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Dose-response relationships between phytopathogenic bacteria and their hosts

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Abstract

Theories on the relationships between the challenge dose of phytopathogenic bacteria and the quantal response of the host plant are discussed and applied to experimental results. In a number of cases the extent of disease was directly proportional to the dose, which is an indication of independent action. Departures from linearity in the relationships were ascribed to *inter alia* a shortage of multiplication sites, antagonism amongst the cells of the pathogen and heterogeneity of the tested host plants with respect to susceptibility. In a few host-pathogen combinations, dose-response relationships showing an upward curve were found; this is probably an indication of facultative synergism. The relationships found in homologous and heterologous host-pathogen combinations were similar. The implications of the above findings for the quantitative study of factors affecting the susceptibility of plants to bacterial infection are discussed.

Additional keywords: infectivity titrations, bacterial interactions.

Introduction

In inoculation experiments with plant pathogenic bacteria a series of plants of a given host usually respond consistently only if challenged by several cells of the pathogen. This poses the question whether the inoculum dose must exceed or be equal to a certain numerical threshold, because it is only by mutual co-operation of a number of bacteria that a response of the host can be induced (numerical threshold theory), or instead whether the inoculum dose must be high, because only a certain proportion of the cells of the pathogen hit a multiplication site and are able to multiply and produce symptoms (theory of independent action) (Meynell and Meynell, 1975; Van der Plank, 1975).

In the mathematical treatment of both theories, that is given below, it is presupposed that the outcome of the infection process is decided once the pathogenic bacteria have reached their final sites, presumably within a short time after inoculation.

Numerical threshold theory. According to the numerical threshold theory the resistance of the host must be overcome at the inoculation site by the co-operative action of a number of cells of the pathogen before these can multiply. In an infectivity titration one would expect that no response would be induced as long as the challenge dose is smaller than the threshold value, whereas a challenge dose higher than or equal

to this value would induce a response in all the inoculation sites. One must, however, take into account that it is practically impossible to apply an exact number of cells of the pathogen to the inoculation site in such a way, that they all contribute to the saturation of the host defence.

If in an experiment the inoculum consists of m bacteria of the pathogen suspended in water and p is the probability that a bacterium reaches a site, where it contributes to the saturation of the host defence after the plant has been inoculated, then $pm = a$ is the average number of bacteria that saturate the host defence per site and the Poisson equation $P(r) = e^{-a}a^r/r!$ gives the probability that a site receives in fact $r = 0$, $r = 1$, $r = 2 \ldots$ bacteria, that saturate the host defence. If the threshold value is t, the probability, P (healthy), that the inoculation is unsuccessful will be the sum of the probabilities P(r) taken over the values $r = 1, 2 \ldots (t - 1)$, because, by definition, these numbers of cells are too small to overcome the host defence.

The probability that the inoculation is successful is given by

$$P(\text{diseased}) = 1 - \sum_{r=0}^{t-1} \frac{e^{-a} \cdot a^r}{r!} \qquad (1)$$

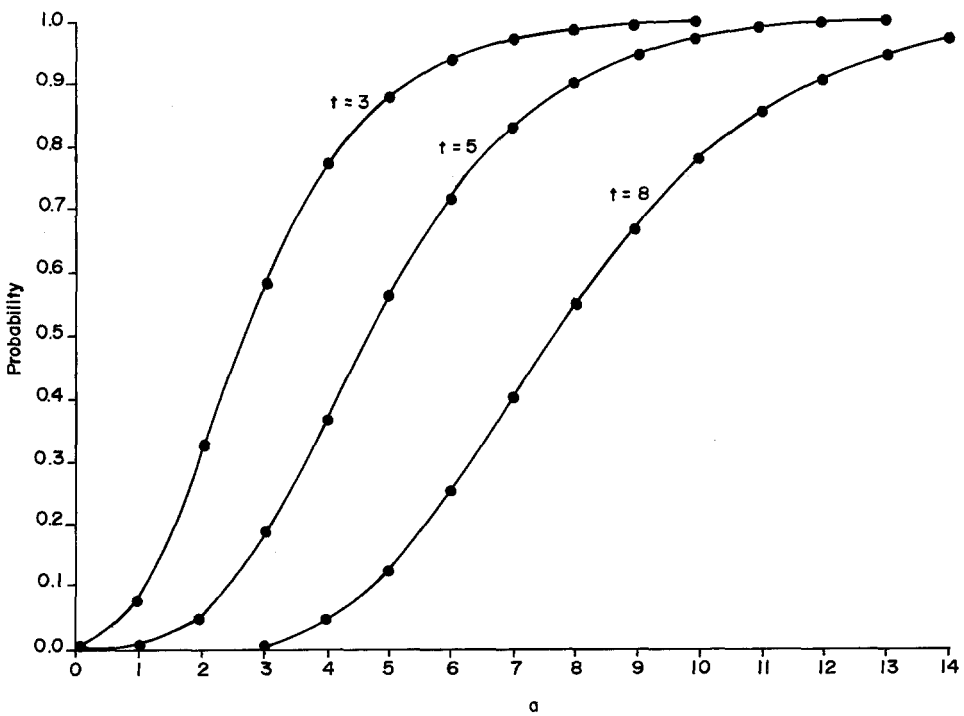

Fig. 1. Relationships between the probability of a successful inoculation and the average number of cells a of the pathogen that saturate the host defence per inoculation site if the threshold values (t) equal 3, 5 and 8 respectively.

In Fig. 1 the probabilities of successful inoculations are presented if $a = 1, 2 \ldots 14$, and the threshold values are 3, 5 and 8 respectively. The value of t is dependent on the virulence of the pathogen, the susceptibility of the host and the environmental conditions.

Because the dose is proportional to m and m is proportional to *a*, if *p* is constant, the probability can be plotted against the dose instead of *a* without changing the essential shape of the curve. If we equate by approximation probability with proportional frequency, then more of less S-shaped curves depicting the dose-response relationship, similar to those in Fig. 1, can be expected, if the numerical threshold theory is correct, provided that the plants are homogeneous with respect to susceptibility and the numbers of inoculation sites are sufficiently high.

Theory of independent action. This theory assumes that
(a) the cells of the pathogen act independently; and
(b) if one invading cell hits a multiplication site in the plant, it will multiply and produce symptoms.

If the probability that a cell of the pathogen hits a multiplication site equals λ and the dose equals 1, the probability that the inoculation is unsuccessful, which means that no symptoms develop, is equal to $1 - \lambda$. After subsequent inoculation with a second cell of the pathogen at the same inoculation site the probability, that both inoculations are unsuccessful, will be $(1 - \lambda)^2$, if one assumes that the site does not change in susceptibility. If the total dose equals n, the probability that all inoculations are unsuccessful equals $(1 - \lambda)^n$, which is the theoretically expected proportion of inoculation sites, that do not show a response after inoculation with n cells of the pathogen per site.

If the number of inoculation sites is sufficiently high and y is the observed proportion of successful inoculations, then by approximation $1 - y = (1 - \lambda)^n$. The same formula was given by Druett (1952) and Peto (1953) for dose-response relationships in animals.

If the cells of the pathogen act independently and the inoculation sites do not change in susceptibility, it is of no consequence whether the cells are applied one by one or simultaneously in one dose (n).

The formula may be written:
$\ln(1 - y)^{-1} = n\ln(1 - \lambda)^{-1}$ or if $p = \ln(1 - \lambda)^{-1}$, then
$$\ln(1 - y)^{-1} = pn \qquad (2)$$

Values of $\ln(1 - y)^{-1}$ are given by Van der Plank (1963, Table 3). The table shows that for small values, y is almost equal to $\ln(1 - y)^{-1}$.

Thus, if the theory of independent action is correct, the line depicting the relationship between $\ln(1 - y)^{-1}$ and the dose (n) is straight and passes through the origin.

Plotting $\ln(1 - y)^{-1}$ instead of y against the dose is in fact a transformation for multiple infection (Gregory, 1948). It is assumed that there are several multiplication sites (possibly places on the cell walls, where pathogenic bacteria can attach themselves and multiply) at one inoculation site, thus multiple infection means in this context that one inoculation site is invaded by more than one cell of the pathogen that hit a multiplication site.

In the above it was tacitly assumed that the cells of the pathogen had more or less the same virulence and that the inoculation sites were homogeneous for susceptibility.

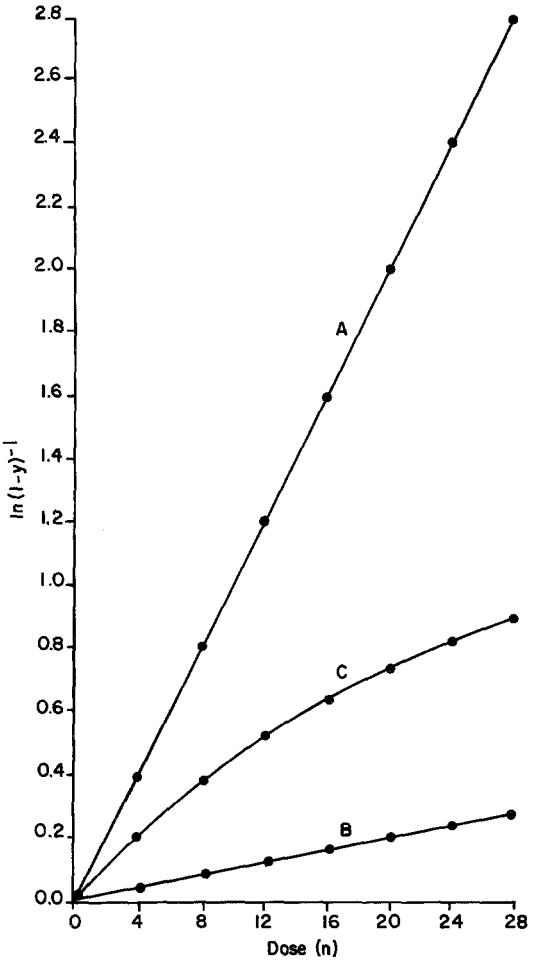

Fig. 2. Dose-response relationships for three host-pathogen systems. A: host homogeneous with respect to susceptibility, p = 0.10. B: ditto, p = 0.01. C: mixture of equal numbers of A and B. (n = dose, y = proportion of diseased plants, $p = \ln(1 - y)^{-1}/n$).

Figure 2 depicts the dose-response relationships between a certain pathogen and a group (C) of plants (each inoculated at one site) consisting of a mixture of equal numbers of two groups (A) and (B) with p values equal to 0.10 and 0.01, respectively. For the homogeneous groups these relationships are rectilinear and the lines pass through the origin, but the dose-response relationship of the mixture curves to the right.

Another test for independent action is carried out by plotting the probit of the proportion of successful inoculations against the logarithm of the dose expressed by multiples of ED_{50} (the dose causing 50% successful inoculations). Peto (1953) calculated that the tangent of the slope (b), near ED_{50} equals 2.0003, if the cells act independently and the plants are homogeneous with respect to susceptibility.

Experimental evidence

The correctness of the above theories can be tested by comparing graphs of the plotted

results of infectivity titrations with the lines in Fig. 1 and 2 or by calculating the value of b, as defined above.

In a number of infectivity titrations found in the literature, the results were taken as *proportions* of diseased plants out of the total number of plants or – generally speaking – proportions of successful inoculations out of the total number of inoculations. In other experiments the *numbers* of successful inoculations were recorded. Most of the work, that is discussed here, was done with homologous host pathogen combinations, but in other studies attention was given to heterologous combinations.

The present review covers dose-quantal response relationships only. The degree of symptom development, such as diameter of lesions, degree of wilting and size of galls, is not considered.

Infectivity titrations in which the proportions of successful inoculations were recorded (homologous combinations). Pods of green beans cv. Strike, were inoculated with *Xanthomonas campestris* pv. *phaseoli* (Smith) Dye, by pricking them with the needle of a microsyringe and simultaneously applying a droplet of inoculum (Boelema, unpublished data). The relationship between the dose and the response, i.e. in this context the proportion of successful inoculations transformed for multiple infection, is depicted in Fig. 3. The relationship is rectilinear and the line passes through the origin. A similar relationship was found in infectivity titrations with *Pseudomonas syringae* pv. *phaseolicola* (Burkholder) Young et al. (Psp) in green bean leaves, cv. Thor (Boelema, 1984). Ercolani (1967) inoculated four-week-old tomato seedlings by placing a suspension of *Corynebacterium michiganense* pv. *michiganense* (Smith) Jensen (Cmm) in a slit cut in the stem. For the cultivars Fiorentina and Roma the response was directly proportional to the dose, when allowing for experimental error (Van der Plank, 1975, p.26).

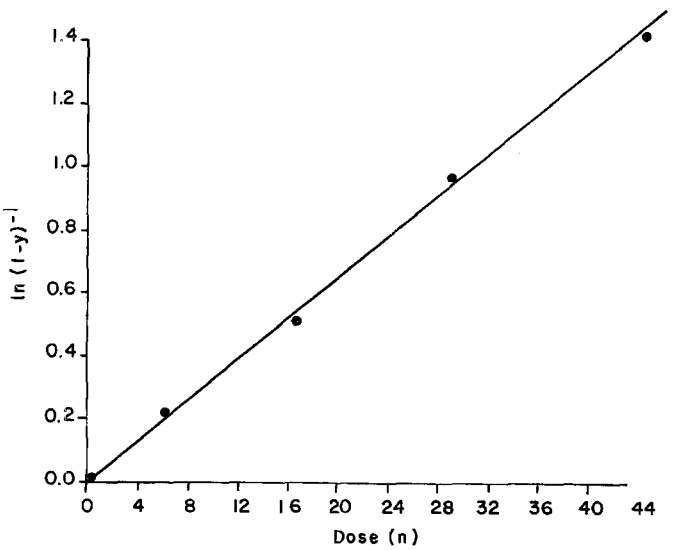

Fig. 3. Dose-response relationship between *Xanthomonas campestris* pv. *phaseoli* and green bean pods cv. Strike (y = proportion of successful inoculations).

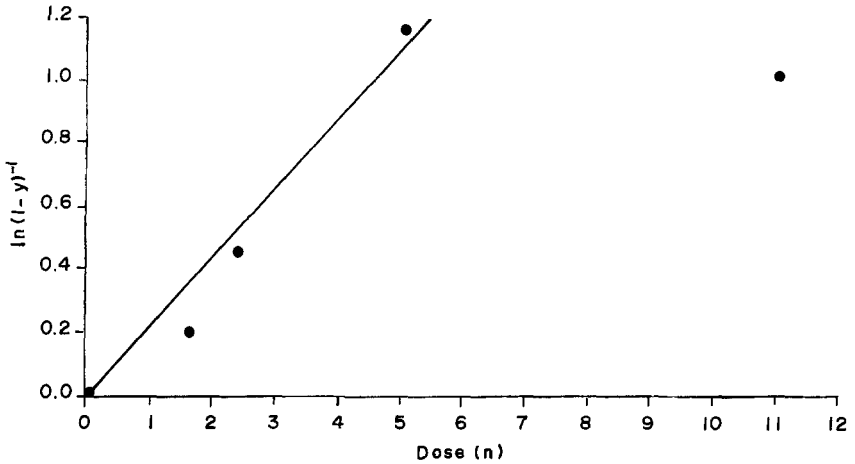

Fig. 4. Dose-response relationship between *Corynebacterium michiganense* pv. *michiganense* and tomato seedlings, cv. Roma VF (y = proportion of successful inoculations).

Ercolani (1973) did a number of other infectivity titrations. The following pathogen-host combinations were studied: *Pseudomonas syringae* pv. *lachrymans* (Smith and Bryan) Young et al. in cucumbers, *P. s.* pv. *morsprunorum* (Wormald) Young et al. in cherries, *P. s.* pv. *syringae* Van Hall in pears, *P. s.* pv. *tabaci* (Wolf and Foster) Young et al. in tobacco, *P. s.* pv. *tomato* (Okabe) Young et al. (Pst) in tomato and Psp in green beans. In these experiments the bacterial suspensions were infiltrated into the leaves by close atomizing. Ercolani calculated the tangents of the probit slopes for the infectivity and found values of approximately 2 in some of these homologous pathogen – host combinations. Other values, however, were smaller.

Boelema (1976) inoculated 36-day-old tomato seedlings, cv. Roma VF with Cmm by excising the oldest true leaf near the stem and immediately applying a droplet of freshly prepared inoculum to the wound by means of a microsyringe. The response was directly proportional to the inoculum dose up to a dose of approximately 5 cells per plant. The response to a dose of 11 cells was considerably lower than that calculated by an extrapolation from the dose-response line for the lower doses (Fig. 4). Thyr (1968) did similar experiments. An analysis of his data, where no interval occurred between preparation of the inoculum and inoculation, shows that the response was directly proportional to the dose up to an inoculum dose of 5 cells per wound. The response to a dose of 50 cells was much lower than that calculated by extrapolation from this line.

Green beans, cv. Galatin 50, were inoculated by pricking the leaves with the needle of a microsyringe and applying a droplet of Psp inoculum at the same time (Boelema, 1984). The dose-response relationship is depicted in Fig. 5, which shows a curve bent to the right. Pérombelon (1972) inoculated discs of potato tubers with serial dilutions of a suspension of *Erwinia carotovora* pv. *atroseptica* (Van Hall) Dye (Eca) under anaerobic conditions. An analysis of his data shows that the dose-response relationship also can be depicted as a line curving to the right. Analysis shows a similar pattern

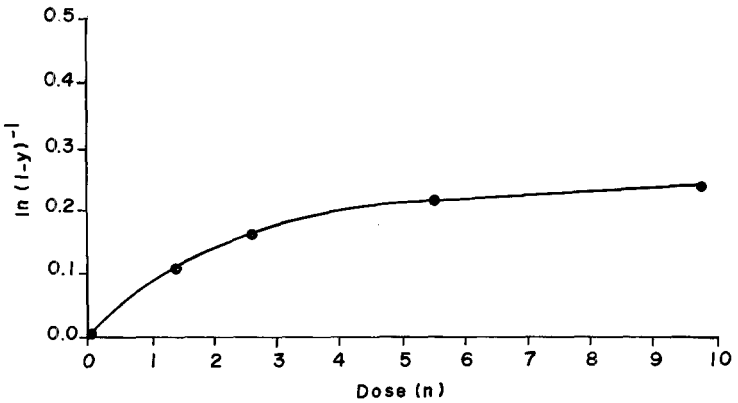

Fig. 5. Dose-response relationship between *Pseudomonas syringae* pv. *phaseolicola* and green bean leaves, cv. Gallatin 50 (y = proportion of successful inoculations).

in the data of Crosse et al. (1972), obtained in their study of the response of apple leaves to graded inoculum doses of *E. amylovora* (Burrill) Winslow et al., deposited on the severed ends of the main veins. A curvature to the right was found by analyzing the data obtained by Ercolani (1967) in an infectivity titration with Cmm inoculated into the stems of tomatoes of the line C 1402 (Van der Plank, 1975, p. 26).

Basu (1966) sprayed a Cmm suspension on uninjured leaves of 2-to-3-week-old tomato seedlings. He did similar experiments with Pst and *X. campestris* pv. *vesicatoria* (Doidge) Dye. In all three cases the dose-response line showed a strong curvature from the origin to the right.

The results of an infectivity titration with Cmm in the tomato line P.I. 330727, inoculated according to the excised leaf method, showed a curve that was bent upwards (Boelema, 1976). In an infectivity titration with Psp (prick inoculation) in green bean pods, cv. Gallatin 50, a similarly shaped curve was found (Fig. 6) (Boelema, 1984). Pérombelon (1972) not only carried out experiments with potato discs kept under

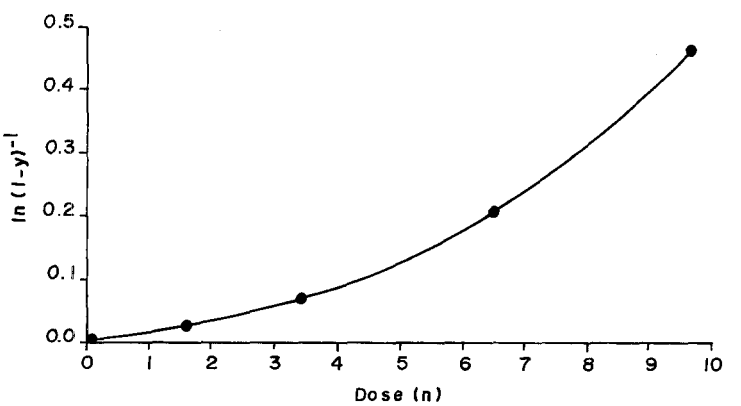

Fig. 6. Dose-response relationship between *Pseudomonas syringae* pv. *phaseolicola* and green bean pods, cv. Gallatin 50 (y = proportion of successful inoculations).

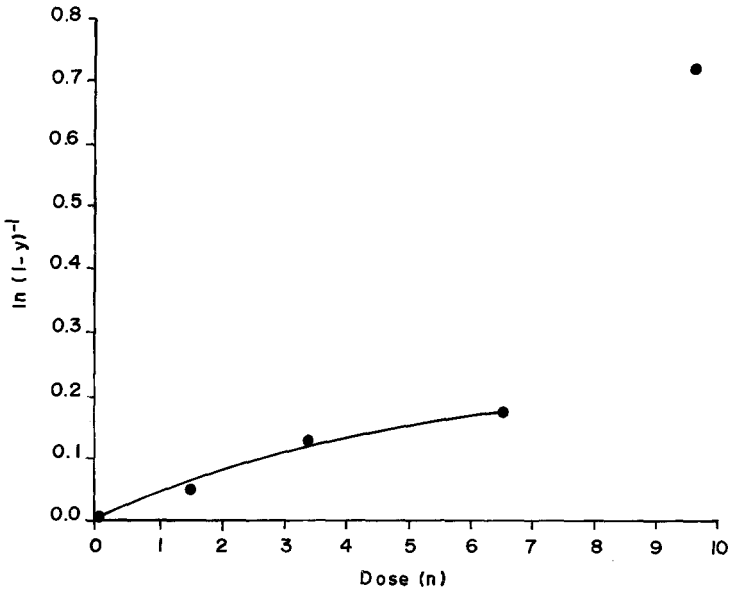

Fig. 7. Dose-response relationship between *Pseudomonas syringae* pv. *phaseolicola* and green bean pods, cv. Thor (y = proportion of successful inoculations).

anaerobic conditions after inoculation, as mentioned above, but also with potato discs kept under aerobic conditions. An analysis of the data of the latter experiment shows a dose-response line with a slightly upward curvature up to an inoculum dose of 91 cells.

In the same series of experiments as above (Boelema, 1984) with Psp in green bean pods a dose-response relationship for pods of the cultivar Thor was found, which is depicted in Fig. 7. The author interpreted the relationship as a line curved to the right for the lower doses, and a response tot the highest dose (9.6), which is much greater than could be predicted by extrapolation of the curved line.

Hildebrand (1942) inoculated tomato plants with *Agrobacterium radiobacter* pv. *tumefaciens* (Smith and Townsend) Kerr et al. (Art) by introducing graded doses of the pathogen into shallow or deep wounds in the stems. An analysis of his results shows that the dose-response relationships were rectilinear in the dose range from 1 to 10 cells of the pathogen, with a positive intercept on the ordinate axis. It can be calculated that for shallow wounds the intercept was 0.084 ± 0.006 and for deep wounds 0.246 ± 0.051.

Infectivity titrations in which the numbers of successful inoculations were recorded (homologous combinations). Essenberg et al. (1979) inoculated susceptible cotton leaves with *Xanthomonas campestris* pv. *malvacearum* (Smith) Dye (Xcm) by means of vacuum infiltration. They found that the response – in this context the number of water-soaked areas per cm^2 – was directly proportional to the dose, up to where the spots coalesced and the counting of individual areas became impossible. In infectivity titrations with Art in primary leaves of 'Pinto' beans, Lippincott and Heberlein

(1965) found that the numbers of tumours per leaf were directly proportional to the numbers of bacteria in the inoculum, up to approximately 100 tumours per leaf induced by 8×10^7 cells of the pathogen. The leaves were inoculated by stroking them with a glass-rod, after they had been dusted with carborundum and a droplet of 100 μl of inoculum had been put on each leaf. With increasing dose, however, the line depicting the dose-response relationship showed a curvature to the right.

The dose-response relationship between Cmm and tomato leaves was studied by Layne (1968). He dusted the leaves with carborundum and rubbed the inoculum in by means of a pad of cheesecloth soaked in the bacterial suspension. The line depicting the relationship between the dose and the number of spots shows a curvature from the origin to the right and flattens at higher doses.

A curvature to the right in the line depicting the dose-response relationship was also found in an experiment in which cherry trees were inoculated with cherry strains of *P. syringae* pv. *morsprunorum* (Wormald) Young et al., by pulling off the leaves in autumn and applying a drop of suspension of the pathogen to the freshly exposed scars (Crosse and Garrett, 1970).

Civerolo (1975) inoculated peach leaves with *Xanthomonas campestris* pv. *pruni* (Smith) Dye by means of close atomizing. He described part of his data as a rectilinear relationship between the average number of lesions, which develop at inoculation sites, and the density of the pathogen in the inoculum up to 5×10^4 colony forming units (CFU) per ml, which corresponds to a dose of 500 CFU/site. An alternative interpretation of the responses to the lower and perhaps more natural doses is possible. Starting from the origin the line through the responses of the lower doses shows a curvature to the right up to a dose of approximately 60 CFU. The response to the next dose is much greater than predicted by extrapolation from this line. Thus, this might be a relationship similar to that depicted in Fig. 7.

Infectivity titrations with heterologous host-pathogen combinations. Klement et al. (1964) injected suspensions of several *Pseudomonas* species into tobacco leaves. Viewing the leaves under a 10 to 12 $\times$ magnification they found that the number of local lesions was directly proportional to the number of cells of the pathogen injected, if the combination was heterologous and the inoculum density did not exceed 10^6 cells ml^{-1}.

Leaves of a cotton cultivar with genes for vertical resistance to Xcm were infiltrated with this pathogen and the affected palisade cells stained with Azure B (Essenberg et al., 1979). The tissue was examined under the microscope. The number of brown cell clusters was found to be directly proportional to the dose, up to approximately 2.5×10^3 cells of the pathogen per cm^2 leaf. At higher doses, however, the number of brown cell clusters was smaller than could be predicted by extrapolation from the dose-response line.

Ercolani (1973) did the experiments with *Pseudomonas* spp., as described above, not only with homologous, but also with heterologous host-pathogen combinations. From the results of these infectivity titrations he calculated probit slopes which were considerably greater than 2.

Values of λ. From the results of some infectivity titrations I calculated the probabilities that cells of certain pathogens hit the infection sites. In infectivity titrations

with Cmm in tomatoes it was found that one out of an average of 2.2 to one out of an average of 6.8 cells of the pathogen could initiate a complete infection process (Boelema, 1976). Ercolani (1967) studied the same host-pathogen combination, but used a different inoculation method. A value for λ of 1 : 415 ('Roma') and 1 : 901 ('Fiorentina') can be calculated from his data. In infectivity titrations with Psp in beans a value for λ of 1 : 26 was found for leaf infections and 1 : 39 for pod infections (Boelema, 1984). Lippincott and Heberlein (1965) calculated that 10^5 to 10^6 cells of Art were required to initiate one tumour on a 'Pinto' bean leaf.

Discussion

If the cells of the pathogen act independently, the inoculation sites are homogeneous, their numbers sufficiently high and the sites do not change in susceptibility, if higher doses are used, i.e. a sufficient number of multiplication sites are present per inoculation site, then the relationship between the dose and the proportion of successful inoculations, transformed for multiple infection, will theoretically be rectilinear and the line will pass through the origin, i.e. the response is directly proportional to the dose. In a number of host-pathogen systems this was indeed the case and it can therefore be concluded that the cells acted independently. A similar conclusion can be drawn for those cases in which the tangent of the probit slope for the infectivity was approximately 2.

Deviations from linearity must be due to interaction among the cells of the pathogen, heterogeneity of the inoculation sites, a limited number of multiplication sites and/or inaccuracy in delivering precise doses of the pathogen. Furthermore, if the number of inoculation sites is too low, the points will not fit the line because then the variance is too high. Finally, because an experiment has to be terminated after a certain time, not all successful inoculations may have been scored as such. If the relationship between 'successful inoculations' and those that are scored as 'successful inoculations' is dependent on the dose, deviations from linearity of the dose-response lines will occur. Ercolani (1973) ascribed negative deviations from the theoretical tangent value of 2 of the probit slopes to heterogeneity of the host plants.

Different types of deviations from linearity were found. In some host-pathogen combinations the response was directly proportional to the lower doses, but the response to the highest dose was considerably lower than predicted by extrapolation from the line. In these cases the cells of the pathogen probably acted independently at the lower doses and antagonistically at the higher doses or the number of multiplication sites was limited.

A smooth curvature from the origin to the right might be an indication of increasing antagonism among the cells of the pathogen with the dose, of a strong heterogeneity of the infection sites, or of a limited number of multiplication sites. In one host-pathogen combination (Fig. 7) the relationship was interpreted as a curved line for the lower doses indicating antagonism. The response to the highest dose was much stronger than could be predicted by extrapolation and this would indicate synergism, assuming that the interpretation was correct.

The lines with a strong upward curvature, for instance the line in Fig. 6, might be explained by the numerical threshold theory. Assuming that $a = 0.25n$ and $t = 3$ (Equation 1) in the dose-response relationship between Psp and beans (Boelema, 1984)

and a = n and t = 3 in the Cmm-tomato system (Boelema, 1976), the experimental results of the infectivity titrations fit the theoretical curves, when allowing for experimental error. Seen in the context of the other data discussed here, however, it is more likely that upward curves are an indication of facultative synergism.

An analysis of Hildebrand's (1942) data obtained in his work on crown gall showed a line with a positive intercept on the ordinate. this would imply that a zero dose caused a response, which is of course impossible. An explanation might be that some of the growths classified as tumours might have been callus.

In experiments where numbers instead of proportions were recorded similar relationships were found. In these cases a transformation for multiple infection is not possible. Nevertheless in certain host-pathogen combinations dose-response relationships were found that could be depicted by a straight line passing through the origin. Disease was directly proportional to the dose. This is an indication that the (unknown) number of multiplication sites was high relative to the response and therefore $y \sim \ln(1 - y)^{-1}$. Curvatures to the right may be due to a limited number of multiplication sites or to antagonism among the cells of the pathogen.

The values of λ varied considerably. One would expect this because the probability that a multiplication site is hit depends on the virulence of the pathogen, the quality and number of multiplication sites, methods of inoculation and environmental factors.

The results of the infectivity titrations conducted by Klement et al. (1964) and Essenberg et al. (1979) with heterologous host-pathogen combinations did not differ essentially from those with homologous host-pathogen combinations. Ercolani (1973), however, concluded from his experiments that there was an essential difference between dose-response relationships in heterologous and homologous host-pathogen combinations. He described the first by the hypothesis of co-operative action and the latter by the hypothesis of independent action. The interpretation of Ercolani's results was queried by Van der Plank (1975), because Ercolani had apparently not used a microscope to detect incompatible reactions within a single host cell. The criticism seems justified because it is apparently characteristic for the hypersensitive reaction that it leads to the collapse of a limited number of plant cells, invisible to the naked eye, if small doses are used (Turner and Novacky, 1974; Essenberg et al., 1979; Holliday, 1981).

Two types of analysis have been applied to experimental results covered by this review. The one is a simple test. The response is plotted against the dose on an arithmetic scale. If the response is given as a proportion it is transformed for multiple infection. The other is the log dose-probit response analysis, which is more complicated. Moreover it has the essential drawback that only a range near ED_{50} (Peto, 1953) is used for the calculation of the tangent of the slope in the test for independent action. The present review, however, shows that responses to doses considerably lower than ED_{50} may be of great value in the interpretation of the dose-response relationship.

For more elaboration on the probit idea the reader is referred to Ercolani's recent review (1984). In the Introduction it was stated that the present treatment of the subject is based on the assumption that the outcome of the infection process is decided once the pathogenic bacteria have reached their final sites, presumably within a short time after inoculation. If this assumption is incorrect, a treatment of the subject according

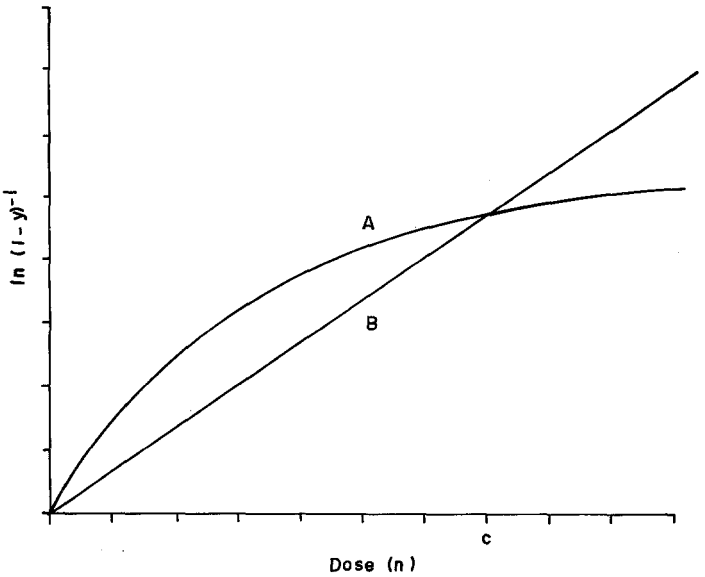

Fig. 8. Dose-response relationships between a pathogen and two hypothetical cultivars A and B of its host plant (y = proportion of successful inoculations).

to the birth-death model may be a better approach. This possibility was explored by Ercolani (1984).

Deviations from linearity in dose-response relationships have a practical implication for the quantitative study of factors affecting the susceptibility of plants to bacterial pathogens. For instance in a test to assess differences in susceptibility among cultivars, the dose-response relationship may be rectilinear in one cultivar, whereas it may show a curvature to the right in the other cultivar (Fig. 8). The graph shows that the response of cultivar A to a dose $<c$ is higher than that of B, but at a dose $>c$ the response of B is higher. Before conclusions can be drawn from this hypothetical test one has to decide which dose agrees more with doses resulting from natural infection in the field. It seems likely that this will be the smaller dose, at least early in an epidemic.

From this review it is concluded that quantitative studies of host-pathogen relationships should include the performance of infectivity titrations with low doses. This, however, poses a problem, because plant pathogenic bacteria in aqueous suspensions of low densities may die rapidly (Strider, 1967; Thyr, 1968; Essenberg et al., 1970; Boelema, 1984). A method therefore needs to be developed to keep the doses applied constant during the time that the plants of one treatment are inoculated.

According to Van der Plank (1975) all existing mathematical models of epidemics assume that susceptible sites in healthy tissues are inexhaustible. One also must conclude that this may not be the case for plant pathogenic bacteria.

Samenvatting

Verbanden tussen de inoculumdoses van fytopathogene bacteriën en de responses van hun waardplanten

Na een bespreking van de theorieën over de relatie tussen het aantal fytopathogene bacteriën per inoculatie en de al-of-niet respons – waarbij dus de grootte van de individuele respons niet in acht genomen wordt – worden deze theorieën getoetst aan de resultaten van proeven die in de literatuur beschreven zijn. Onderscheid wordt gemaakt tussen proeven waarin de proporties en die waarin de aantallen geslaagde inoculaties bepaald zijn. Verder is een vergelijking gemaakt tussen homologe en heterologe combinaties van waardplant en pathogeen.

Waar de proporties geslaagde inoculaties werden gemeten, vond ik in een aantal gevallen evenredigheid tussen dosis en respons, mits een transformatie werd toegepast voor veelvoudige infectie. Dit is een aanduiding dat de cellen van het pathogeen elkaar niet beïnvloeden. Afwijkingen van dit rechtlijnig verband kunnen o.a. worden toegeschreven aan antagonisme tussen de cellen van het pathogeen, aan heterogeniteit in vatbaarheid van de waardplanten of aan een beperkt aantal vermeerderingsloci.

In drie gevallen werd een naar boven gekromde lijn gevonden, hetgeen waarschijnlijk wijst op facultatief synergisme.

In proeven waarin de aantallen geslaagde inoculaties gemeten werden, vond ik in de literatuur evenredige en dus rechtlijnige verbanden met afbuigingen naar rechts voor responses tot te hogere doses. De afwijkingen van rechtlijnigheid kunnen hier worden toegeschreven aan een beperkt aantal vermeerderingsloci of aan antagonisme tussen de cellen van het pathogeen.

Geen essentieel verschil in dosis-respons verhoudingen werd gevonden tussen homologe en heterologe waardplant-pathogeen combinaties.

De implicaties van bovengenoemde resultaten voor de kwantitatieve studie van factoren die de vatbaarheid van planten voor infectie door bacteriën bepalen, worden besproken.

References

Basu, P.K., 1966. Conditions for symptomatological differentiation of bacterial canker, spot and speck on tomato seedlings. Can. J. Pl. Sci. 46: 525-530.

Boelema, B.H., 1976. Infectivity titrations with *Corynebacterium michiganense* in tomato seedlings. I. The effect of the age of the seedlings at the time of inoculation. Phytophylactica 8: 41-46.

Boelema, B.H., 1984. Infectivity titrations with race 2 of *Pseudomonas syringae* pv. *phaseolicola* in green beans *(Phaseolus vulgaris).* Phytophylactica 16: 327-329.

Civerolo, E.L., 1975. Quantitative aspects of pathogenesis of *Xanthomonas pruni* in peach leaves. Phytopathology 65: 258-264.

Crosse, J.E. & Garrett, C.M.E., 1970. Pathogenicity of *Pseudomonas morsprunorum* in relation to host specificity. J. gen. Microbiol. 62: 315-327.

Crosse, J.E., Goodman, R.M. & Shaffer, Jr., W.H., 1972. Leaf damage as a predisposing factor in the infection of apple shoots by *Erwinia amylovora.* Phytopathology 62: 176-182.

Druett, H.A., 1952. Bacterial invasion. Nature (London) 170: 288.

Ercolani, G.L., 1967. Bacterial canker of tomato. II. Interpretation of the aetiology of the quan-

tal response of tomato to *Corynebacterium michiganense* (E.F. Sm). Jens. by the hypothesis of independent action. Phytopathologia Medit. 6: 30-40.
Ercolani, G.L., 1973. Two hypotheses on the aetiology of response of plants to phytopathogenic bacteria. J. gen Microbiol. 74: 83-95.
Ercolani, G.L., 1984. Infectivity titration with bacterial plant pathogens. Ann. Rev. Phytopathol. 22: 35-52.
Essenberg, M., Cason, Jr., E.T., Hamilton, B., Brinkerhoff, L.A., Gholson, R.K. & Richardson, P.E., 1979. Single cell colonies of *Xanthomonas malvacearum* in susceptible and immune cotton leaves and the local resistant response to colonies in immune leaves. Physiol. Pl. Path. 15: 53-68.
Gregory, P.H., 1948. The multiple-infection transformation. Ann. appl. Biol. 35: 412-417.
Hildebrand, E.M., 1942. A micrurgical study of crown gall infection in tomato. J. agric. Res. 65: 45-59.
Holliday, M.J., Keen, N.T. & Long, M., 1981. Cell death patterns and accumulation of fluorescent material in the hypersensitive response of soybean leaves to *Pseudomonas syringae* pv. *glycinea*. Physiol. Pl. Path. 18: 279-287.
Klement, Z., Farkas, G.L., & Lovrekovich, L., 1964. Hypersensitive reaction induced by phytopathogenic bacteria in the tobacco leaf. Phytopathology 54: 474-477.
Layne, R.E.C., 1968. A quantitative local lesion bio-assay for *Corynebacterium michiganense*. Phytopathology 58: 534-535.
Lippincott, J.A. & Heberlein, G.T., 1965. The quantitative determination of the infectivity of *Agrobacterium tumefaciens*. Am. J. Bot. 52: 856-863.
Meynell, G.G. & Meynell, E., 1975. Theory and practice in experimental bacteriology. Second Edition, Cambridge University Press Cambridge, London, New York, Melbourne.
Pérombelon, M.C.M., 1972. A quantitative method for assessing virulence of *Erwinia carotovora* var. *carotovora* and *E. carotovora* var. *atroseptica* and susceptibility to rotting of potato tuber tissue. In: Maas Geesteranus, H.P. (Ed.), Proceedings of the Third International Conference on Plant Pathogenic Bacteria, Wageningen: 14-21 April, 1971. Pudoc, Wageningen, p. 299-303.
Peto, S., 1953. A dose-response equation for the invasion of micro-organisms. Biometrics 9: 320-335.
Strider, D.L., 1967. Survival studies with the tomato bacterial canker organism. Phytopathology 57: 1067-1071.
Turner, J.G. & Novacky, A., 1974. The quantitative relation between plant and bacterial cells involved in the hypersensitive reaction. Phytopathology 64: 885-890.
Thyr, B.D., 1968. Bacterial canker of tomato: inoculum level needed for infection. Pl. Dis. Reptr 52: 741-743.
Van der Plank, J.E., 1963. Plant diseases: epidemics and control. Academic Press, New York/London.
Van der Plank, J.E., 1975. Principles of plant infection. Academic Press, New York/San Fransisco/London.