S. Diab et al. (1982) Phytoparasitica 10(3): 183-191.

STUDIES OF INFECTION WITH *XANTHOMONAS CAMPESTRIS* PV. *VESICATORIA*, CAUSAL AGENT OF BACTERIAL SCAB OF PEPPER IN ISRAEL

S. DIAB,* Y. BASHAN** and Y. OKON*

Xanthomonas campestris pv. *vesicatoria*, the causal agent of bacterial scab of pepper, was isolated in several regions in Israel. When artificial inoculation was practiced, pathogen growth was enhanced by high temperatures (30-36°C), and an inoculum concentration of 10^6 colony-forming units (CFU) per ml was optimal for symptoms to develop on plants. Pre-inoculation treatments such as wounding the leaves by rubbing them with carborundum powder or spraying them with diluted wax solvents, markedly increased disease severity, but were not essential. Pre-inoculation conditioning at two different relative humidity levels (R.H. ≅ 100% or R.H. < 40%) did not affect disease severity. Young leaves were more severely affected following infection than older leaves. Disease severity was similar with several isolates of *X. campestris* pv. *vesicatoria*.

KEY WORDS: Epidemiology; *Capsicum annum*; infection studies; *Xanthomonas campestris* pv. *vesicatoria*; bacterial spot of pepper; bacterial scab of pepper.

INTRODUCTION

Bacterial scab (leaf spot) of pepper is caused by *Xanthomonas campestris* pv. *vesicatoria* (4) and results in crop damage in Israel mainly in the spring and summer (18).

The first infection symptoms to appear on the leaves are light- or dark-green scab-like lesions. These lesions subsequently become necrotic, with a light brown center and a small chlorotic zone. In heavily infected peppers, there is intense defoliation which results in fruit damage due to sun exposure. In the Jordan Valley, where the daytime temperatures in the summer sometimes rise above 40°C, the disease can

Publication of the Agricultural Research Organization. Received Feb. 2, 1982; received in final form June 15, 1982. No. 523-E, 1982 series.

* Dept. of Plant Pathology and Microbiology, The Hebrew University of Jerusalem, Faculty of Agriculture, Rehovot.

** Div. of Plant Pathology, ARO, The Volcani Center, Bet Dagan.

also be found in relatively warm winters (day temperature 25°-30°C). In most parts of the world it usually develops at an intermediate temperature range (22°-27°C) and under high relative humidity (R.H.) (4, 5, 9, 10, 12). Studies by Volcani (18), and preliminary field observations, have shown that bacterial scab appears mainly in once-a-week sprinkle-irrigated fields, in which the plant leaves remain dry most of the day because of the prevailing arid conditions (temp. > 30°C; R.H. < 40%). These conditions are usually adverse for multiplication of bacterial pathogens on the surface of leaves (8, 15).

The purpose of this study was to explore the factors affecting infection of pepper by *X. campestris* pv. *vesicatoria*, in order to establish a system that will enable further studies.

MATERIALS AND METHODS

Twenty strains of *Xanthomonas campestris* pv. *vesicatoria* were isolated from infected pepper plants in the summer from four areas, namely, the Yizre'el Valley, the Lakhish region, the Jordan Valley, and the Jericho area. The bacteria were isolated on nutrient agar (Difco) plates supplemented with 0.15 g/liter sodium deoxycholate. The pathogens were kept on nutrient agar slopes at room temperature (20°-40°C) and transferred to fresh medium weekly. To prevent loss of pathogenicity, the pepper leaves were inoculated with the pathogen and the bacteria were reisolated at least once a month according to the leaf enrichment method (6, 14). Also lyophilized infected leaves containing a viable pathogen population were kept dry in glass boxes with silica gel (1).

An isolate of *X. campestris* pv. *vesicatoria* from the Jericho area was used in most of the infection studies. Bacterial populations were counted as described previously (3).

Pepper plants cv. 'Ma'or', susceptible to bacterial scab, were used in all experiments. The experiments were carried out in humid chambers (20 x 30 x 40 cm) made of polyethylene bags, placed either in a fully controlled environment growth chamber (30° ± 2°C, 16 h light, 75 W/m², 8 h darkness) or phytotron, or in a mist chamber [3 sec mist every 30 min, 30° ± 2°C, daylight (3)]. Seeds were obtained from Hazera Co., Haifa. Experiments were conducted two or three times in a completely randomized fashion with ten replicates. Other susceptible cultivars (California, Zohar, Zahov-Naharia) were used also.

Growth conditions, inoculum preparation and inoculation procedures were as described elsewhere (2, 11).

After inoculation, plants were further irrigated by drip irrigation, which left the foliage dry.

Growth of bacteria in liquid medium was monitored in 100-ml Erlenmeyer flasks equipped with side arms, each containing 25 ml nutrient broth. The inoculated flasks were incubated at temperatures ranging from 15° to 37°C on a rotary shaker, and tur-

bidity readings were done at 420 nm with a Junior 11 Coleman spectrophotometer. Doubling time was calculated from a mid-log phase culture.

The disease index was estimated using the four mature upper leaves of each plant (19). The index scale was: 0 = no symptoms, 1 = 2-5 scabs (spots) together or spread over the leaf; 2 = 6-10 scabs; 3 = more than 11 scabs on each leaf. All plants were examined 8 days after inoculation; four plants constituted a replicate. The number of scabs per leaf was counted separately and the mean of the four leaves was considered as the disease index of the plant.

RESULTS

Effect of inoculum concentration of X. campestris *pv.* vesicatoria *on bacterial scab symptoms in pepper*

Pepper plants were sprayed to run-off with pathogen suspension at final inoculum levels between 10^2 and 10^8 colony-forming units (CFU)/ml. Control plants were sprayed with sterile water. Disease index was determined 8 days after incubation in a humid chamber. Pepper plants react to stress by defoliation of the lower leaves; therefore, percentage of defoliation and day of appearance of first scab were also recorded. The optimum level for efficient artificial inoculation with this isolate was with 10^6 CFU/ml (Table 1). Scab symptoms which developed were similar to those observed in naturally infested fields. Percentage of defoliation was low (20%). In addition, infection could be achieved at very low inoculum levels (10^2-10^3 CFU/ml), but after longer periods of time and with a lesser severity.

TABLE 1
EFFECT OF INOCULUM CONCENTRATION OF *XANTHOMONAS CAMPESTRIS* PV. *VESICATORIA* ON BACTERIAL SCAB SYMPTOMS IN PEPPER PLANTS
(Disease indices were recorded 8 days after inoculation, and defoliation percentage after 14 days.)

Inoculum concentration (CFU/ml)	*Disease index (0-3)*	*Defoliation (%)*	*Symptom appearance (days)*
0	0 e*	0 d	0
10^2	0.048 e	0 d	9
10^3	0.147 e	0 d	9
10^4	0.52 d	3.0 c	8
10^5	1.48 c	7.5 c	7
10^6	2.36 b	14.7 b	6
10^7	2.64 a	19.1 b	5
10^8	2.72 a	38.3 a	5

* Numbers followed by different letters in the same column differ significantly at $P = 0.05$.

Effect of mechanical and chemical pretreatments on disease severity

Prior to pepper infection by spraying with 10^6 CFU/ml, the following pretreatments were carried out. Pepper leaves were rubbed with carborundum powder (300 grid), or punctured with sterile needles, or sprayed with diluted wax solvent, *e.g.* chloroform, acetone and NaOH (0.1%, 0.1% and 0.001 N, respectively), in order to facilitate penetration. Control plants were sprayed with sterile water or with the wax solvents, or they were mechanically wounded, or not pretreated. Later, plants were transferred to a humid chamber or to a mist chamber.

Each of the pretreatments significantly increased the number of scab lesions per leaf and the percentage of defoliation (Fig. 1, A and B). However, *X. campestris* pv.

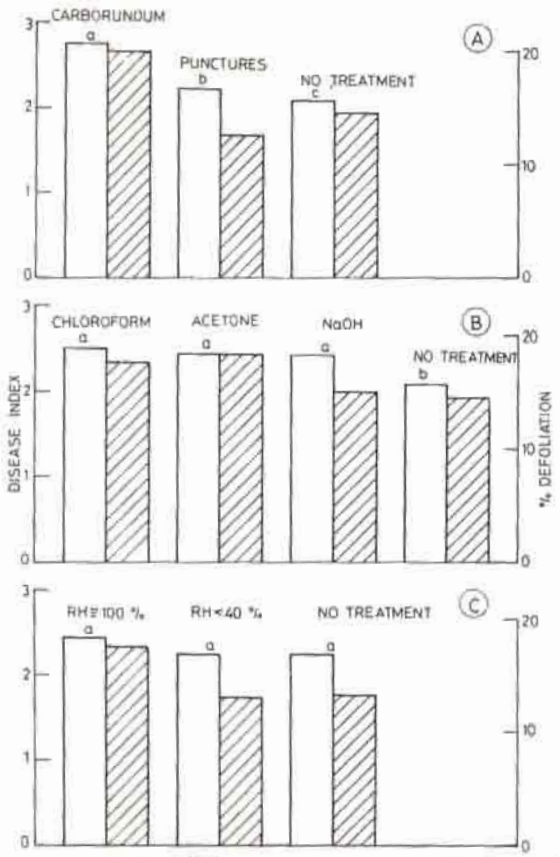

Fig. 1. Effect of different pretreatments on disease severity of bacterial scab of pepper caused by *Xanthomonas campestris* pv. *vesicatoria*. A. Mechanical pretreatments. B. Chemical pretreatments. C. Environmental pretreatments. Hatched areas refer to defoliation. Different letters at tops of columns (within the same graph) indicate significant difference ($P = 0.05$).

vesicatoria was capable of infecting pepper plants also without mechanical or chemical pretreatments, provided the plants were later subjected to periodic mist for 8 days. No difference in disease severity was observed between incubation in a humid chamber and a mist chamber.

Pre-infection relative humidity and disease severity

Pepper plants were maintained at R.H. = 40% in controlled chambers or at R.H. = 100% in humid chambers made of polyethylene bags for 24 h, sprayed as described previously, and incubated in a humid chamber or in a growth chamber at 40% R.H. for 8 days. Control plants were sprayed with sterile water and incubated under the same conditions.

Subjecting plants to different R.H. conditions prior to inoculation had no effect on the number of lesions per leaf or on the percent of defoliation (Fig. 1 C). Plants kept at R.H. = 100% after inoculation did show disease symptoms, but infected plants incubated under 40% R.H. for 8 days showed no disease symptoms.

Effect of method of injection on disease severity

Two methods of injection were examined: (*a*) Direct injection of 0.1 ml bacterial suspension into leaves, using a hypodermic needle; and (*b*) slow injection of 2 ml bacterial suspension into the stem (2). Inoculum levels were 10^4 or 10^9 CFU/ml. One-half of the plants were incubated for 7 days in a growth chamber at R.H. $< 40\%$ and the second half at R.H. = 100%. Control plants were either sprayed with the same suspensions or with sterile water until run-off, or were injected with sterile water. This experiment showed that injection of 10^4 CFU *X. campestris* pv. *vesicatoria*/ml to pepper leaves after a 9-day period of incubation at R.H. $< 40\%$ or at R.H. = 100% resulted in localized necrosis. Injection of the same dose into the stem caused no usual symptoms on leaves. However, spraying of leaf surfaces with the same suspensions and incubation at R.H. $\cong$ 100% resulted in a disease index of 0.2. Injection of leaves with higher bacterial concentrations (10^9 CFU/ml) caused either severe necrosis (when the plants were incubated at R.H. $< 40\%$) or plant collapse (at R.H. = 100%). Injection into the stem did not result in any apparent symptoms. However, spraying the plants with the same suspension led to severe disease (disease index = 3.0) only in plants incubated at R.H. = 100%.

Infection of pepper plants with different isolates

Twenty summer strains of *X. campestris* pv. *vesicatoria*, obtained from four regions in Israel, were tested for their ability to initiate bacterial scab of pepper. No significant differences were found among the isolates tested. Isolates obtained from the Yizre'el Valley, the Lakhish region, the Jericho area and the Jordan Valley caused a disease index of 1.42, 1.29, 1.53 and 1.8, respectively.

Effect of leaf age at time of infection on disease severity

Pepper plants with four, six or ten mature leaves corresponding to 1.5, 2 and 3 months after seeding, respectively, were inoculated with 10^6 CFU/ml. Plants were either incubated for 8 days in a humid chamber at R.H. = 100%, or treated in petri

dishes by the leaf enrichment method – three leaves per plate, four replicates (6, 14).

Disease index for mature inoculated leaves and for young leaves (which developed to full size during the incubation period) was monitored. It was found that *X. campestris* pv. *vesicatoria* developed better on young leaves of pepper and that leaf development and disease severity increased concomitantly.

TABLE 2
DISEASE INDEX OF YOUNG LEAVES AND MATURE LEAVES ON THE SAME PEPPER PLANT INOCULATED WITH *XANTHOMONAS CAMPESTRIS* PV. *VESICATORIA*
(Experiment repeated two times, with three replicates.)

Plant age (months)	*Disease index (0-3)*	
	Young leaves	*Mature leaves*
1.5	2.62 a*	0.97 b
2	2.39 a	0.48 b
3	2.55 a**	0.12 c

* Numbers followed by different letters in the same column differ significantly at $P = 0.05$.
** Incubation in petri dishes.

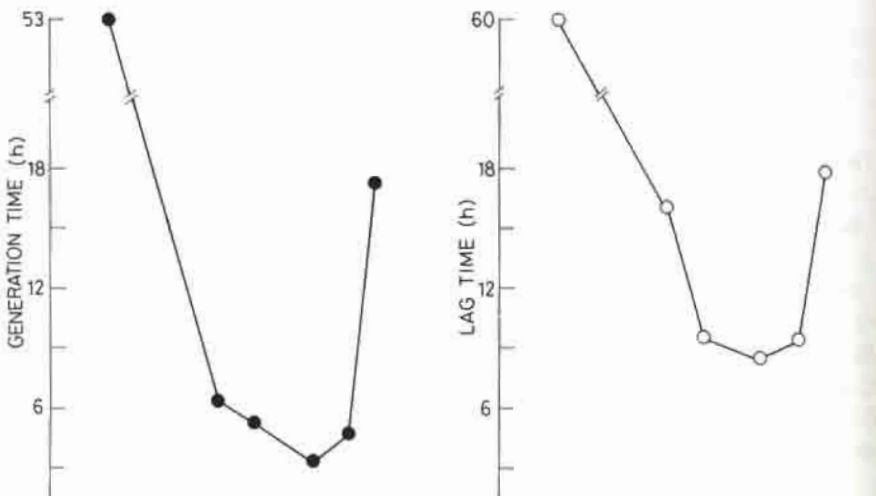

Fig. 2. Generation time (left) and lag time (right) of *Xanthomonas campestris* pv. *vesicatoria* in liquid culture.

Effect of temperature on growth of X. campestris *pv.* vesicatoria in vitro *and on disease development*

The effect of incubation temperature on the growth of *X. campestris* pv. *vesicatoria* (Jericho isolate) in nutrient broth liquid culture (Difco; 8 g/l) was studied at 15°, 24°, 27°, 32°, 35° and 37°C in a rotary shaker. This isolate was sprayed on pepper plants, as described previously, and the plants were then transferred inside humid chambers to a phytotron and incubated for 8 days at day/night temperatures of 17°/12°, 22°/17°, 27°/22°, 32°/27° and 36°/36°C; R.H. was ≅ 100%.

The pathogen grew in cultures at temperatures ranging from 24° to 37°C, with an optimum at 32°C. Doubling time was 3.5 h at 32°C and lag time was 8 h (Fig. 2). Maximum disease index was observed at 27°/32°C and 22°/27°C (Fig. 3). Similar results were obtained with the other susceptible cultivars tested.

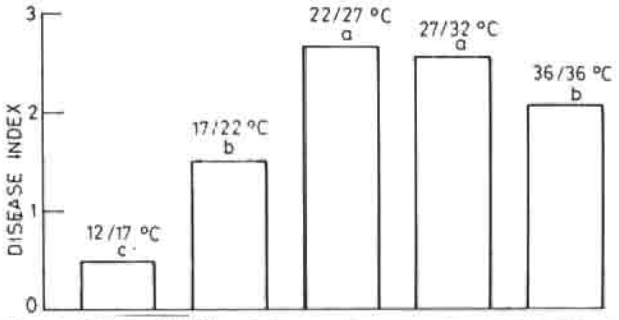

Fig. 3. Effect of different temperatures on disease severity of bacterial scab of pepper caused by *Xanthomonas campestris* pv. *vesicatoria*. Different letters at tops of columns indicate significant difference ($P = 0.05$).

DISCUSSION

Pepper is one of the most important crops of the Israeli export vegetable market, and bacterial scab of pepper causes serious damage to the fruit. To date, no comprehensive study has been reported of the factors affecting infection by this disease although a few studies of artificial inoculation under different conditions are available (9, 10, 13, 17). We have shown that in order to obtain clear scab symptoms under our experimental conditions it is necessary to use a high concentration of *X. campestris* pv. *vesicatoria* isolates. However, some symptoms (disease index = 0.05) could also be obtained with a low inoculum dose of the pathogen. Although disease was increased by factors which commonly favor bacterial infections, such as tissue damage or injection, the isolates were also capable of causing symptoms when sprayed on the leaves of pepper plants, incubated under high R.H., but which had not been subjected to prior high R.H. treatment.

The results of this study also confirmed previous work (7, 10, 16) on bacterial scab of tomato and chili, suggesting that *X. campestris* pv. *vesicatoria* is more likely to attack young leaves, which develop to full size after infection.

Information in the literature concerning optimal temperatures for disease development indicated that symptoms of bacterial scab developed mainly at intermediate temperatures (22°-27°C) (4, 5, 7, 9, 10, 12). The data presented in this work indicated that Israeli isolates caused the most marked disease symptoms under phytotron conditions at temperatures of 27°-32°C, and even at 36°C plants showed high disease severity.

A direct significant correlation between ratio of disease index in plants/generation time of bacteria in liquid cultures on the one hand, and temperature on the other hand was obtained in the temperature range of 15°-32°C ($Y = 0.04x - 0.652$; $r = 96.6$). Above these temperatures (32°-37°C) there was no correlation. It is not yet known whether the Israeli isolates of *X. campestris* pv. *vesicatoria* originated from foreign isolates and became adapted to the local warm conditions or whether they are endemic.

ACKNOWLEDGMENTS

This work was partially supported by grant No. 823/026 from the Agricultural Research Organization, Ministry of Agriculture, Israel, and by grant No. I-214-80 from the United States–Israel Binational Agricultural Research and Development Fund (BARD).

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