CHAPTER 19

Measure of Life-Cycle Traits of a Biotrophic Pathogen

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PREPARATION

The R statistical software (6) is required for this exercise. If R is not available, any other statistical software can be used for the data analysis. The data files LAT-01.dump, SPR-01.dump, and SPR-5a.dump are provided in the supplemental files for this chapter. An R script, chap19.R, is also provided to help download the data and draw the figures. An answer key containing detailed answers to the questions and additional procedures is included in another R script file named chap19-answ.R. It will take about 2–3 h to complete this exercise.

INTRODUCTION

For many plant pathogens, a gene-for-gene relationship determines whether or not a pathogen genotype is compatible with (able to infect) a host genotype. Pathotypes can be defined according to this compatibility relationship as "phenotypes for qualitative pathogenicity." The pathotype of an isolate indicates whether or not it is able to infect a given host genotype. However, all compatible pathotypes do not cause the same amount of disease on a given variety. Some are more aggressive than others and express a higher rate of reproduction (see exercise 3). For biotrophic fungal pathogens, aggressiveness is usually separated into elementary traits of the pathogen life cycle, such as infection efficiency, latent period, sporulation rate, infectious period, and lesion size. In this chapter, we focus on latent period, spore production, and lesion size. A review of aggressiveness (including definitions, a discussion of the aggressiveness components, and their relation to pathogen fitness is given in Lannou (3). To understand how the life-cycle traits contribute to the rate of disease increase, the reader is referred to Sackett and Mundt (7) and van den Bosch et al. (9). The exercises in this chapter are based on data obtained on *Puccinia triticina*, a biotrophic pathogen that causes wheat leaf rust. A more complete analysis of the data can be found in Pariaud et al. (5).

Latent Period

For fungal pathogens, the latent period is the time interval between infection and onset of sporulation from that infection. Latent period is an important parameter, since it is related to the generation time of the pathogen and thus strongly influences the rate of epidemic development and pathogen fitness. The latent period is usually measured by inoculating a leaf (or any susceptible plant organ) with spores and estimating the time at which the resulting lesions produce new spores. In artificial inoculations, however, not all lesions start sporulation at the same time, and the variability in observed latent period among individual lesions must be taken into account. Several methods can be used to measure the latent period. The time from inoculation to first sporulation (observation of the first lesion that sporulates) is a possibility but is generally considered imprecise. A better measure of latency is the time needed for half of the final number of lesions to start sporulation (T50) (see Knott and Mundt [2]). For estimating T50, the number of sporulating lesions must be counted at regular time intervals to establish the dynamics of the transition from latency to sporulation. The time required for 50% of the lesions to become infectious can then be estimated by linear interpolation between the two closest values (Fig. 19.1). But a more precise method, proposed by Shaner (8), is to fit a model to the transition from latency to sporulation and

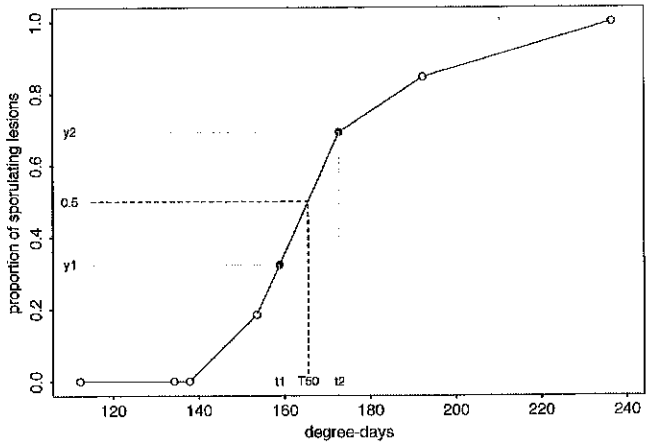

Fig. 19.1. Estimation of latent period (T50, the time at which 50% of the lesions sporulate). The curve shows the transition of infections from latency to sporulation over time (in degree-days).

estimate T50 from that model. This has the advantage of using the whole dataset and thus all the available information. These methods are illustrated in exercise 1.

The biological development of an organism usually depends on temperature, and this is true for leaf rust lesions as well. To compare different experiments, it is thus recommended that the latent period be expressed in thermal time (4). Thermal time, usually expressed in degree-days, is an accumulation of temperature units above a threshold (base) temperature over a specified period of time. For instance, if the average temperature is 25°C for 2 days and 20°C for the following 4 days, the resulting thermal time is (2 × 25) + (4 × 20) = 130 degree-days (with a threshold at 0°C).

Sporulation Rate

The sporulation rate is the number of spores produced per lesion (or per unit area of lesion) per time unit. The number of spores produced is an important fitness component for the pathogen, since it determines its transmission capacity. In practice, spores can be collected from an infected leaf and either weighed or counted. *P. triticina* produces spores continually during the infectious period, but the rate at which the spores are produced is high for young lesions and decreases gradually until the lesion dies.

Lesion Size

Lesion size is another quantitative trait that is commonly measured as an aggressiveness component. Lesion size is generally defined as the area of the surface that produces the spores. For *P. triticina*, lesion size remains relatively limited, but it can dramatically increase in other species such as *Phytophthora infestans* or *Puccinia striiformis*. In those cases, lesion size accounts for a large part of the quantitative development of epidemics, and lesion growth rate is a key factor of pathogen competition for available host tissue (1).

Environment

Aggressiveness components can be affected by environmental constraints. We have seen that the latent period varies with temperature and can be judiciously expressed in thermal time. For biotrophic pathogens such as *P. triticina*, the host plant can be considered the local environment for the lesion development, and the physiological status of the host may affect the pathogen development. An example is given of the effect of the leaf nitrogen content on the pathogen spore production capacity (exercise 2). Another important constraint that affects the measurement of aggressiveness components is the local lesion density. The development of a lesion on a leaf can be strongly affected by the density of surrounding lesions, and this density-dependence effect may complicate the comparison of different pathogen genotypes. This is illustrated in exercise 2.

PROCEDURE AND EVALUATION

Empirical data on lesion development and spore production in leaf rust are used in these exercises. In the experiment, flag leaves of adult wheat plants in a greenhouse were inoculated with leaf rust spores. Three pathotypes were compared for their aggressiveness levels. Each pathotype was represented by different isolates sampled in different years and at different places in order to capture the within-pathotype variability. Several plants were inoculated with each isolate to replicate the measurements. The measured variables considered here are the latent period, the number of spores produced per lesion, and the lesion size.

1. Estimating Lesion Latency

A subset of the latency data is used in this exercise. In the experiment, the number of sporulating lesions was determined about every 12 h for 8 days. The data are in file LAT-01.dump provided in the supplemental files for this chapter.

a. Plot the proportion of sporulating lesions as a function of thermal time (degree-days) on each inoculated leaf. Use the graph to directly estimate T50.

b. Estimate T50 by linear interpolation between the two closest values (as in Figure19.1).
c. Fit a logistic or a Gompertz function to the data, and use the function to estimate T50. Check how the function fits the different datasets. The form of these functions is indicated in the script in the file chap19.R.
d. Compare the different methods. Which one seems the most efficient for estimating T50?

2. Estimating Spore Production

In this exercise, we compare the spore production of three isolates, each of a different pathotype. Adult wheat leaves were inoculated, and the spores were collected regularly until the end of the infectious period. Spore production per lesion is defined as the total number of spores produced during the lesion life span. The measure was realized by weighing all of the spores produced by an inoculated leaf and dividing the figure by the number of infections on that leaf. Two fertilization regimes were applied to the plants: either a standard fertilization level (N+) or a reduced fertilization level (N–). Several leaves (15) were inoculated for each isolate × fertilization regime combination in order to replicate the measurements. The data are in file SPR-01.dump.

a. Visualize the data by drawing a boxplot of spore production per lesion for each isolate and each plant fertilization level. Comment on the graphs.
b. Test for differences among isolates with an ANOVA. Which effects should be included in the ANOVA model?
c. Look at the residual distribution, and comment on the validity of the ANOVA model.

It is known that, for biotrophic pathogens, lesion development may be affected by the lesion density on the infected leaf. We now introduce this density effect into the analysis.

d. Draw a graph of spore production per lesion as a function of lesion density. What is the effect of lesion density on spore production, and how do you explain it?
e. How can you take into account the density effect in the data analysis? We can assume that the density effect is the same for all isolates.
   i. Introduce the density effect into the analysis; check the residuals.
   ii. Explain the differences from the previous model.
   iii. What is the effect of the fertilization regime? Explain this effect.
f. We might want to estimate the average spore production per lesion for the different isolates. Is it reasonable to simply calculate mean values from the dataset? Explain why or why not and propose a solution.
g. The requirements of the linear model are not always fulfilled. A possible alternative to the regular ANOVA is suggested in the script in chap19-answ.R that is included with the answer key. See also Chapters 14 and 15 for a Bayesian approach.

3. Analysis of Spore Production Per Lesion

Here we compare the spore production of leaf rust lesions of three different pathotypes. The measurements were performed for several isolates for each pathotype. The isolates are supposed to represent the pathotype diversity in the field population. The measurements were replicated six times for each isolate. As before, adult wheat leaves were inoculated with the isolates, and the spores were collected regularly until the end of the infectious period. Three variables were estimated: spore production per lesion (mg), lesion size (mm^2), and spore production capacity (defined as the spore production per unit of sporulating tissue, in mg/mm^2). Lesion size was estimated by image analysis. The data are in file SPR-5a.dump.

a. Visualize the data for the three measured variables.
b. Compare the three pathotypes for each variable.
c. What kind of information is provided by decomposing spore production per lesion into lesion size and spore production per square millimeter of sporulating tissue?

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FURTHER READING

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