were provided. Long periods at low RH irreversibly prevented the pathogen from initiating disease, even if high RH was provided later.

Additional key words: bacterial leaf spot, epidemiology, phytopathogenic bacteria.

Relative humidity (RH) is the key factor that determines the development of many bacterial diseases in vegetables (2,3,6,7, 11,12,25). High RH (>90%) in general and free water on leaf surfaces in particular (fog, irrigation water, dew) help the pathogen reach the infection site and enhance its multiplication at the onset of disease development (14,16,17,19). Furthermore, free water also favors secondary infection of healthy plants by most bacterial pathogens under field conditions (1). On the other hand, long periods of low relative humidity, rainless and dewless seasons, especially when accompanied by high temperatures, retard the development of plant diseases (1,15,25).

It is known that Israeli isolates of the causal agent of bacterial scab of pepper, *Xanthomonas campestris* pv. *vesicatoria* (Doidge, 1920; Dye, 1978b) prefer relatively high temperatures (optimum) 30–32 C under RH ≅ 100%). It can be found in late spring and summer pepper fields in semiarid regions and is more abundant in fields with sprinkle irrigation than with drip irrigation (5,21,22).

The purpose of this work was to determine the precise relationship between humidity and development of bacterial scab (leaf spot) of pepper.

MATERIALS AND METHODS

Organisms, growth conditions, isolation from plants, and preparation of inoculum. Isolates of *X. campestris* pv. *vesicatoria* were obtained in the summer from infected pepper plants growing in the Yezreel and the Jordan Valleys, the Lachish region, and the Jericho area. The bacteria were isolated on nutrient agar (Difco) plates supplemented with 0.15 g sodium deoxycholate per liter. The pathogens were kept on nutrient agar slants at room temperature (20–24 C) and transferred weekly to fresh medium. To prevent loss of pathogenicity, the pathogen was inoculated into pepper leaves and reisolated at least once a month according to the leaf enrichment method (8,9,18). No significant differences in symptom production were found between the four isolates. Therefore, an isolate of *X. campestris* pv. *vesicatoria* from the Jericho area was used in all experiments. Bacterial populations were counted as previously described (4). Pepper plants (four true leaves), cultivar 'Ma'or', susceptible to bacterial scab, were used in all experiments carried out either in humid chambers made of polyethylene bags placed in an environmentally fully controlled growth chamber (30 ± 2 C, 16 hr light, 10,000 lux, and 8 hr darkness) or in mist chambers (3 sec of mist every 30 min, 30 ± 2 C, day light). Seeds were obtained from "Hazera" Co., Haifa, Israel. Experiments were conducted three times in a completely randomized fashion in 10 replicates. Growth conditions, inoculum preparation, and inoculation procedures were as described elsewhere (1,13).

Disease index. The disease index was estimated according to Yunis et al (24) using the four mature upper leaves of each plant.

Constant relative humidity chambers. Saturated solutions (1 L each) of different salts were put separately in 20-L flat plastic tanks to cover the bottom 1-cm deep. Stainless steel stands (10 cm high) were placed in the solution and inoculated pepper leaves placed on water agar in petri dishes were put on the top of the stands. The covers of the petri dishes were removed, and the tanks were sealed with a polyethylene sheet and transferred to the growth chamber. The vapor pressure of each salt solution at 30 C created constant relative humidity in the tank. The salts used were $NaNO_2$ for RH = 60%; NaCl for RH = 75%; KCl for RH = 85%; K_2SO_4 for RH = 96%; and water for RH ≅ 100% (with free moisture on leaves) (23). Relative humidity in growth chambers, moist chambers, mist chambers, and in tanks was measured with a hygrothermograph.

Intermittent high and low RH treatments. Two groups of pepper plants (50 plants per group) were inoculated with *X. campestris* pv. *vesicatoria*, and the plants were incubated under different RH conditions (RH ≅ 100% or RH <40%) for different periods of time. At the end of each incubation period, disease severity was monitored on the newly developed leaves of each group. Plants incubated under RH ≅ 100% were later incubated at RH <40% and vice versa. The following treatments were arranged: Group a: RH ≅ 100% for 8 days; RH <40% for 25 days; RH ≅ 100% for 8 days; and RH <40% for 25 days. Group b: RH <40% for 9 days; RH ≅ 100% for 10 days; RH <40% for 15 days and RH ≅ 100% for 8 days. Inoculated control plants were maintained at either RH ≅ 100% or RH <40% throughout. Uninoculated control plants were sprayed with sterile water and transferred intermittently to the different RH.

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RESULTS

Effect of intermittent high and low relative humidity on bacterial scab. The limiting humidity for development of bacterial scab on pepper plants incubated for long periods of time was RH <40% (Fig. 1). Plants exposed to dry conditions following infection did not develop any symptoms; even when later transferred to high RH conditions, disease severity was very low in these plants. Plants maintained at RH ≅ 100% (with free moisture on leaves) after inoculation showed severe symptoms, but long dry periods decreased disease severity to a minimum. Even further incubation under wet conditions did not favor disease, and disease severity did not reach the level obtained shortly after inoculation (disease index >2.5).

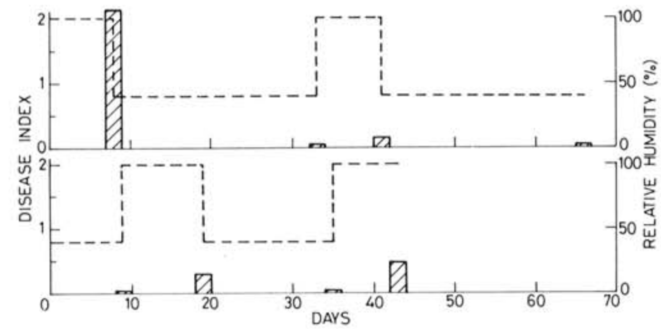

Fig. 1. Effect of intermittent high and low relative humidity (RH) on bacterial scab of pepper. Top, group a; bottom, group b; ------ represent changes in RH. Columns represent disease severity. The experiment was done three times. Results are from one experiment.

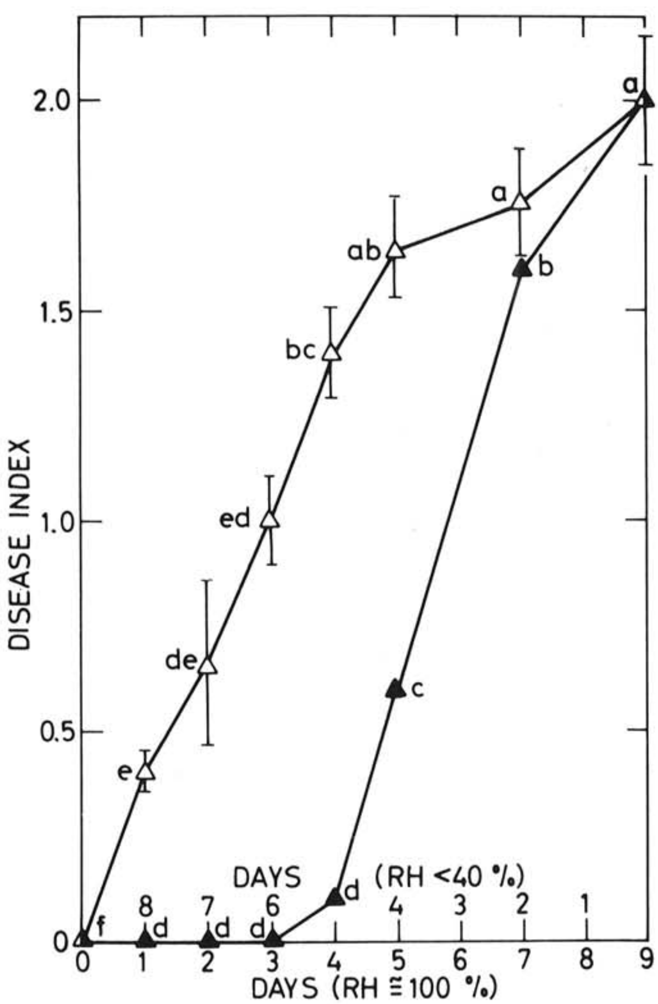

Fig. 2. Accumulating influence of high relative humidity on disease severity. △——△ = Disease index 9 days after infection; ▲——▲ = Disease index from the day the plants were removed to low RH. Different letters in the graph show significant differences at $P = 0.05$. Experiment was done three times. Results are from one experiment.

Cumulative effects of high relative humidity. One hundred pepper plants were inoculated with *X. campestris* pv. *vesicatoria* and transferred to RH ≅ 100%. Every day for 9 days, a group of 10 plants was transferred to a growth chamber where RH was <40%. Disease was assessed in each group the day the plants were removed to low RH conditions and 9 days after infection. Control plants were given the same treatments. Accumulation of high RH periods resulted in a parallel increase in disease severity (Fig. 2). Even after short incubation periods at high RH, plants developed disease symptoms. When successful infection was achieved, disease symptoms developed at least 5 days after infection; in those cases short, dry periods had no effect on the rate of symptom development.

Relationship between development of scab in pepper plants and length of incubation period under high relative humidity. The results of the previous experiment indicated that short periods of high RH were required for disease establishment. An attempt was made to determine the minimum duration of high RH required for disease development. Five groups of plants (20 plants each) were infected with the pathogen and incubated under RH ≅ 100% for periods varying from 0 to 4 days. Every day, one group was transferred to RH <40% for 7 days to prevent increase in disease severity. After the dry period, all plants were incubated for 6 days at RH ≅ 100%. Disease indices were determined after the dry period and after the wet period. Controls were as previously described. Primary infection with *X. campestris* pv. *vesicatoria* required only short periods of time (1–2 days) (Table 1). Disease development was prevented during the dry period, but the pathogen, which was established in the plant, caused severe disease symptoms under the reestablished high RH conditions.

Minimal incubation period at maximum relative humidity required for disease development. Following the observation that only one or two days of incubation at high RH were sufficient for disease development, shorter periods of incubation were also examined.

Six groups (20 plants each) of pepper plants were inoculated with *X. campestris* pv. *vesicatoria*. Every 24 hr, each group was incubated in a moist chamber for one of the following periods of time: 0, 1, 2, 3, 4, 6, or 24 hr. During the remaining time, the plants were grown in a nonmisting growth chamber (RH <40%). Control plants were sprayed with sterile water and incubated for the same periods. Disease was rated 9 days after inoculation.

Increasing the daily misting period resulted in an increase in disease severity (Fig. 3). Nevertheless, very short periods of misting, (eg, 1–2 hr/day) were sufficient for the pathogen to cause symptoms.

TABLE 1. Effect of incubation at relative humidity (RH) ≅ 100% after infection on development of bacterial scab of pepper

Incubation at RH ≅ 100% post infection (days)	Incubation at RH <40% after incubation at RH ≅ 100% (days)	Disease index after incubation at RH <40%	Disease index after a second 6-day incubation period at RH ≅ 100%
0	9	0.00 c[y]	0.28 d[z]
1	8	0.41 b	1.21 c
2	7	0.62 b	1.70 b
3	6	1.00 a	2.30 a
4	5	1.30 a	2.50 a

[y]Values (in the same column) followed by different letters differ significantly, $P = 0.05$.

[z]Disease indices ranged from 0 to 3, with 3 representing 10 or more scabs per leaf and 0 representing no symptoms. Disease was monitored in the four upper leaves of each plant.

Determination of the minimal relative humidity required for successful infection. Detached pepper leaves in petri dishes (15 per replication in five replicates) or pepper plants (four true leaves, five plants per replication, five replicates) were either inoculated with the pathogen by the leaf enrichment method (8,18) or sprayed with a suspension of the pathogen (1), respectively. The infected, detached leaves or plants were incubated for 7 days in humid chambers at RH of 60, 75, 85, 96, or 100%. Control leaves and plants were resprayed with sterile water and incubated under the same conditions. No disease symptoms were observed in plants or leaves incubated at relative humidity levels <85% (Table 2). Increase in RH also increased disease severity.

Cumulative effect of low relative humidity on disease severity. Because we found that RH <40% is a limiting factor for disease development, the effect of accumulation of days at RH <40% was tested.

Pepper plants were inoculated with *X. campestris* pv. *vesicatoria* and incubated under RH <40% for 0 to 9 days. Every day a group of 20 plants was transferred to RH ≅ 100% for 9 days, when disease severity was measured. Control plants were incubated under RH <40% or RH ≅ 100% throughout the experimental period.

Incubating infected pepper plants at RH <40% resulted in a reduction in severity of disease even when the infected plants were later incubated in RH conditions optimal for disease development (Fig. 4). Three days of incubation at RH <40% had the maximum effect, and further increases in the incubation period at RH <40% did not further reduce disease severity.

DISCUSSION

Bacterial scab of pepper is a typical hot season disease in Israel (5,21). The climatic conditions prevailing in the pepper-growing regions (high temperatures, short periods of high relative humidity, and no rain) might enhance disease. Therefore, the effect of the relative humidity and free moisture on the leaves on disease development was tested in our study.

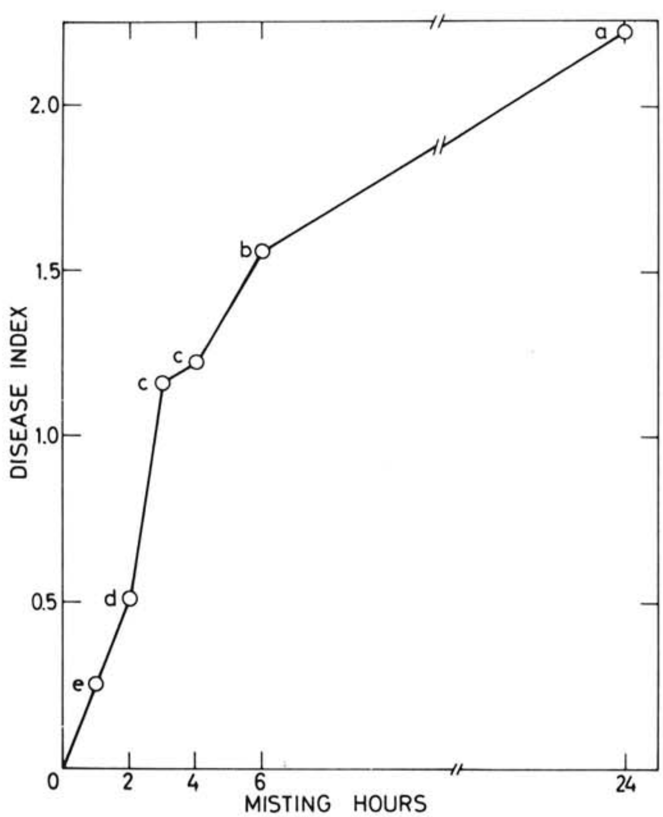

Fig. 3. Minimal incubation period at maximum relative humidity required for disease development. Different letters in the graph show significant differences at $P = 0.05$. Experiment was done twice. Results are from one experiment.

The evidence provided here shows that once *X. campestris* pv. *vesicatoria* is present inside pepper leaves, unfavorable RH conditions may delay disease temporarily, but they cannot prevent its later development. The same phenomenon is known to occur with other bacterial pathogens (10).

As the length of the dry periods between high RH periods increases (3–4 wk), the ability of the pathogen to initiate disease under high RH conditions is irreversibly damaged. Since mature pepper plants are less susceptible to infection by *X. campestris* pv. *vesicatoria* (5,20), a long dry period immediately after inoculation

TABLE 2. Effect of different relative humidities on severity of bacterial scab of pepper

Relative humidity (%)	Disease index	Scabs per leaf
60	0[x] d[y]	0[z]
75	0.20 d	0
85	1.05 c	4
96	1.85 b	8
100	2.55 a	11

[x] Disease indices ranged from 0 to 3, with 3 representing 10 or more scabs per leaf and 0 representing no symptoms. Disease was monitored in the four upper leaves of each plant.

[y] Different letters following values indicate values that differ significantly at $P = 0.05$.

[z] Results obtained from detached leaves maintained in petri dishes by the leaf enrichment method.

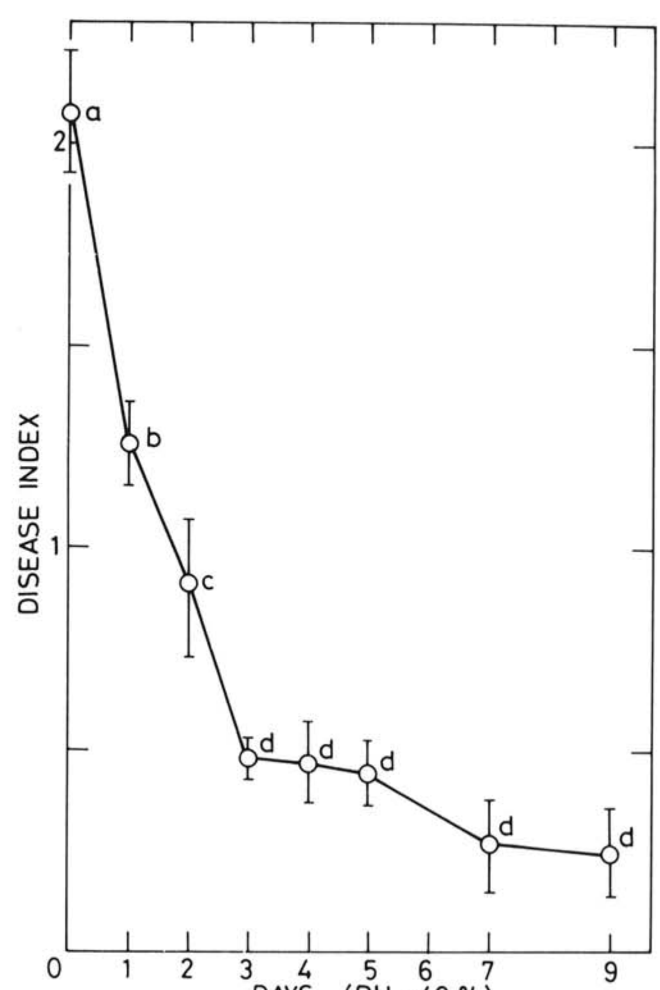

Fig. 4. Accumulating effect of low relative humidity on disease severity. Different letters in the graph show significant differences at $P = 0.05$. Experiment was done three times. Results are from one experiment.

prevents the later development of disease. Furthermore, exposing diseased plants to a long period of low RH prevents further disease development, even if the plants are later reincubated for long periods under high RH.

On the other hand, the relatively short period of free moisture (RH $\cong$ 100%), a few hours per day for 1–2 days, was enough for Israeli isolates of *X. campestris* pv. *vesicatoria* to cause disease. Thus, there is a potential for disease development in sprinkle-irrigated pepper crops in arid areas, and irrigation systems other than sprinkling may be recommended.

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