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.
We thank L. V. Madden very much for his helpful comments and notes on the paper and also the two reviewers for their valuable suggestions.
Accepted for publication 19 May 1993.

ABSTRACT


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 densi-
ties. 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 ex-
plained. Infection probability (IP) is the probability that a sin-
gle 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 syner-
gistic 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 susceptibil-
ity, 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 \( \xi \) with mean \( m \), i.e.,

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

(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

\[
\bar{p} = \prod_{n=0}^{\infty} P(A_n) \cdot P(B_n)
\]  

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

\[
\bar{p} = \prod_{n=0}^{\infty} \frac{\exp(-m)m^n}{n!} (1 - IP)^n
\]

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

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, \ldots 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 \( p = 1 - y \), and

\[
m \cdot IP = -\ln(1 - y)
\]  

(3)

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

\[
m = -\ln(1 - y)
\]  

(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 \( y \) and \( m \) is not stressed (Appendix).

Suppose there are two treatments, I and II, in an inoculation
experiment with inoculum densities \( x \) and \( kx (k > 1) \), respec-
tively. If the average number of spores loaded on one unit of I is \( m \),
then the average number of spores loaded on one unit of II is \( km \).
We use \( y_1 \) and \( y_2 \) to denote the fraction of infected units
in I and II, respectively. From equation 3,

\[
IP(x) = -\ln(1 - y)/m
\]

\[
IP(kx) = -\ln(1 - y)/km
\]

Then, we have

\[
\frac{IP(x)}{IP(kx)} = k \frac{\ln(1 - y_1)}{\ln(1 - y_2)}
\]  

(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:

\[
f(x, kx) < 1: \text{synergism,}
\]

\[
f(x, kx) = 1: \text{nil (i.e., no interaction),}
\]

\[
f(x, kx) > 1: \text{antagonism.}
\]

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
\( x_1 > x_2 \)).

**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 near vertically 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 cham-
ber 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, pre-
specified 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 symptoms (i.e., pin-point, faintly semitranslucent
flecks) appeared, numbers of flecks and numbers of disease leaves
were counted every day. In treatments of low inoculum densities,
all the inoculated leaves were examined. In treatments of higher

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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 cm², and disease flecks larger than 0.1 cm² 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 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(0, 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(0, 100) > 1 is not affected, since the mean f(0, 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 x₂ > x₁ and y₂ < y₁. Since x₂ = kx₁ (assuming k > 1) and 1 > y₁ > y₂ > 0, therefore, ln(1 - y₁) is always greater than ln(1 - y₂).

So Vanderplank's antagonism of spores in terms of our discrimination function at two x's would be:

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

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

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

With this scenario, how does one interpret 1 ≤ f(x, kx) ≤ k? We think that “antagonism” should be defined by the comparison of the IP of spores under different inoculum densities, as is done for synergism. As long as the IP decreases 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, k₁x, k₂x, ..., kₙx, we can get the estimate of f(x, kₙx) 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

$$Y/N = 1 - e^{ax}$$

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

$$a = -\frac{\ln(1 - 0.1)}{1000}$$

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 a₁ and in part 2 a₂, then a₁, a₂, and a should be equal if this parameter reflects susceptibility. However, using the above formula in each of the two groups, we have

$$a₁ = a₂ = -\frac{\ln(1 - 0.1)}{500} = 2a$$

| TABLE 1. Results of inoculation of wheat with Puccinia striiformis at different inoculum densities |
| --- | --- | --- |
| Replicate | Inoculum density x (mg/4,225 cm²) | f(x, 10x)² (mg/4,225 cm²) |
| 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 |
| 10 | 0.005 | 0.414 |
| 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 | ... |

²The fraction of infected sites.
³The 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