Chlorosis and Ethylene Production in Pepper Leaves Infected by *Xanthomonas campestris* pv. *vesicatoria*

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ABSTRACT

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An unusual pepper (*Capsicum annuum*) line, designated P-107, was selected from descendants of a cross between a plant of PI 271322 and cultivar Early Calwonder (ECW). Leaves of P-107 yellowed rapidly compared to leaves of ECW after inoculation with the bacterial spot pathogen (*Xanthomonas campestris* pv. *vesicatoria*). Ethylene evolution, multiplication of the bacterium, and electrolyte leakage increased in leaves of P-107 and ECW after inoculation. The pattern of increase for each parameter was similar in both pepper types. However, chlorosis occurred more rapidly in P-107 leaves than in ECW leaves after exposure to ethylene or infiltration with ethephon. The differential rate of chlorosis of the two pepper types after exposure to ethylene and after inoculation with *X. campestris* pv. *vesicatoria* is evidence that the chlorotic zone surrounding necrotic lesions of bacterial spot is associated with ethylene production in the diseased leaves.

Additional key words: bacterial spot of pepper.

The yellowing of tissue (chlorosis) around the necrotic spots caused in leaves of pepper (*Capsicum annuum* L.) by *Xanthomonas campestris* pv. *vesicatoria* (Doidge) Dye is a common symptom of the bacterial spot disease. The yellowing usually increases in area with time and often encompasses the whole leaf. Abscission of yellowed leaves often follows.

Leaves of some pepper plants obtained from seed from the USDA Plant Introduction Station, Experiment, GA, became yellow more rapidly than did leaves of plants of commercial cultivars after inoculation with the bacterial spot pathogen. This differential response provided an opportunity to investigate the causal factors for the yellowing symptom associated with bacterial spot.

Yellowing occurs after treatment of plant leaves with ethylene (1, 3). Reports on the production of ethylene in diseased plants and the association of ethylene with symptoms of disease have been reviewed (7). An association of ethylene production has been made with epinasty, leaf abscission, and premature fruit ripening (4–6). However, no report was found that dealt specifically with the causal relationships of ethylene and the yellowing symptom associated with a leaf-spot pathogen.

The purpose of the research reported here was to determine if production of ethylene increased in diseased pepper leaves and if an association existed between the chlorosis caused in pepper leaves by *X. campestris* pv. *vesicatoria* and that caused by ethylene in commercial and chlorosis-prone pepper plants.

MATERIALS AND METHODS

Plants in the F_2 generation from a cross of a plant of *Capsicum annuum* 'PI 271322' with a plant of *C. annuum* 'Early Calwonder' were selected for rapid yellowing of leaves after inoculation with *X. campestris* pv. *vesicatoria* (Fig. 1). Progeny of the selected F_2 plants were again inoculated and a line within which all plants were susceptible and responded with rapid yellowing was selected. This line was designated P-107 and seeds of several plants were combined for production of plants for this work. The test plants were in the F_4 generation. Plants of Early Calwonder (ECW) were used to represent a standard commercial cultivar.

Plants about 15 cm tall were transplanted to 8-L plastic pots, each of which contained 4 kg of Metromix 500 (W. R. Grace, Cambridge, MA 02140). A water-soluble fertilizer (20-20-20, NPK) was added weekly at 1.4 g per plant. The plants were flowering before leaves were inoculated or infiltrated. Leaves below the first fork of the plant were used. The plants were kept in a glasshouse at 25–35 C.

A strain of *X. campestris* pv. *vesicatoria* was cultured in nutrient broth, centrifuged to form a pellet, resuspended in sterile tap water, and standardized in a 1-cm-diameter tube to a turbidity equivalent to $A = 0.3$ at 600 nm wavelength. The resulting suspension contained about 3×10^8 cells per milliliter and was diluted to obtain 10^4 cells per milliliter. Leaves were completely infiltrated with the latter suspension by injection with a hypodermic syringe and needle. Some leaves were infiltrated by the same procedure with 240 $\mu g \cdot ml^{-1}$ ethephon, a source of ethylene. Control leaves were infiltrated with sterile tap water.

Leaves were removed from plants at designated intervals after inoculation with bacteria, or infiltration with ethephon or water. Ethylene evolution, chlorophyll content, bacterial concentration and electrolyte leakage were determined with each leaf. For

Fig. 1. Leaves of P-107 (left) and Early Calwonder pepper (ECW) inoculated 10 days previously at four locations with *Xanthomonas campestris* pv. *vesicatoria* at a concentration of $\sim 10^8$ cells per milliliter. The P-107 leaf became yellow, but the ECW leaf remained green.

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determinations of ethylene evolution, a leaf was weighed, placed in a test tube of 34.5 ml volume, sealed with a serum cap, and kept in the light at 23 C. Samples were withdrawn hourly over a 3-hr period. Ethylene was determined with a gas chromatograph with a flame-ionization detector and an activated alumina column. Chlorophyll content was determined by the Arnon procedure (2). Only total chlorophyll data are reported. Bacterial concentration and electrolyte leakage were determined as previously described (11).

For determinations of ethylene sensitivity, leaves were detached and their petioles were placed in test tubes, each of which contained 10 ml of water. Each test tube, with its leaf, was placed in a 0.95-L Mason jar. A 50-ml beaker containing 25 ml of 1N NaOH was also placed in each jar to absorb CO_2. Enough ethylene was added to half of the sealed jars to reach a concentration of 10 nl·ml^{-1} in each jar. The other half of the jars received no ethylene and served as controls. The jars were kept in an area with light intensity of 110 μE·m^{-2}·sec^{-1} and a 14-hr daylength. The ethylene content in all jars was checked at the beginning of the experiment and daily until the end of the experiment.

Each experiment was repeated, but mean values of four replicates of only one of the experiments are given. The results of the duplicate experiment were similar.

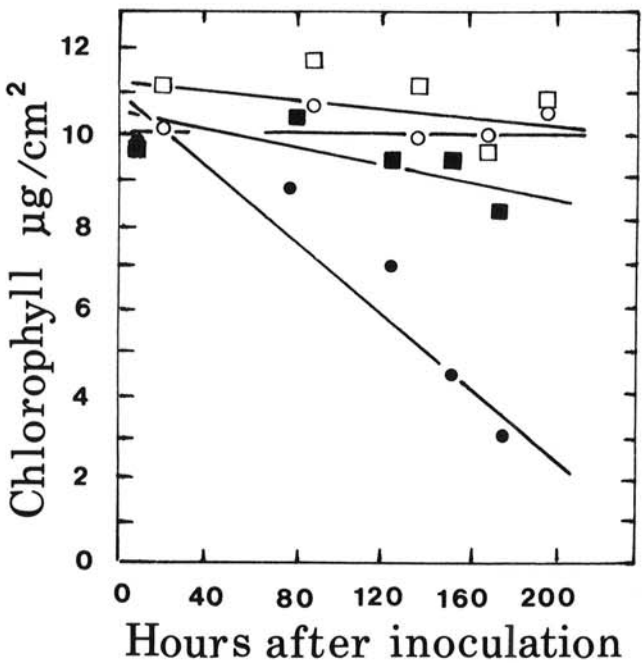

Fig. 2. Regression lines for chlorophyll content versus time in P-107 (circles) or Early Calwonder (squares) pepper leaves inoculated with *Xanthomonas campestris* pv. *vesicatoria* (closed) or infiltrated with water (open).

RESULTS

Chlorophyll decreased in inoculated leaves of P-107 plants, but did not decrease in control leaves of P-107, which were infiltrated with water only (Fig. 2). A slight decrease in chlorophyll occurred in ECW leaves after water infiltration, and a slightly greater loss of chlorophyll occurred after inoculation. However, chlorophyll loss in inoculated leaves of P-107 was much more rapid than in inoculated leaves of ECW.

The conductance of water containing inoculated leaf disks of P-107 increased 34 μmhos·hr^{-1} immediately after inoculation and 58 μmhos·hr^{-1} at the last assay period, 190 hr after inoculation. With inoculated tissue of ECW, conductivity changed 41 μmhos·hr^{-1} at the beginning and 112 μmhos·hr^{-1} after 190 hr of incubation. Leakage of electrolytes from control leaves of P-107 and ECW was slightly less in both types of pepper at the last assay than in the assay made immediately after inoculation.

Bacterial populations in the inoculated leaves of P-107 and ECW were similar at each of the five sampling times. *X. campestris* pv. *vesicatoria* increased from 4.5×10^2 colony-forming units (cfu) per square centimeter of leaf tissue to 1.5×10^8 cfu in P-107 leaves. The bacterium increased from 5.8×10^2 cfu to 1.3×10^8 cfu per square centimeter of leaf tissue in ECW leaves. Populations were high enough to cause visible necrosis with both pepper lines only at the last determination, which was 190 hr after inoculation.

A sharp increase in the evolution of ethylene occurred in the inoculated leaves of both P-107 and ECW after 125 hr of incubation (Fig. 3). After 149 hr of incubation, ethylene evolution dropped in inoculated P-107 leaves, but continued to increase in

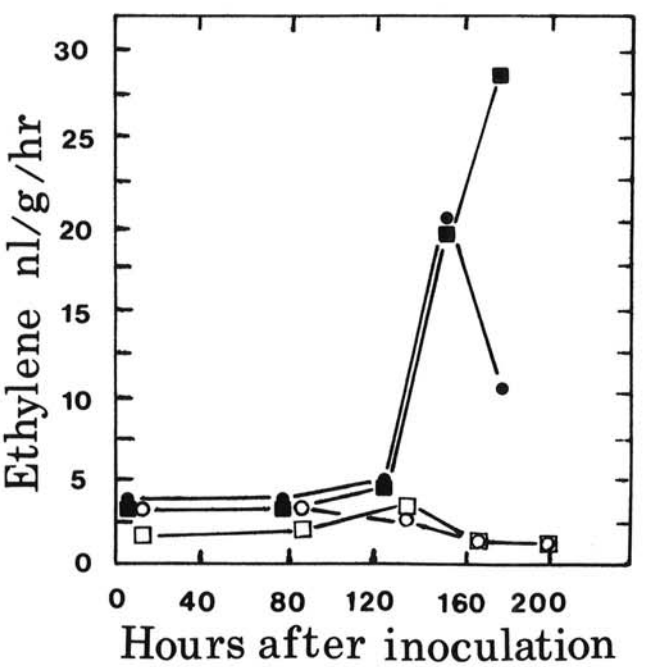

Fig. 3. Time course of ethylene evolution from P-107 (circles) or Early Calwonder (squares) pepper leaves inoculated with *Xanthomonas campestris* pv. *vesicatoria* (closed) or infiltrated with water (open).

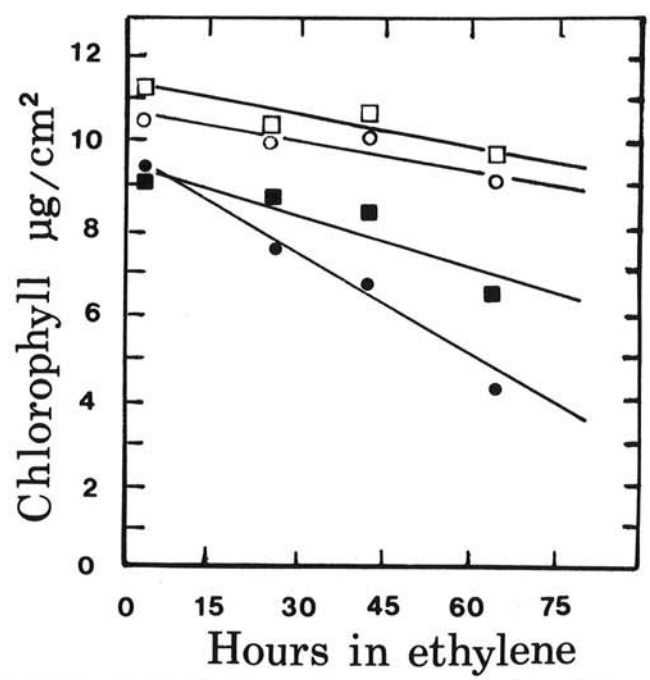

Fig. 4. Regression lines for chlorophyll content versus time in P-107 (circles) or Early Calwonder (squares) pepper leaves placed in an atmosphere of 10 ppm ethylene (closed) or without ethylene (open).

inoculated ECW leaves. Ethylene evolution did not increase in water-infiltrated leaves of P-107 or ECW over the 190-hr test period.

Chlorophyll decreased more rapidly in detached P-107 leaves than in detached ECW leaves placed in closed jars with an atmosphere of 10 $nl \cdot ml^{-1}$ of ethylene (Fig. 4). Chlorophyll also decreased in both P-107 and ECW leaves that were detached and placed in jars with no ethylene added to the atmosphere, but the rate of decrease in leaves of both types was about the same in the atmosphere without added ethylene. Thus, leaves of P-107 were more sensitive to ethylene than were leaves of ECW.

Leaves of P-107 were also more sensitive than were leaves of ECW to loss of chlorophyll after infiltration of attached leaves with ethephon (Fig. 5). Chlorophyll decreased in water-infiltrated leaves only slightly during the 120-hr test. Electrolyte leakage from treated leaves did not increase after infiltration of either type of leaf with either ethephon or water during the test.

DISCUSSION

The necrotic and chlorotic symptoms associated with diseased plants have been thought to be caused by toxins, but epinasty and defoliation were attributed to ethylene-stimulated growth regulator imbalances (12). Some types of chlorosis associated with disease, however, may be stimulated by ethylene. The evidence for that presented here is based on the differential response of the pepper types. Yellowing, whether induced by *X. campestris* pv. *vesicatoria* or by exogenously supplied ethylene, was more pronounced in the yellowing-sensitive plants than in plants of a commercial cultivar.

The amount of ethylene evolved after inoculation of the two types of leaves with *X. campestris* pv. *vesicatoria* was different only at the last determination. The decline in ethylene evolution in P-107 leaves at that time was probably because they were chlorotic. The ethylene evolution data at the last determination probably was not important, because differential chlorosis had already occurred. The major reason leaves of P-107 become yellow more rapidly than ECW leaves after inoculation with *X. campestris* pv. *vesicatoria* seems to be that P-107 leaves are more sensitive to the effects of ethylene produced in the leaves. The reason for the differences in sensitivity are not clear at present. Sensitivity is probably not associated with resistance to the pathogen, because bacterial multiplication and electrolyte leakage occurred about equally in both types of plants.

The source of ethylene in diseased pepper leaves was not determined, but others have speculated that ethylene is produced by the plant even though a pathogen may produce ethylene (8). Production of ethylene in nutrient broth cultures of *X. campestris* pv. *vesicatoria* has not been detected, but some ethylene is produced if methionine is added to the nutrient broth (*unpublished*). Production of small amounts of ethylene by *X. campestris* pv. *vesicatoria* may be important, however, because ethylene was found to stimulate ethylene production in citrus leaves (9). This may occur in pepper, too.

Mechanical injury is known to stimulate ethylene production in leaves (10). Pathogenic bacteria may mimic such injury in the development of lesions. The differential chlorosis in the two pepper types after inoculation does not appear to be caused by differential injury, however. A relatively low number of bacteria was infiltrated into leaves in this work. Little visible necrosis and only a small increase in electrolyte leakage occurred during the course of the experiments. Based on electrolyte leakage, less injury occurred in inoculated P-107 leaves than in inoculated ECW leaves. However, injury was not extensive in either plant type during the course of the experiments.

Relative insensitivity to ethylene is common among commercial cultivars of bell pepper. This trait apparently is inherited and may have been selected by plant breeders unknowingly when selections were made against damage caused by pathogenic organisms.

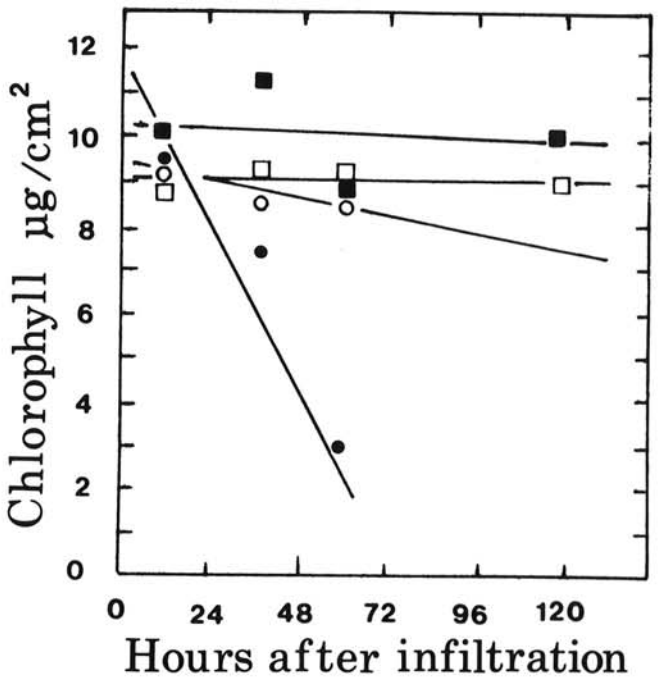

Fig. 5. Regression lines for chlorophyll content versus time in cultivars P-107 (circles) or Early Calwonder (squares) pepper leaves infiltrated with ethephon at 240 $\mu g \cdot ml^{-1}$ (closed) or water (open).

Ethylene insensitivity may be advantageous for increased yields when diseases occur on plants. On the other hand, relative insensitivity to ethylene may be responsible for the poor fruit-ripening that occurs in bell peppers. Field research is needed to assess the economic importance of ethylene insensitivity in bell pepper.

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