CROP PROTECTION (1985) 4 (1), 77–84

Crop loss of pepper plants artificially infected with *Xanthomonas campestris* pv. *vesicatoria* in relation to symptom expression

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ABSTRACT. Crop losses in peppers artificially infected with *Xanthomonas campestris* pv. *vesicatoria* (XCV) were determined during 3 years of field experiments in three areas of Israel. Direct losses of 23–44% in fruit yield were recorded when severe leaf infection occurred or was induced at an early stage of plant growth. Yield losses and disease index were markedly lower in plants inoculated at later stages and near maturation. Indirect losses in severely infected fields were mainly due to shedding of leaves and exposure of fruits to sun. In this case, up to 95% of the fruits lost their commercial value. In artificially infected symptomless plants with massive endogenous populations of XCV in the leaves, a loss of 24% in yield was measured, compared with plants free from an endogenous pathogen population.

Introduction

Bacterial scab of pepper (leaf spot) caused by *Xanthomonas campestris* pv. *vesicatoria* (Doidge 1920) Dye 1978b is a widespread disease found all over the world (Leben, 1962; Shekhawat and Chakravarti, 1976; Diab, Bashan and Okon, 1982). Assessment of crop losses in the field caused by this disease under various environmental conditions has not been extensively studied. Preliminary observations made by personnel of the extension service of the Ministry of Agriculture in Israel (unpublished data) have indicated that the incidence and severity of bacterial scab disease in Israel does not follow any particular pattern. In some years the disease was widespread and apparently caused fruit loss and leaf damage, whereas in other years the damage in the same area was negligible. Moreover, in a

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0261–2194/85/01/77–08$03.00

given season, adjacent fields with similar cultivars and similar environmental and agricultural conditions often sustained different degrees of damage. If a field is damaged, the diseased plants are generally localized around defined centres spread randomly throughout the field. Scab symptoms are not easily detected in the field, as they appear on the lower portion of the large dense foliage plant. Diseased leaves are usually shed, and the plant appears healthy. Chemical treatment is applied only when symptoms are obvious, although at this stage treatment has only a marginal effect in arresting disease development (Azaizeh and Bashan, 1984).

The purpose of this study was to assess crop losses caused by bacterial scab of pepper in artificially infected fields in three regions of Israel.

Materials and methods

Plant growth conditions

Pepper (*Capsicum annuum*) cultivars tested included cv. Ma'or grown in Israel for the export market (fresh), and cv. Zahov-Naharia, grown for the local market (fresh), both of which cultivars are susceptible to bacterial scab (Bashan, Azaizeh and Diab, 1984). Seeds were obtained from Hazera Co., Haifa, Israel. Seedlings were planted in commercial fields in the following locations: in 1980, two experiments were conducted on Terra rosa soil (Rhodoxeralfs) in Daburia in the north-eastern Yizreel Valley, and one on Mediterranean brown forest soil (Haploxeralfs) in Tamra in north-west Galilee (Table 1). In 1981, one experiment was done on brown alluvial soil (Vertisols) chromoxeralfs in Mukeble, Eastern Yizreel Valley; one on Terra rosa soil in Bet-Netofa Valley, central Galilee, and one in Daburia (Tables 2 and 3). In 1982, one experiment was carried out in Daburia (Table 4). Experiments were conducted in a randomized block design with five replicates. Each plot consisted of 90 plants in three rows (forming a garden bed) each 10 m in length with three plants per metre and 60 cm between rows. The fields were sprinkle-irrigated with 15 mm water once a week throughout the season.

Inoculation method

A local isolate of XCV (R-3) was used in all experiments and inoculum was prepared according to Diab, Bashan and Okon (1982). Plant leaves were inoculated by spraying a suspension of XCV (10^7 colony-forming units (CFU)/ml) until runoff. In order to maintain high humidity on leaves, the plots were sprinkle-irrigated for 3 days after inoculation (15 minutes' irrigation each day during the cool hours of the evening).

Disease index on leaves

The index scale was 0 = no symptoms; 1 = 2–5 scabs together or spread over the leaf; 2 = 6–10 scabs; 3 = more than 11 scabs on each leaf. The four mature upper leaves of each plant were used for determination of disease index. Three samples were taken per plot, each consisting of four plants. 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. The mean of four plants was the disease index of the sample and the mean of samples was the disease index of the replicate.

Fruit symptoms were very rare during the 3 years of field experiments and therefore were not counted.

In order to establish different levels of disease development, in the experiment conducted in Daburia in 1982 plants were sprayed weekly with a 0·3–0·5% suspension of a bacteriocide: either Kocide 101 (77% $Cu(OH)_2$; Kocide Corp., Houston, Texas), or Coprox 50 (87% $CuCl_2 \cdot 3Cu(OH)_2$; Makhteshim, Beer Sheva, Israel) at a rate of 350 ℓ/ha.

Measurements of endophytic bacteria, dry weight and yield

Endophytic populations of XCV were counted in leaves by the method of Sharon, Okon, Bashan and Henis (1982). Leaves (1 g fresh weight) were immersed in 3% (w/v) NaOCl for 5 min, washed five times with sterile distilled water and homogenized in 20 ml of saline solution (8·5 g NaCl) in a sterile Omni-mixer (Sorvall). Serially diluted suspensions (0·1 ml) were spread on nutrient agar plates supplemented with 200 mg/ℓ sodium deoxycholate (applied after autoclaving). Colonies were counted after 48 h incubation at 30°C.

Plant material was dried at 80°C for 7 days in a forced-air oven and then weighed.

Field yield of pepper cv. Ma'or was from a single harvest (in the 1980–81 experiments) carried out 5 days before commercial harvest of the field. In cv. Zahov-Naharia (1982 experiment), the first three harvests were weighed. The yield from all experiments consisted of fruits taken from a 9 m section of each row, leaving a 0·5 m border on the plot edges.

Statistical analysis

Because diseased plants tend to be localized around centres in the field, the disease index of the plots was not normally statistically distributed. The field data were therefore transformed to a normal distribution according to Hald (1952). The transformed data were analysed using Duncan's multiple range test with significance between treatments at $P \leqslant 0{\cdot}05$.

Results

Effects of plant age on infection and yield

Three field experiments were conducted in 1980. In each experiment, the plants were inoculated at different stages of growth. The leaf disease index was estimated 30 days after inoculation. Fruits were harvested at the end of the growing season. In two experiments in summer 1980, one near Tamra and one near Daburia, the plants were inoculated 80 and 60 days after planting, respectively. The plants became moderately infected (disease index =0·05 and 1·1, respectively), but there was no significant loss of fruit yield (Table 1). However, in a third experiment conducted in an adjacent field in Daburia and inoculated 15 days after planting, the disease index was higher (disease index = 1·7) and there was a significant decrease in yield of 23·3% compared with non-inoculated controls (Table 1).

TABLE 1. Effect of time of inoculation with *X. campestris* pv. *vesicatoria* in the field on yield

Time of inoculation (days after planting)	Location and time of harvest	Inoculation (+, −)	Disease severity 30 days after inoculation*	Yield (kg/9 m row)	Yield (as percentage of control)
15	Daburia, Yizreel Valley, December 1980	+	1·7 ±0·13†	14·1b‡	77·7
		−	0	18·14a	100
60	Daburia, November 1980	+	1·1 ±0·17	13·28a	97·8
		−	0	13·57a	100
80	Tamra, North-western Galilee, August 1980	+	0·05 ±0·03	15·40a	98·7
		−	0	15·60a	100

*Disease index 0–3.
†SE of the means of 5 replicates.
‡Numbers followed by different letters (in each experiment) differ significantly at $P \leq 0{\cdot}05$.

Two more field experiments were carried out in 1981 near Mukebla and in the Bet-Netofa Valley. The fields were inoculated at different stages of plant ontogeny. The disease index was estimated 37 days after inoculation and then weekly for 4 weeks. Fruit yield was determined from a single harvest at the end of the growing season. In the experiment near Mukebla, the disease index obtained was relatively high in young plants and a direct crop loss of 24·6% of the yield was detected. Disease index and fruit loss decreased with the maturity of the plants (Table 2). In addition, in plots that were severely infected, over 70% of foliage and over 90% of the harvested fruits were affected by exposure to the sun.

In Bet-Netofa Valley, 15% of the plants showed a low disease index (<0·5 units). The rest of the plants did not display any symptoms of bacterial scab although the field was inoculated twice. Leaf samples taken from inoculated plants (five replicates from each plot, 10–15 young leaves, five times during the growing season) indicated that endophytic populations of XCV were present ($2{\cdot}4 \pm 0{\cdot}51 \times 10^6$ CFU/g fresh weight of leaves). No endophytic XCV population could be detected in leaves taken from non-inoculated control plots. Despite a much lower disease index in the Bet-Netofa experiment, the direct loss of 24% of the yield was comparable with that in the Mukebla experiment.

Observations on the relationship between disease severity, plant weight and yield in naturally infected fields

Disease centres and healthy areas were selected and marked early in the season in naturally infected fields, adjacent to the 1982 experimental field in Daburia. At the

TABLE 2. Relationship between leaf age, disease severity and yield of pepper plants cv. Ma'or inoculated with XCV under field conditions

Time of inoculation	Location and date	Disease severity after 37 days*	Changes in disease severity after additional 28 days*	Yield (kg/9 m row)	Yield (as percentage of control)	Relationship between mean D.I. and mean yield (r)	Line formula y=yield x=disease index
After seedling establishment	Mukebla	2·47a†	−0·57	11·89a†	75·41	−0·93	$y=14{\cdot}47-1{\cdot}36x$
Flowering	Eastern	0·95b	−0·4	12·87a	81·53	−0·88	$y=14{\cdot}06-1{\cdot}18x$
First mature fruit	Yezreel Valley	0·59b	−0·09	13·54ab	85·73	−0·91	$y=14{\cdot}12-1{\cdot}63x$
Uninoculated control	1981	0c	0	15·79b	100	ND	—
After seedling establishment	Bet-	0·49a	0·21	9·27a	76·13	−0·96	$y=11{\cdot}6-6{\cdot}07x$
Flowering	Netofa	0·43a	−0·12	10·59ab	87·00	−0·99	$y=11{\cdot}17-5{\cdot}65x$
First mature fruit	Valley 1981	0·24a	0·04	11·26ab	92·50	−0·99	$y=11{\cdot}33-5{\cdot}78x$
Uninoculated control		0a	0	12·18b	100	ND	—

*Disease index 0–3.
†Numbers followed by different letters (in each column and in each experiment) differ significantly at $P=0{\cdot}05$.
ND—Not determined.

end of the growing season, samples were taken from three centres (three samples per centre, ten plants per sample). The results clearly revealed that severe disease was associated with a large reduction in plant weight and fruit yield (Table 3). Indirect severe damage was caused to the fruits from sun exposure (~95% of fruits). Similar observations were made in 1980 and 1981 in three regions.

TABLE 3. Relation between disease severity, plant dry weight and yield in cv. Ma'or plants taken from naturally diseased centres and from healthy areas in Daburia in 1982

Sampling	Disease severity*	Dry weight of 10 plants (g)	Fruit yield of 10 plants (g)	Yield (as percentage of control)
Healthy plants from healthy area	0	505·2 ±40†	3900 ±160	100
Diseased plants from disease centres	3·0	200·6 ±50	2190 ±110	56·15

*Disease index 0–3.
†SE.

Effect of different severities of disease on yield

Different levels of disease in the field were obtained in Daburia in 1982, by spraying copper compounds after inoculation. Plots were harvested once at the end of the season.

Table 4 shows that yield decreased with an increase in disease severity. In the inoculated plots that were not treated with copper compounds, all the leaves were shed and over 90% of fruits suffered from sun exposure.

Discussion

Our work has demonstrated that bacterial scab of pepper can cause significant losses in Israeli fields.

As a result of the unusual appearance and distribution of the disease in the field, as indicated earlier, it was not possible to predict a natural appearance of the disease in a given field, even if the agroclimatological conditions were favourable for disease outbreak in the susceptible cultivar used. Moreover, it was very difficult to mark plots in a given field in size and number sufficient to be subjected to proper statistical analysis: artificial inoculation was, therefore, essential. The inoculation was performed in such a manner that the appearance of disease symptoms was similar to that resulting from the natural infections in adjacent fields. As disease development was recorded throughout the season always on the new leaves, the artificial inoculation served only as a primary source of pathogen and the initial inoculum level had negligible effects on later stages of disease development. Thus, it seems to us that the results regarding crop loss obtained from artificial inoculation with XCV were relevant in the field.

Table 4. Effect of different disease severities on yield in pepper plants (cv. Zahov-Naharia) inoculated with *X. campestris* pv. *vesicatoria*

Chemical treatment	Location and date of harvest	Disease severity after 60 days*	Yield per 10 plants (kg)	Yield (as percentage of control)	Line formula and r value
Inoculated control, unsprayed†		2·17a‡	5·26a	100	
Inoculated plants with:					
Coprox 0·3%	Daburia,	1·66b	5·72ab	108·7	
Coprox 0·5%	October–	1·22b	5·82ab	110·6	$y=8{\cdot}4-1{\cdot}28x$
Kocide 0·3%	November	1·05b	6·32b	120·1	$r=-0{\cdot}91$
Kocide 0·5%	1982	0·91b	7·02b	133·4	

*Disease index 0–3.

†Non-inoculated controls were infected by natural secondary infections from inoculated areas throughout the growing season.

‡Numbers followed by different letters in each column differ significantly at $P=0{\cdot}05$.

Reduction in yield can be caused by direct damage, which lowers photosynthate production, plant biomass and fruit production, and can lead to a 44% yield loss in extreme cases (disease index >2·0), or indirect damage due to exposure of fruits to the sun following leaf shed, which caused almost total crop loss.

Direct damage from symptoms on fruits was very rarely observed during the 3-year experimental period in Israel.

Direct positive correlations were demonstrated between disease severity and yield, whether different levels of disease were obtained by inoculating plants at different stages of growth or by treatments with copper compounds after inoculation. Significant economic damage was detected only when the fields were infected at early stages of growth. This stage is known for its susceptibility to infection with XCV (Shekhawat and Chakravarti, 1976; Diab, Bashan and Okon, 1982). Treatment with copper compounds after inoculation significantly reduced crop loss.

The phenomenon of symptomless plants with endogenous pathogen populations in their leaves has been reported for XCV in both tomato and pepper (Leben, 1962; Leben, 1981; Bashan, Diab and Okon, 1982). We have shown that plots with little apparent disease but massive endogenous XCV population can suffer losses similar to those in plots with severe symptoms. If this is a widespread phenomenon, it complicates decision-making for the farmer, who relies on visual inspection of the field to evaluate disease progress and to implement control measures.

We therefore conclude that bacterial scab of pepper can be of relatively major economic importance in warm areas such as Israel, although the disease is not easily detected in the field.

Acknowledgements

We thank growers F. Hatib, A. Abo-Afif, F. Ibrahim, F. Azaizeh and A.A. Azaizeh for free cultivation of the experimental fields. This work was partially supported by grant no 823/026 of the Ministry of Agriculture Israel and by grant No. I-214-80 of the United States–Israel Agricultural Research and Development Fund (BARD).

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Accepted 2 August 1984