Stochastic Variation and Probabilistic Model – Estimating Frequency of Fungicide Resistant Phenotype in Plant Pathogen Population

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Abstract

Methodological aspects of stochastic variation were considered on example of bioassay for estimation of fungicide resistance (KADISH & COHEN 1988). The corresponding probabilistic model for measuring frequency of fungicide resistant phenotype in a plant pathogen population was developed. Unpredictable relationships between estimates from the model and the experimental results were observed.

Keywords: fungicide resistance; metalaxyl; *Phytophthora infestans*; stochastic variation; probabilistic model

INTRODUCTION

Most experimental data are of a random nature, and different methods of statistical analysis are used to process them. There are experiments with an implicit form of randomness, and unobvious stochastic variation is not taken into account. The simple analysis based on average numbers may lead to deviation from reliable estimates and unfounded "statistically based" conclusions and interpretations. In such experiments probabilistic models can help to obtain robust estimates. The bioassay for quantitative estimation of fungicide resistance (KADISH & COHEN 1988) is a striking example of such an experiment. The purpose of this paper is to develop the corresponding probabilistic model and to emphasize methodological significance to account for the stochastic nature of the experiment. The estimates from the model are compared with the experimental results to show unpredictable relationships between them.

A natural sporangial population of the fungal pathogen can be subdivided into two classes with respect to fungicide sensitivity: a) fungicide resistant individuals (R-type, R-sporangia), and b) fungicide sensitive individuals (S-type, S-sporangia). Individuals of the two types are able to grow and sporulate on plant tissues placed on water without fungicide, whereas only R-types would grow and sporulate on tissue treated with this fungicide. The following method was proposed for estimating the frequency of R-sporangia in a mixed population in the case of metalaxyl resistance in *Phytophthora infestans* (KADISH & COHEN 1988). Tuber disks are inoculated with droplets of inoculum suspension of a fixed size, which contain R- and S-sporangia. The same number of disks is placed on water and on a solution of fungicide. Then the frequency of R-sporangia (R-frequency) in this mixed population can be computed by dividing the number of disks supporting fungal sporulation on a solution of fungicide by the number of disks supporting fungal sporulation on water.

Two points are encountered if the experimental values of R-frequency are calculated according to the proposed method: a) how to take into account the fact that the number of sporangia per droplet of inoculum may vary while droplets are of equal size and are from the same inoculum suspension; b) how to take into account the fact that ability to induce new infection by a single sporangium or a group of a few sporangia is not absolute. These two issues reflect a random nature of the experiments, and the corresponding stochastic variations should be taken

into consideration. A suitable probabilistic model will further be developed for computing the estimates of actual frequency of R-sporangia.

The model

Let λ be the average number of all resistant and sensitive sporangia in a droplet of inoculum suspension (λ is determined in experiment), and *f* be the actual frequency of R-sporangia. Then ρ = λ*f* is the average number of R-sporangia in a droplet, and $\nu = \lambda - \rho = (1 - f)\lambda$ is that for S-sporangia. The numbers of all sporangia, R-sporangia and S-sporangia in an arbitrary droplet of inoculum suspension are random variables, which are Poisson distributed (FELLER 1968) with parameters λ, ρ and ν, respectively. Then a droplet of suspension contains *n* sporangia of both kinds, *k* R-sporangia and *m* S-sporangia with probabilities

$$\pi(\lambda; n) = \frac{\lambda^n}{n!} e^{-\lambda}, \quad \pi(\rho; k) = \frac{\rho^k}{k!} e^{-\rho}$$

$$\text{and} \quad \pi(\nu; m) = \frac{\nu^m}{m!} e^{-\nu}$$

respectively. If any droplet includes *n* sporangia, then *n+1* different combinations of *k* R-sporangia and $m = n - k$ S-sporangia for $k = 0, 1, \ldots, n$ are possible.

Let *k* R-sporangia and *m* S-sporangia induce infection with probabilities r_k and s_m, respectively. These infection efficacies are determined from experiments, and they also subject to stochastic variation. Numbers *k* of R-sporangia and *m* of S-sporangia in a droplet are so-called random variables, and $r(k) = r_k$ and $s(m) = s_m$ are the random functions of random variables *k* and *m*, respectively. The expectation that one inoculum droplet causes infection on a target treated with the fungicide has the form

$$p_{\text{fung}} = \sum_{k=0}^{\infty} r_k \pi(\rho; k) \tag{1}$$

We assume that R- and S-sporangia act independently of one another. If an inoculum droplet is comprised of *k* R-sporangia and *m* S-sporangia, the probability that the inoculation will result in no infection is $(1-r_k)(1-s_m)$. Then the expectation of infection on a non-protected target due to inoculation by one droplet of inoculum equals

$$p_{\text{water}} = 1 - \sum_{k=0}^{\infty} \sum_{m=0}^{\infty} (1 - r_k)(1 - s_m)\pi(\rho; k)\pi(\nu; m) =$$

$$= 1 - e^{-\lambda} \sum_{k=0}^{\infty} \sum_{m=0}^{\infty} (1 - r_k)(1 - s_m) \frac{\rho^k}{k!} \frac{\nu^m}{m!} \tag{2}$$

The experimental (observed) value of R-frequency, *F*, is computed by dividing the number of protected targets by that of non-protected which support fungal sporulation. The relative numbers of protected and non-protected targets supporting sporulation are, in fact, the expectations of obtaining infection on targets placed on fungicide, p_{fung}, and water, p_{water}, respectively. Thus, the observed value *F* of R-frequency equals the following ratio:

$$F = p_{\text{fung}} / p_{\text{water}} \tag{3}$$

The value of ρ ($0 < \rho < \lambda$ if $0 < F < 1$) can be found as a solution of equation (3) by substituting into (3) the expressions for p_{fung} and p_{water} from (1) and (2), respectively, and the experimentally obtained values of *F*, λ, r_k and s_m. One can prove that there is only one solution of (3) which meets the condition $0 < \rho < \lambda$, and the cases $F = 0$ and $F = 1$ result in ρ= 0 and ρ = λ, respectively. Then the estimate of actual value of R-frequency for the corresponding experimentally observed value *F* is the calculated value $f = \rho/\lambda$.

Analysing the frequency of a metalaxyl-resistant subpopulation

The data from bioassay of KADISH and COHEN (1988) for three mixed sporangial model populations MS1 + MR1, MS2 + MR2 and MS3 + MR3 of metalaxyl-sensitive (MS) and metalaxyl-resistant (MR) field isolates of *Phytophthora infestans* were used to verify the model. Four average concentrations of sporangia in a droplet of inoculum suspension from their data (λ = 1.5625, 3.125, 6.25 and 12.5 sporangia per droplet, sp/dr) were considered. The values *F* of observed frequency of MR-sporangia (the model's R-sporangia) were set up for tabulating. Numerical solutions of equation (3) provided the estimates of the actual values *f* of MR-sporangia frequency. The first thirty terms of the infinite series (1) and (2) were considered, because the remaining terms are negligibly small. It was assumed that probabilities r_k and s_m equal *1* for relatively large *k* and *m* in line with the data from infection efficacy experiments.

The results for the mixed population MS1+MR1 are presented in Figure 1. The least integral deviation of the calculated frequency, *f*, from the observed frequency, *F*, was revealed for λ = 6.25 sp/dr. However, for example, a better point agreement for $F = 0.90$ was obtained for λ = 3.125 sp/dr. All four λ-values did not provide a good approximation of MR2-frequency in MS2 + MR2 population; the observed frequencies considerably overestimated the actual (calculated) ones (data is not shown).

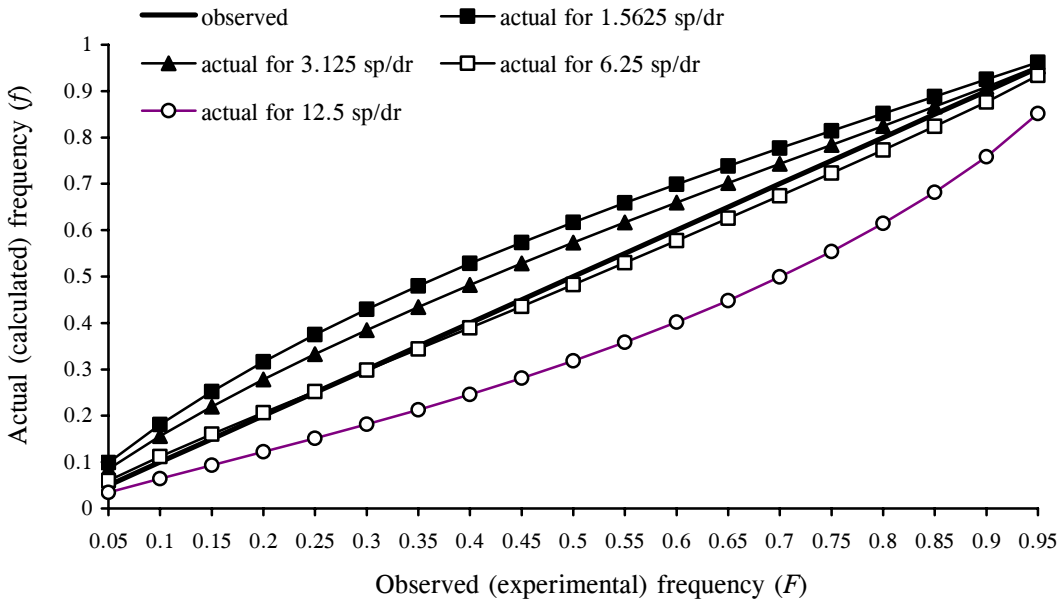

Figure 1. Calculated frequency as the estimate of actual frequency of metalaxyl resistant subpopulation MR1 in the mixed sporangial population MS1 + MR1

KADISH and COHEN (1988) used the linear regression model with zero intercept to analyze conformity between the observed and actual frequencies of MR-sporangia. Goodness of fit with this model was significantly dependent on the average number λ of sporangia per droplet of inoculum. Moreover, this kind of statistical analysis is not relevant due to its integral nature, whereas the goal is to minimize the discrepancy between the observed value and the unique actual value. Goodness of fit with the linear regression model does not necessarily imply good point estimation for any actual value (see Figure 2 in KADISH & COHEN 1988).

CONCLUSIONS

The model takes into account the stochastic variation hidden in experiments. The estimate of the model (calculated frequency) is robust; it does not depend on the average number λ of sporangia per droplet of inoculum. To the contrary, the correspondence between experimentally observed and actual frequency is very sensitive to parameter λ; optimal values of λ, if they exist, should be found in a number of experiments in order to obtain relatively accurate assessments of R-frequency. Application of the model may improve the estimates, raise reliability of the results and reduce the experimental part of the bioassay.

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