Discriminating Synergism and Antagonism of Spores Within Species of Phytopathogenic Fungi

Ping Sun and Shimai Zeng

Department of Plant Protection, Beijing Agricultural University, Beijing, 100094, People's Republic of China.

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ABSTRACT

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Synergism and antagonism of spores of phytopathogenic fungi can be detected by comparison of the changes in the infection probabilities at different inoculum densities. In this paper, we describe a model, developed from the formula of the multiple infection transformation and probability theory, that can be used to discriminate the synergism and antagonism of spores. A function was derived that allows the easy evaluation of synergism and antagonism. The model was used to analyze the experimental data of *Puccinia striiformis* spores and winter wheat. The definition of antagonism is broader than that used by Vanderplank.

Additional keywords: random distribution, spore load.

Synergism and antagonism of spores within species of phytopathogenic fungi have been previously studied (1,5,6,10,13). The synergistic and antagonistic interactions between different species of phytopathogenic fungi have also been studied (3,8,9). Vanderplank (12) summarized the literature and described the two interactions of spores by using dose-response types of curves. Nevertheless, there is not yet a quantitative method to discriminate the interactions. Researchers often count the number of deposited spores (X) and the corresponding number of diseased sites (Y) in a certain area on the host plant and use the change of the ratio Y/X at different inoculum densities to estimate the synergism

or antagonism (7,11,12). The defect in this method is that the effect of multiple infection is ignored. A more precise method to detect the different interactions should be based on counting the total number of spores (Y') that have succeeded in infection and then comparing the ratios Y'/X at different inoculum densities. Unfortunately, Y' and X are very difficult to determine.

In this paper, we describe a model used to detect the synergism or the antagonism of spores that was developed from the formula for multiple infection transformation. In our model, it is not necessary to determine Y' or X, but only Y.

MATERIALS AND METHODS

Model development. The infection probability of spores, the synergism of spores, and the antagonism of spores are first explained. Infection probability (IP) is the probability that a single spore induces an infection. The symbol $IP(x)$ is the IP at the inoculum density x. If there are two inoculum densities, x_1 and x_2, $x_1 < x_2$ and $IP(x_1) < IP(x_2)$, then there is a synergistic interaction in spores. If $x_1 < x_2$ but $IP(x_1) > IP(x_2)$, then there is an antagonistic interaction in spores.

Suppose that all the locations on the host plant capable of becoming infected (i.e., disease units) are uniform in susceptibility, that the positioning of spores is random, and that all spores are uniform in virulence, aggressiveness, and viability. Then the number of spores loaded on one disease unit can be considered a Poisson random variable ξ with mean m, i.e.,

$$P(\xi = n) = \frac{\exp(-m)m^n}{n!} \quad n = 0, 1, 2, \ldots \tag{1}$$

in which n is the number of spores deposited. Let $\bar{p}$ denote the probability that a unit will remain uninfected, which can be defined as

$$\bar{p} = \sum_{n=0}^{\infty} P(An) \cdot P(Bn) \tag{2}$$

in which An denotes the event that there are n spores loaded on the unit, and Bn denotes the event that all the n spores failed to cause an infection. $P(An)$ is equal to $P(\xi = n)$ of equation 1 and $P(Bn)$ equals $(1 - IP)^n$ on the basis of the binomial distribution. Equation 2 then can be written as

$$\bar{p} = \sum_{n=0}^{\infty} \frac{\exp(-m)m^n}{n!}(1-IP)^n$$

$$= \exp(-m) \sum_{n=0}^{\infty} \frac{[m(1-IP)]^n}{n!} .$$

Since the summation term is a Taylor Series Expansion of $\exp[m(1 - IP])$, then

$$\bar{p} = \exp(-m \cdot IP) .$$

Let y be the fraction of units that are infected. An infected unit could have 1,2,3, . . . infections. Then y can be used as an estimate of the probability that a unit will be infected, and $1 - y$ can be used as an estimate of the probability that a unit will not be infected. Then $\bar{p} = 1 - y$, and

$$m \cdot IP = -\ln(1 - y) . \tag{3}$$

Equation 3 expresses the interaction between the y, IP, and m. When $IP = 1$, equation 3 becomes

$$m = -\ln(1 - y) . \tag{4}$$

In equation 4, m is still the average number of spores loaded on one unit, but since $IP = 1$, m can be thought of as the average number of infections in one unit. Equation 4 is the well-known formula of Gregory's multiple infection transformation (4).

The measurements of y and m are related to the operational definition of "disease unit" adopted by the researcher in disease measurement. When the disease unit is an infected site on a leaf, y is the fraction of sites infected, and m is the average number of spores loaded on one site. When the disease unit is a leaf of the host, y is the fraction of infected leaves, and m is the average number of spores loaded on one leaf, and so on. Equation 3 also appears in other literature (12), but the consistency of the measurements of y, m, and the disease unit has not been stressed (Appendix).

Suppose there are two treatments, I and II, in an inoculation experiment with inoculum densities x and kx ($k > 1$), respectively. If the average number of spores loaded on one unit of I is m, then the average number of spores loaded in one unit of II is km. We use y and y_k to denote the fraction of infected units in I and II, respectively. From equation 3,

$$IP(x) = -\ln(1 - y)/m,$$
$$IP(kx) = -\ln(1 - y_k)/km.$$

Then, we have

$$\frac{IP(x)}{IP(kx)} = k \frac{\ln(1 - y)}{\ln(1 - y_k)} . \tag{5}$$

We use $f(x, kx)$ to denote the right-hand side of equation 5 and name it the discrimination function.

The discrimination function (equation 5) is the ratio of infection probabilities of spores at two inoculum densities. The following relations based on these definitions can be stated:

$f(x, kx) < 1$: synergism,
$f(x, kx) = 1$: nil (i.e., no interaction),
$f(x, kx) > 1$: antagonism.

The discrimination function can be used to detect the synergism or antagonism between any two spore densities, x_1 and x_2, because we can find a constant $k > 1$ that makes $x_2 = kx_1$ (assuming $x_2 > x_1$).

Experiments and data analysis. Inoculum experiments on spores of yellow rust caused by *Puccinia striiformis* Westend. on wheat (Yanda-1885, a susceptible cultivar) were conducted to test the model. A spore-settling tower with an inner height of 200 cm and an area of 4,225 cm^2 (65 × 65 cm) was constructed with an iron sheet. A shallow square tray made of sheet iron with an area of 4,225 cm^2 could be slid into the oblong opening at the bottom of the tower, and a small inoculation hole was opened on one side of the vertical wall just above the entrance of the drawer.

In the greenhouse, wheat seedlings were grown in trays of garden soil (about 1,000 seedlings per tray). When all of the second leaves were entirely emerged, a seedling tray was slid into the bottom of the settling tower and inoculated with fresh spores mixed with talc powder. Inoculum was dispersed from a small duster by properly fitting the nozzle of the duster into the inoculation hole so that the nozzle was in the center of the square horizontally, and the opening of the nozzle pointed upward. The pressure for dusting was supplied by an air pump. When the pressure gauge was 196 kPa, the switch was opened, and the inoculum was sprayed for 10 s. Then the nozzle was immediately taken out, and the inoculation hole was covered again. The inoculum was allowed to settle on the nearly vertical leaves for 5 min. The seedling tray was removed, and 10 seedlings were taken from each tray for measurement of leaf area and estimation of spore load. The other plants were kept in the tray and incubated in a moist chamber for 24 h at 9–11 C. Inoculum density was set by the total number of dry spores dispersed upon the whole area of 4,225 cm^2, prespecified as 0.1, 1, 10, 100 mg. The study was done four times.

After incubation, the seedlings were kept in a greenhouse where day and night temperatures varied between 5 and 22 C. Once the first disease symptom (i.e., pin-point, faintly semitranslucent flecks) appeared, numbers of flecks and numbers of diseased leaves were counted every day. In treatments of low inoculum densities, all the inoculated leaves were examined. In treatments of higher

densities, only a random sample of 100 inoculated leaves from each replicate was examined.

To make certain that the proportion of spore density on leaves for the four inoculum densities was equal to the corresponding proportion of predetermined inoculum densities, the spores in 10 fields of collodion prints from both sides of the sampled leaves were counted to estimate spore density. The leaf area was roughly determined by 0.9 × width × length. The average number of spores per leaf at the four inoculum densities (0.1, 1, 10, and 100 mg) was 0.6, 4.5, 50, and 527, respectively. A random sample of 10 inoculated leaves from each replicate was used.

The average leaf area was 2.35 cm^2, and disease flecks larger than 0.1 cm^2 could no longer be distinguished because of the coalescence of lesions. A maximum number of infection sites on a leaf was tentatively given as 20.

Results are given in Table 1. Values of $f(x, kx)$ with $k = 10$, i.e., $f(x, 10x)$, for the first three densities, i.e., f(0.1, 1) and f(1, 10), indicate a synergistic interaction among spores, $f(x, 10x) < 1$. Between the inoculum densities of 10 and 100 there was an antagonistic interaction, $f(x, 10x) > 1$. Student t tests were performed on the $f(x, 10x)$ data. The tests indicated that the mean $f(x, 10x)$ for the first three densities was less than one at $P < 0.1$. The mean final $f(x, 10x)$ was greater than one at $P < 0.05$.

Errors in estimations of the $f(x, 10x)$ in our experiments are not small. For example, the four values of f(10, 100) are 2.12, 2.84, 4.85, and 1.48, respectively. Although it is difficult to find the causes of the errors, our conclusion that $f(10, 100) > 1$ is not affected, since the mean f(10, 100) was significantly greater than 1.

RESULTS AND DISCUSSION

The term "infection" used in this paper means not only the successful penetration but also the resulting disease; hence, the synergism and antagonism tested here refer to both penetration and colonization. There are differences between our definition of antagonism and that described by Vanderplank. Although Vanderplank did not give an exact definition of an antagonistic interaction, his idea may be interpreted in the following manner (12).

Antagonism would occur in the Vanderplank scenario if $x_2 > x_1$ and $y_2 < y_1$. Since $x_2 = kx_1$ (assuming $k > 1$) and $1 > y_1 > y_2 > 0$, therefore, $\ln(1 - y_1)$ is always greater than $\ln(1 - y_2)$. So Vanderplank's antagonism of spores in terms of our discrimination function at two x's would be:

$$f(x_1, kx_1) = k \frac{\ln(1-y_1)}{\ln(1-y_2)} > k .$$

Then the following relations based on Vanderplank's scenario can be defined:

$f(x, kx) < 1$: synergism,
$f(x, kx) > k$: antagonism.

With this scenario, how does one interpret $1 \leq f(x, kx) \leq k$? We think that "antagonism" should be defined by the comparison of the *IP*s of spores under different inoculum densities, as is done for synergism. As long as the *IP* decreased with the increase of inoculum density, it should be considered antagonism. Vanderplank's definition of antagonism is a special case of our broader definition.

Our discrimination function can be thought of as an extension of Baker's work (2). Baker gave an idealized inoculum density-disease intensity (ID-DI) curve to describe the relation between the disease incidence and the inoculum density. The idealized ID-DI curve was divided into three phases: 1) the true logarithmic phase, in which the inoculum density is low and the increase in disease is small in response to increasing units of inoculum; 2) the synergistic phase, in which increase in inoculum may result in synergistic action; and 3) the transitional phase, in which antagonism occurs as multiple infections increase and propagules begin to compete for limited sites.

Baker's ID-DI curve gives a general description of the interaction between inoculum density and the infection probability of a spore. Theoretically, our discrimination function makes it easy to see how the infection probability of a spore varies with the increase of inoculum density. If we use an inoculum density x as a standard and do a series of inoculum experiments at densities $x, k_1x, k_2x, \ldots, k_nx$, we can get the estimate of $f(x, k_ix)$ from $i = 1$ to n.

Finally, although our model can be used conveniently to detect synergism or antagonism, the complexity of interaction of plant pathogens might be beyond the capability of a simplified mathematical model to reveal. Further studies are needed to verify the general validity of our discrimination function.

APPENDIX

The inconsistency of the measurements of y and m can be confusing. For instance, in Vanderplank (12), it is stated that

$$Y/N = 1 - e^{ax} \qquad \text{(A1)}$$

in which Y is the number of infections that occur in any uniform group of oranges, x is the number of spores of *Penicillium digitatum* per group of oranges, N is the total number of wounds in the group (i.e., disease units as used here), and a is a parameter reflecting the susceptibility of the wounds to infection.

If x is defined in this way, then a cannot be used to reflect the susceptibility of spores of *P. digitatum*. For instance, suppose there are 10 infections, 100 wounds, and 1,000 spores in this group. Then, with equation A1, the estimate of a is

$$a = \frac{-\ln(1 - 0.1)}{1{,}000} .$$

Because a is the parameter that reflects the susceptibility of wounds, it should be independent of x if there are no synergistic or antagonistic interactions between spores.

Now divide the uniform group of the example into two equal parts, part 1 and part 2. In each of the two parts there will be five infections, 50 wounds, and 500 spores. If the a in part 1 is denoted a_1 and in part 2 a_2, then a_1, a_2, and a should be equal if this parameter represents susceptibility. However, using the above formula in each of the two groups, we have

$$a_1 = a_2 = \frac{-\ln(1 - 0.1)}{500} = 2a .$$

TABLE 1. Results of inoculation of wheat with *Puccinia striiformis* at different inoculum densities

Replicate	Inoculum density x (mg/4,225 cm^2)	y[a]	$f(x, 10x)$[b]
1	0.1	0.001	0.646
	1	0.016	1.033
	10	0.148	2.118
	100	0.531	...
2	0.1	0.0003	0.600
	1	0.005	0.414
	10	0.117	2.840
	100	0.356	...
3	0.1	0.0004	0.247
	1	0.015	0.721
	10	0.192	4.846
	100	0.356	...
4	0.1	0.0008	0.878
	1	0.009	0.715
	10	0.113	1.483
	100	0.553	...

[a] The fraction of infected sites.
[b] The value of $f(x, 10x)$, i.e., $f(x, kx)$, $k = 10$, was calculated with equation 5. Each value of $f(x, 10x)$ is based on the current and the following inoculum densities.

So the use of equation A1 can lead to a wrong result if x is not defined in terms of spores per disease units. If x is defined as the average number of spores loaded in one disease unit (i.e., in one wound), then the confusion disappears.

Suppose that the average number of spores in one wound is c, that we then divide the group into equal two parts, and that the average number of spores loaded in one wound is still c in the two parts. We then have

$$a_1 = a_2 = a = \frac{-\ln(1 - 0.1)}{c} .$$

LITERATURE CITED

1. Allen, P. J. 1965. Metabolic aspects of spore germination in fungi. Annu. Rev. Phytopathol. 3:313-342.
2. Baker, R. 1971. Analyses involving inoculum density of soilborne plant pathogens in epidemiology. Phytopathology 61:1280-1292.
3. Dickinson, C. H. 1979. External synergisms among organisms inducing disease. Pages 97-111 in: Plant Disease: How Pathogens Induce Disease. J. G. Horsfall and E. B. Cowling, eds. Academic Press, New York.
4. Gregory, P. H. 1948. The multiple infection transformation. Ann. Appl. Biol. 35:412-417.
5. Macko, V., Staples, R. C., Renwick, J. A. A., and Pirone, J. 1972. Germination self-inhibitors of uredospores. Physiol. Plant Pathol. 2:347-455.
6. Marte, M. 1971. Studies on self-inhibition of *Uromyces fabae* (Pers.) de Bary. Phytopathol. Z. 72:335-343.
7. Petersen, L. J. 1959. Relations between inoculum density and infection of wheat by uredospores of *Puccinia graminis* var. *tritici*. Phytopathology 49:607-614.
8. Powell, N. T. 1979. Internal synergisms among organisms inducing disease. Pages 113-131 in: Plant Disease: How Pathogens Induce Disease. J. G. Horsfall and E. B. Cowling, eds. Academic Press, New York.
9. Rowe, R. C., Riedel, R. M., and Martin, M. J. 1985. Synergistic interactions between *Verticillium dahliae* and *Pratylenchus penetrans* in potato early dying disease. Phytopathology 75:412-418.
10. Stanbridge, B., and Gay, J. L. 1969. An electron microscope examination of surfaces of the uredospores of four races of *Puccinia striiformis*. Trans. Br. Mycol. Soc. 53:149-153.
11. Vanderplank, J. E. 1967. Epidemiology of fungicidal action. Pages 63-92 in: Fungicides. D. C. Torgeson, ed. Academic Press, New York.
12. Vanderplank, J. E. 1975. Principles of Plant Infection. Academic Press, New York.
13. Wood, R. K. S. 1967. Physiological Plant Pathology. Blackwell Scientific Publications, Oxford.