

## Ethylene production in pepper (*Capsicum annuum*) leaves infected with *Xanthomonas campestris* pv. *vesicatoria*

ANAT BEN-DAVID, YOAV BASHAN† and YAACOV OKON

*Department of Plant Pathology and Microbiology, The Hebrew University of Jerusalem, Faculty of Agriculture, P.O. Box 12, Rehovot 76100, Israel*

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*Xanthomonas campestris* pv. *vesicatoria*, the causal organism of bacterial scab in pepper (*Capsicum annuum*) produced small amounts of ethylene when grown under low oxygen tensions in liquid culture. Ethylene was produced by bacterial scab lesion tissue in pepper, but not by bacterial speck lesion tissue in tomato caused by *Pseudomonas syringae* pv. *tomato* or by angular leaf spot lesion tissue in cucumber, caused by *P. syringae* pv. *lachrymans*. Direct correlations were found between ethylene production in diseased plants and the number of bacteria in the tissue and between the initial inoculum and leaf abscission and disease development. Young susceptible pepper leaves produced more ethylene than mature, less susceptible leaves after inoculation and the ethylene was produced mainly in the distal parts of the leaf blade around developing necrotic spots. Spraying with methionine increased ethylene production and disease severity, whereas spraying with indole acetic acid or aminooxyacetic acid reduced ethylene production, disease severity and leaf-abscission. It is suggested that the ethylene produced during bacterial scab infection contributes to the development of disease symptoms including leaf abscission.

### INTRODUCTION

During normal plant growth the amounts of ethylene produced are usually relatively low, but slight peaks of production occur mainly around germination, when the plant is mature and during leaf abscission [14]. It is known that stress factors such as chemical treatments, irradiation, drought, infestation with pests, diseases and mechanical injuries cause increases in ethylene synthesis [14, 25]. Some bacteria, such as *Pseudomonas solanacearum* [18], *Xanthomonas campestris* pv. *citri* [11], *Pseudomonas syringae* pv. *phaseolicola* and *Erwinia rhapontici* [12], are capable of synthesizing ethylene in axenic culture. Thus some of the ethylene produced in infected plants may be produced by the pathogen.

The relationship between ethylene production and pathogenesis is complex and not fully understood [14]. The ethylene produced in banana plants attacked by *P. solanacearum* induces early fruit maturation [10]. In *Fusarium* disease of tomato, bud necrosis and inhibition of vegetative growth [19], or wilting and leaf abscission, are induced by ethylene [13]. In cauliflower, increases in ethylene synthesis were found in

†Present address: Department of Plant Genetics, The Weizmann Institute of Science, Rehovot 76100, Israel

Abbreviations used in text: AOA, aminooxyacetic acid; IAA, indole-3-acetic acid.

plants attacked by *Erwinia carotovora* or by *X. campestris* pv. *campestris*, but these bacteria failed to produce ethylene in culture [16]. A relationship between ethylene production and pathogenesis has also been reported for citrus trees infected with *X. campestris* pv. *citri* [11].

Leaf-abscission in healthy plants is known to be related to ethylene production, and thought to be caused by a decrease in auxin levels brought on by the inhibition of auxin synthesis, or by translocation or degradation of auxin [6, 17]. Abscission of diseased leaves is common in plants infected with fungi [20, 24], but less common with bacterial infections [11]. Recent studies have demonstrated that the symptom of bacterial scab in pepper (*Capsicum annuum*), caused by *X. campestris* pv. *vesicatoria*, which causes the greatest proportion of the economic loss of the crop, is the loss of leaves, since it results in damage to fruits by exposing them to sunlight [5, 9]. Recently, Stall & Hall [23] reported that the chlorotic zone surrounding the necrotic lesions of bacterial scab is associated with ethylene production,

The purpose of this study was to examine ethylene production in leaves of *C. annuum* infected with *X. campestris* pv. *vesicatoria*, particularly in relation to leaf abscission.

## MATERIALS AND METHODS

### *Organisms and growth conditions*

*Xanthomonas campestris* pv. *vesicatoria* (Doidge) Dye, (R-3) isolated from infected pepper plants [9] was used in all experiments. *Pseudomonas syringae* pv. *tomato* (Okabe) Young, Dye and Wilkie, (WT-1) causal agent of bacterial speck of tomato, *P. syringae* pv. *lachrymans* (Smith and Bryan) Young, Dye and Wilkies (Bet-Dagan) causal agent of angular leaf spot of cucumber, *Oidiopsis taurica* Tepper, causal agent of powdery mildew of pepper, and the red spider mite *Tetranychus cinnabarinus* L. were used in one experiment. Pepper plants (*Capsicum annuum* L.) cv. "Ma'or", highly susceptible to bacterial scab [4] were used in all experiments. In one experiment, tomato plants (*Lycopersicon esculentum* Mill) cv. "VF-198", susceptible to *P. syringae* pv. *tomato*, and cucumber plants (*Cucumis sativus* L. (cv. "Bet-Alfa", susceptible to *P. syringae* pv. *lachrymans*, were used.

Symptoms were assessed on the four fully expanded upper leaves of each plant, on a scale from 0–3, where 0 = no symptoms, 1 = 2–5 lesions together or spread over the leaf, 2 = 6–10 lesions per leaf, and 3 = 11 or more lesions per leaf. The plants were assessed 8–10 days after inoculation. The mean of the assessment for the four leaves was used as the disease index (D.I.) of the plant.

Conditions for plant growth, and the procedures for inoculum preparation, inoculation of whole plants, and pathogenicity tests were as described previously [9].

### *Media*

For all purposes, bacteria were grown in a yeast-peptone liquid medium [9] supplemented with 10 g of sucrose and 1.5 g of CaCl<sub>2</sub> (YPS medium). Nutrient-broth medium supplemented with 200 mg l<sup>-1</sup> sodium deoxycholate was used for the isolation of bacteria from infected pepper leaves according to the method of Bashan & Assouline [3].

### *Ethylene determination*

The four fully expanded upper leaves beneath the growing tip of the plant were sampled. Two to four leaves were placed in 21 ml rubber-stoppered bottles and incubated at 30 °C

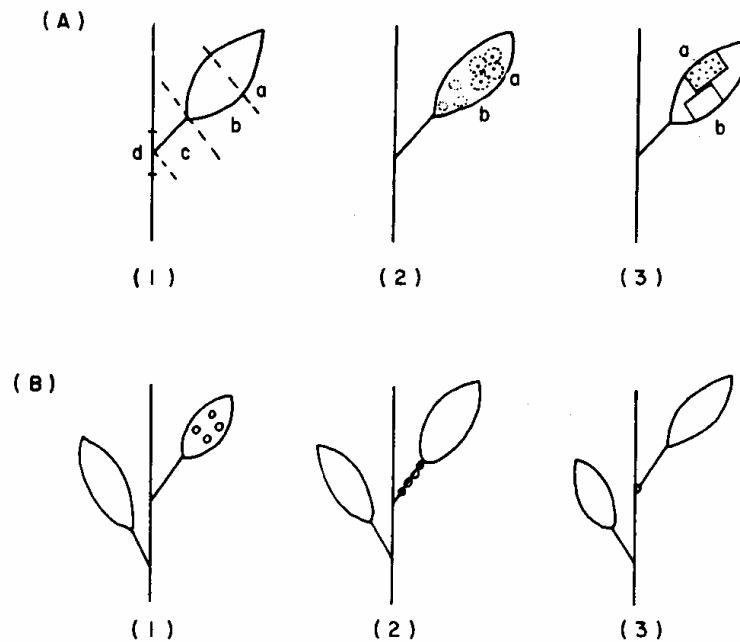


FIG. 1. Diagrams illustrating inoculation sites and sites which were sampled to test for ethylene production.

(A) Leaves were inoculated by spraying with  $10^8$  cfu ml<sup>-1</sup> and were sampled at different sites. (1) a, Distal half of lamina; b, proximal half of lamina; c, petiole; d, connection between petiole and stem. (2) a, Leaf discs with one to three lesions; b, leaf discs without symptoms. (3) a, Infected leaf area (four to ten lesions); b, control area without symptoms.

(B) Agar discs containing bacteria were applied to different parts of the leaf. (1) Four discs on the lamina. (2) Four discs on the petiole. (3) One disc in the axil of the leaf.

for 24 h. Ethylene production over a 24 h period was measured using a gas chromatograph (Gow-Mac Instrument Co., series 750) fitted with a flame ionization detector. The rate of ethylene production was measured as pmol g<sup>-1</sup> dry weight h<sup>-1</sup> using purified ethylene as a standard.

Leaves for dry weight determinations were dried in a forced draught oven at 80 °C for 48 h and weighed after cooling.

#### *Procedures for inoculating plants at precise locations*

Four agar discs covered with bacteria (4 mm in diameter) were cut from a 24 h culture on solid YPS medium and placed face down on the leaf blade, the leaf petiole or the junction between leaf petiole and stem, as required (Fig. 1). After inoculation the plants were incubated in a humid chamber at  $30 \pm 2$  °C for 4 days. Ethylene production was measured in 1 cm<sup>2</sup> sections from the leaf, or in 1 cm lengths of petiole, cut from the sites where the inoculum disc was applied and in the tissues surrounding these sites (Fig. 1).

#### *Determination of bacterial populations from infected leaves*

One hundred pepper leaves showing varying degrees of disease severity were collected from inoculated plants at the time of symptom appearance. Ethylene levels were determined in whole leaves as described above. The bacterial population was determined in two discs cut with an 8 mm cork borer from each leaf according to Sharon *et al.* [22]. Leaves of different ages and varying symptom severity were assessed as follows.

TABLE I  
Effect of leaf age on ethylene production and disease severity

Leaf position	Ethylene production <sup>a</sup> (pmol g <sup>-1</sup> dry weight h <sup>-1</sup> )		Mean disease index
	Leaves inoculated with <i>X. campestris</i> pv. <i>vesicatoria</i>	Non-inoculated leaves	
Lower leaves	264Aa <sup>b</sup>	296Aa	0.25A
Central leaves	302Aa	175Aa	0.50A
Upper leaves	677Ba	97Ab	2.62B

<sup>a</sup>Ethylene was determined over a 24 h period, 5 days after inoculation.

<sup>b</sup>Significance calculated using Duncan's multiple range test. Numbers in each column followed by different capital letters differ significantly at  $P \leq 0.05$ . For each leaf position numbers followed by different lower case letters differ significantly at  $P \leq 0.05$ .

(a) Pepper plants with five to six true leaves were inoculated with *X. campestris* pv. *vesicatoria*. Ten days after inoculation, 200 diseased leaves were selected according to their D.I. at intervals of 0.5 D.I. units (approx. 30 leaves per D.I., see Fig. 4).

(b) Pepper plants with 14 true leaves were inoculated with *X. campestris* pv. *vesicatoria*. Five days later, samples were taken from the first two leaves above the cotyledons, the sixth and seventh leaves, and the leaves immediately beneath the growing tip (Table 1).

#### *Stresses imposed on pepper plants*

In order to compare the effect of physical stress with pathogen induced stress, pepper plants were subjected to one of the following treatments. Leaves were rubbed with carborundum powder (300 grid) or punctured with ten sterile needles; plants were incubated for 24 h at 4 °C or 4 h at 40 °C; plants were maintained without irrigation at  $30 \pm 2$  °C for 48 hrs until the growing tip began to wilt; plants were inoculated with *X. campestris* pv. *vesicatoria*, *Oidiopsis taurica* or *Tetranychus cinnabarinus*. Leaves were sampled for the determination of ethylene production immediately after completion of the treatment and again one and five days later.

#### *Ethylene production by the pathogen in culture*

(a) Bacteria were inoculated onto slants of YPS medium or YPS medium supplemented with 10 mM methionine. After 24 h incubation at 30 °C, the slants were sealed with rubber stoppers. Ethylene production was measured 24 h after sealing. (b) Bacteria were grown in YPS liquid medium with or without 10 mM methionine for 24 h, after which 5 ml of bacterial suspension were transferred to 21 ml rubber stoppered bottles. Ethylene production was recorded after 24 h incubation at 30 °C. (c) One millilitre of a bacterial suspension was mixed with 19 ml YPS medium in rubber stoppered 100 ml bottles and incubated for 48 h at 30 °C. Reductions in dissolved oxygen during the course of incubation were measured using a galvanometric oxygen electrode (NBS model M1016-0208) fitted to a Servomex oxygen analyser. Ethylene production was determined after incubation. Sterile media were used as controls.

*Ethylene cells*

Four pepper plants with six fully expanded leaves were placed in hermetically sealed 11 litre glass flasks. One millilitre of purified ethylene was injected through a rubber bridge into each flask. Half of the flasks were covered with aluminium foil to exclude the light and, with the others, were incubated in a temperature controlled glasshouse at  $30 \pm 2$  °C. Flasks without added ethylene served as controls.

*Chemical treatments*

The following substances were dissolved in distilled water supplemented with 0.01% Tween 20: 1 mM indole-3-acetic acid (IAA) (Sigma) containing 0.1% acetone; 1 and 0.1 mM aminooxyacetic acid (AOA) (Sigma) and 10 mM methionine. The solutions were sprayed on to the leaves until run off and the leaves then inoculated after the solutions on the leaf surfaces had dried.

*Experimental design*

All experiments were replicated and repeated two to four times. The replicates, arranged in a randomized design, consisted of 10 plants, 10 plant parts, 10 flasks or four ethylene cells. The results given are from a representative experiment.

**RESULTS***Ethylene production in culture by three phytopathogenic bacteria*

Three bacterial pathogens (*X. campestris* pv. *vesicatoria*, *P. syringae* pv. *tomato* and *P. syringae* pv. *lachrymans*) were tested for their ability to produce ethylene in culture. All three isolates failed to produce ethylene in culture either under aerobic or fully anaerobic conditions, but, when the oxygen tension was reduced to between 0.01 and 0.05 atm, all three produced small amounts, ranging from 222 to 1022 pmol ethylene h<sup>-1</sup> per 20 ml of bacterial suspension containing 10<sup>9</sup> cfu ml<sup>-1</sup>.

*Ethylene production in relation to disease and leaf abscission*

Leaf abscission from pepper plants incubated in ethylene cells was evaluated. Percentage leaf drop 24 h after ethylene injection was  $62 \pm 4\%$  and increased to  $72 \pm 3\%$  after 48 h in illuminated cells, while in dark cells percentage leaf drop after 24 h was  $50 \pm 6\%$ , and after 48 h was  $77 \pm 4\%$ .

Pepper, tomato and cucumber plants were inoculated with *X. campestris* pv. *vesicatoria*, *P. syringae* pv. *tomato* or *P. syringae* pv. *lachrymans*. After symptom development in each host inoculated with its compatible pathogen, leaves from both the compatible and the two incompatible combinations were sampled for ethylene determinations. Pepper leaves inoculated with *X. campestris* pv. *vesicatoria* produced  $3710 \pm 127$  pmol ethylene g<sup>-1</sup> dry weight h<sup>-1</sup> compared to  $105 \pm 15$  pmol ethylene g<sup>-1</sup> dry weight h<sup>-1</sup> in uninoculated plants or in pepper plants inoculated with the incompatible pathogens *P. syringae* pv. *tomato* or *P. syringae* pv. *lachrymans*. The amount of ethylene produced by tomato or cucumber, whether involving compatible or incompatible combinations was negligible, reaching a maximum of no more than  $250 \pm 40$  pmol ethylene g<sup>-1</sup> dry weight h<sup>-1</sup>. The amounts of ethylene produced by non-inoculated tomato and cucumber plants was too low to be detected by the method used.

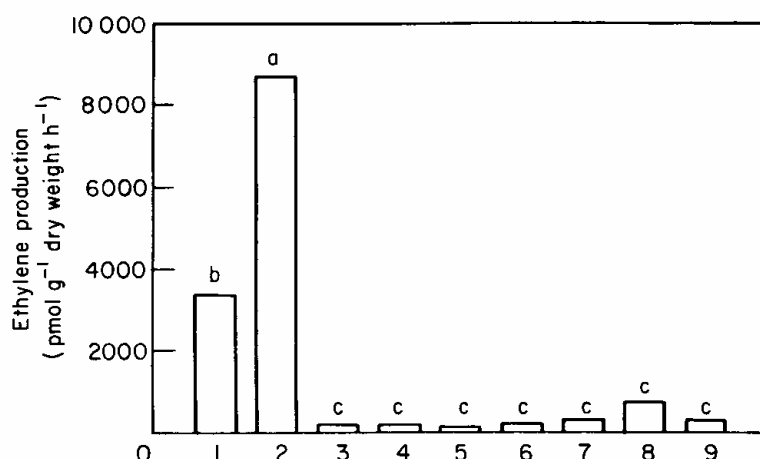


FIG. 2. Effects of different stress factors on ethylene production in young leaves. 1, inoculated with *X. campestris* pv. *vesicatoria*; 2, inoculated with *Oidiopsis taurica*; 3, infested with red spider mites (*Tetranychus cinnabarinus*); 4, water stress; 5, wounded by rubbing with carborundum; 6, incubated at 40 °C for 4 h; 7, punctured with needles; 8, incubated at 4 °C for 24 h; 9, untreated control. Differences between column 1 and 2 were significant from each other and from all other columns,  $P \leq 0.05$ . None of the other differences between columns 3 to 9 were significant. Ethylene production was determined over a 24 h period 5 days after inoculation or treatment.

Pepper plants were subjected to several forms of stress as well as that due to inoculation with *X. campestris* pv. *vesicatoria*. Samples taken immediately after the imposition of the stress or inoculation and up to 24 h later evolved negligible amounts of ethylene. Figure 2 shows that only inoculated pepper plants were producing significant quantities of ethylene after 5 days. Except for exposure to low temperatures (4 °C), none of the stress treatments, as indicated by measurements made 1 and 5 days later, had any effect on ethylene production over the 5 day period subsequent to treatment. Exposure to 4 °C induced a slight increase in ethylene production ( $746 \pm 153$  pmol ethylene g<sup>-1</sup> dry weight h<sup>-1</sup>) after 5 days.

#### *Ethylene production in the diseased plant*

Ethylene production and bacterial multiplication were monitored during disease development. The rate of ethylene production increased with time after inoculation [Fig. 3(a)]. Similarly, the *X. campestris* pv. *vesicatoria* population in the tissues increased with time [Fig. 3(b)]. After 7 days, the disease index of the infected plants was  $2.97 \pm 0.1$ .

The relationship between the number of bacteria in the leaf and ethylene production was examined in two experiments. In one, pepper leaves were inoculated with *X. campestris* pv. *vesicatoria* at concentrations of  $10^3$ ,  $10^5$ ,  $10^7$  and  $10^9$  cfu ml<sup>-1</sup> and leaves were examined for ethylene production 6, 8 and 13 days after inoculation. Bacterial scab symptoms appeared after 5 days in plants inoculated with  $10^7$  and  $10^9$  cfu ml<sup>-1</sup>, after 7 days in those inoculated with  $10^5$  cfu ml<sup>-1</sup>, and 9 days after inoculation with  $10^3$  cfu ml<sup>-1</sup>. Figure 3(c) shows a positive linear correlation between initial inoculum concentration and ethylene production, at all three sampling times, with the highest rates of ethylene production occurring on the 8th day following inoculation. In another experiment, infected leaves were sampled 6, 8 and 13 days after inoculation, and the bacterial population within them, together with their capacity to produce ethylene, was determined. A significant quadratic relationship ( $r = 0.969$  at  $P \leq 0.001$ ) was found

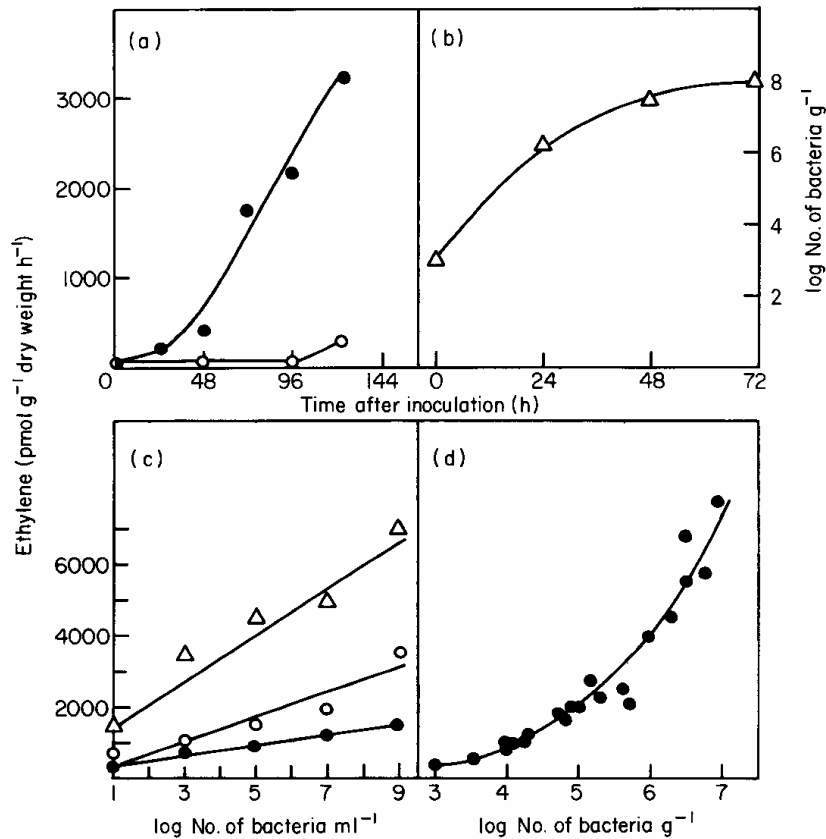


FIG. 3. Ethylene production in infected tissues of pepper plants in relation to time after inoculation and bacterial growth.

(a) Ethylene production in relation to time after inoculation: ●, inoculated plants; ○, non-inoculated control. (b) Bacterial multiplication in leaves in relation to time after inoculation. Each point is the mean of 10 replicates. (c) Regression lines of the relationship between inoculum concentration and ethylene production in leaves at different times after inoculation. In all cases the linear regressions are significant at  $P \leq 0.05$ . ○, 6 days after inoculation,  $y = 0.279x + 0.206$ ;  $r = 0.857$ ; △, 8 days after inoculation,  $y = 0.578x + 1.369$ ;  $r = 0.977$ ; ●, 13 days after inoculation,  $y = 0.125x + 0.206$ ;  $r = 0.99$ . Each point is the mean of 10 replicates. (d) Relationship between ethylene production and bacterial population in infected tissues 8 days after inoculation.  $y = 0.552x^2 - 3.866x + 7.361$  at  $3 < x < 7$ ,  $r = 0.969$  significant at  $P \leq 0.001$ . Each point is the mean of five replicates.

between the actual number of *X. campestris* pv. *vesicatoria* in infected tissue as determined at sampling time and ethylene production eight days after inoculation [Fig. 3(d)].

#### *Relations between disease severity, leaf age and ethylene production*

Tissues from pepper leaves showing different disease severities were analysed for ethylene production. Figure 4 shows a positive linear correlation ( $r = 0.924$ ,  $P \leq 0.05$ ) between disease severity and ethylene production. In addition, an analysis of ethylene production by leaves of different ages, showed that the more susceptible young leaves also produced the most ethylene on infection. Inoculation of mature leaves did not stimulate increased ethylene production (Table 1).

#### *Ethylene production sites in relation to chlorosis, necrosis and leaf abscission*

Inoculated and non-inoculated plant parts were sampled for ethylene production as indicated in Fig. 1. Table 2 shows that the major site of ethylene production is the leaf

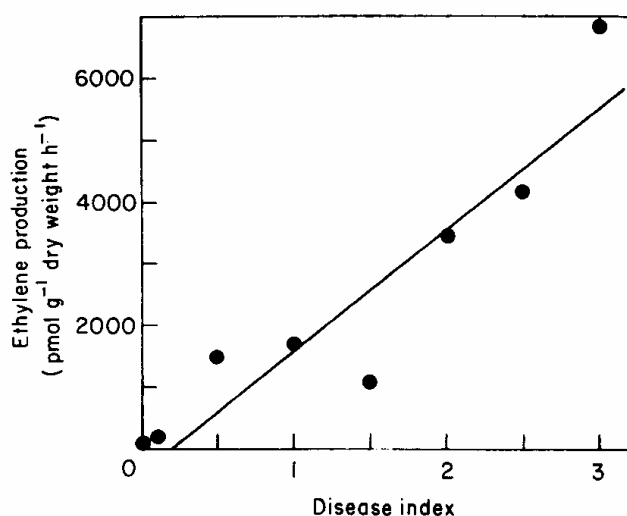


FIG. 4. Relationship between disease severity and ethylene production in pepper plants. Each point represents the mean of 10 replicates. Ethylene production and the disease index were determined 10 days after inoculation.  $y = 1.914x - 0.1801$ ;  $r = 0.924$  significant at  $P \leq 0.05$ .

TABLE 2  
*Ethylene production in relation to development of lesions in X. campestris pv. vesicatoria infected leaves*

Site	(See Fig. 1A)	Ethylene <sup>a</sup> ( $\text{pmol g}^{-1}$ dry weight $\text{h}^{-1}$ )
Distal half of the lamina	-1a	1385B <sup>b</sup>
Proximal half of the lamina	-1b	723C
Petiole	-1c	0D
Connection between petiole and stem	-1d	0D
Leaf discs with symptoms	-2a	2575B
Leaf discs without symptoms	-2b	558C
Diseased tissue	-3a	5193A
Apparently healthy tissue from diseased leaves	-3b	213C
Non-inoculated leaf		91C
Non-inoculated petiole		0D

<sup>a</sup>Ethylene production was determined over a 24 h period, 8 days after inoculation.

<sup>b</sup>Numbers followed by different letters differ significantly at  $P \leq 0.05$ .

blade, particularly the distal part. No detectable level of ethylene was produced by petioles or stems. This experiment showed a relationship between necrotic sites and ethylene production. Further evidence for a relationship between ethylene production and necrosis, chlorosis and leaf abscission was obtained by inoculating different parts of the plant [Fig. 1(b)]. Table 3 demonstrates that ethylene production was stimulated only at sites where bacteria were inoculated.

#### *Effects of IAA and AOA on ethylene production and disease severity*

Groups of inoculated pepper plants were treated with IAA at the same time as they were inoculated, or 2 or 4 days later. Figure 5(a) shows that all treatments with IAA reduced both disease severity and ethylene production. However, both parameters were monitored only after 7 days, at which time leaf abscission was minimal (approx. 2%). In a further experiment, exogenous IAA was sprayed on 1 h before inoculation at various



TABLE 3  
*Relationships between ethylene production, chlorosis, necrosis and leaf abscission in infected plants*

Site of inoculation	Necrotic leaves <sup>a</sup> (%)	Chlorotic leaves <sup>a</sup> (%)	Leaf abscission <sup>a</sup> (%)	Ethylene <sup>b</sup> (pmol g <sup>-1</sup> dry weight h <sup>-1</sup> )	
				Leaf blade	Leaf petiole
Leaf blade (1) <sup>c</sup>	90a <sup>d</sup>	0.3b	11b	3573a	0b
Leaf petiole (2) <sup>c</sup>	0b	42a	30a	809b	2900a
Connection between petiole and stem (3) <sup>c</sup>	0b	21b	2b	168b	3700a
Non-inoculated leaves	0b	0c	0c	240b	0b

<sup>a</sup>200 leaves were sampled.

<sup>b</sup>Ethylene production was determined over a 24 h period, 8 days after inoculation.

<sup>c</sup>See Fig. 1(b) for details of tissues inoculated and analysed.

<sup>d</sup>In each column numbers followed by different letters differ significantly at  $P \leq 0.05$ , calculated using Duncan's multiple range test.

concentrations ( $10^{-3}$ ,  $5 \times 10^{-4}$  and  $5 \times 10^{-5}$  M). All treatments significantly reduced ethylene production from about 8000 to 3500 pmol g<sup>-1</sup> dry weight h<sup>-1</sup>, percentage leaf abscission from 60% to 30% compared to the untreated controls and disease index from 2 to 1. Treatment with AOA 1 h before inoculation significantly decreased disease severity and ethylene production, but had no effect on leaf abscission [Fig. 5(b)].

## DISCUSSION

Ethylene production by leaf tissue during the development of bacterial infections is not a common phenomenon [11, 15, 23]. Observations in this study and by Stall & Hall [23], on bacterial scab of pepper, indicate the production of considerable amounts of ethylene during disease development. No such production was observed with bacterial speck of tomato or angular leaf spot of cucumber. Therefore this study concentrated on ethylene production in pepper in relation to disease severity (as expressed by necrotic symptoms) and leaf abscission.

Studies with a number of plant species suggest that increased ethylene synthesis and leaf abscission are reactions to both biotic and abiotic stresses [7, 14, 25]. However, we found that, of a variety of physiological and environmental stresses including attack by mites, only infection by *O. taurica* and *X. campestris* pv. *vesicatoria* induced significant production of ethylene in *C. annuum*. In both cases, when disease severity and ethylene production increased, leaf abscission occurred.

Since ethylene evolution only began several days after inoculation, its production is different from "wound ethylene" which is released shortly after mechanical injury or treatment with chemicals [25].

It is known that young leaves are more severely infected by *X. campestris* pv. *vesicatoria* than old ones [9]. The rate of ethylene production was found to be higher in young infected leaves, although mature healthy leaves of pepper usually produced more ethylene than young healthy leaves.

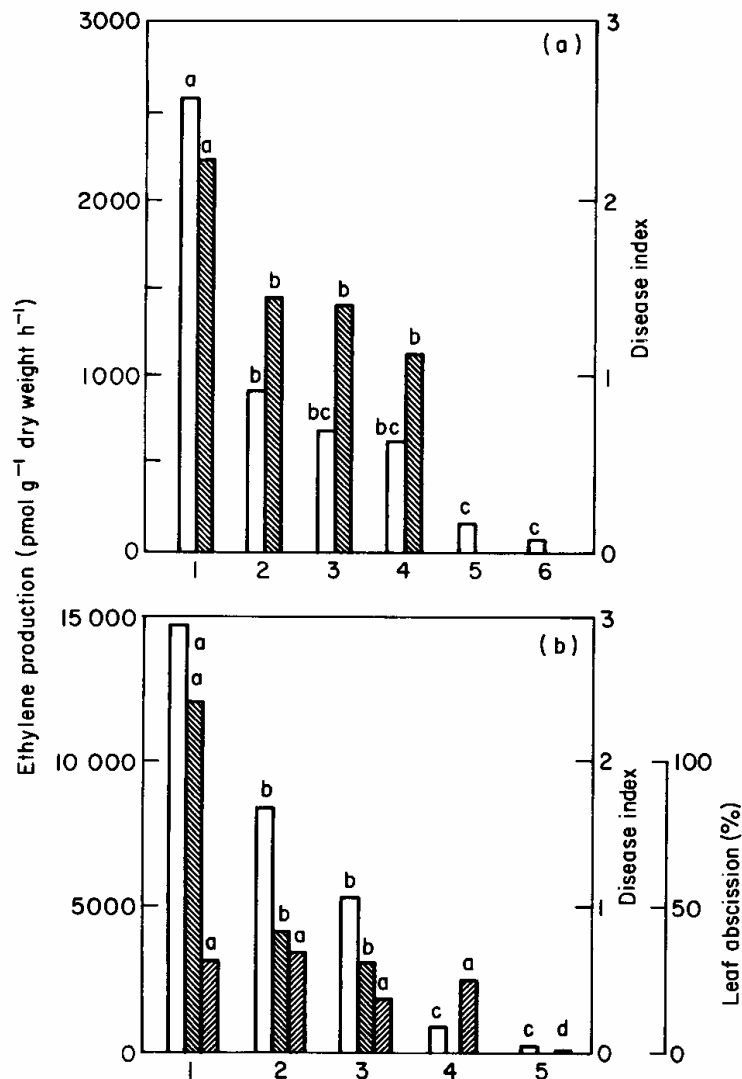


FIG. 5 (a) The effect of time of application of indole-3-acetic acid (IAA) to plants on ethylene production and disease severity. 1, Inoculated untreated plants; 2, IAA applied at time of inoculation; 3, IAA applied 2 days after inoculation; 4, IAA applied 4 days after inoculation; 5, healthy plants treated with IAA at the same time as group 2 plants; 6, healthy plants.

(b) The effect of aminooxyacetic acid (AOA) on ethylene production, leaf-abscission and disease severity; 1, inoculated, untreated plants; 2, 1 mM AOA applied 1 h before inoculation; 3, 0.1 mM AOA applied one hour before inoculation; 4, healthy plants treated with AOA; 5, healthy controls. Ethylene production was measured over a 24 h period 7 days after inoculation.

□, Ethylene production; ▨, Disease index; ▩, Leaf abscission. Related columns with different letters are significantly different at  $P \leq 0.05$ ; calculated using Duncan's multiple range test.

It has been reported that the polyphagous phytopathogenic bacterium *P. solanacearum* [10] as well as some strains of *P. syringae* pv. *phaseolicola* and *E. rhapontici* [12] produce ethylene in culture. We found small quantities of ethylene to be produced by *X. campestris* pv. *vesicatoria* in liquid culture but only when it was grown under reduced oxygen tensions. The addition of methionine to aerobic cultures did not induce ethylene production. These results are not in accord with those of Stall & Hall [23], who observed ethylene production by aerobic cultures. It is concluded that the ethylene is produced by the plant but that its production is triggered by the pathogen. However, the mechanism which triggers production is unknown. Increased ethylene production paralleled disease

development, the number of bacteria in the initial inoculum and the number of bacteria in the tissue [21]. Ethylene production was at its maximum 8 days after inoculation, when the bacterial population had reached a level of  $10^8$  cfu g<sup>-1</sup> tissue and when there was visible necrosis. These findings are similar to those of Goto *et al.* [11], with citrus leaves infected with *X. campestris* pv. *citri*.

Diab *et al.* [9] demonstrated a relationship between leaf abscission and disease severity. In this study we tried to locate the sites of ethylene synthesis in relation to leaf abscission. When the inoculum was applied near the base of the petiole (abscission site), both leaf-abscission and ethylene production increased, as occurs in citrus leaves infected with *X. campestris* pv. *citri* [11]. In most experiments, it was shown that ethylene was produced from tissues where bacteria were present. Leaf abscission occurred during the later stages of disease development. This abscission has also been demonstrated with powdery mildew of pepper [20]. Therefore, it may be suggested that leaf abscission is induced by plant produced ethylene, the production of which is triggered by bacterial activity.

Contradictory results concerning the relationships between IAA and ethylene have been reported. On the one hand, exogenous IAA has been reported to stimulate or have no effect on ethylene production [1, 8] while, on the other hand, antagonistic actions between these hormones have been reported [8, 14]. In pepper plants, the rate of ethylene production is known to be related to auxin levels in the tissue [20], and so applications of high concentrations of IAA may prevent ethylene production and thus leaf-abscission and so give some control of the more severe aspects of bacterial scab disease. The application of AOA, a known ethylene inhibitor, similarly decreased ethylene production and disease severity. However, AOA is not a very specific ethylene inhibitor and can inhibit many pyridoxyl phosphate mediated reactions.

At the present moment bacterial scab is only partially controlled by chemicals [2]. Treatments with IAA may provide some control of the disease, by alleviating symptom production rather than reducing pathogen growth.

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