Physiological Responses of Susceptible and Resistant Cucumber to Erwinia tracheiphila

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ABSTRACT

Bacteria multiplied rapidly in susceptible cucumber plants inoculated with *Erwinia tracheiphila*. Initial wilt of lower leaves occurred after 2 days. Histological examination showed that the bacteria migrated upward through the xylem of stem and petiole en masse, and plugging coincided with progressive wilt of adjacent leaves. Vascular deterioration was first seen 6 days after inoculation, by which time the plants were completely wilted. No wilt, plugging, or vascular deterioration were observed for resistant plants. Isolation assays demonstrated virulent bacteria in resistant plants for 10 days after inoculation, but the bacteria disappeared by the time the plants reached maturity. Transpiration decreased 1 day after initial wilt, and continued to decrease progressively as wilt developed. Respiration increased 2 days after wilt, and increased progressively for 10 days. Only slight and delayed transpiration and respiration responses occurred in resistant plants, which indicated limited multiplication. No evidence for pectolytic or cellulotytic enzyme production in vitro or in vivo was observed. Vascular plugging and wilt preceded physiological response and vascular deterioration; therefore, the primary wilting mechanism was considered to be bacterial plugging. Resistance was thought to involve a nutritional or bacterial inhibition principle that prevented continued multiplication and characteristic susceptible wilt symptoms. Phytopathology 61:518-522.

Additional key words: Cucumis sativus, bacterial wilt.

Bacterial wilt caused by *Erwinia tracheiphila* is an endemic and sometimes serious disease of commercial cucumbers in Wisconsin (17). The historical significance of this disease dates back to the early investigations of E. F. Smith (14) and the highly specialized insect vector-pathogen relationship described by Rand & Cash (13). Although deterioration of the host xylem occurs in advanced stages of pathogenesis (20), mechanical plugging by masses of bacteria is generally considered the primary cause of wilting (3, 14, 20). Wilt symptoms coincide with decrease in transpiration 4-5 days after inoculation. Early increase in transpiration prior to wilt indicative of phytotoxin action on leaf permeability has not been found (3, 20).

It is not known whether xylem deterioration is the result of mechanical pressure alone, due to multiplication of the bacteria, or if macerating enzymes are involved. Smith (15) found no polyglycosidase (β-glycosidase) or pectinmethylesterase activity in culture filtrates of *E. tracheiphila*.

Commercial wilt-resistant cucumber cultivars are not available; however, a source of monogenic resistance has been reported (11, 19). This resistance has been incorporated into Wisconsin scab mosaic-resistant breeding lines (9).

Physiological and histological comparisons have not been made between susceptible and resistant cucumber plants. The objectives of this investigation were to study alterations in transpiration, respiration, and pathological deterioration of xylem caused by *E. tracheiphila* in order to gain information on pathogenesis and the nature of resistance.

MATERIALS AND METHODS.—Wilt-susceptible cucumber (*Cucumis sativus* L. 'Wisconsin SMR 18') and a homozygous-resistant breeding line (WR18) were used for all studies. Plants were grown in steamed quartz sand in 6-inch porcelain crocks and watered as needed with Hoagland's nutrient solution (5). All experiments were carried out at 28 ± 2 C in the greenhouse under a 14-hr photoperiod provided by Gro-Lux fluorescent lamps supplemented with incandescent lamps providing approx 1,400 ft-c at bench level.

The isolate of *E. tracheiphila* (9) was obtained from infected, field-grown cucumbers at Madison, Wis., in 1962. Since this organism loses pathogenicity quickly in culture (2), virulence was maintained by repeatedly inoculating small, susceptible cucumber plants every 10-14 days with plant juice expressed from infected plants (12). Inoculum was prepared in a similar manner. Serial dilution plate counts established inoculum concentrations in the juice to be approx 2×10^6 cells/ml.

Inoculation and isolation procedures.—Inoculations were made by stem puncture into each of the five bicollateral bundle ridges at the base of the second internode of 3-week-old plants. Assessment of bacterial multiplication and distribution was conducted by isolating from stem, hypocotyl, and root tissues 6 and 10 days after inoculation (12). All isolates obtained were tested for pathogenicity using a cucumber seedling assay (9, 12). Quantitative estimation of bacterial populations in vivo was not attempted.

Wilt symptoms appeared on the lowermost leaves of susceptible plants 2 days after inoculation; wilting progressed upward until the plants were completely wilted

after 6 days. Wilt symptoms were never observed on inoculated resistant plants, although a slight stunting was noted. Virulent bacteria were isolated from all stem internodes of both susceptible and resistant plants 6 days after inoculation, but not from hypocotyl or root tissue. *Erwinia tracheiphila* was never isolated from inoculated resistant plants that were permitted to reach maturity.

Effect of infection on transpiration.—Susceptible and resistant plants were carefully removed from sand culture and the roots gently washed free of sand with tap water. The root system of each plant was then immersed in Hoagland's nutrient solution in 1-liter flasks. The flasks were covered to inhibit the growth of algae. After a 48-hr equilibration period, five susceptible and five resistant plants were inoculated by stem puncture. Five plants of each cultivar were inoculated with sterile water in the same manner and served as controls. At 24-hr intervals for a period of 8 days, wilt development and volume of water transpired were recorded. The volume taken up by five plants/inoculation treatment was used to compute transpiration rates expressed as ml/plant per day. The experiment was performed twice.

Transpiration rate increased as leaf area increased for 3 days after inoculation in susceptible plants, then decreased sharply to near zero at 6 days (Fig. 1-A). Transpiration rate continued to increase normally during the 8-day experimental period in the controls. Only a slight decrease in transpiration occurred in inoculated resistant plants compared to the resistant control.

Effect of infection on respiration.—Susceptible and resistant plants grown in sand culture were stem inoculated as described above. Plants of each cultivar were inoculated with sterile water and served as controls. Two days after inoculation, and continuing for 8 days thereafter, respiration rate of stem tissue was measured by conventional Warburg technique (10, 16). Twelve stem tissue discs (1 mm thick and totaling approx 200 mg fresh wt) from the third internode were placed in 0.01 M sodium phosphate buffer (pH 6.5) and evacuated at 20 lb./square inch vacuum to remove the bacteria from the xylem (10). The discs were washed in buffer and placed in 15-ml reaction vessels containing 0.2 ml of 20% (w/v) potassium hydroxide in the center well, and 2.9 ml of the same buffer used above in the main compartment. Following a 15-min thermal equilibration period, endogenous respiration was measured at 15-min intervals for 2 hr at 30 C in the dark. At the termination of each experiment, the discs were blotted and dried to constant weight in a forced air oven at 90 C. Each treatment was performed in duplicate, and respiration rates from two separate experiments were computed and expressed as μliters oxygen uptake per mg dry wt per hr.

Four days after inoculation, an increase in respiration rate of stem tissue was apparent in the inoculated susceptible plants (Fig. 1-B). Respiration continued to increase, and doubled after 7 days compared to controls. A slight increase occurred in inoculated resistant tissue 6-10 days after inoculation.

Prevalence and distribution of bacteria in relation to wilt.—Inoculated and control plants of both cultivars were examined histologically at the onset of wilt symptoms and again 6 days after inoculation. The plants

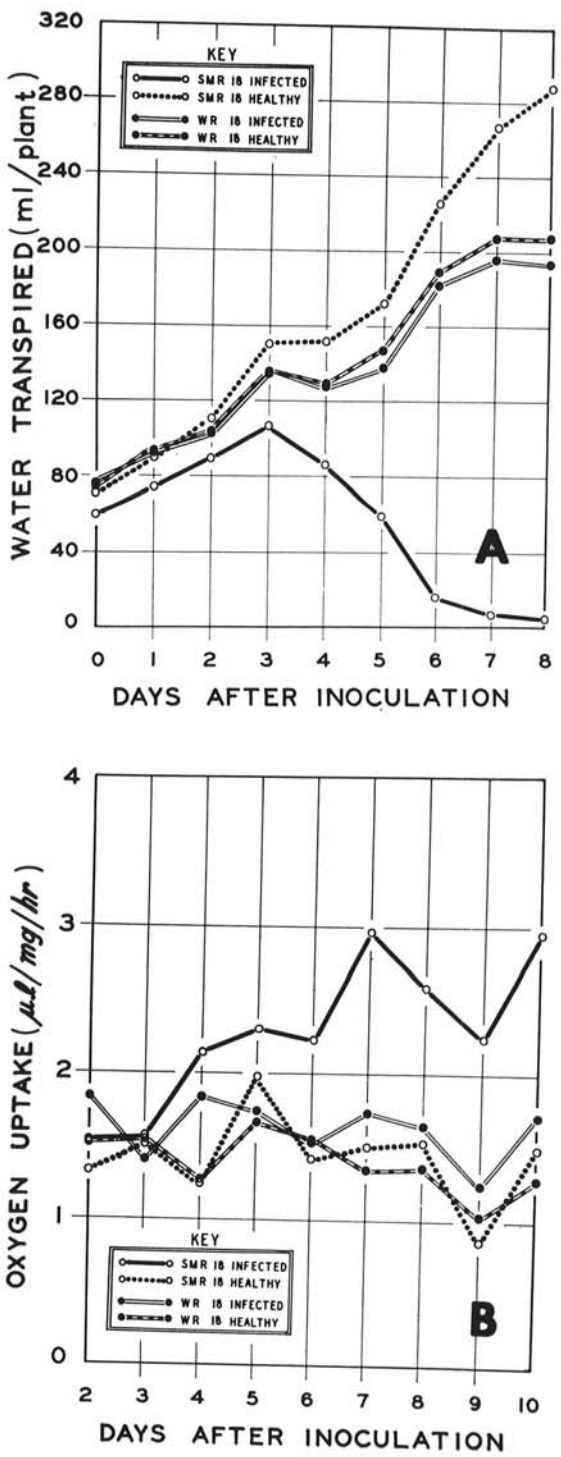

Fig. 1. Effect of *Erwinia tracheiphila* on transpiration and respiration of wilt susceptible (SMR-18) and resistant (WR-18) cucumber plants; **A)** transpiration rate; and **B)** respiration rate.

were cut at sand level, the leaves removed, and the stems, petioles, and roots immediately fixed in Formalin-acetic acid-alcohol. Duplicate plants were prepared for each inoculation treatment. The tissues were dehydrated in a tertiary-butyl alcohol series and embedded in paraffin. Twenty-μ longitudinal sections were cut on a rotary microtome, mounted in Canada balsam, and stained with safranin-fast green procedure (8).

Bacterial masses were abundant in the lower stem xylem of susceptible plants 2 days after inoculation, and were found in all internodes after 6 days. Bacterial masses or plugs in stem and petiole xylem were coincident with the progressive wilt of adjacent leaves. Vascular deterioration (Fig. 2) did not occur until 5-6 days after inoculation, after which the bacteria broke through the xylem walls and spread into the parenchyma (4). Gums or deposits separate from the bacteria were not found. Bacteria and xylem deterioration were never observed in hypocotyl or root tissue of susceptible plants, or in any part of inoculated resistant plants during the 11-day experimental period. Vascular browning or necrosis was never observed in inoculated susceptible or resistant plants.

Hydrolytic enzyme analysis.—Preliminary assays for the presence of macerating enzymes were conducted using culture filtrates of *E. tracheiphila* and extracts of susceptible plants 6 days after inoculation. Bacteria were grown in shake culture at 28 C in nutrient dextrose broth containing 2.5% peptone. Crude enzyme preparations were prepared by centrifuging the culture at 12,100 *g* for 1 hr and retaining the supernatant. Infected stem tissue was homogenized in 0.01 M sodium phosphate buffer (pH 5.5) at 4 C, strained through cheesecloth, and centrifuged at 12,100 *g* for 30 min. A portion of the enzyme preparation from each source was boiled for 10 min and centrifuged, and the supernatant used as a control. All preparations were passed through Seitz filters and adjusted to pH 5.5 prior to assay. Polygalacturonase (PG) and cellulase (Cx) activity were determined as loss of viscosity of sodium polypectate and carboxymethylcellulose substrates, respectively, following the procedure of Husain & Kelman (7).

Assays for PG and Cx activity in culture filtrates and infected plant extracts were negative, and this approach was not pursued further.

DISCUSSION.—In the susceptible plants, *E. tracheiphila* multiplied rapidly following inoculation and proceeded en masse upward through the xylem. Plugging of stem and petiole xylem coincided with the progressive wilt of the adjacent leaf. Our study showed that the bacteria were confined to the xylem for 4-5 days

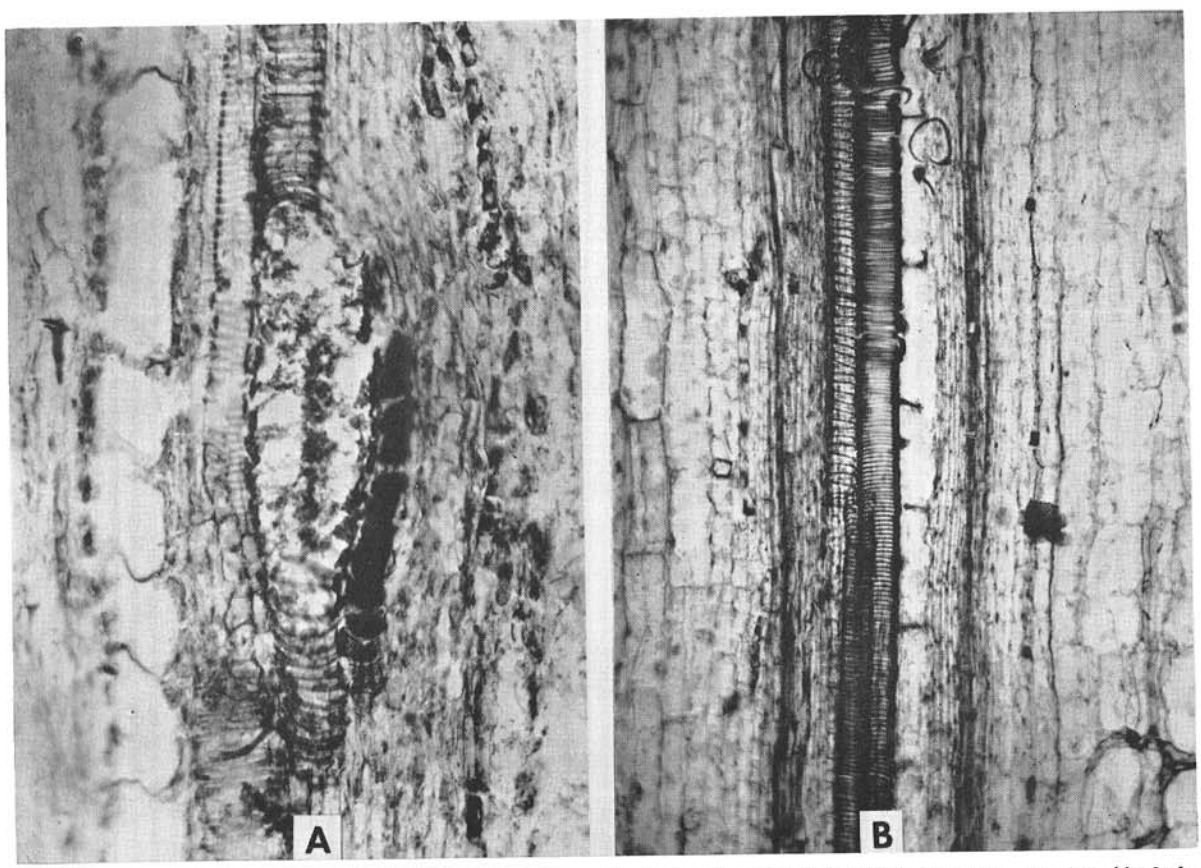

Fig. 2. Longitudinal sections of cucumber stem 6 days after inoculation with *Erwinia tracheiphila*, showing **A)** dark-staining bacterial masses and xylem deterioration in susceptible (SMR-18) cucumber; and **B)** lack of bacterial masses and vascular deterioration in resistant (WR-18) cucumber plants. (×400)

after inoculation, which agrees with Yu (20). Bacteria were isolated from all stem internodes of resistant plants 10 days after inoculation, but no plugs or wilt symptoms were ever observed. All isolates of *E. tracheiphila* from resistant plants were pathogenic using the cucumber seedling assay, indicating that the resistance mechanism had no effect on pathogen virulence per se.

Alteration of transpiration pattern of inoculated susceptible plants followed those reported previously (3, 20). The slight decrease for inoculated resistant plants could indicate limited bacterial multiplication during the experimental period. The role of phytotoxic metabolites of *E. tracheiphila* in wilt induction and transpiration alteration is doubtful, since previous attempts to demonstrate such materials have proved negative (3, 20). Decreased viscosity of xylem sap by bacterial extracellular polysaccharide has been suggested as a mechanism to explain wilt caused by *Pseudomonas solanacearum* (6). *Erwinia tracheiphila* is encapsulated (1), but has never been reported to produce free, extracellular polysaccharides in culture or in vivo. These considerations strongly indicate that transpiration decrease and wilt of cucumber plants results from blockage or plugging of the transpirational stream resulting in irreversible wilt and death of the plant.

Vascular deterioration was not considered an important factor in initial pathogensis or wilt induction, as it occurred after complete and irreversible leaf damage. We detected no pectolytic or cellulolytic enzyme activity in culture filtrates or infected stem tissue, which agreed with Smith (15). Sodium polypectate and carboxymethylcellulose were not included in the culture medium, so the possibility of inducible hydrolytic enzymes could not be ruled out entirely. The most probable mechanism of xylem deterioration was mechanical pressure due to mass action by bacterial multiplication.

Endogenous respiration increased in susceptible stem tissue 2 days after onset of wilt symptoms and 1 day after initial decrease of transpiration (Fig. 1-B). Since xylem deterioration had not yet occurred, and the bacteria had been removed from the stem tissue by evacuation, the initial increase was considered due to host response. Respiration continued to increase up to 10 days, and probably reflected xylem deterioration from 6-10 days after inoculation. The slight increase in resistant stem tissue after 7 days indicated a delayed host response due to differences in bacterial multiplication.

Maine (10) showed that alteration in respiration of plants inoculated with *P. solanacearum* resulted from increased polyphenol oxidase activity, and was concomitant with vascular browning and necrosis. Although polyphenol oxidase activity was not determined in our studies on cucumber wilt, vascular browning was never observed in inoculated susceptible or resistant plants at any stage of disease development.

Resistance may have a nutritional basis. *Erwinia tracheiphila* requires high levels of organic nitrogen for opt growth (14, 18, 20). Wei et al. (18) showed that the wilt index increased as the balanced nutritional level available to susceptible plants increased. Our studies were conducted at a nutritional level favorable for wilt development in susceptible plants. The resistant cucumber plant may not, however, provide the proper nutrients for sustained bacterial multiplication. This possibility should be investigated further.

Our physiological and histological data support earlier theories that bacterial wilt of susceptible cucumber results primarily from mechanical plugging (3, 14, 20). The sequence of pathological events, under the experimental conditions used, were as follows. The lower leaves showed initial wilt and bacterial masses were present in the xylem 2 days after inoculation. Rapid and progressive decrease of transpiration occurred after 3 days. Four days after inoculation, respiration of stem tissue increased. By the 6th day, plants were completely wilted and vascular deterioration was evident. During this period, histological examination showed the bacterial masses to be associated with progressive wilt, while physiological response appeared after plugging had occurred.

In vivo population estimates were not attempted, but isolation studies demonstrated that *E. tracheiphila* multiplied and survived for at least 10 days in resistant plants. Wilt and pathological responses characteristic of the susceptible reaction were delayed or did not occur at all. Resistance was thought to involve a nutritional or bacterial inhibition principle that prevented wilt and ultimately resulted in the disappearance of *E. tracheiphila* from the resistant host.

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