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Influence of Environmental Conditions on Reactions Induced by Infiltration of Bacteria into Plant Leaves

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Injection of bacteria into tobacco leaves resulted in several reactions: typical hypersensitivity (HR), "greasy" hypersensitivity, delayed necrosis, chlorosis, hypertrophy, and occasionally leaflet formation. The type of reaction depended upon the species of bacteria used, the numbers injected into the leaf, and the environmental conditions under which the tests were conducted.

Most phytopathogenic fluorescent pseudomonads, xanthomonads, and several erwinias caused typical hypersensitivity when introduced in large numbers, delayed necrosis when in moderate numbers, and chlorosis when in small numbers. "Greasy" hypersensitivity was induced by many of the soft-rotting pathogens. Under low light conditions, agrobacteria caused hypertrophy and leaflet formation and, occasionally, death. The temperature at which plants were maintained both before and after injection greatly affected the type of host response with most organisms. High temperatures favor induction of HR by *Pseudomonas solanacearum;* low temperatures induction by *P. syringae* and *P. phaseolicola.* Preconditioning plants at 16°C before infiltration and maintaining plants at 32°C after infiltration enhanced HR formation by xanthomonads in most cases. The rapidity and effect of temperature on HR caused by xanthomonads differed among four varieties of tobacco. *Nicotiana glutinosa* was the only tobacco in which HR was induced by all xanthomonads. Expression of HR in this variety, however, took 36 to 48 hours to be expressed, instead of the more usual 6 to 20 hours. The numbers of bacteria required for HR under optimum conditions ranged from approximately 1.0×10^6 for *P. syringae* to 1.5×10^8 for *P. phaseolicola, P. glycinea,* and *P. savastanoi* among the pseudomonads, and to 3.0×10^9 for *Erwinia amylovora.*

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Influence of Environmental Conditions on Reactions Induced by Infiltration of Bacteria into Plant Leaves[1]

Abstract. The number of bacteria needed to cause the hypersensitive (HR) or other necrotic responses in plant leaves was determined often by the environmental conditions under which the plant was grown prior to and after intromission of the bacteria into the intercellular spaces. Low temperatures reduced the number of *Pseudomonas syringae, P. phaseolicola,* and *P. savastanoi* cells required for HR whereas they increased the cell numbers required with *P. solanacearum.* Temperature and the tobacco species used affected induction of HR by xanthomonads. The amount of light influenced the type of response induced by agrobacteria with a necrotic response occurring under conditions of darkness.

INTRODUCTION

A HYPERSENSITIVE REACTION (HR), induced when large numbers of certain bacteria are introduced into the intercellular spaces of plant leaves (Klement *et al.,* 1964) is routinely used to aid in the identification of bacterial pathogens (Lelliott *et al.,* 1966). This reaction is characterized by rapid necrotization, wherein the leaf tissue collapses and dries out about eight to 24 hours after infiltration of the bacteria. The induction of the HR is typical of many pseudomonads, xanthomonads, and *Erwinia amylovora.*

Although considerable reliance is placed in the HR to help identify bacterial plant pathogens, little is known about the parameters of the reaction. Questions that commonly arise include the following: (i) Are all pathogens equal in their ability to cause hypersensitivity? (ii) How do environmental factors such as temperature and light affect the reaction? (iii) Is the HR easily recognized, or are there other reactions which might be confused with the HR? (iv) Do all plants behave similarily upon injection of an incompatible pathogen into their intercellular spaces? Answers to these questions are needed to fully evaluate the reliability of the HR as a method of identification. Consequently, these studies were conducted to help answer the above questions and to assist in defining what constitutes an HR.

Temperature effect upon HR reactions

Previous studies indicated that high temperatures favored the development of the HR by xanthomonads (Schroth and Hildebrand, 1967) and *Pseudomonas solanacearum* (Sands *et al.,* 1970), the latter being confirmed by Lozano and Sequeira (1970). In contrast, unpublished results have been cited (Klement and Goodman, 1967) which indicate that temperatures of 36°C or higher prevent the HR. Thus, an investigation was begun to examine the effect of temperature in more detail.

[1] Submitted for publication March 10, 1971.

TABLE 1

STRAINS OF PATHOGENS USED IN THIS STUDY AND THEIR SOURCES

Pathogen	Strain designation	Origin
Agrobacterium		
A. tumefaciens	CG14	ICPB* TT103
A. tumefaciens	CG41	ICPB TT131
A. tumefaciens	CG45	Eu-7, received from R. S. Dickey, Cornell, N.Y., 1964
A. tumefaciens	CG68	UCBPP isolated from rose, Berkeley, Calif.
A. tumefaciens	CG69	UCBPP isolated from eucalyptus, 1969
A. tumefaciens	Eu-8	Received from R. S. Dickey, Cornell, N.Y., 1964
A. tumefaciens	Kerr 22	Received from C. I. Kado, Davis, Calif., 1969
A. tumefaciens	Kerr 27	Received from C. I. Kado, Davis, Calif., 1969
A. radiobacter	AR-15	ICPB TR102
A. rhizogenes	HR-1	ICPB TR108
Erwinia		
E. amylovora	FB-1	UCBPP, isolated from cotoneaster, 1967
E. amlyovora	FB-9	UCBPP, isolated from apple, Orinda, Calif., 1966
Pseudomonas		
P. coronafaciens	#8	NCPPB 1348
P. glycinea	Glyc 564	Received from C. Leben, Wooster, Ohio, 1968
P. glycinea	R-4	Received from B. W. Kennedy, St. Paul, Minn., 1967
P. lachrymans	PL-1	NCPPB 277
P. phaseolicola	HB-16	G-2, received from R. G. Grogan, Davis, Calif., 1967
P. phaseolicola	HB-28	Race 1, received from J. I. Natti, Geneva, N.Y., 1967
P. phaseolicola	HB-36	UCBPP, isolated from bean, Salinas, Calif., 1964
P. savastanoi	OK-22	NEAP E-4
P. solanacearum	#3	B139, received from I. Buddenhagen, 1967
P. solanacearum	#8	H1527-SFR, received from I. Buddehnagen, Honolulu, Hawaii, 1968
P. solanacearum	#10	P-28-T, received from I. Buddenhagen, Honolulu, Hawaii, 1968
P. syringae	S-3	UCBPP, isolated from pear, Berkeley, Calif., 1963
P. syringae	S-9	UCBPP, isolated from plum, Santa Rosa, Calif., 1957
P. syringae	S-36	UCBPP, isolated from pear, Orinda, Calif., 1967
P. syringae	620	Received from C. Leben, Wooster, Ohio, 1968
P. tomato	P. tom. 1	UCPBB, isolated from tomato, Salinas, Calif., 1967
P. tomato	P. tom. 8	UCPBB, isolated from tomato, 1961
Xanthomonas		
X. campestris	XC-2	UCPBB
X. dieffenbachiae	XD-1	UCPBB, isolated from dieffenbachiae, San Francisco, Calif., 1967
X. fragariae	XE-32	UCPBB, isolated from strawberry, Salinas, Calif., 1966
X. fragariae	XF-102	ICPB
X. incanae	SI-3	503, received from M. T. Lai, Sacramento, Calif., 1970
X. phaseoli	XP-2	ICPB
X. pruni		UCPBB
X. vesicatoria	XVG-36	UCPBB
X. vitians	X. lett.	UCPBB, isolated from lettuce, Salinas, Calif., 1964
Xanthomonas sp.	X. ti.	Received from A. H. McCain, Berkeley, Calif.

*Abbreviations used:
NEAP—Estacaco Agronomica Nacional (Maria de Lourdes d'Oliveria), Oerias, Portugal.
ICPB—International Collections of Phytopathogenic Bacteria, Department of Bacteriology (M. P. Starr), University of California, Davis.
NCPPB—National Collection of Plant Pathogenic Bacteria, Plant Pathology Laboratory (R. A. Lelliott), Harpenden Herts, England.
UCBPP—Department of Plant Pathology, University of California, Berkeley.

Combined with this investigation were studies to determine the effect of preconditioning of the host upon the HR and of the numbers of bacteria required to induce HR.

Four suspensions (turbidity reading of 160 on a Klett-Summerson colorimeter using a green filter #54 and dilutions of 1/10, 1/100, and 1/1000) were prepared of cells of various pseudomonads, xanthomonads, and erwinias (Table 1) grown on King's medium

B (King *et al.*, 1954) or YDCP (Leben *et al.*, 1970). These suspensions were injected with a hypodermic syringe into alternate intercostal areas of leaves of Glurk (*Nicotiana glutinosa* L. × *N. tabacum* L. [Samsun × Xanthi] cv. Turkish, nc. tobacco plants which had been preconditioned under natural light for four days or longer in constant temperature (± 2°C) cubicles of 16, 21, 27, and 32°C. Immediately after injection, the plants were moved to different temperature cubicles. The degree of HR which occurred was determined after 24, 48, 72, and 168 hours.

The response to changes in temperature were divided into there general types: those where temperature had no effect on HR under the range tested (Type I), those where higher temperatures generally enhanced HR (Type II), and those where lower temperature generally enhanced the reaction (Type III). These three types of responses are illustrated in table 2 by the data obtained for *P. tomato* strain P. tom. 8 (Type I), *P. solanacearum* #8 (Type II), and *P. syringae* S-9 (Type III). Preconditioning also had an effect upon the HR. For example, more bacteria often were required to induce HR in the Type III response at all incubation temperatures when the plants were preconditioned at 32°C. Another effect noted was that the HR ofter tended to be less severe if plants were maintained at the same temperature before and after infection of the bacteria.

Different strains differed in their ability to induce HR. The bacteria were divided into groups depending upon the temperature response type of HR they produced and the minimum number of cells required to produce complete killing of the infiltrated portion of the leaf within 72 hours was determined (table 3). Examination of the groupings indicated that the number of bacteria required for HR, and the temperature response type generally were characteristic for each species. For example, the two strains of *Erwina amylovora* and the two strains of *P. tomato* produced a Type I response. However, it generally took 20 to 200 times as many cells of *E. amylovora* as cells of *P. tomato* to induce HR. Individual strains of species differed somewhat from each other, both in the number of cells needed to induce the HR and in their specific response to temperature changes. The same number of cells of *P. syringae* isolates S-3 and S-9 caused HR when plants were kept at 32°C or moved to 32°C from 27° or 21°C; but when moved to 32° from 16°C, ten times more cells of S-3 than of S-9 were required for HR.

The bacteria were grouped according to the general type of response they induced on the basis of all of the data obtained, including the additional dilutions and the length of time and amount of killing which occurred.

Host variety effect upon injection reaction

Xanthomonad strains (table 1) were used to determine the effect of host variety upon the HR, and of the interaction with temperature. Four tobacco varieties were used: Glurk, *N. tabacum, N. rustica* L., and *N. glutinosa.* All plants were preconditioned in the constant-temperature greenhouse cubicles for four days prior to injection with suspensions of 160 Klett units of the xanthomonads. The pre- and post-infiltration temperatures used in this test were 16° to 16°C, 16° to 32°C, 32° to 16°C, and 32° to 32°C.

The various tobacco varieties all reacted differently in these tests with respect both to the strain of the pathogen used and to the effect of temperature (table 4). In general, incubation of plants at 16°C, regardless of the preconditioning temperature, prevented the HR. Preconditioning the plants at 16°C and incubating them at 32°C after injection was most favorable for the HR.

TABLE 2

EFFECT OF TEMPERATURE CHANGES UPON THE DEGREE OF HYPERSENSITIVE REACTION OCCURRING AFTER THE INJECTION OF VARIOUS DILUTIONS OF BACTERIAL SUSPENSIONS INTO GLURK TOBACCO LEAVES

Type reaction	Bacteria number/ml	Temperature changes															
		32° C*				27° C				21° C				16° C			
		32° C†	27° C	21° C	16° C	32° C	27° C	21° C	16° C	32° C	27° C	21° C	16° C	32° C	27° C	21° C	16° C
Type I	10^9	++++‡	++++	++++	++++	++++	++++	++++	++++	++++	++++	++++	++++	++++	++++	++++	++++
(*P. tomato* strain	10^8	++++	++++	++++	++++	++++	++++	++++	++++	++++	++++	++++	++++	++++	++++	++++	++++
P. tom. 8)	10^7	+	+	+	++	+	++	−	++	−	+	++	−	+	+	−	+
	10^6	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−
Type II	10^9	++	++++	++++	++++	++	++++	++++	++++	++++	++++	++++	++++	++++	++++	++++	++++
(*P. solanacearum*	10^8	++	++++	++++	+	++	++++	++++	+	++++	++++	+++	+	+	+	+	−
strain #8)	10^7	++	++	−	−	++	++	−	−	−	−	−	−	−	−	−	−
	10^6	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−
Type III	10^9	++++	++++	++++	++++	++++	++++	++++	++++	++++	++++	++++	++++	++++	++++	++++	++++
(*P. syringae*	10^8	++++	++++	++++	++++	++++	++++	++++	++++	++++	++++	++++	++++	++++	++++	++++	++++
strain S-9)	10^7	−	−	−	+	−	++++	++++	++++	−	++++	++++	++++	++++	++++	++++	+++
	10^6	−	−	−	−	−	+	+	−	−	+	+++	++++	−	+++	++++	+

*Temperature before injection.
†Temperature after injection.
‡Rating of reactions: ++++ = complete killing within 24 hours; +++ = ⅓ or more killing within 24 hours; ++ = complete killing within 72 hours; + = partial killing within 72 hours; − = no killing.

TABLE 3

TYPE OF TEMPERATURE RESPONSE AND NUMBER OF BACTERIA REQUIRED TO CAUSE NECROSIS OF THE ENTIRE INJECTED GLURK TOBACCO LEAF SECTIONS WITHIN 72 HOURS

Bacteria strain	Number	32° C*				27° C				21° C				16° C			
		32° C†	27° C	21° C	16° C	32° C	27° C	21° C	16° C	32° C	27° C	21° C	16° C	32° C	27° C	21° C	16° C
Erwinia																	
E. amylovora (FB1)	3.2×10^9‡	9§	9	9	9	9	9	9	9	9	9	9	9	9	9	9	9
E. amylovora (FB9)	2.4×10^9	9	9	9	9	9	9	9	9	9	9	9	9	9	9	9	9
Pseudomonas																	
P. tomato (P. tom. 1)	1.0×10^9	9	8	8	8	8	8	8	7	8	8	8	8	8	8	8	8
P. tomato (P. tom. 8)	8.7×10^8	9	8	8	7	8	7	8	8	8	8	7	8	8	8	8	8
P. coronafaciens #3	8.5×10^8	7	7	7	7	7	7	7	7	7	7	7	7	7	7	7	7
P. lachrymans (PL-1)	1.3×10^9	7	7	7	7	7	7	7	7	7	7	7	7	7	7	7	7
Pseudomonas																	
P. solanacearum #8	1.1×10^9	7	7	8	9	7	7	8	9	8	8	8	9	8	9	9	>9
P. solanacearum #10	1.3×10^9	8	8	8	>9	8	8	8	>9	8	8	8	>9	8	8	8	>9
Pseudomonas																	
P. glycinea (Glyc 564)	1.2×10^9	9	8	8	8	>9	9	8	8	>9	9	8	8	>9	9	8	8
P. glycinea (R-4)	1.6×10^9	9	9	8	8	9	8	8	8	9	8	8	8	9	9	8	8
P. phaseolicola (HB-1b)	1.3×10^9	>9	>9	9	9	>9	>9	9	9	>9	9	9	9	>9	9	9	8
P. phaseolicola (HB-28)	1.0×10^9	>9	9	9	9	>9	9	9	8	>9	8	8	8	9	8	8	8
P. phaseolicola (HB-36)	1.4×10^9	>9	>9	9	8	>9	>9	8	9	>9	9	8	9	>9	9	8	8
P. savastanoi (OK-22)	1.8×10^9	>9	>9	9	9	>9	>9	9	9	>9	9	8	9	9	8	8	9
P. syringae (S-3)	1.0×10^9	8	8	7	7	8	8	7	7	8	7	7	7	8	8	7	7
P. syringae (S-9)	8.7×10^8	8	8	8	8	8	7	7	7	8	7	7	6	7	7	6	7
P. syringae (S-36)	1.1×10^9	9	8	8	8	9	8	8	8	9	8	8	8	9	8	8	8
P. syringae (S-620)	1.0×10^9	8	8	7	7	8	7	7	7	8	7	7	7	8	7	7	6
Xanthomonas																	
X. fragariae (XF-32)	1.7×10^9	>9	9	9	>9	>9	9	9	8	9	8	9	7	9	>9	9	9
X. fragariae (XF-102)	2.4×10^9	>9	9	9	>9	9	8	8	8	8	8	8	8	8	8	8	9

*Temperature before injection.
†Temperature after injection.
‡Number/ml in standardized suspension of 160 Klett units (green filter) as measured with an electronic particle counter (Coulter counter Mod. B).
§Number of bacteria/ml to nearest power of ten in the suspension infiltrated into the intercellular spaces of tobacco leaves.

TABLE 4

THE EFFECT OF TEMPERATURE CHANGES UPON KILLING RESPONSE INDUCED IN DIFFERENT TOBACCO VARIETIES BY INFILTRATION OF LEAVES WITH XANTHOMONADS

Strains	Tobacco variety															
	Glurk				*Nicotiana tobacum*				*Nicotiana rustica*				*Nicotiana glutinosa*			
	16*–16†	32–16	16–32	32–32	16–16	32–16	16–32	32–32	16–16	32–16	16–32	32–32	16–16	32–16	16–32	32–32
X. phaseoli (XP-2)‡	–§	–	–	–	–	–	–	–	–	–	–	–	–	–	+	++
X. dieffenbachiae (XD-1)	–	–	–	–	–	–	–	–	–	–	+	++	–	–	–	++
X. pruni	–	–	++++	+	–	–	+	–	–	–	+	–	–	–	+	++
X. incanae (XI-3)	–	–	–	–	–	–	–	–	–	–	+	+	–	–	++	++
X. campestris (X. camp.)	–	–	++++	–	–	+	+	–	–	–	–	+	–	+	++	++
X. vesicatoria (XVG-36)	–	–	++++	++	–	–	++++	++++	–	–	++++	+++	–	–	++	++
X. fragariae (XF-32)	++++	++	++++	–	–	+	++++	–	+	+	+++	++++	–	–	++	+++
X. fragariae (XF-102)	++++	+	++++	–	–	+	++++	–	–	–	+	+	–	–	++	++
X. vitians (X. lett.)	–	–	++++	–	–	+	++++	+	–	–	++++	++++	–	–	++	++
Xanthomonas sp. (X. ti.)	–	–	++++	+	–	–	++++	++	–	–	++	++	–	–	++	++

*Temperature before infiltration.
†Temperature after infiltration.
‡O.D. of bacterial suspensions was 160 Klett units using a green filter.
§Rating of reactions: ++++ = complete killing within 24 hours; +++ = ⅓ or more killing within 24 hours; ++ = complete killing within 72 hours; + = partial killing within 72 hours; – = no killing.

However, maintaining the plants at 32°C before and after injection generally prevented HR in Glurk and *N. tabacum,* whereas HR was severe in the *N. rustica* and *N. glutinosa* varieties. The most sensitive indicator plant appeared to be *N. glutinosa,* although the HR in this plant was delayed, and death usually occurred after approximately 40 rather than after the common 18 hours.

Effect of light upon reactions induced by *Agrobacterium tumefaciens* and other bacteria

Inoculation of plant leaves using the carborundum inoculation method has resulted in the formation of tumors (Lippincott and Heberlein, 1965). Therefore it is of interest to determine the effect of injection of *A. tumefaciens* and related bacteria into leaves. For comparison, several pseudomonads and xanthomonads were also tested. The tests were conducted primarily in growth chambers, although one series was conducted in a greenhouse under constant temperature of 27° ± 2°C. The growth chambers were programmed for temperatures of 16° or 27°C with lighting of 150 or 300 ft-c on a continuous, or alternating, 12-hour on/12-hour off, or total-darkness basis. Glurk tobacco plants were maintained for four days before infiltration at the conditions under which the tests were to be conducted. The bacterial suspensions were prepared from 24- to 48-hour cultures grown on King's medium B or YDCP and standardized at 200 Klett units (green filter). Reactions generally were recorded nine to 14 days but, occasionally up to 30 days after infiltration.

The reaction of Glurk tobacco to the injection of a cell suspension of agrobacteria into leaves depended upon the environmental conditions under which the plants had been maintained after injection. Every isolate, whether virulent or avirulent, produced some killing in the infiltrated leaf panel under conditions of total darkness and temperature of 27°C (table 5). On the other hand, no reactions were observed when the plants were maintained under continuous light (150 ft-c) at 27°C. Marked chlorosis occurred under several conditions. A thickening of leaves inoculated with certain isolates of *A. tumefaciens* was observed in plants maintained in the greenhouse at 27°C during the winter, but not during the summer. Microscopic examination indicated that the thickening was due to hypertrophy, not hyperplasia. Occasional tumors occurred on some of these leaves, and plantlets were rarely formed. The tumors and plantlets appeared to originate from vein tissue and not from leaf lamina tissue.

The reactions induced by the xanthomonads were also influenced by light. As with the agrobacteria, there appeared to be more killing under conditions of continuous darkness. Light appeared to have little effect on the reactions caused by the pseudomonads.

Host reactions types resulting from injection of bacteria into plant leaves

It became obvious during the preceding tests that HR is not the only alternative to a true pathogenic response which may occur upon injection of bacteria into plant leaves. Usually the various reactions and conditions under which the reactions occurred were characteristic for a given group of bacteria, and, consequently, they can be used to a limited extent as an aid to identification of a pathogen. Occasionally they can be confused with a true pathogenic response, and, consequently, observations of host reactions must be interpreted with caution. A summary of the reactions which were observed and their characteristics is presented in table 6, as an aid to identification of pathogens and interpretation of plant responses to the infiltration of bacteria.

TABLE 5

EFFECT OF LIGHT AND TEMPERATURE UPON REACTIONS INDUCED IN GLURK TOBACCO LEAVES UPON INJECTION OF A SUSPENSION* OF AGROBACTERIA AND OTHER BACTERIA INTO THE INTERCELLULAR SPACES

Strain	Greenhouse 27° C		Continuous light			Intermittent light			Continuous darkness	
			150 ft-c		300 ft-c	150 ft-c		300 ft-c	15° C	27° C
	Winter	Summer	15° C	27° C	15° C	15° C	27° C	15° C		
Agrobacterium										
A. tumefaciens (CG14)	TT†	–	–	–	–	C	C	–	+	+
A. tumefaciens (CG41)	T	–	–	–	–	C	C	C	+	+
A. tumefaciens (CG45)	TT	–	–	–	–	C	C	C	+	+
A. tumefaciens (CG68)‡	C	–	–	–	–	C	C	–	+	++
A. tumefaciens (Kerr22)	C	–	C	–	C	C	C	C	+	++
A. tumefaciens (Kerr27)	TT	–	C	–	C	C	C	C	+	++
A. tumefaciens (CG69)	C	–	–	–	–	C	C	–	+	+
A. tumefaciens (Eu-8)	TT	–	–	–	–	–	T	C	–	T+
A. rhizogenes (HR-1)	C	–	–	–	C	C	++	C	–	++
A. radiobacter (AR-15)	+	–	–	–	–	C	+	CC	+	+
Pseudomonas										
P. syringae (S-9)	++	++	++	++	++	++	++	++	++	++
P. coronafaciens (#3)	++	++	++		++	++		++	++	
P. phaseolicola (HB-28)	+	+	+		++	+		+	++	
P. solanacearum (#3)	++	++	+		+	+		+	++	
Xanthomonas										
X. campestris (XC-2)	C	+	C	Not tested	++	++	Not tested	++	++	Not tested
X. pruni	C	+	C		C	C		C	++	
X. phaseoli (XP-2)	–	C	–		C	C		C	++	
X. vesicatoria (XVG36)	C	C	C		C	C		+	++	
X. incanae (XI-3)	++	C	+		++	++		+	++	
X. dieffenbachiae (XD-1)	C	C	C		+	+		C	++	
X. vitians (X. lett.	++	C	++		++	++		++	++	
X. fragariae (XF-32)	++	++	++		++	++		C	++	
X. fragariae (XF-102)	++	++	++		C	C		C	++	
Xanthomonas sp. (X. ti.)	C	C	C		C	C		C	+	

*O.D. of suspensions were 200 Klett units (green filter).

†Rating of reactions: ++ = complete killing; + = partial killing; – = no reaction; C = chlorosis; T = thickening; and TT = thickening and tumors. Results were read nine to 14 days after injection, except for TT reactions which were observed 20 to 30 days after infiltration.

‡Avirulent.

TABLE 6

REACTION OF TOBACCO (GLURK) LEAVES INDUCED BY INJECTION OF SUSPENSIONS OF INCOMPATIBLE PATHOGENS INTO THE INTERCELLULAR SPACES OF LEAVES

Reaction type	Characterization of reaction
Hypersensitivity (typical)	Reaction characterized by rapid necrosis (within 6–20 hours after injection) of infiltrated portion of leaf. The killed section of the leaf remains green or becomes slightly brown. This reaction occurs after infiltration of large numbers of *Erwinia amylovora*, occasionally other erwinias including some isolates of *E. quercina* and *E. carotovora*, all species of the *Pseudomonas syringae* group (Sands *et al.*, 1970), *P. solanacearum* and many xanthomonads. Occurrence of the reaction is sensitive to light and temperature conditions. Usually associated with incompatible pathogens, but can occur also with compatible pathogens.
Hypersensitivity ("greasy")	Reaction characterized by rapid necrosis (12–20 hours after injection) of infiltrated portion of leaves. Large numbers of cells are required. The killed section of the leaf becomes "greasy" in appearance. This reaction occurs often—but not always—with some isolates of the *Erwinia* and nonfluorescent pseudomonad, soft-rotting groups.
Delayed necrosis	Reaction characterized by a slow necrosis (30–192 hours after injection) of infiltrated portion of leaf. The killed section often becomes chlorotic before dying. The color of the dead section is brown. This reaction occurs with most strains which cause a typical HR when only moderate numbers of cells are infiltrated, or if environmental conditions not as favorable for HR are selected. In addition, injection of *A. tumefaciens* or *A. rabiobacter* will produce this result if the injected plants are kept under low light or total darkness.
Hypertrophy and leaflet formation	This reaction occurs with isolates of *A. tumefaciens* and *A. radiobacter* under moderate light. Hypertrophy is common, whereas leaflet formation is observed only rarely.
Chlorosis	This reaction is induced by all bacteria including *saprophytes* and pathogens if sufficient numbers are introduced into the leaf. Numbers, however, must be fewer than those required to cause one of the killing reactions.

Discussion

The HR induced by bacteria has been described as a rapid necrosis of tissue occurring 8 to 24 hours after introduction or large numbers of a pathogen into the intercellular spaces of the plant. Although the onset of the HR can be delayed, it still is characterized by the rapidity with which the infiltrated tissue collapses and dies. We found, however, that in many cases a slow necrosis will occur under certain conditions. Sometimes this involves only the selection of a different plant. Thus the questions arise whether this reaction can be called HR and whether nonpathogens might induce the same reaction under certain conditions. We did not test nonpathogens, but it is conceivable that many with the proper manipulation could cause death in plants. An example of this was established by Lovrekovich and Lovrekovich (1970) who demonstrated that with proper manipulation, *P. fluorescens* can kill a tobacco leaf.

Our results indicate that tests for HR should be conducted under several conditions if any reliance is to be placed on statements that an organism does or does not cause HR. Rather, such a statement may be a reflection of the range of conditions under which HR is induced by an organism. Thus our original report that *E. quercina* does not induce HR (Hildebrand and Schroth, 1967) was erroneous, as we now find HR induction by this organism after only slight changes were made in conditions under which the tests were conducted. Certainly many of the xanthomonads appear to induce the HR under a much

narrower range of conditions than most pseudomonads. An exception to this appears to be *X. fragariae* which induces HR under a range of conditions similar to that of pseudomonads.

Our findings also provide evidence that different pathogens have different systems causing reactions which can be grouped under the term HR. In some cases, this evidence concerns the appearance of the dead plant part such as the "greasy" versus "typical" HR. Further indications that different pathogens have different systems for inducing HR and that this directly involved interactions with the host plant were provided by the findings that the number of bacteria required for HR and the specific response to temperature changes were characteristic for various species of the pathogen. The characteristic responses to temperature could not be ascribed solely to an effect of temperature upon the pathogen because preconditioning the host at specific temperatures had a significant effect upon the induction of HR. This preconditioning effect was particularly pronounced when xanthomonads were tested against Glurk tobacco. HR in this case was enhanced if the plant had been preconditioned at what appears to be an unfavorable (for HR) temperature before being moved to the favorable temperature. In contrast, HR exhibited a tendency to be more severe if the plants were preconditioned at a temperature favorable for HR when pseudomonads were tested. A final indication that different bacteria have different systems for causing HR is the effect of light upon the reaction. Although darkness increases the lethal effect of xanthomonads, it apparently has little effect on the HR caused by pseudomonads.

Several possibilities concerning the mechanisms of killing are suggested by examination of the reactions which occurred. The association of soft-rotting organisms, both erwinias and pseudomonads, with the "greasy" HR suggests that pectolytic or similar enzymes may be a factor. A possibility with *A. tumefaciens* is the involvement of auxins. Formation of IAA or similar auxins by *A. tumefaciens* could result in the hypertrophy observed. Perhaps under the condition of darkness the levels of auxin formed became toxic and the infiltrated parts of the leaf were killed.

The HR is a test which has been extensively used in the recognition of phytopathogenic bacteria. It appears, however, that its full potential in this area has not been reached. Currently, each laboratory uses a different variety grown under a different set of conditions to test for HR. This lack of standardization perhaps does not affect results in many cases if only the capacity of an organism to induce HR is being tested. On the other hand, the ability of some organisms to cause HR is greatly influenced by the conditions under which tests are conducted, and considerable variation in results among laboratories would be expected. Furthermore, it appears that different species of bacteria react to different conditions in a characteristic manner, and that these characteristic reactions could be used in species identification. The full potential of these tests, however, will not be realized until laboratories standardize the condition under which HR is tested.

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