Two Hypotheses on the Aetiology of Response of Plants to Phytopathogenic Bacteria

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SUMMARY

Graded doses of six phytopathogenic bacteria, namely *Pseudomonas lachrymans*, *P. morsprunorum*, *P. phaseolicola*, *P. syringae*, *P. tabaci* and *P. tomato*, were introduced into the leaves of one of their natural (homologous) hosts and of other (heterologous) plants. Probit slopes for the infectivity of any of the six bacteria did not exceed the value $b = 2$ in the homologous combinations, but were much greater than 2 in the heterologous ones. In the homologous combinations, the mean probability, P , per inoculated bacterium of multiplying to induce host response was invariant to inoculum dose, d . Hence the probability, Q , that a host would not respond after inoculation with d bacteria was given by the simple exponential, $Q = e^{-Pd}$. In the heterologous combinations, the value of P increased with increasing d .

When a streptomycin-sensitive and a streptomycin-resistant variant of the same bacterium were mixed together in a 1:1 proportion in the challenge dose, plants responding after inoculation with a heterologous pathogen yielded a mixture of both variants in the same proportion, whereas those responding to inoculation with a homologous pathogen yielded either a 1:1 mixture of both variants or a large predominance of a single variant, depending on whether the dose contained more or less than 1 ED₅₀. It was concluded from these experiments that the relationships between inoculated cells of homologous and heterologous bacteria during growth *in vivo* were described best by the hypothesis of independent action and the hypothesis of co-operative action, respectively.

INTRODUCTION

The definitions of host range and host specificity, as applied to phytopathogenic bacteria, have been open to many questions following repeated demonstrations that these bacteria, when inoculated under suitable conditions, are able to multiply and induce symptoms not only in their natural (homologous) hosts, but also in quite unrelated (heterologous) plants (Klement & Lovrekovich, 1961, 1962; Klement, Farkas & Lovrekovich, 1964; Ercolani & Crosse, 1966). Differences have been established in the pattern of growth *in vivo* and the mechanism of induction of symptoms between homologous and heterologous combinations (Ercolani & Crosse, 1966; Klement & Goodman, 1967*a*), but the relationships between growth data and dose/response data in different systems have not received adequate attention.

Many plants respond predictably with visible symptoms after inoculation with phytopathogenic bacteria only when the challenge dose contains more than one bacterial cell. Therefore the response observable in such plants might be assumed to be the result of either (i) the action of one random inoculated bacterium which chances to generate a large enough

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progeny *in vivo* to cause infection, or (ii) the joint action of several inoculated bacteria, all of which contribute to overcome plant defences. Possibilities (i) and (ii) are the basic postulates of two well-known models of infection, i.e. the hypothesis of independent action and the hypothesis of co-operative action, respectively (Meynell & Stocker, 1957). The results of most titration experiments on animals suggest that living micro-organisms are independent after inoculation, whereas co-operation occurs between drug molecules *in vivo* (see Meynell, 1957; Meynell & Meynell, 1970). Comparable information is lacking for plants. However, *Corynebacterium michiganense* and *Erwinia carotovora* have been reported to act independently during infection of tomato plants and invasion of potato tuber discs respectively (Ercolani, 1967*a, b*; Pérombelon, 1971).

This paper describes the application of the hypothesis of independent action and the hypothesis of co-operative action to the aetiology of response of some plants to homologous and heterologous phytopathogenic *Pseudomonas* spp. A preliminary proposal for the applicability of the two hypotheses has been advanced (Ercolani, 1968*a*).

METHODS

Test plants. These were plants of bean (*Phaseolus vulgaris*) 'Borlotto di Vigevano', sweet cherry (*Prunus avium*) 'Bigarreau Moreau', cucumber (*Cucumis sativus*) 'SMR 18', pear (*Pyrus communis*) 'Kaiser', tobacco (*Nicotiana tabacum*) 'Burley Ky 16', and tomato (*Lycopersicon esculentum*) 'Ace'. Bean, cucumber, tobacco and tomato plants were raised from surface-sterilized seed sown in Vermiculite, transplanted individually at the seedling stage into pots of coarse silica sand, and grown in a room with 50 to 60 % r.h., 20000 lux provided by fluorescent daylight lamps (Osram-L 40 W/20 R and 40 W/15 R) for a 12-h photoperiod, day temperature of 23 ± 2 °C and night temperature of 16 ± 2 °C. To prepare cherry and pear plant material, cuttings of either species, which had just completely rooted under mist, were obtained from a commercial nursery, transplanted individually into pots of coarse silica sand, and grown further in a greenhouse with 70 to 80 % r.h., approximately 10000 lux on a 12-h photoperiod in addition to sunlight, and a temperature of 24 ± 4 °C during the day and 16 ± 3 °C at night. After transplanting, all plants were watered daily with the nutrient solution of Hoagland & Snyder (1933) and grown for definite periods before they were used in inoculation experiments: 2 weeks for the beans, 4 weeks for the cucumbers and the tomatoes, 6 weeks for the tobacco plants, and 8 weeks for the cherries and the pears. To ensure that the plants did not harbour any unwanted micro-organisms, random samples were taken periodically and plated on nutrient agar containing 5 % sucrose (NAS), on which all bacteria included in this study (see next paragraph) form characteristic colonies (Crosse, 1959; Lelliott, Billing & Hayward, 1966).

Bacterial cultures. The following cultures, representative of six fluorescent phytopathogenic *Pseudomonas* nomenspecies, were used initially: *P. lachrymans* NCPPBI425, isolated originally by Z. Klement from cucumber in Hungary in 1958; *P. morsprunorum* CM8, isolated from necrotic leaf spots on sweet cherry trees near Bologna, Italy, in 1965; *P. phaseolicola* IL3, from halo blight lesions on dwarf bean plants 'Borlotto di Vigevano' near Bologna, Italy, in 1962; *P. syringae* SB4, from blighted blossoms on pear trees 'Kaiser' near Modena, Italy, in 1965; *P. tabaci* TL2, from wildfire lesions on the leaves of tobacco plants 'Burley Ky 16' near Piacenza, Italy, in 1965; *P. tomato* PT4, from tomato fruits affected with bacterial speck at Parma, Italy, in 1965.

A streptomycin-sensitive clone (Sm^s) and a streptomycin-resistant, non-streptomycin-dependent clone (Sm^r) of each culture were used in the experiments described below. The

Sm^s clones were isolated at the beginning of the work from discrete lesions produced by inoculating each of the original cultures on the leaves of its homologous host. The Sm^r clones were selected by plating thick suspensions of Sm^s bacteria on plates of nutrient agar (NA) containing 400 µg streptomycin/ml. All cultures were maintained on nutrient agar + 2 % glycerol (NAG) slopes in screw-capped vaccine bottles and stored at 4 °C.

Sm^s and Sm^r variants of each bacterium had the same growth rate *in vitro* and *in vivo*, and did not affect each other's specific growth rate when they were cultured together. The ED₅₀s (see below) of the variants of each pathogen for the homologous host did not differ significantly.

Preparation of inocula. Exponentially growing cultures were prepared by subculturing the bacteria on fresh NAG slopes twice at daily intervals and incubating at 25 °C. The 24 h growth from the second slope was quickly suspended in sterile distilled water (SDW), washed by centrifuging and resuspending twice in SDW, and finally adjusted nephelometrically to a concentration of 10⁸ viable bacteria/ml by reference to a calibration curve relating scattered light to viable cell counts. The stock suspensions thus prepared were rapidly diluted with SDW to the required concentration, and the resulting suspensions were used immediately to challenge the plants according to the techniques described below.

In the experiments designed to determine dose/response relationships, the doses of inoculum were equally spaced by a dilution factor of either 10^{-0.5} (in host/pathogen combinations of the homologous type) or 10^{-0.2} (in heterologous combinations) between an upper and lower limit corresponding to an expected response of approximately 0.9 and 0.1 as indicated by pilot experiments. In the experiments intended to determine the composition of bacterial populations in plants responding to inoculation, equal volumes of two suspensions containing an estimated concentration of 10⁸ viable cells/ml of either the Sm^s or the Sm^r variant were mixed together and then diluted to give a number of selected doses. Plate counts were always made to determine the exact composition of all suspensions. In the experiments with mixtures of Sm^s and Sm^r bacteria, doses were expressed as number of ED₅₀s (see below), the latter being computed on the summed results of the experiments reported in Table 1. Pilot experiments indicated that the ED₅₀ of a 1:1 mixture of Sm^s and Sm^r variants of the same pathogen did not differ from that of either variant alone.

Inoculation techniques. Each plant was enclosed in a plastic bag, uncovered after 4 h and inoculated immediately as described by Ercolani & Crosse (1966). Bacterial suspensions were applied to the fifth lowest leaf of the plants, with the exception of the beans which were inoculated on the lowest trifoliate leaf, and cucumbers which were challenged on the third lowest true leaf. In infectivity titration experiments, each concentration of inoculum was administered to a separate group of 30 plants. In other experiments with mixtures of Sm^s and Sm^r bacteria, plants were inoculated in groups of 10 or 20 to each dose level, depending on whether the challenge dose contained more or less than 1 ED₅₀ (see below).

Inoculation procedures were standardized to ensure that leaves of comparable size were inoculated on each plant, and that each leaf was infiltrated uniformly with bacterial suspensions. Calibration experiments with suspensions containing 10⁸, 10⁵ and 10⁷ bacteria/ml, using the homologous pathogen, showed (i) that the mean inoculum dose, i.e. the mean number of bacteria introduced into each leaf, was in all three dose groups a constant fraction of the number of bacteria/ml of inoculum, namely 0.104 of that number in pear, 0.126 in cherry, 0.313 in bean, 0.590 in tomato, 0.802 in cucumber, and 0.920 in tobacco leaves; and (ii) that within each dose group, the range of individual inoculum doses introduced into the leaves of any one plant species was invariably narrower than the 95 % confidence limits of the number of bacteria in the corresponding mean inoculum dose as estimated by the

method of Seligman & Mickey (1964) using a dilution factor of 10^{-3} and inoculating 20 tubes at each dilution level. Based on these results, all leaves of any one plant species in any one dose group were considered for practical purposes to have received an equal dose of inoculum, the size of which was computed directly from the known concentration of inoculum by using the fractional factors given above.

Response records. Quantal responses only were taken by recording the number of inoculated healthy and diseased leaves daily up to 20 days after challenge. Water-soaking of infected tissues, gradually followed by typical field symptoms, were observed only in homologous combinations. Symptoms induced by heterologous bacteria consisted of the sudden appearance of dry necrotic areas, suggestive of a hypersensitive plant reaction (Klement & Goodman, 1967a).

Determination of dose/response relationships. Plant response records were subjected separately for each infection experiment to probit analysis (Finney, 1971). The median effective dose (ED₅₀) and the slope of the log dose/probit response curve (*b*) were estimated by maximizing the likelihood functions, and the precision of the estimates was determined by using the variance formulae.

Determination of bacterial clones in plants responding. This was done in the inoculation experiments with mixtures of Sm^s and Sm^r variants. As soon as any leaf responded, it was cut away 1 cm from the stem, surface-sterilized for 4 min in a 2 % (w/v) aqueous solution of chloramine T containing 0.01 % sodium sulphosuccinate as wetting agent, washed in running water and three changes of SDW, blotted dry, suspended in 10 ml of sterile 0.05 M-phosphate buffer (pH 6.5), and macerated for 5 min in an M.S.E. micro-homogenizer running at 12000 rev./min. The homogenate was held for approximately 1 h at room temperature with periodical shaking before making tenfold dilutions, each of which was spread in 0.1 ml amounts on the surface of five NAS plates. Plates developing 20 to 200 colonies after 4 days at 25 °C were replicated with a velvet pad (Lederberg & Lederberg, 1952) on the surface of a plate of plain NA and a plate of NA containing 400 µg streptomycin/ml. Between 234 and 680 colonies were scored as Sm^s or Sm^r for each inoculation experiment.

From 12 plants found to contain either variant alone, one Sm^s and one Sm^r clone of each pathogen were isolated and re-examined for their growth rate *in vitro* and *in vivo*, their ED₅₀ for the homologous host, and their effect on the specific growth rate of the other variant in mixed culture. No differences could thus be detected between these re-isolates and the original cultures.

RESULTS

Infectivity titration experiments. The results of the probit analysis of plant response records are shown in Table 1. The data for the heterologous combinations are based on the results obtained with all dose groups, whereas only the results recorded for those dose groups which gave a response of between 0.3 and 0.7 are incorporated in the figures given for the homologous combinations. When more extreme responses were included in the analysis for the homologous combinations, the upper and lower branches of the resulting probit diagrams showed an upward bias so that the log dose/probit response relationship assumed a distinctly curvilinear trend. However, an excellent fit of a straight line to the complete set of experimental data for both the heterologous and the homologous combinations was obtained when $\log(-\ln \text{response})$ was plotted versus log dose, as, for instance, in the so-called Weibull analysis (Peto, 1953; Armitage, 1959; Shortley & Wilkins, 1965).

In all plant/pathogen combinations the ED₅₀ values of the homologous bacteria were at least 100-fold lower than those of the heterologous ones. However, an accurate estimate of

Table 1. Dose/response relationships observed in infectivity titration experiments

Pathogen	Host	Combination type	Expt no.	log ED 50	b
<i>Pseudomonas lachrymans</i> (Sm ^s)	Cucumber	Homologous	1	1·881 ± 0·295	1·943 ± 0·104
			2	1·921 ± 0·295	1·872 ± 0·093
			3	1·785 ± 0·301	2·001 ± 0·101
	Tobacco	Heterologous	1	4·740 ± 0·324	12·242 ± 2·318
			2	4·771 ± 0·312	14·245 ± 2·849
	Tobacco	Heterologous	1	4·785 ± 0·288	11·982 ± 2·702
<i>P. lachrymans</i> (Sm ^r)	Cucumber	Homologous	1	1·854 ± 0·305	1·904 ± 0·091
	Tobacco	Heterologous	1	4·785 ± 0·288	11·982 ± 2·702
<i>P. morsprunorum</i> (Sm ^s)	Cherry	Homologous	1	1·983 ± 0·328	1·801 ± 0·094
			2	2·003 ± 0·269	1·784 ± 0·096
	Pear	Heterologous	1	4·203 ± 0·295	12·535 ± 2·482
			2	4·160 ± 0·293	14·140 ± 2·781
<i>P. morsprunorum</i> (Sm ^r)	Cherry	Homologous	1	1·978 ± 0·293	1·746 ± 0·088
	Pear	Heterologous	1	4·198 ± 0·323	13·480 ± 3·112
<i>P. phaseolicola</i> (Sm ^s)	Bean	Homologous	1	1·276 ± 0·320	1·864 ± 0·104
			2	1·203 ± 0·311	1·939 ± 0·096
			3	1·444 ± 0·278	1·877 ± 0·088
	Tomato	Heterologous	1	4·483 ± 0·311	10·725 ± 1·750
			2	4·513 ± 0·288	10·550 ± 2·277
			3	4·539 ± 0·275	11·485 ± 2·300
	Cucumber	Heterologous	1	4·788 ± 0·281	10·702 ± 2·156
			1	4·764 ± 0·307	11·100 ± 2·204
	Tobacco	Heterologous	1	4·788 ± 0·281	10·702 ± 2·156
			1	4·764 ± 0·307	11·100 ± 2·204
	Bean	Homologous	1	1·398 ± 0·270	1·848 ± 0·093
	Tomato	Heterologous	1	4·496 ± 0·300	10·254 ± 2·058
<i>P. phaseolicola</i> (Sm ^r)	Pear	Homologous	1	2·048 ± 0·304	1·724 ± 0·084
			2	2·091 ± 0·317	1·739 ± 0·096
	Cherry	Heterologous	1	3·849 ± 0·312	8·723 ± 1·665
			2	3·876 ± 0·328	9·460 ± 1·811
	Bean	Heterologous	1	4·072 ± 0·311	8·852 ± 1·444
	Cucumber	Heterologous	1	4·584 ± 0·302	9·951 ± 1·712
	Tomato	Heterologous	1	4·130 ± 0·291	8·051 ± 2·132
	Tobacco	Heterologous	1	4·551 ± 0·324	9·262 ± 2·144
	Pear	Homologous	1	2·075 ± 0·285	1·708 ± 0·087
	Cherry	Heterologous	1	3·819 ± 0·288	7·929 ± 1·880
	Pear	Homologous	1	2·075 ± 0·285	1·708 ± 0·087
	Cherry	Heterologous	1	3·819 ± 0·288	7·929 ± 1·880
<i>P. syringae</i> (Sm ^s)	Pear	Homologous	1	2·048 ± 0·304	1·724 ± 0·084
			2	2·091 ± 0·317	1·739 ± 0·096
			1	3·849 ± 0·312	8·723 ± 1·665
			2	3·876 ± 0·328	9·460 ± 1·811
	Bean	Heterologous	1	4·072 ± 0·311	8·852 ± 1·444
	Cucumber	Heterologous	1	4·584 ± 0·302	9·951 ± 1·712
	Tomato	Heterologous	1	4·130 ± 0·291	8·051 ± 2·132
	Tobacco	Heterologous	1	4·551 ± 0·324	9·262 ± 2·144
	Pear	Homologous	1	2·075 ± 0·285	1·708 ± 0·087
	Cherry	Heterologous	1	3·819 ± 0·288	7·929 ± 1·880
	Pear	Homologous	1	2·075 ± 0·285	1·708 ± 0·087
	Cherry	Heterologous	1	3·819 ± 0·288	7·929 ± 1·880
<i>P. syringae</i> (Sm ^r)	Tobacco	Homologous	1	0·603 ± 0·271	1·914 ± 0·094
			2	0·602 ± 0·312	1·838 ± 0·093
			3	0·779 ± 0·307	1·937 ± 0·078
			4	0·843 ± 0·272	1·900 ± 0·077
	Cucumber	Heterologous	1	4·584 ± 0·272	8·127 ± 1·249
			2	4·636 ± 0·311	9·105 ± 1·810
			3	4·622 ± 0·317	7·801 ± 1·692
	Bean	Heterologous	1	4·433 ± 0·297	8·910 ± 2·002
			2	4·501 ± 0·297	9·127 ± 1·898
	Tomato	Heterologous	1	4·314 ± 0·302	8·241 ± 1·314
	Tobacco	Homologous	1	0·634 ± 0·281	2·015 ± 0·078
<i>P. tabaci</i> (Sm ^s)	Tobacco	Homologous	2	0·770 ± 0·276	1·956 ± 0·082
			1	4·606 ± 0·288	8·543 ± 1·928
	Cucumber	Heterologous	1	4·606 ± 0·288	8·543 ± 1·928
	Tomato	Homologous	1	1·936 ± 0·325	1·803 ± 0·091
<i>P. tabaci</i> (Sm ^r)	Tobacco	Homologous	2	1·856 ± 0·295	1·754 ± 0·094
			3	1·880 ± 0·279	1·778 ± 0·096
			1	4·584 ± 0·316	12·174 ± 2·445
	Bean	Heterologous	2	4·529 ± 0·306	13·122 ± 2·567
			3	4·545 ± 0·321	12·270 ± 2·223
	Cucumber	Heterologous	1	4·729 ± 0·278	12·702 ± 2·385
	Tobacco	Heterologous	1	4·692 ± 0·290	14·105 ± 2·630
	Tomato	Homologous	1	1·950 ± 0·301	1·746 ± 0·085
	Bean	Heterologous	1	4·546 ± 0·329	12·370 ± 2·185

Fig. 1. For legend see opposite.

the relative infectivity of different bacteria for each host could not be obtained by direct comparison of the respective ED₅₀ values, because of the broad differences in the corresponding values of b (Table 1). Thus, for the reasons given in the Discussion, differences in the relative infectivity of homologous and heterologous bacteria were more marked at the lower doses of inoculum.

Probit slopes for infectivity of different bacteria for each plant species were never significantly greater than 2 in the homologous combinations and invariably much greater than 2 in the heterologous ones, suggesting that inoculated bacteria were acting independently in the former combination type and co-operating in the latter (Peto, 1953; Meynell & Stocker, 1957).

Cucumber and tobacco plants gave a fairly uniform susceptible response to *Pseudomonas lachrymans* ($b > 1.872$) and *P. tabaci* ($b > 1.838$), respectively, but progressively increasing amounts of heterogeneity in susceptibility to the homologous pathogens were present in bean, cherry, tomato and pear plants. The degree to which individual plants differed in their resistance to heterologous bacteria appeared to be correlated with the identity of the bacterium involved, rather than to the botanical identity of the plant itself. In this respect, more uniform responses were induced by *P. morsprunorum*, *P. lachrymans* and *P. tomato* ($b > 12$) than by *P. tabaci* and *P. syringae* ($b < 10$), with *P. phaseolicola* ranging between the two groups.

Mixed inoculation experiments. Fig. 1 to 3 show the composition of bacterial populations isolated from the plants that responded to inoculation with a mixture of Sm^s and Sm^r variants of the same pathogen. Plants responding after inoculation with more than 1 ED₅₀ of the mixture were generally found to comprise both variants in approximately the same proportion as they were in the inoculum. Only a small number of plants given few ED₅₀s of a mixture of the Sm^s and Sm^r variants of the homologous pathogen contained a predominance of one variant. Plants challenged with 1 ED₅₀ or less yielded different results, depending on the type of plant/pathogen combination studied. In the heterologous combinations, the composition of bacterial populations was always similar to that of the challenge dose, but in the homologous ones a single variant was generally present alone or with a small proportion of the other variant. When all plants responding to inoculation in each experiment were considered together, the overall frequency of isolation of each variant proved to be approximately equal to the proportion in which the same variant was present in the inoculum, thus confirming that no detectable difference existed between the infectivity of the Sm^s and Sm^r variants of the same pathogen.

The observed rates of occurrence of Sm^s and Sm^r variants in plants responding to inoculation with different doses of the homologous pathogen were compatible with the predictions of the hypothesis of independent action, but not with those of the hypothesis of co-operative action (Meynell & Stocker, 1957), and the reverse was true of the plants given different doses of a heterologous pathogen. This supports the conclusion, reported above, of the experiments in which the slope of the log dose/probit response curve was measured.

Fig. 1. Proportion of a streptomycin-sensitive clone (shown in white) and a streptomycin-resistant clone (shown in black) of *Pseudomonas phaseolicola* and *P. tomato* in the leaves of bean and tomato plants responding to inoculation with graded doses of a mixture of the two variants. (a) *Pseudomonas phaseolicola* on bean (homologous combination); (b) *P. tomato* on bean (heterologous); (c) *P. phaseolicola* on tomato (heterologous); (d) *P. tomato* on tomato (homologous). Each column represents one leaf. The column at the extreme left shows the proportions of the two variants in the inoculum (I) and that at the extreme right their overall rate of recovery (R) in the plants responding. The doses of inoculum (expressed as number of ED₅₀s) and the ratio of responding to inoculated plants are also indicated at the bottom of each graph.

Fig. 2. Proportion of a streptomycin-sensitive clone (shown in white) and a streptomycin-resistant clone (shown in black) of *Pseudomonas lachrymans* and *P. tabaci* in the leaves of cucumber and tobacco plants responding to inoculation with graded doses of a mixture of the two variants. (a) *Pseudomonas lachrymans* on cucumber (homologous combination); (b) *P. tabaci* on cucumber (heterologous); (c) *P. lachrymans* on tobacco (homologous); (d) *P. tabaci* on tobacco (homologous). See Fig. 1 for further explanations.

Fig. 3. Proportion of a streptomycin-sensitive clone (shown in white) and a streptomycin-resistant clone (shown in black) of *Pseudomonas morsprunorum* and *P. syringae* in the leaves of cherry and pear plants responding to inoculation with graded doses of a mixture of the two variants. (a) *Pseudomonas morsprunorum* on cherry (homologous combination); (b) *P. syringae* on cherry (heterologous); (c) *P. morsprunorum* on pear (heterologous); (d) *P. syringae* on pear (homologous). See Fig. 1 for further explanations.

DISCUSSION

The results of infectivity titration and mixed inoculation experiments performed in this study combine to show that the aetiology of response of plants to challenge by homologous and heterologous pathogens is substantially different. The outcome of infection of any of the six plant species tested with the selected homologous pseudomonad pathogen always fulfilled the expectations of a system to which the hypothesis of independent action was applicable. Since the hypothesis is stochastic in nature, in such systems the host response may be produced by the progeny of only one of the inoculated bacteria. Thus, the findings of this study corroborate the few previous reports of a single phytopathogenic bacterium being sufficient to infect its homologous host (Hildebrand, 1937; Thyr, 1968). Infectious units of *Agrobacterium tumefaciens* may consist of single bacteria (Hildebrand, 1942; Lippincott & Heberlein, 1965; Manigault & Beaud, 1967), but the diversity of the mechanism of pathogenesis involved therein sets the relationship between inoculated bacteria in that system quite apart, conceptually at least, from that in most other natural plant/pathogen combinations. In contrast with their behaviour in their homologous hosts, single bacteria of any of the six pathogenic pseudomonads used in this study proved inherently incapable of infecting heterologous hosts. Although the 'individual effective dose' necessary for induction of symptoms by any of the heterologous pathogens varied to some extent from plant to plant, as indicated by the observed probit slopes, the values of the latter were always much in excess of 2, indicating that plant responses resulted from the joint action of several of the inoculated bacteria. This is consistent with the large existing body of evidence which indicates that symptoms are only observed in heterologous combinations following introduction of heavy doses of inoculum into the plant (Logan, 1960; Klement & Goodman, 1967*a*; Hildebrand & Riddle, 1971).

Why phytopathogenic bacteria act independently in their homologous hosts but co-operate to induce response in the heterologous ones has to be left open to speculation at the present time. Evidently, within the tissues of any plant given the same number of ED₅₀s of either a homologous or a heterologous pathogen, interaction between inoculated bacteria is more likely to occur in the latter case, in which the resulting concentration of bacteria in the tissues is higher. The production of typical disease symptoms in homologous combinations is strictly a function of bacterial multiplication *in vivo*, and attainment of certain population thresholds is a prerequisite for symptom production in such systems (see Ercolani, 1968*b*). In heterologous combinations, symptoms of the so-called hypersensitive type appear after a period of predictable and comparatively short duration even if the bacteria are killed as early as 2 or 3 h after inoculation (Klement & Goodman, 1967*b*; Klement, 1971). Although the mechanism of its induction is not clear, hypersensitivity in its present acceptance appears to be primarily a host reaction triggered by the bacteria during a short 'induction period' immediately following inoculation (Klement, 1971). Since co-operation *in vivo* is characteristic, in animals at least, of inanimate agents like drug molecules (Meynell, 1957), the interaction between inoculated heterologous bacteria in plant tissues might simply reflect the joint cumulative effect of some bacterial component on the plant. Sequeira & Ainslie (1969), Ercolani (1970), Slesman, Perley & Hoitink (1970) and Gardner & Kado (1972) have reported on the possible occurrence of such components in the surface layers of phytopathogenic bacteria.

When very high numbers of bacteria are introduced into a host plant, homologous pathogens will also be likely to interact and to some extent co-operate to give infection. In this case, however, co-operation would be of a somewhat different kind since it would depend

on the resultant overloading of the plant tissues with an overwhelming dose, and not on the inherent phytopathogenic apparatus of the micro-organisms involved. In this respect, it would seem appropriate that future studies on this subject include varieties of the same plant species, whose expected response to inoculation with the same pathogen would be either of the compatible or of the hypersensitive type.

Although dose/response relationships for all systems included in this study invariably assumed a linear form when they were plotted on a metamer log dose/log ($-\ln$ response) scale, the slope of individual plots differed depending on the type of plant/pathogen combination involved. In the homologous combinations, probit slopes $b \simeq 2$ were transformed into Weibull slopes of approximately 1, indicating (i) that the mean probability, P , per inoculated bacterium of multiplying *in vivo* to induce response was invariant to the average concentration, d , of bacterial cells in the standard inoculum received by the plants (Peto, 1953; Armitage, 1959), and (ii) that the underlying relationship between the proportion of uninfected plants, Q , and the bacterial concentration, d , was a simple exponential, $Q = e^{-Pd}$, with e being the base of natural logarithms (Druett, 1952; Peto, 1953). In the heterologous combinations, probit slopes $b \gg 2$ were transformed into Weibull slopes considerably greater than 1, thus indicating that P values increase with the average concentration of bacteria in the inoculum dose, d .

It therefore appears that the relative infectivity of homologous and heterologous bacteria for any given plant can only be estimated with some approximation by studying dose/response relationships over a considerably broader range of inoculum doses than has been used customarily in phyto-bacteriological experiments so far. Although the most appropriate dosage for this kind of titrations is obviously dependent on the inoculum potential (*sensu* Horsfall, 1932) of different pathogens in the field, from the practical standpoint comparable variations of the dose of inoculum will cause the proportion of responding plants to change more dramatically in heterologous combinations. Also, whenever, as in this study, the definition of the ED₅₀ or other critical dose is given at the level of individual leaves, variations in leaf size between plants of different species will make comparisons of the infectivity of the same bacterium for different plants difficult.

The occurrence of a minority of one variant along with a majority of the other variant in plants responding to inoculation with 1 ED₅₀ or less of the homologous pathogen can probably be explained, in this as in other mixed inoculation experiments (Meynell & Stocker, 1957; Ercolani, 1967*b*), by a terminal breakdown of host resistance at a late stage of invasion by the progeny of one cell of the dominant variant. In a few control experiments in which leaves expected to respond to challenge with 1 ED₅₀ were sampled at different times up to 2 days before the earliest anticipated date of appearance of symptoms, only one variant was almost invariably found. Where both variants co-existed, their proportion was always close to 1:1, suggesting that the leaf in question was abnormally susceptible. As a result of differences in individual host resistance, the observed rate of occurrence of both variants together is known to be greater than expected in plants responding to inoculation with low doses and smaller than expected in those given high doses (Meynell & Stocker, 1957). In this respect it might be significant that tomato and pear plants, which appeared to give a somewhat heterogeneous response to their homologous pathogens as indicated by b values of 1.746 to 1.803 and 1.708 to 1.739 for *P. tomato* and *P. syringae* respectively, were also those which yielded the highest number of leaves containing either a single variant after inoculation with more than 1 ED₅₀, or both variants after inoculation with 1 ED₅₀ or less. However, these results should be considered with caution, because the amount of heterogeneity in the resistance of the plant material was not very great.

For practical purposes, it is usually taken as a matter of course that homologous and heterologous pathogens be thus defined with respect to the plant from which they were isolated originally. However, these assumptions might cause some degree of confusion in laboratory work if a bacterium happened to be isolated from a hypersensitive lesion developed on a non-host plant under abnormal ecological conditions in the field. The results of this study suggest that the homologous or heterologous nature of plant/pathogen interactions may in fact be recognized by simply determining whether challenging bacteria are acting independently or co-operating during growth *in vivo*. Even those modifications of the basic mechanism of invasion of plant tissue by homologous bacteria, which may occur when the inoculum dose is raised above 1 ED₅₀ (Ercolani, 1972), will not affect the discriminative power of the techniques used in this study to recognize different types of plant/pathogen combination.

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