Differentiation of Races of Pseudomonas solanacearum by a Leaf Infiltration Technique

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ABSTRACT

The three known races of *Pseudomonas solanacearum* could be differentiated by the reaction of tobacco leaves following infiltration with bacterial suspensions containing at least 3.5×10^7 cells/ml. Race 1 isolates caused no visible symptoms on inoculated leaves after 24 hr, but a dark-brown necrotic lesion surrounded by a yellow halo appeared after 36 hr in the infiltrated area. By 60 hr, the bacteria had invaded the adjoining tissues and vascular elements. By 8 days after infiltration, there was extensive wilting, yellowing, and necrosis of the leaf tissues. In contrast, race 2 isolates induced a hypersensitive reaction (HR) by 10-12 hr after infiltration. At this time the infiltrated area was water-soaked, and slightly chlorotic, but became thin, white, and translucent by 60 hr. The reaction was limited to the infiltrated area. Race 3 isolates caused only a yellowish discoloration of the infiltrated area by 48 hr after inoculation.

The populations of race 1 and race 3 isolates increased within the tobacco leaf tissues during the first 48 hr after infiltration, but declined by 72 hr. The populations of race 2 isolates, which induced the HR, remained unchanged during the first 6 hr after infiltration and decreased sharply thereafter; by 48 hr, no live bacteria could be detected.

In leaves exposed to 900 ft-c for 48 hr, the HR was less intense than at 1,800 ft-c. In total darkness for 48 hr, the HR was not induced by race 2, but a spreading necrotic reaction, similar to that induced by race 1 in the light, was obtained. Increased periods of exposure to darkness were correlated with increased multiplication of the bacteria, and their spread into adjacent tissues.

Sterile fluids extracted from tobacco leaves during the initial symptoms of the HR inhibited the growth of both incompatible and compatible isolates of *P. solanacearum*. Phytopathology 60:833-838.

Several attempts have been made to group *Pseudomonas solanacearum* E. F. Sm. isolates into biotypes, varieties, or races on the basis of differences in physiological characteristics (1, 4, 5, 6), bacteriophage specificity (5), pathogenicity (2, 8), and serological properties (15, 16).

Buddenhagen et al. (2) separated isolates into three races, differing mainly in host range, as follows: race 1, pathogenic to tobacco, tomato, and many other solanaceous plants, and certain diploid bananas (*Musa* groups BB or AA) (17); race 2, pathogenic to triploid bananas (*Musa* groups AAA, AAB, and ABB) and *Heliconia* spp.; race 3, pathogenic to potato and tomato, but only weakly pathogenic to other solanaceous crops. Therefore, the three races could be determined on the basis of their pathogenicity to three differential hosts, tobacco, banana, and potato. In addition, colony appearance on Kelman's tetrazolium chloride (TZC) medium (7) could be used as an aid to differentiate the three races.

The use of differential hosts to determine races of *P. solanacearum* has the following drawbacks: (i) some strains from potato and peanut are only weakly pathogenic to tobacco (8), although from other physiological characteristics they clearly belong to race 1; (ii) some banana (race 2) and potato (race 3) strains are weakly pathogenic to tobacco, producing some of the symptoms obtained with certain race 1 strains; and (iii) other races could be excluded due to the narrow range of differentials used. Thus, additional methods to increase the accuracy and speed of characterizing isolates of *P. solanacearum* would be useful. The reaction of tobacco leaves following infiltration with bacterial suspensions may provide such a method. Preliminary results (14) indicated that a hypersensitive reaction (HR) was obtained in tobacco leaves infiltrated with incompatible strains of *P. solanacearum*. The HR in the tobacco leaf is characterized by rapid death of cells in contact with incompatible bacterial plant pathogens (10, 11, 12).

The present study was undertaken to determine (i) the race specificity of the HR induced by *P. solanacearum*; (ii) the inoculum concentration and the environmental conditions necessary for the reaction; and (iii) the changes in bacterial populations in compatible and incompatible reactions.

MATERIALS AND METHODS.—Seeds of *Nicotiana tabacum* L. 'Bottom Special' were sown on vermiculite, and seedlings were grown for 30 days. Each seedling was transplanted into a 6-inch pot containing coarse silica sand. Hoagland's nutrient solution was applied daily, and plants were grown either in a controlled environmental growth chamber at 28 C, 50% relative humidity (RH), and 1,800 ft-c on a 12-hr photoperiod; or in the greenhouse at 28 C (± 8 C) and 800 ft-c on a 12-hr photoperiod.

Twenty-six isolates of *P. solanacearum*, including representatives of all major races, and five avirulent mutants from isolates K 60, S 110, and S 109 were used (Table 1). Each isolate was streaked on TZC medium (7). After 48-hr incubation at 32 C, wild type colonies of each isolate were selected on the basis of their fluidity, color, and morphology; they were stored in sterile distilled water to reduce mutant formation.

When the tobacco plants were 30 to 45 days old,

TABLE 1. Origin of isolates of *Pseudomonas solanacearum* and reactions induced in tobacco leaves by these isolates 60 hr after infiltration with 1.6×10^8 cells/ml

Race	Isolate no.	Location	Host	Reaction type[a]
1	K 60	Wake Co., North Carolina	Tomato	N
1	K 74	Worth Co., Georgia	Tomato	N
1	K 105	Quincy, Florida	Tobacco	N
1	K 136	Trinidad	Tomato	N
1	S 123	Coto, Costa Rica	*Eupatorium odoratum*	N
1	S 213	Paraiso, Costa Rica	Potato	N
1	S 221	Nairobi, Kenya	Potato	N
1	S 225	Lupuna, Peru	Tomato	N
1	S 236	Nambour, Australia	Tomato	N
1	S 245	Atherton, Australia	Potato	N
1	S 247	Santander, Colombia	Tobacco	N
2	S 147	Coto, Costa Rica	*Heliconia* sp.	HR
2	S 210	Ibague, Colombia	Plantain	HR
2	S 215	Venezuela	Banana	HR
2	S 222	Timicuro, Peru	Banana	HR
2	S 223	Timicuro, Peru	Banana	HR
2	S 228	Buena Vista, Honduras	Banana	HR
2	S 253	Isla Padre, Peru	Banana	HR
3	K 56	Israel	Potato	Y
3	S 205	Tibaitata, Colombia	Potato	Y
3	S 206	Las Palmas, Colombia	Potato	Y
3	S 207	Popayan, Colombia	Potato	Y
3	S 238	Toowoomba, Australia	Potato	Y
3	S 243	Nambour, Australia	Potato	Y
3	S 246	Kingaroy, Australia	Potato	Y
?	S 208	Tibaitata, Colombia	Potato	HR
	B 1		Mutant from K 60	HR
	B 2		Mutant from K 60	HR
	B 1-1		Mutant from K 60	Y
	M 1		Mutant from S 110	Y
	M 2		Mutant from S 109	Y

[a] N = Dark necrotic area surrounded by a yellow halo; HR = hypersensitive reaction (evident 12 hr after inoculation); Y = yellowish discoloration of the infiltrated area.

from date of transplanting, the fully expanded leaves were infiltrated by injecting water suspensions of bacteria into the intercellular spaces with a hypodermic syringe fitted with a fine (No. 30) needle (10). An area, 3 to 5 cm^2, in each of 8 to 12 intercostal areas of each leaf, was infiltrated with a suspension of 1.6×10^8 bacterial cells/ml. At least 10 leaves were infiltrated with each isolate. For purposes of comparison, tobacco leaves were also infiltrated with suspensions of 1.6×10^8 cells/ml (as determined by standard serial dilution techniques) of each of the following species of bacteria: *Pseudomonas lachrymans* (Smith & Bryan) Carsner (Wis. B-73), *Xanthomonas axonopodis* Starr & Garces (ICPB No. XA127), and *Escherichia coli* (Migula) Castellani & Chalmers (NDA W3110). Leaf reactions were recorded after 60-hr incubation under the greenhouse conditions described above.

RESULTS.—*Tobacco leaf reactions following bacterial infiltration.*—Initial experiments involved leaf infiltration with isolates K 60 (race 1, pathogenic to tobacco), S 210 (race 2, nonpathogenic to tobacco), and S 207 (race 3, nonpathogenic to tobacco). Significant differences in the reaction of tobacco leaf tissues were obtained as follows:

1) Isolate K 60 (race 1) induced a dark-brown necrotic reaction in the infiltrated area by 36 hr after infiltration. A distinct yellow zone formed at the spreading edge of the lesion (Fig. 1). The lesion became progressively darker, and the yellow halo surrounding the dark central area became more noticeable by 48 hr. By 60 hr, the bacterium had invaded the tissues adjoining the infiltrated area and had moved along the veins and veinlets, which darkened. The bacteria became systemic throughout the plant within 8 to 10 days after infiltration, and there was extensive wilting, yellowing, and necrosis of the leaf tissues.

2) Isolate S 210 (race 2) induced a rapid HR in the infiltrated area. By 10-12 hr after infiltration, the leaf tissues were water-soaked, slightly chlorotic, and translucent. By 24 hr, the affected parenchyma cells were bleached and water-soaked; the affected area was sharply delimited from the noninoculated tissues by a definite border. Water was rapidly lost, and the affected areas became thin, white, and translucent by 60 hr after infiltration (Fig. 1).

3) Leaves infiltrated with isolate S 207 (race 3) remained symptomless until 48 hr after inoculation, when a yellowish discoloration of the infiltrated area appeared. The degree of yellowing increased slightly by 72 hr, but the size of the lesion remained constant and the bacteria did not invade adjoining tissue even by 96 hr after infiltration (Fig. 1).

In further experiments, the 26 isolates listed in Table 1 were infiltrated into tobacco leaves. All race 1 isolates caused the compatible, dark-brown necrotic reaction previously described for isolate K 60; all race 2 isolates induced the HR described for isolate S 210; and all race 3 isolates caused the yellowish discoloration described for isolate S 207 (Table 1).

Isolate S 208, and the avirulent mutants from K 60, B 1 and B 2, also induced the HR. In contrast, the avirulent strain B 1-1, also a mutant from K 60, and the avirulent strains M 1 and M 2, obtained from pathogenic isolates of race 2, caused a chlorotic reaction similar to that induced by race 3 isolates (Table 1).

Pseudomonas lachrymans caused an HR similar to that induced by race 2 isolates, isolate S 208, or the

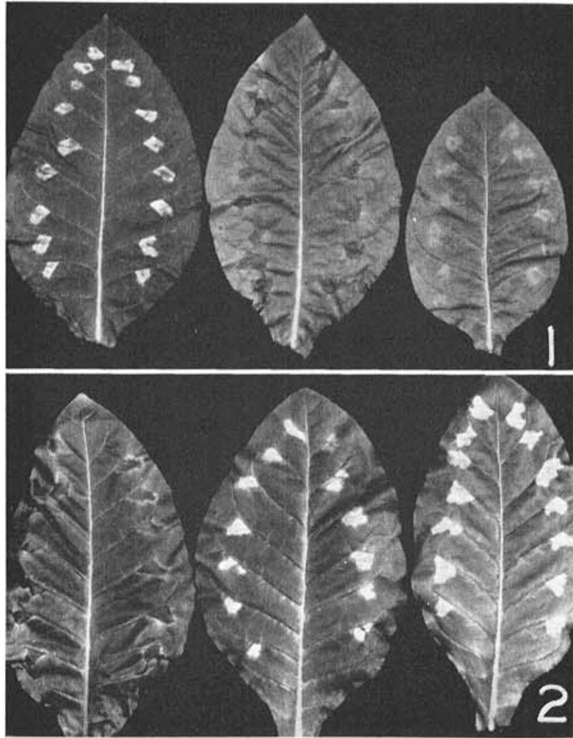

Fig. 1-2. 1) Tobacco leaf reactions 60 hr after infiltration with *Pseudomonas solanacearum* suspensions of 1.6×10^8 cells/ml. (left) Hypersensitive reaction (HR) induced by S 210 (incompatible race 2); (center) dark brown necrotic reaction (N) induced by K 60 (compatible race 1); (right) yellowish discoloration (Y) induced by S 207 (incompatible race 3). **2)** Effect of light intensity on the HR induced by the incompatible isolate S 210 of *P. solanacearum* (race 2) in tobacco leaves after 48 hr. (left) Dark-brown necrotic reaction induced when the leaf was exposed to total darkness after infiltration; (center) reaction obtained at low light intensity (900 ft-c); (right) the HR induced by this isolate under high light intensity (1,800 ft-c).

avirulent mutants B 1 and B 2. On the other hand, by 48 hr after infiltration, *X. axonopodis* and *E. coli* induced only a chlorotic reaction similar to that induced by race 3 isolates and certain avirulent mutants. The results indicate that the HR is induced only by certain specific bacteria, and is not a property of all plant pathogenic species.

Effect of inoculum concentration.—To determine the effect of inoculum concentration on the reactions induced by *P. solanacearum*, tobacco leaves were infiltrated with 8.5×10^7, 1.6×10^8, 6.0×10^8, and 3.5×10^9 cells/ml of two isolates each of race 1 (K 60 and S 213), race 2 (S 210 and S 228), and race 3 (S 206 and S 207). Each bacterial suspension was infiltrated into 8-10 different intercostal areas of each of three leaves. Plants were maintained in growth chambers, and reactions were recorded every 6 hr up to 48 hr after inoculation. The typical necrotic, spreading lesion obtained with race 1 isolates appeared from 24 to 36 hr after infiltration, depending on the cell population (Table 2); only the highest population (3.5×10^9 cells/ml) induced the reaction in 24 hr. Race 2 isolates, on the other hand, induced the HR by 10-12 hr after infiltration at all bacterial populations used (Table 2). The HR could be induced with populations of S 210 as low as 3.5×10^7 cells/ml.

Race 3 isolates induced chlorosis at the three lower populations by 48 hr after infiltration (Table 2). However, at the highest population (3.5×10^9 cells/ml), a reaction similar to the necrotic reaction characteristic of race 1, but without the outer yellow halo, was observed by 36 hr after infiltration.

Population changes in compatible and incompatible reactions.—To determine the changes in bacterial populations within the leaf tissues after infiltration, each of 10 tobacco leaves was infiltrated with a heavy bacterial suspension (approximately 10^8 cells/ml) of isolates K 60 (race 1), S 210 (race 2), and S 207 (race 3). The plants were maintained at 28 C in a growth chamber under continuous light (1,800-2,000 ft-c). Five 1-cm^2 discs were removed with a cork borer from the center of each infiltrated area at 6-, 12-, or 24-hr intervals up to 72 hr after inoculation, and the bacterial population of each disc was determined (10) as follows: each disc was comminuted with a TenBroeck tissue grinder in 1 ml sterile distilled water, the supernatant fluid was diluted with distilled water in a standard logarithmic series, and a 0.1 ml sample from each dilution up to 10^{-10} was pipetted onto TZC medium in a petri dish and spread over the surface with a bent glass rod. Individual colonies typical of the isolate were counted after 48-hr incubation at 32 C. The populations of both K 60 and S 207 increased during the first 48 hr after infiltration, but declined by 72 hr. Isolate S 210, which

TABLE 2. Reactions of tobacco leaf tissue to infiltration with different populations of isolates from races 1, 2, and 3 of *Pseudomonas solanacearum* at 28 C

Isolate no.	Race	Host reaction[a] at hr after infiltration:															
		12	24	36	48	12	24	36	48	12	24	36	48	12	24	36	48
		8.5×10^7 cells/ml				1.6×10^8 cells/ml				6.0×10^8 cells/ml				3.5×10^9 cells/ml			
K 60	1	—	—	N	N	—	—	N	N	—	—	N	N	—	N	N	N
S 213	1	—	—	N	N	—	—	N	N	—	—	N	N	—	N	N	N
S 210	2	HR	HR	HR	HR	HR	HR	HR	HR	HR	HR	HR	HR	HR	HR	HR	HR
S 228	2	HR	HR	HR	HR	HR	HR	HR	HR	HR	HR	HR	HR	HR	HR	HR	HR
S 206	3	—	—	—	Y	—	—	—	Y	—	—	—	Y	—	—	N′	N′
S 207	3	—	—	—	Y	—	—	—	Y	—	—	—	Y	—	—	N′	N′

[a] — = No visible reaction; N = dark brown necrotic area surrounded by a yellow halo; HR = hypersensitive reaction; Y = yellowish discoloration of the infiltrated area; N′ = dark brown necrotic area, with no halo.

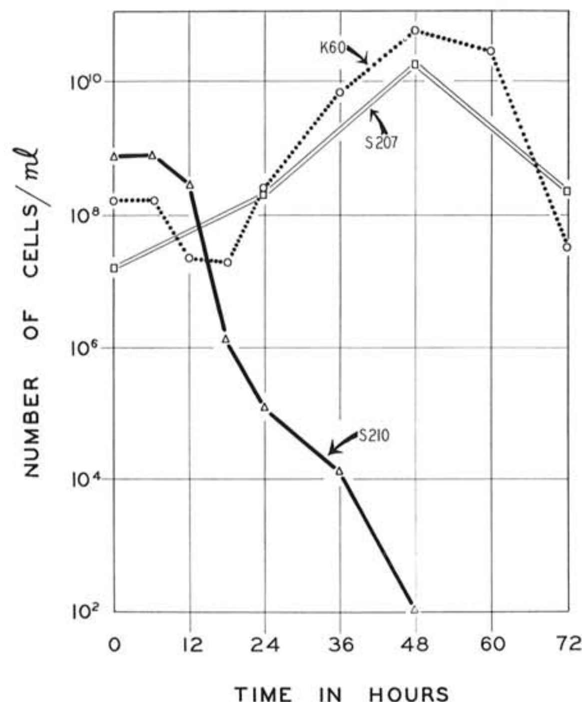

Fig. 3. *Pseudomonas solanacearum* population changes in tobacco leaves exhibiting compatible and incompatible reactions: K 60 (compatible race 1), S 210 (incompatible race 2), and S 207 (incompatible race 3). Cells/ml = cells/cm^2 leaf area.

induces the HR, did not increase in numbers during the first 12 hr after infiltration, the population remaining almost constant, but decreased sharply after 12 hr. By 48 hr after infiltration, it was not possible to detect any live cells of *P. solanacearum* in the tissues (Fig. 3).

Effect of temperature on the compatible and incompatible reactions.—To determine if reactions caused by the compatible (race 1) and incompatible (race 2) strains of *P. solanacearum* were affected by temperature, leaves of 10 tobacco plants (approximately 70 panels) were infiltrated with 6.0×10^8 cells/ml of isolates K 60 or S 210. The plants were placed in growth chambers maintained at 28, 24, 20, and 16 C for a 12-hr photoperiod (1,800 ft-c). The dark-brown necrotic reaction characteristic of K 60 (race 1) was observed by 36 hr after infiltration at 28 C, but only after 48 hr at 24 C, or 72 hr at 20 C. The reaction was not obtained after 96 hr at 16 C. The HR induced by isolate S 210 (race 2) was observed 10-12 hr after infiltration at 28 C and 24 C, but only after 24 hr at 20 C or after 72 hr at 16 C (Table 3).

Effect of light.—To determine the effect of light on the HR, tobacco leaves were infiltrated with suspensions of 6.0×10^8 cells/ml of isolate S 210, and the plants were maintained at 28 C under 1,800 or 900 ft-c provided by Sylvania Grolux, General Electric cool-white fluorescent tubes, and Mazda incandescent lamps with photoperiods varying from 0 to 48 hr. In some experiments, inoculated entire leaves, or half leaves, were covered with aluminum foil to provide complete darkness for various periods. To allow for gas exchange within the covered leaves, the openings at the base and the tip of each leaf were loosely plugged with cotton. In leaves exposed to high light intensity (1,800 ft-c), the HR was observed by 12 hr after infiltration. At the lower light intensity (900 ft-c), however, the reaction was less intense than that at 1,800 ft-c, and was characterized by a brown necrotic center surrounded by a slightly yellow halo and undefined margins. This was unlike the sharply defined lesions of the HR obtained at high light intensity. When leaves were placed in total darkness for 48 hr, the infiltrated areas became darker, with indefinite margins, and were surrounded by a yellow halo (Fig. 3). This reaction to a normally incompatible race 2 isolate in total darkness was comparable to that observed in the light after infiltration with compatible race 1 isolates.

In some experiments, tobacco leaves were infiltrated with isolate S 210 and immediately covered with aluminum foil for periods of 6 to 48 hr. The longer the exposure to darkness, the greater the bacterial multiplication and spread from the infiltrated area into adjacent tissues (Table 4). In leaves exposed to 48 hr of darkness, high bacterial populations were found in the infiltrated areas and in the adjoining discolored areas. The spread of the bacteria was arrested as soon as the leaves were exposed to light; the recently invaded areas showed the characteristic HR after 24-hr exposure to light. In any 48-hr period, initial incubation of the leaves for 12 hr or less in the dark, followed by 36 hr or more in the light, resulted in death of *P. solanacearum* in the infiltrated areas. When the period of darkness increased from 18 to 48 hr, followed by a decreasing light period, however, bacterial populations

TABLE 3. Effect of temperature on reactions of tobacco leaves after infiltration with isolates K 60 (race 1) and S 210 (race 2) of *Pseudomonas solanacearum*

Isolate no.	Temp	Host reaction[a] at hr after infiltration:						
		12	24	36	48	60	72	96
K 60 (race 1)	28 C	—	—	N	N	N	N	N
	24 C	—	—	—	N	N	N	N
	20 C	—	—	—	—	—	N	N
	16 C	—	—	—	—	—	—	—
S 210 (race 2)	28 C	HR	HR	HR	HR	HR	HR	HR
	24 C	HR	HR	HR	HR	HR	HR	HR
	20 C	—	HR	HR	HR	HR	HR	HR
	16 C	—	—	—	—	—	HR	HR

[a] N = dark necrotic area surrounded by a yellow halo; HR = hypersensitive reaction; — = no visible reaction.

TABLE 4. Populations of isolate S 210 (race 2) of *Pseudomonas solanacearum* in infiltrated tobacco leaves incubated under various periods of light (1,800 ft-c) and darkness for 48 hr

Period of darkness	Period of light	Bacteria/ml[a] after 48 hr
hr	*hr*	*no.*
0	48	0.0
6	42	0.0
12	36	0.0
18	30	4.0×10^2
24	24	6.1×10^3
30	18	6.2×10^4
36	12	4.0×10^5
42	6	6.2×10^6
48	0	1.4×10^7

[a] 1 ml = 1 cm^2 leaf area.

increased almost linearly from 4.0×10^2 to 1.4×10^7 cells/ml (Table 4).

In other experiments the procedure was reversed; leaves were exposed to varying periods of light, followed by periods of darkness. Twenty-four hr of continuous light immediately after infiltration resulted in death of the bacteria within the leaf. Moreover, when leaves were exposed to 0, 6, 12, or 18 hr of light immediately following infiltration and then to 48 hr of darkness, the populations of bacteria remaining in the center of the infiltrated area were 4.5×10^7, 6.8×10^6, 5.0×10^6, and 3.2×10^2 cells/ml, respectively.

To compare the pathogenicity to tobacco of isolates S 210 and K 60 under light and dark conditions, 20 plants were stem-inoculated with each isolate, or injected with sterile distilled water. One-half the plants was kept in a growth room under total darkness at 28 C and 60% RH, while the other half remained in a growth room at 28 C under continuous light (1,800 ft-c) and 50% RH. All plants inoculated with isolate S 210 and maintained in the light remained symptomless even after 15 days. Plants inoculated with isolate S 210 and maintained in the dark had wilted by 8 days after inoculation. Plants inoculated with the compatible isolate K 60 were killed after 10-12 days, whether under continuous light or darkness. Control plants did not wilt under either environmental condition.

Effect of relative humidity.—To determine the effect of RH on the development of the compatible or incompatible reactions, three leaves of each of 10 tobacco plants were inoculated with either K 60 (race 1) or S 210 (race 2) isolates. Five plants inoculated with each isolate were incubated in growth rooms under 1,800 ft-c of continuous light and either 87-92% or 50% RH. The change in appearance of the infiltrated areas was recorded every 6 hr up to 60 hr. The differences in ambient humidity did not visibly affect either the compatible necrotic reaction or the HR. The rapidity of induction of the HR, and the color and over-all appearance of the lesions, were similar at both RH values used.

Inhibitory substances produced during the HR.—To determine the toxicity of intercellular fluids to bacteria, liquid was extracted every 2 hr, during a 24-hr period, from leaves infiltrated with distilled water, heat-killed bacteria, or viable cells of isolates K 60 (race 1) or S 210 (race 2), following the method of Klement (9). Approximately 20 g of infiltrated tissue were centrifuged (1,020 *g* for 10 min at 4 C), and the fluids obtained were kept at 4-8 C for 1 hr after sterilizing them by Millipore (0.45 μ pore size) filtration. The sterility of the solution was checked by incubation on standard TZC medium. Melted TZC medium (without tetrazolium salts) was seeded with K 60 or S 210 cells (approximately 10^4 cells/ml) and allowed to harden in petri dishes. Samples of the leaf extracts (0.01-0.03 ml) were then (i) absorbed onto sterile filter paper discs which were placed on the surface of the medium; and (ii) dispensed into small glass cylinders which were inserted in the medium. The presence or absence of a clear zone around the discs or cylinders was recorded after 48-hr incubation at 30 C. An inhibitory effect on the growth of both K 60 and S 210 isolates was observed only with the sterile fluid extracted from leaves infiltrated with live S 210 cells. This inhibitory extract was obtained by 12 hr after infiltration, at a time when the HR first became noticeable. Maximum inhibition, as determined by the diam (5 mm) of the clear zone surrounding the assay disc or cylinder, was observed at 18-20 hr after infiltration. The inhibitory substances in leaf extracts decreased as the host cells collapsed, and were not present by 24 hr after infiltration.

The undiluted, sterile leaf fluids that produced the maximum inhibitory effect on bacteria in culture also induced a reaction similar to the HR when infiltrated into tobacco leaves.

DISCUSSION.—The differences in reactions of tobacco leaves to infiltration with isolates of *P. solanacearum* can be used as a simple and quick means to determine the race of any particular virulent isolate of this pathogen. The race may be determined within 48 hr after infiltration, when the reactions characteristic to each race become evident. The effectiveness of this technique, however, is dependent upon the population in the inoculum, the period of exposure to light, and the temp of incubation, since these factors evidently influence the type of reaction obtained.

Of all the virulent isolates of *P. solanacearum* studied, only one, S 208, which presumably belongs in race 3, induced an unexpected reaction (HR) in tobacco leaf tissues. However, Lozano (13) reported that this particular potato isolate was unusual in its high pathogenicity to banana, although its sensitivity to antibiotics and other physiological characteristics were like those of other race 3 isolates. This isolate may represent a link between races 2 and 3, and may constitute a distinct separate race.

The reactions of the tobacco leaf to infiltration with races of *P. solanacearum* were similar to those induced by other compatible and incompatible plant pathogenic bacteria and by saprophytic bacteria (10, 11). With *P. solanacearum*, however, the incompatible reaction (HR) was induced not only by virulent isolates nonpathogenic to tobacco, but by two avirulent mutants (B 1 and B 2) as well. A slightly chlorotic reaction was induced by race 3 isolates nonpathogenic to tobacco, several avirulent mutants, *X. axonopodis*, and *E. coli*. The

latter reaction was reportedly produced only by saprophytic bacteria (10, 11), but our results indicate that it is not restricted to these bacteria. The markedly different reactions of tobacco leaves to *P. solanacearum* indicate that they are induced by specific groups of bacteria which have particular structural or biochemical factors in common, and can or cannot multiply within host tissues. It is clear that the concept that any incompatible host-bacterium combination terminates in an HR (12) cannot be supported in the case of *P. solanacearum* and *X. axonopodis.*

The HR induced by *P. solanacearum* appears to depend on (i) cell numbers in the infiltrated area, since at low populations (less than 3.5×10^7 cells/ml) the HR was not induced or was delayed; (ii) photoperiod, probably because the synthesis of substances that induce the HR, or their effect on tobacco cells, appears to be light-dependent (in the absence of the HR, tobacco tissues become an ideal substrate for a normally incompatible isolate of *P. solanacearum*); Klement & Goodman (12), on the other hand, were of the opinion that light had no effect on the HR in tobacco; (iii) temperature, since exposure to 16 C delayed the onset of the HR up to 72 hr after infiltration.

The population changes after infiltration of tobacco leaf tissues indicate that only race 1 of *P. solanacearum* is able to multiply and spread. Race 3 isolates, which do not induce the HR, were able to multiply in the infiltrated tissues, but were unable to spread into adjacent tissues and did not cause necrosis. The factor(s) responsible for resistance to this particular race may be different from those that result in the HR. In contrast, race 2 isolates were unable to multiply in the tobacco leaf, possibly as a result of the rapid HR they induce. Additional evidence for the effectiveness of the HR is the fact that in the dark, where the HR is not induced, a race 2 isolate (S 210) was able to spread and kill tobacco plants.

The population changes of the three races of *P. solanacearum* observed in tobacco leaf tissues were not in agreement with those reported for other compatible and incompatible bacterial plant pathogens. With the incompatible isolate S 210, there was no demonstrable increase in numbers by 6 hr after infiltration, in contrast with reports indicating a rapid initial increase of other incompatible bacterial pathogens (10, 11). However, the populations used in our investigations were considerably higher than those used by other workers.

The HR in tobacco leaf tissues is possibly a defense mechanism induced by plant pathogens that produce a specific inducing factor or factors. The host apparently responds to this factor, producing substances that are toxic to both the pathogen and the plant cells. The collapse of the leaf tissues begins by 12 hr after infiltration with the incompatible bacterium, in agreement with previous reports (3, 10, 11), suggesting that this is the period required for the synthesis of toxic substances at concentrations sufficient to cause death of both host and pathogen cells. The presence of toxic substances which affect both host and pathogen, in sterile fluids extracted from leaves 12-20 hr after infiltration, supports the above conclusions. Similar inhibitory substances have been extracted from leaves of pepper cultivars hypersensitive to *X. vesicatoria* after infiltration with this organism (18, 19). The nature of the toxic substances from tobacco leaves is currently under investigation.

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