Association of Host Plasma Membrane K^+/H^+ Exchange with Multiplication of *Pseudomonas syringae* pv. *syringae* in *Phaseolus vulgaris*

Merelee M. Atkinson and C. Jacyn Baker

Research plant pathologists, USDA-ARS, Microbiology and Plant Pathology Laboratory, Beltsville, MD 20705.
We thank A. Lemanski for technical assistance and A. Collmer, S. W. Hutcheson, and J. Loper for manuscript review.
This research was supported in part by United States Department of Agriculture Competitive Grant 85-CRCR-1-1779.
Accepted for publication 30 March 1987 (submitted for electronic processing).

ABSTRACT

Atkinson, M. M., and Baker, C. J. 1987. Association of host plasma membrane K^+/H^+ exchange with multiplication of *Pseudomonas syringae* pv. *syringae* in *Phaseolus vulgaris*. Phytopathology 77:1273-1279.

Pseudomonas syringae pv. *syringae*, a bean pathogen, induced plasma membrane K^+ efflux/net H^+ influx exchange and multiplied rapidly in bean leaf tissue. Nine bacterial mutants previously selected for impaired ability to induce a hypersensitive response and the K^+/H^+ response in tobacco were also examined. Mutants unable to induce K^+/H^+ exchange in bean did not multiply within this host, whereas weak, moderate, and strong inductions were associated with slow, moderate, and rapid population growth, respectively. Time course experiments indicated that induction of K^+/H^+ exchange preceded the onset of bacterial population growth. Continued operation of the exchange increased host intercellular fluid pH from approximately 5.5 to 7.5. Infiltration of host intercellular spaces with pH 7.0–8.0 buffers promoted growth of a nonpathogenic bacterial mutant, whereas pH 6.0 buffer inhibited growth of the wild-type strain. These results suggest that increased pH of host intercellular fluids resulting from K^+/H^+ exchange promotes bacterial multiplication. In view of the role of H^+ gradients in active transport of sucrose, amino acids, and inorganic ions across the plasmalemma, we hypothesize that increased intercellular pH leads to increased nutrient levels in intercellular spaces where bacteria reside.

Additional key words: brown spot, ion transport.

Pseudomonas syringae pv. *syringae* causes bacterial brown spot on bean (7). The disease is characterized by small necrotic lesions on bean foliage with or without chlorotic halos. As with many bacterial pathogens, the molecular mechanisms that determine pathogenicity of this organism are not well understood. Many strains of this bacterium produce a toxin, syringomycin, which can significantly increase virulence of the bacterium (14,22). However, this toxin is not required for pathogenicity and does not account for the host specificity of the pathogen (14,22). Additional pathogenicity factor(s) must therefore be present.

Several lines of evidence suggest that mechanisms controlling disease susceptibility are related to those controlling hypersensitivity. Many compatible pathogens, including *P. s.* pv. *syringae*, induce necrotic lesions and symptoms that are also associated with hypersensitivity (2,8,19,25). Klement has argued that the mechanism of development is the same but that symptoms and necrosis develop more rapidly or at a lower inoculum level in incompatible interactions (17,19). The ability to cause hypersensitive necrosis in tobacco has even been used as a criterion for pathogenicity of *Pseudomonas* species (16). Finally, genetic evidence suggests that at least one gene required for induction of the hypersensitive response (HR) is also required for pathogenicity. Mutants of *P. s.* pv. *phaseolicola* (12,20), *P. s.* pv. *tomato* (9), *P. solanacearum* (6,21), and *Erwinia amylovora* (5) that have lost pathogenicity as well as the ability to induce the HR have been reported. These results, as a whole, indicate that the molecular basis for disease susceptibility may be related to that for hypersensitivity.

The HR of tobacco to *P. s.* pv. *pisi* has been recently shown to proceed through the activation of a plasma membrane K^+ efflux/H^+ influx exchange (3). This exchange causes a severe loss of K^+, but not other ions, from plant cells and is accompanied by an equimolar net influx of H^+. We have recently shown that induction of the HR in tobacco by *P. s.* pv. *syringae* is also closely associated with induction of K^+/H^+ exchange (4). Specifically, Tn*5* insertion mutants of this bacterium were independently selected for inability to induce the HR and the K^+/H^+ exchange in tobacco. Without exception, loss of one trait was associated with loss of the other. The purpose of this study was to investigate the association of K^+/H^+ exchange induction by wild-type and mutant strains with the ability to multiply in *Phaseolus vulgaris*. Because our wild-type strain was found to be a weak pathogen on bean, we also tested this association with a highly virulent strain. A preliminary report of this work has been published (1).

MATERIALS AND METHODS

Bacteria. *P. s.* pv. *syringae* strain 61, isolated from wheat by M. Sasser (University of Delaware), has been found to be weakly virulent on bean. A nalidixic acid-resistant strain was selected and used for transposon mutagenesis. The details of this procedure and the selection of eight Tn*5* insertion mutants with impaired ability to induce the HR or K^+/H^+ response in tobacco are described elsewhere (4). All mutants were prototrophic and showed growth rates in minimal medium comparable to those of the wild-type strain. Most mutants possessed a single Tn*5* insertion site. Mutant strains B1 and C4 induced both the HR and K^+/H^+ exchange but not as strongly as the wild-type strain. Strains B2, B3, B4, B5, B6, and B7 induced little or no K^+/H^+ exchange and did not induce the HR. *P. s.* pv. *syringae* strain Y30, isolated from and highly virulent on bean, was obtained from D. J. Hagedorn (University of Wisconsin).

Bacterial inocula for experiments were prepared from 16- to 20-hr cultures grown at 30 C on King's B agar containing 25 μg/ml of nalidixic acid (for wild-type, strain 61), 25 μg/ml of nalidixic acid and 40 μg/ml of streptomycin (for mutants, strain 61), or unamended King's B agar (for strain Y30). Bacteria were suspended in 1 mM MES (2-(*N*-morpholino)ethanesulfonic acid) adjusted to pH 6.0 with NaOH, washed once by centrifugation at 10,000 *g* for 10 min at 25 C, then resuspended in fresh buffer of the same composition to the appropriate inoculum density. To test the effect of intercellular fluid pH on bacterial multiplication in bean leaves, washed bacteria were instead resuspended in 50 mM MES-Tris, pH 6.0; 50 mM HEPES (*N*-(2-hydroxyethyl)

piperazine-*N'*-2-ethanesulfonic acid)-Tris, pH 7.0; or 50 mM HEPES-Tris, pH 8.0.

K^+/H^+ exchange. The first primary leaves from 2- to 3-wk-old bean plants (*P. vulgaris* moderately resistant cultivar Pinto 111 or susceptible cultivar Tendercrop) were used for all experiments. Upper leaf surfaces were rubbed gently with a cotton swab dipped in an aqueous suspension of 600-grit Carborundum to facilitate ion movement through the cuticle. Leaf disks (0.55 cm^2, approximately 0.01 g per disk) were cut with a brass cork borer and transferred to 50-ml beakers containing bacterial suspensions or buffer controls. Disks were infiltrated under vacuum, blotted dry, and air-dried for approximately 30 min. For each treatment or replicate, 10 disks were transferred to 10 ml of assay medium (0.5 mM MES-Tris, 0.5 mM $CaCl_2$, pH 6.0) in a 50-ml beaker. Beakers were incubated at 27 C with rotation at 160 rpm.

Net K^+ and H^+ fluxes of disks were determined by measuring changes in K^+ concentration and pH of the assay medium at 2-hr intervals for 12 hr. One-half milliliter of assay medium was removed from each beaker at designated time intervals and later analyzed by atomic absorption spectroscopy for K^+ concentration; increased concentration was interpreted as net K^+ efflux and decreased concentration, as net K^+ uptake by bean disks. A combination electrode with calomel reference was used to measure pH of the entire assay medium (10 ml) in the presence of leaf disks; increased pH was interpreted as net H^+ influx and decreased pH, as net H^+ efflux by bean disks. For quantification of H^+ fluxes, pH measurements were compared to an acid-base titration curve for the assay medium. Because control (uninoculated) leaf disks typically showed a substantial net H^+ efflux and K^+ influx, most flux data are presented as the numerical difference between bacterial treatments and buffer controls.

A nalidixic acid-susceptible wild-type of strain 61 was used to determine the effect of this antibiotic (an inhibitor of prokaryotic DNA replication and therefore bacterial multiplication) on induction of net H^+ influx. Nalidixic acid (25 μg/ml) was added to bacterial suspensions 30 min before infiltration into leaf disks. Aliquots (0.1 ml) of these suspensions plated onto King's B medium containing 25 μg/ml of nalidixic acid produced no colonies except for an apparent mutation rate of approximately 10^{-8}. Following infiltration, disks were air-dried and transferred to assay medium that also contained 25 μg/ml of nalidixic acid. Net H^+ influx was measured as described above.

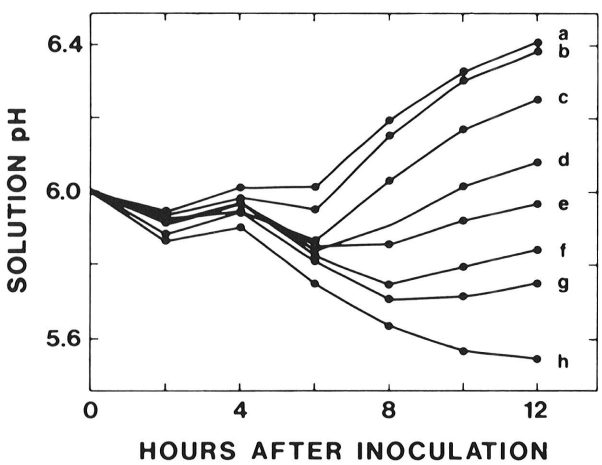

Fig. 1. Effect of *Pseudomonas syringae* pv. *syringae* (wild-type, strain 61) inoculum density on pH of solutions containing inoculated bean (cv. Pinto 111) leaf disks. Uninoculated (control) disks (h) acidified the solution owing to net H^+ efflux. Increased solution pH relative to the control (h) indicates bacterial activation of H^+ uptake by bean tissue. Disks were vacuum-infiltrated at −0.5 hr with bacterial suspensions and incubated in 0.5 mM MES-Tris pH 6.0, 0.5 mM $CaCl_2$. Bacterial inoculum density was 5.0, 2.5, 1.0, 0.5, 0.25, 0.10, 0.05, 0.00 (a–h) × 10^8 cfu/ml. Data are means of two replicates not varying by more than 0.09 pH unit.

Bacterial populations and foliar symptoms. Unless noted otherwise, these studies were conducted with intact plants. The moderately resistant bean cultivar Pinto 111 or the susceptible cultivar Tendercrop were used for all experiments. Bacterial suspensions were infiltrated into the first primary leaves of 2- to 3-wk-old bean plants using a syringe and a 26.5-gauge hypodermic needle. Infiltrated areas were approximately 3 cm^2. Leaves were rinsed with H_2O to remove bacteria from leaf surfaces. Plants were grown in a greenhouse and were transferred to growth chambers after inoculation. Growth chamber conditions were 80 C and 85% humidity during the day and 70 C and 65% humidity at night, with a 16-hr photoperiod.

For population experiments, 0.55 cm^2 disks were cut from leaves with a cork borer at 0- to 48-hr intervals after inoculation. Each disk was ground in a sterile mortar and pestle with 1 ml of 25 mM KPO_4, 0.1 mM NaCl, pH 7.0. Bacterial content of homogenates was determined by dilution plate count. Dilutions were made in sterile grinding buffer and aliquots plated onto King's B medium plates with nalidixic acid (wild-type, strain 61), nalidixic acid and streptomycin (mutants, strain 61), or unamended (strain Y30). For determination of foliar symptoms, inoculated leaf areas were inspected for chlorotic and necrotic symptoms 24 and 48 hr after inoculation and intermittently thereafter for 7–10 days.

To simultaneously monitor K^+/H^+ exchange and bacterial multiplication, bacterial populations were also measured in leaf disks incubated in assay medium. For these studies, one disk was removed from each beaker whenever a pH measurement and an aliquot for K^+ measurement were taken. Bacterial content of these disks was determined as described above.

RESULTS

K^+ efflux/H^+ influx. Uninoculated (control) bean tissue showed a substantial net H^+ efflux (acidification of medium) (Fig. 1). Because of this inherent and opposite H^+ flux, bacterial induction of H^+ influx was first observed as a decreased efflux relative to the control (Fig. 1). A measurable response was obtained with as few as 5×10^6 cfu/ml, whereas 2.5×10^8 gave a near-maximum response. For clarity, all K^+/H^+ exchange data, with the exception of data shown in Figure 1, represent the difference between ion fluxes of inoculated and control bean disks.

The wild-type of strain 61 induced net plasma membrane H^+ uptake and K^+ efflux (Fig. 2) in bean leaf disks. The onset of K^+/H^+ exchange occurred less than 2 hr after infiltration of leaf tissue with 2×10^8 cfu/ml. Initial rates of approximately 3 μmol of K^+ or H^+ per gram (fresh weight) of leaf tissue per hour were maintained for approximately 6 hr, after which rates were increased to 8–10 μmol per gram per hour. Rates gradually declined after 8 hr. Relative to the controls, the total K^+/H^+ exchange within 12 hr after inoculation amounted to approximately 40 μmol of each ion per gram (fresh weight) of leaf tissue. Total K^+ content of bean leaf tissue was approximately 100 μmol/g.

Mutants C4 and B2 of strain 61 induced moderate and low rates of K^+/H^+ exchange, respectively (Fig. 2); B3 induced no K^+/H^+ exchange. Survey of the remaining five mutant strains indicated that four failed to induce net H^+ influx and one, B1, induced low rates of H^+ influx (Table 1).

Induction of K^+/H^+ exchange by strains 61 and Y30 was compared on the moderately resistant bean cultivar Pinto 111 and the susceptible cultivar Tendercrop (Fig. 3). On Tendercrop, induction of K^+/H^+ exchange by strain 61 was abrupt and maximum rates were reached quickly, whereas induction by strain Y30 was slower and reached maximum rates gradually. On Pinto 111, induction of K^+/H^+ exchange by both strains was more abrupt than on Tendercrop and induction by strain 61 was slightly faster than that by strain Y30.

The effect of bacterial cell growth on assay medium pH was tested to determine whether this made a significant contribution to the observed alterations in medium pH. The addition of 10^7 cfu/ml (wild-type, strain 61) to assay medium did not alter medium pH even after incubation for 12 hr. In assay medium supplemented with 10 mM sucrose, however, bacteria acidified the medium

slightly, from pH 6.07 to 5.86. It is conceivable, therefore, that bacterial metabolism resulted in a small underestimation of H^+ uptake by bean disks. In any case, bacterial metabolism was clearly not responsible for alkalinization of the assay medium by inoculated leaf disks.

Bacterial population size and foliar symptoms. Population sizes of wild-type and mutants B2, B3, and C4 of strain 61 were monitored intermittently over a 48-hr period after inoculation of bean leaves (Fig. 4). All bacterial strains showed an initial drop in apparent population size 6 hr after inoculation. Thereafter, the behavior of various bacterial strains differed markedly and was correlated with efficiency of K^+/H^+ exchange induction. Wild-type bacteria multiplied rapidly between 6 and 24 hr after inoculation, reaching levels 100-fold greater than at 0 hr; populations leveled off or declined slightly between 24 and 48 hr, however. Strains C4 and B2 showed moderate and slow population growth between 6 and 48 hr, respectively, whereas populations of B3 declined steadily over the 48-hr experimental period. Leaf tissue infiltrated with the wild-type strain at 5×10^7 cfu/ml developed a necrotic lesion within 24 hr that became surrounded by a small chorotic rim within 10 days. Strain C4 induced pronounced chlorosis but no necrosis, B2 induced weak chlorosis only, and B3 induced no symptoms.

Population size of the remaining five mutant strains was determined immediately after and 20 hr after inoculation (Table 1). We observed population declines for strains B4, B5, B6, and B7 and slow growth of strain B1 over the 20-hr period. B1 induced moderately intense chlorosis of bean leaves and B4, B5, B6, and B7 induced no symptoms.

Population growth of strains 61 and Y30 was compared on the moderately resistant bean cultivar Pinto 111 and the susceptible cultivar Tendercrop (Fig. 5). The two strains showed similar population growth on Pinto 111, but on Tendercrop, strain Y30 reached population densities approximately 10-fold those of strain 61.

On the susceptible host, Tendercrop, the infiltration of 5×10^7 cfu/ml of strain 61 resulted in brown necrotic lesions covering 50% of the infiltrated area within 24 hr. At 10^6 cfu/ml, approximately 25% of the infiltrated area became brown and necrotic within 24 hr. At 10^5 cfu/ml, approximately 30% of the infiltrated area became brown and necrotic within 1 wk after inoculation. Symptoms did not spread beyond the infiltrated area at any inoculum concentration. Strain Y30 (5×10^7 cfu/ml) developed water-soaked lesions within 24 hr that spread within 5 days from the site of infiltration outward to the leaf periphery. Water-soaked areas gradually became dry. Lower inoculum densities (10^5 or 10^6 cfu/ml) induced similar symptoms that began to develop after a lag of approximately 48 hr. On the moderately resistant host, Pinto 111, the infiltration of 5×10^7 cfu/ml of strain 61 induced brown necrotic lesions within 24 hr that did not develop further. A slight chlorosis was sometimes observed within 1 wk after inoculation with 10^6 cfu/ml. No symptoms were observed within 1 wk after inoculation with 10^5 cfu/ml. Symptoms did not spread beyond the infiltrated area at any inoculum concentration. Strain Y30 induced similar symptoms on Pinto 111.

Relationship between K^+/H^+ exchange and bacterial multiplication. To determine their temporal relationship, bacterial multiplication and net H^+ influx were simultaneously measured in leaf disks incubated in assay medium (Fig. 6). As with previous experiments, net H^+ influx began within 2 hr and continued at a constant rate for 6 hr before increasing. Bacterial population size, however, remained nearly constant for 4–6 hr, after which marked increases coinciding with sharp increases in H^+ uptake rate were observed.

Nalidixic acid, an antibiotic that inhibits prokaryotic DNA replication and, therefore, bacterial multiplication, was used to determine whether bacterial multiplication is required for induction of K^+/H^+ exchange. A wild-type strain sensitive to nalidixic acid was used for these experiments. Early induction of net H^+ influx was unaffected by the antibiotic (Fig. 7), but the sharp increase in H^+ influx 6–8 hr after inoculation appeared to be completely suppressed.

Because initial induction of K^+/H^+ exchange preceded, and thus did not require, bacterial multiplication, we investigated the possibility that K^+/H^+ exchange might promote bacterial multiplication. The consequences of increased pH and K^+ concentration of host intercellular fluids caused by K^+/H^+

TABLE 1. Induction of net H^+ influx and foliar symptoms in *Phaseolus vulgaris* 'Pinto 111' by *Pseudomonas syringae* pv. *syringae* wild-type and mutants of strain 61

Bacterial strain	Net H^+ influx (μmol/g)[a]	Bacterial multiplication factor[b]	Foliar symptoms[c]	
			Chlorosis	Necrosis
Wild-type	29.22 ± 0.71	26.9 ± 7.9	+	+
B1	4.45 ± 2.09	3.4 ± 2.3	+	–
B4	−3.98 ± 1.77	0.68 ± 0.69	–	–
B5	0.51 ± 1.41	0.30 ± 0.29	–	–
B6	−0.63 ± 0.85	0.76 ± 0.17	–	–
B7	−1.00 ± 0.71	0.58 ± 0.37	–	–

[a] Measurements were made 12 hr after infiltration of bean disks with 2.5×10^8 cfu/ml.

[b] Bacterial populations at 20 hr/populations at 0 hr; populations at 0 hr ranged from 2 to 6×10^3 cfu/cm^2 leaf tissue.

[c] Recorded 24 hr and 7 days after infiltration of bean leaves with 5×10^7 cfu/ml. Data are means ±1 SD of three replicates.

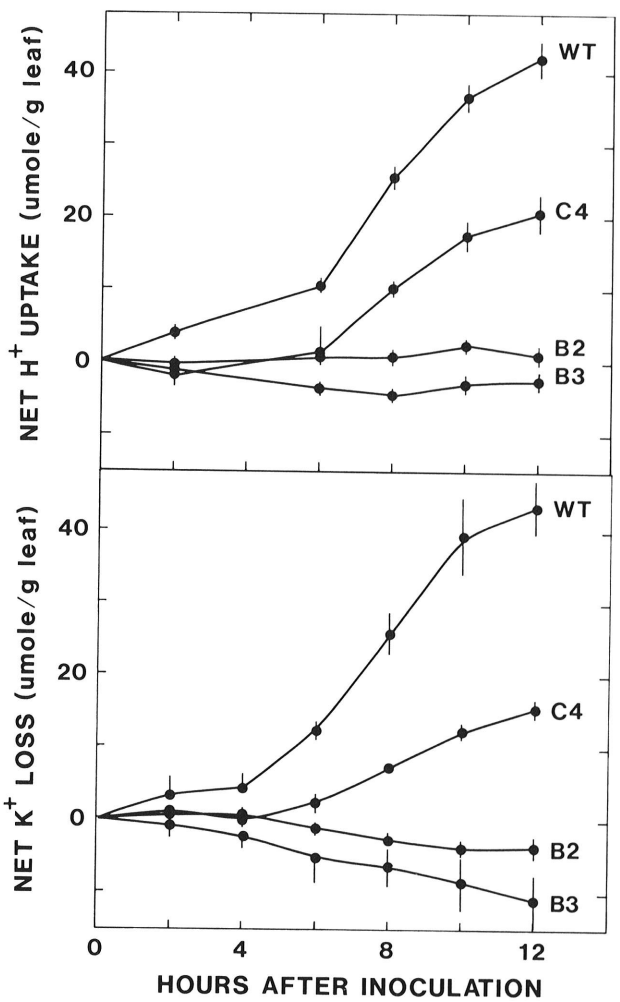

Fig. 2. Effect of *Pseudomonas syringae* pv. *syringae* wild-type and mutants (C4, B2, and B3) of strain 61 on net H^+ and K^+ transport in bean (cv. Pinto 111) leaf disks. Conditions same as described for Figure 1 except that bacterial inoculum density was 2.5×10^8 cfu/ml. Values greater than 0 indicate net H^+ influx or K^+ efflux relative to the control (uninoculated leaf tissue). Values less than 0 indicate net H^+ efflux or K^+ influx relative to the control. Data are means ±1 SD of three replicates.

exchange were specifically considered. To estimate intercellular fluid pH resulting from K^+/H^+ exchange, leaf disks inoculated with wild-type bacteria were incubated in decreasing concentrations of assay buffer. It was necessary to keep some buffer in the assay medium to facilitate pH measurement. Under these conditions, buffer pH approached 7.2–7.6, depending on time after inoculation (Fig. 8). Since hydroxide ions resulting from H^+ uptake must presumably diffuse from the plasmalemma through the host cell wall in order to increase solution pH, we conclude that the pH between the plasmalemma and the cell wall must reach a minimum of 7.2–7.6. Uninoculated leaf disks acidified the medium to approximately 5.5 (Fig. 1).

To determine the effect of intercellular pH on bacterial multiplication, intact leaves were infiltrated with bacterial suspensions made up in 50 mM MES, pH 6.0; 50 mM MES-HEPES, pH 7.0; or 50 mM HEPES, pH 8.0. On the basis of our H^+ flux data (Figs. 1 and 2) and the determination that bean leaf tissue can be infiltrated with approximately 1 ml of solution per gram, we estimated that this buffer concentration was sufficient to maintain intercellular pH at the desired levels for 12 hr after inoculation. Populations of strain B3 declined approximately eightfold in the presence of the pH 6.0 buffer but remained constant or increased up to sixfold at pH 7.0 and 8.0, respectively (Table 2). Wild-type growth was prevented by pH 6.0 buffer, but bacterial populations did not decline significantly below 0-hr levels. Wild-type populations increased 15- to 20-fold in the presence of pH 7.0 and 8.0 buffers.

Medium pH had a small direct effect on bacterial multiplication rates in broth culture (Fig. 9). These differences, however, were not great enough to account for the large differences in growth rates

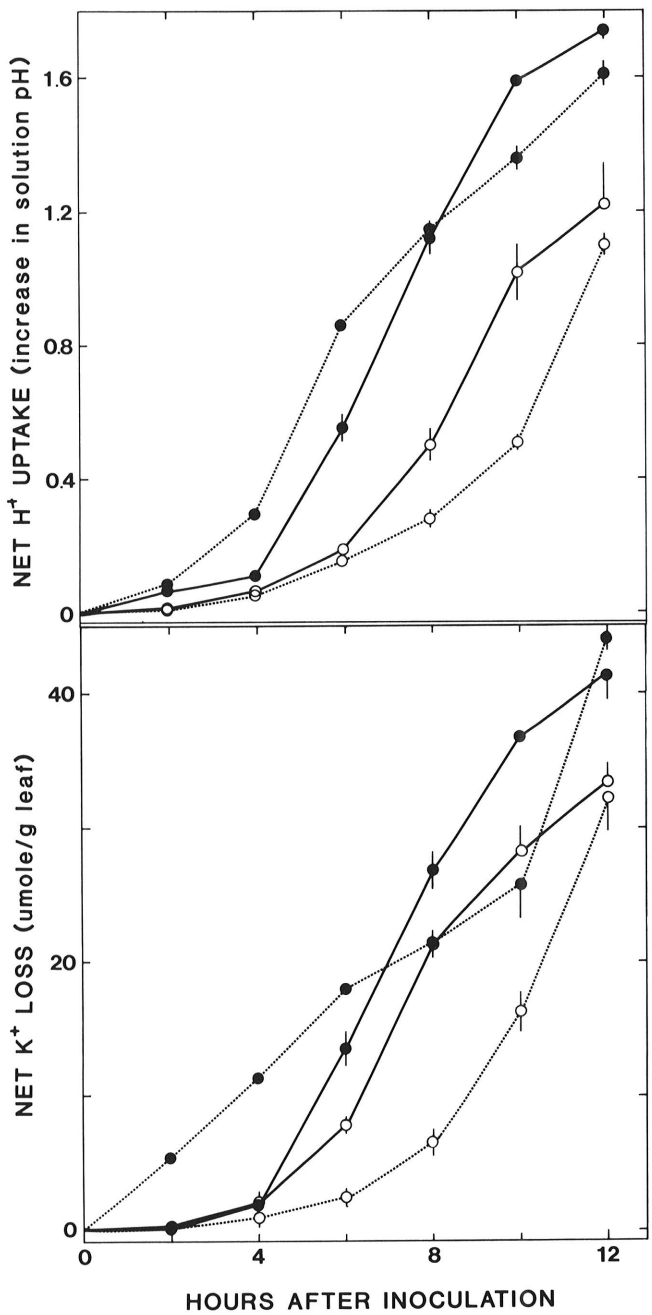

Fig. 3. Effect of *Pseudomonas syringae* pv. *syringae* weakly virulent strain 61 (•) and virulent strain Y30 (o) on net H^+ and K^+ transport in leaf disks from moderately resistant bean cultivar Pinto 111 (solid line) and susceptible cultivar Tendercrop (dotted line). Conditions same as described for Figure 2.

TABLE 2. Effect of buffer pH on multiplication of *Pseudomonas syringae* pv. *syringae* strain 61 in leaves of *Phaseolus vulgaris* 'Pinto 111'

Bacterial strain	Buffer[a]	cfu/disk[b] 0 hr	cfu/disk[b] 13 hr
Wild-type	50 mM MES,[c] pH 6.0	$1.9 \pm 0.6 \times 10^3$	$1.2 \pm 0.3 \times 10^3$
	50 mM HEPES,[d] pH 7.0	$1.7 \pm 0.5 \times 10^3$	$2.8 \pm 1.4 \times 10^4$
	50 mM HEPES, pH 8.0	$2.3 \pm 0.1 \times 10^3$	$4.5 \pm 1.4 \times 10^4$
B3	50 mM MES, pH 6.0	$4.3 \pm 1.0 \times 10^3$	$5.3 \pm 3.5 \times 10^2$
	50 mM HEPES, pH 7.0	$4.8 \pm 0.9 \times 10^3$	$4.2 \pm 2.0 \times 10^3$
	50 mM HEPES, pH 8.0	$5.1 \pm 0.6 \times 10^3$	$2.8 \pm 0.6 \times 10^4$

[a] Bacterial suspensions containing $1–4 \times 10^6$ cfu/ml were prepared in the indicated buffer and injected by syringe into intercellular spaces of bean leaves.
[b] Colony-forming units per 0.5 cm^2 bean leaf disk. Data are means ±1 SD of three replicates.
[c] 2-(*N*-morpholino)ethanesulfonic acid.
[d] *N*-(2-hydroxyethyl)piperazine-*N'*-2-ethanesulfonic acid.

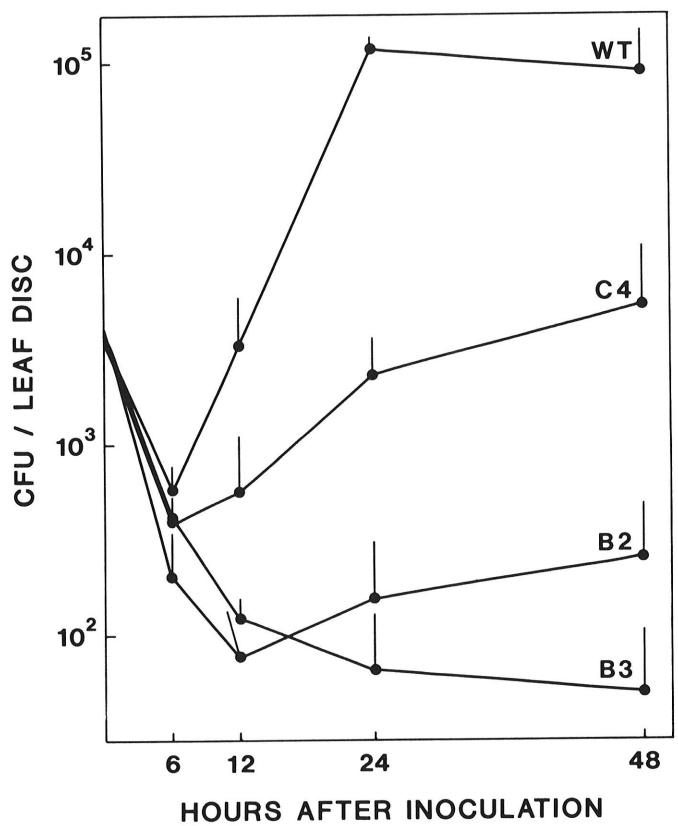

Fig. 4. Multiplication of *Pseudomonas syringae* pv. *syringae* wild-type and mutants (C4, B2, and B3) of strain 61 in bean (cv. Pinto 111) leaves. Bacteria (10^6 cfu/ml) were injected by syringe into the intercellular spaces of leaves at 0 hr. Data are means ±1 SD of three replicates.

observed in leaf tissue. The concentration of buffer in the growth medium (100 mM) was sufficient to maintain the appropriate pH ±0.1 pH unit throughout the 8-hr growth period.

To determine the effect of increased intercellular K^+ concentration on bacterial multiplication, leaves were infiltrated with bacterial suspensions containing various concentrations of KCl. Growth of strain B3 was not promoted by the inclusion of up to 25 mM KCl in the bacterial inoculum (M. M. Atkinson, *unpublished*). However, since K^+ is efficiently taken up by plant cells, intercellular concentrations may have dropped well below 25 mM. Therefore, these results do not rule out the possibility that increased intercellular K^+ concentration promotes bacterial multiplication.

DISCUSSION

We have shown that host plasma membrane K^+ efflux/H^+ influx exchange is closely associated with multiplication of *P. s.* pv. *syringae* and development of foliar symptoms in bean leaf tissue. Evidence supporting this association includes the correlation between rate of K^+/H^+ exchange and rate of bacterial population growth for all bacterial strains tested and the close temporal relationship of these two phenomena.

Our data are consistent with the hypothesis that increased pH of host intercellular fluid resulting from the K^+/H^+ exchange promotes bacterial multiplication in leaf tissue. Several lines of evidence support this interpretation. Time course experiments showed that K^+/H^+ exchange preceded the onset of bacterial population growth. This initial and relatively low rate of K^+/H^+ exchange was not dependent on bacterial multiplication as judged by its insensitivity to nalidixic acid. Finally, neutralization or mild alkalinization of host intercellular spaces with buffers promoted growth of an otherwise nongrowing mutant strain, whereas slightly acidic buffers inhibited wild-type growth. These pH conditions simulated those resulting from bacterium-induced H^+ uptake by host cells (pH 7.0–8.0) and the normally acidic pH of the cell wall spaces (pH 5.5–6.0).

A small portion of the stimulatory effects of increased pH on bacterial populations in bean leaves can be attributed to the direct effect of pH on bacterial growth, since growth rates in culture at pH 6.0–8.0 were approximately twice that at pH 5.5 (M. M. Atkinson, *unpublished*). However, population sizes of B3 or wild-type strains 13 hr after infiltration into bean leaves were 40–50 times larger at pH 8.0 than at pH 6.0, whereas growth rates in culture were comparable. Thus, intercellular fluid pH may affect the plant cell in a manner that promotes bacterial growth.

According to the chemiosmotic theory of plant plasma membrane transport, ATPase-generated H^+ efflux across this

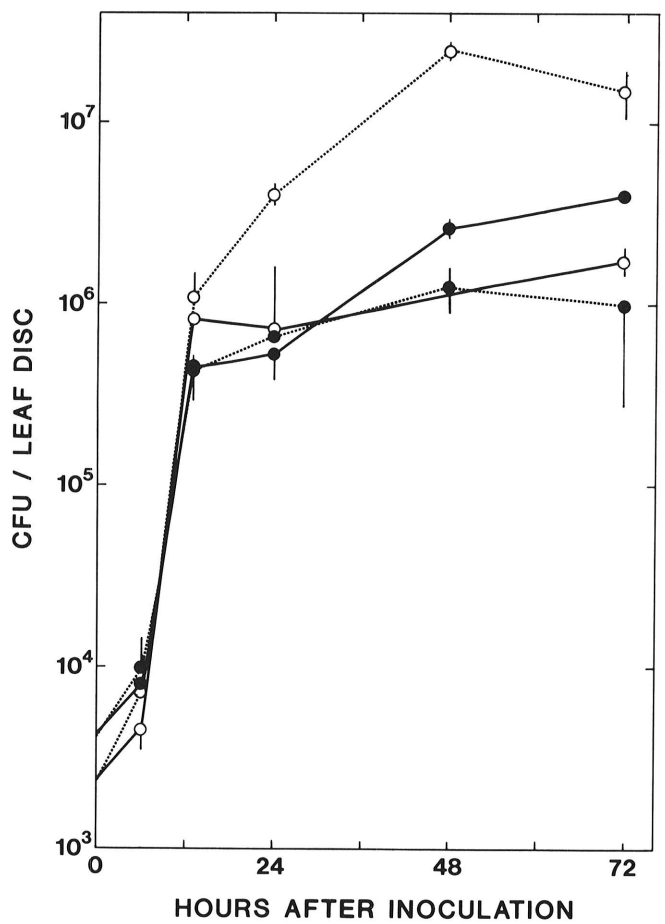

Fig. 5. Multiplication of *Pseudomonas syringae* pv. *syringae* weakly virulent strain 61 (•) and virulent strain Y30 (o) in leaves of moderately resistant bean cultivar Pinto 111 (solid line) and susceptible cultivar Tendercrop (dotted line). Conditions same as described for Figure 4.

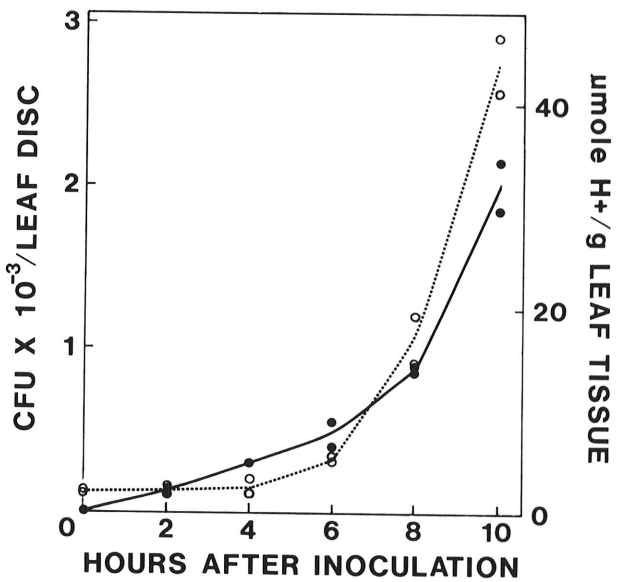

Fig. 6. Time course of net H^+ influx (•) and *Pseudomonas syringae* pv. *syringae* wild-type of strain 61 populations (o) in bean (cv. Pinto 111) leaf disks. Conditions same as described for Figure 2. All data points are given.

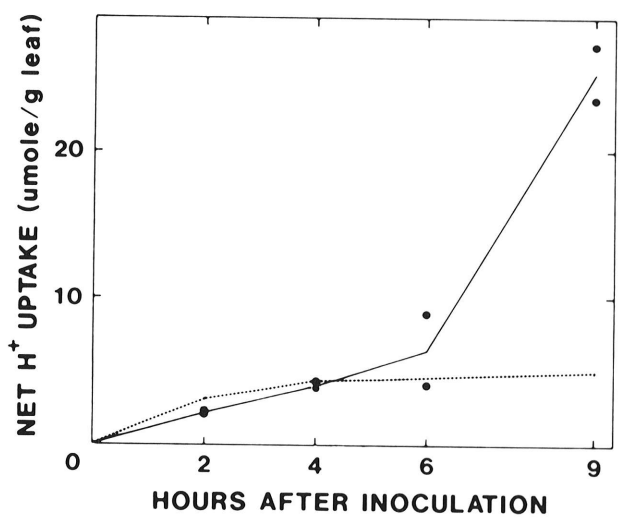

Fig. 7. Effect of nalidixic acid (25 μg/ml) on net H^+ influx in bean (cv. Pinto 111) leaf tissue inoculated with *Pseudomonas syringae* pv. *syringae* (wild-type, strain 61). Conditions same as described for Figure 2. All data points are given. With nalidixic acid, dotted line; without nalidixic acid, solid line.

membrane generates a H^+ gradient (pH 5.5–6.0 outside the membrane and pH 7.0–7.5 inside) which drives the active uptake of sucrose, amino acids, and certain inorganic ions from the intercellular fluids (15,23,24). Specific carrier proteins that cotransport H^+ and various nutrients across the plasmalemma are believed to mediate this process (13,15,23). The destruction, reduction, or reversal of the H^+ gradient by bacterial induction of H^+ influx should lead to the selective accumulation of these nutrients in the intercellular spaces where bacteria reside. It has been shown that neutralization or mild alkalinization of plant intercellular fluids results in the net movement of sucrose out of plant cells into the intercellular fluid (13,26). In support of our hypothesis, we have shown that *P. s.* pv. *syringae* induces a net movement of sucrose into the intercellular fluid of bean tissue and that this is associated with induction of K^+/H^+ exchange (this information will be presented in a separate publication). We suggest that operation of the K^+/H^+ exchange in bean leaves between 0 and 6 hr after inoculation leads to an initiation of bacterial multiplication, possibly resulting from increased nutrient availability. Increased bacterial numbers may induce higher K^+/H^+ exchange rates that further stimulate bacterial multiplication, and so on.

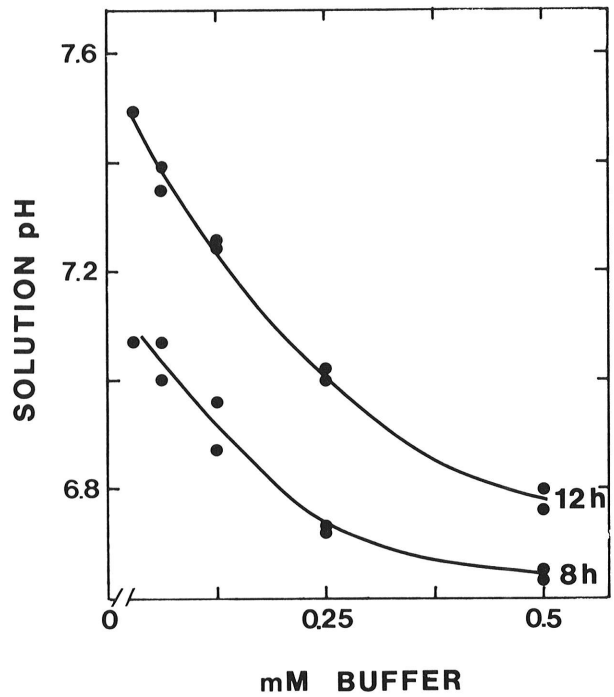

Fig. 8. Estimation of intercellular fluid pH in bean (cv. Pinto 111) leaf disks inoculated with *Pseudomonas syringae* pv. *syringae* (wild-type, strain 61). Conditions same as described for Figure 2 except that assay buffer concentration was reduced from the standard 0.5 mM to 0.025 mM. Maximum pH of solution was considered a low estimate of intercellular fluid pH. All data points are given.

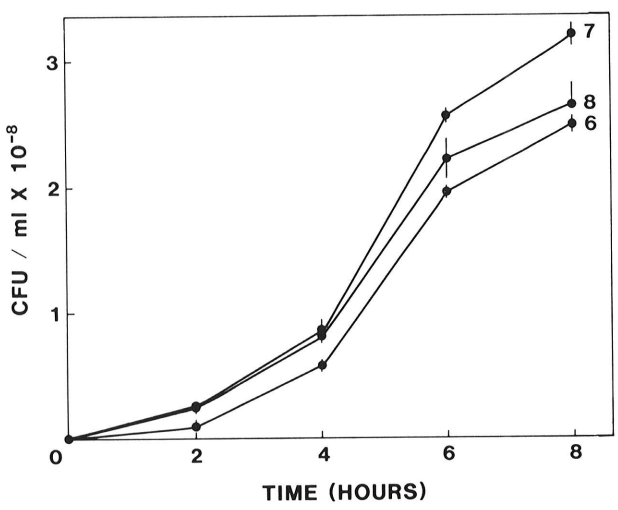

Fig. 9. Effect of medium pH on growth of *Pseudomonas syringae* pv. *syringae* (wild-type, strain 61) in nutrient broth supplemented with 100 mM MES-HEPES, pH 6.0, 7.0, or 8.0. Data are means ±1 SD of three replicates.

Finally, our results and those presented previously (3,4) show that induction of K^+/H^+ exchange is associated with both incompatible and compatible bacterium-plant interactions. Although this may appear contradictory, it is highly consistent with the literature. Studies have shown that electrolyte leakage, respiratory stimulation, and host cell necrosis occur in both incompatible and compatible interactions but generally occur more quickly in the former (2,8,17,19,25). In contrast, these symptoms and necrosis are not induced at all by saprophytic bacteria (2,18). We have found that *P. fluorescens* and *Escherichia coli* do not induce K^+/H^+ exchange in tobacco (M. M. Atkinson, *unpublished*). We would also like to point out that differences between bacterial population growth during compatible and incompatible interactions are also quantitative. In contrast to saprophytes, which normally do not multiply within plant tissues (17,18), incompatible bacteria show significant multiplication resulting in up to 100- to 1,000-fold population increases (our results; 9–11,18). Our comparison of virulent and weakly virulent *P. s.* pv. *syringae* strains on susceptible and resistant hosts indicates that a quantitative or temporal distinction also applies to induction of K^+/H^+ exchange and bacterial multiplication in our system. Further research is required to determine whether quantitative or temporal differences in induction of K^+/H^+ are a cause of, an effect of, or merely associated with the distinction between incompatibility and compatibility.

Several laboratories in addition to ours have isolated *Pseudomonas* or *Erwinia* mutants that are nonpathogenic and also fail to induce the HR in incompatible hosts (5,6,9,12,20,21). In most cases, these mutants were created by single transposon insertions into the bacterial genome, implying that the same gene or group of genes controls both pathogenicity and HR induction. Our results suggest that one function of this gene(s) may be induction of K^+/H^+ exchange. On a physiological level, K^+/H^+ exchange can account for certain aspects of pathogenicity and HR induction. As shown here, the exchange may be necessary for significant population growth. However, as previously discussed, K^+/H^+ exchange is potentially lethal to the plant cell because it causes a major disruption of pH regulation and ionic balance within the cell (3). Further understanding of the K^+/H^+ exchange requires the determination of its molecular basis, the focus of our current research.

In summary, we have demonstrated a close association between bacterial multiplication in bean and induction of host plasma membrane K^+ efflux/H^+ influx exchange. Evidence is presented that this phenomenon promotes bacterial multiplication. Our data do not, however, exclude the existence of additional pathogenicity factors. On the basis of the results described here and in our previous publications, we have developed a testable model that accounts for certain aspects of plant-pathogen specificity concerning disease susceptibility and hypersensitivity of plants to phytopathogenic pseudomonads.

LITERATURE CITED

1. Atkinson, M. M., and Baker, C. J. 1986. Association of host plasmalemma K^+ efflux/H^+ influx exchange with pathogenicity of *Pseudomonas syringae* pv. *syringae* on *Phaseolus vulgaris*. (Abstr.) J. Cell. Biochem. Suppl. 10C, p. J74.
2. Atkinson, M. M., Huang, J.-S., and Knopp, J. A. 1985. Hypersensitivity of suspension-cultured tobacco cells to pathogenic bacteria. Phytopathology 75:1270-1274.

3. Atkinson, M. M., Huang, J.-S., and Knopp, J. A. 1985. The hypersensitive reaction of tobacco to *Pseudomonas syringae* pv. *pisi*: Activation of a plasmalemma K^+/H^+ exchange mechanism. Plant Physiol. 79:843-847.
4. Baker, C. J., Atkinson, M. M., and Collmer, A. 1987. Concurrent loss in Tn*5* mutants of *Pseudomonas syringae* pv. *syringae* of the ability to induce the hypersensitive response and host plasma membrane K^+/H^+ exchange in tobacco. Phytopathology 77:1268-1272.
5. Bauer, D. W., and Beer, S. V. 1986. Cloning of a gene from *Erwinia amylovora* involved in induction of hypersensitivity and pathogenicity. Pages 425-429 in: Proc. Int. Conf. Plant Pathog. Bact. 6th. E. L. Civerolo, A. Collmer, R. E. Davis, and G. A. Gillaspie, eds. Martinus Nijhoff Publishers, Inc., College Park, MD.
6. Boucher, C. A., Barberis, P. A., Trigalet, A., and Demery, D. A. 1985. Transposon mutagenesis of *Pseudomonas solanacearum*: Isolation of Tn*5*-induced avirulent mutants. J. Gen. Microbiol. 131:2449-2457.
7. Burkholder, W. H. 1930. The bacterial diseases of the bean: A comparative study. Cornell Univ. Agric. Exp. Stn. Mem. 127.
8. Burkowicz, A., and Goodman, R. N. 1969. Permeability alterations induced by virulent and avirulent strains of *Erwinia amylovora*. Phytopathology 59:314-318.
9. Cuppels, D. A. 1986. Generation and characterization of Tn*5* insertion mutations in *Pseudomonas syringae* pv. *tomato*. Appl. Environ. Microbiol. 51:323-327.
10. Daub, M. E., and Hagedorn, D. J. 1979. Resistance of *Phaseolus* line WBR133 to *Pseudomonas syringae*. Phytopathology 69:946-951.
11. Daub, M. E., and Hagedorn, D. J. 1980. Growth kinetics and interactions of *Pseudomonas syringae* with susceptible and resistant bean tissues. Phytopathology 70:429-436.
12. Deasey, M. C., Stapleton, M. J., and Matthysse, A. G. 1986. Isolation and characterization of Tn*5* mutants of *Pseudomonas syringae* pv. *phaseolicola* which fail to elicit a hypersensitive response. Pages 444-447 in: Proc. Int. Conf. Plant Pathog. Bact. 6th. E. L. Civerolo, A. Collmer, R. E. Davis, and G. A. Gillaspie, eds. Martinus Nijhoff Publishers Inc., College Park, MD.
13. Delrot, S., and Bonnemain, J.-L. 1981. Involvement of protons as a substrate for the sucrose carrier during phloem loading in *Vicia faba* leaves. Plant Physiol. 67:560-564.
14. Durbin, R. D. 1982. Toxins and pathogenesis. Pages 423-441 in: Phytopathogenic Prokaryotes. Vol. 1. M. S. Mount and G. H. Lacy, eds. Academic Press, New York. 541 pp.
15. Giaquinta, R. T. 1983. Phloem loading of sucrose. Annu. Rev. Plant Physiol. 34:347-387.
16. Klement, Z. 1963. Method for the rapid detection of the pathogenicity of phytopathogenic pseudomonads. Nature 199:299-300.
17. Klement, Z. 1982. Hypersensitivity. Pages 149-177 in: Phytopathogenic Prokaryotes. Vol 2. M. S. Mount and G. H. Lacy, eds. Academic Press, New York. 506 pp.
18. Klement, Z., Farkas, G. L., and Lovrekovich, L. 1964. Hypersensitive reaction induced by phytopathogenic bacteria in the tobacco leaf. Phytopathology 54:474-477.
19. Klement, Z., Hevesi, M., and Sasser, M. 1978. Mechanism of the development of wildfire disease in susceptible tobacco plants. Pages 679-685 in: Proc. Int. Conf. Plant Pathog. Bact. 4th. B. Gilbert-Clarey, ed. Institut National de la Recherche Agronomique, Angers, France.
20. Lindgren, P. B., Peet, R. C., and Panopoulos, N. J. 1986. Gene cluster of *Pseudomonas syringae* pv. *phaseolicola* controls pathogenicity of bean plants and hypersensitivity on nonhost plants. J. Bacteriol. 168:512-522.
21. Message, B., Brostaid, P., Pitrat, M., Schmidt, J., and Boucher, C. 1979. A new class of fluidal mutants of *Pseudomonas solanacearum* unable to induce a hypersensitive reaction. Pages 823-833 in: Proc. Int. Conf. Plant Pathog. Bact. 4th. B. Gilbert-Clarey, ed. Institut National de la Recherche Agronomique, Angers, France.
22. Patil, S. S. 1974. Toxins produced by phytopathogenic bacteria. Annu. Rev. Phytopathol. 12:259-279.
23. Reinhold, L., and Kaplan, A. 1984. Membrane transport of sugars and amino acids. Annu. Rev. Plant Physiol. 35:45-83.
24. Spanswick, R. M. 1981. Electrogenic ion pumps. Annu. Rev. Plant Physiol. 32:267-289.
25. Stall, R. E., Bartz, J. A., and Cook, A. A. 1974. Decreased hypersensitivity to xanthomonads in pepper after inoculations with virulent cells of *Xanthomonas vesicatoria*. Phytopathology 64:731-735.
26. Turgeon, R. 1984. Efflux of sucrose from minor veins of tobacco leaves. Planta 161:120-128.