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Combining experimentation and modelling to estimate primary and secondary infections of take-all disease of wheat

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ABSTRACT

Primary and secondary infections are important processes in the epidemiology of plant diseases but can be difficult to quantify experimentally as they often occur at the same time. This problem is all the more challenging in the case of soil-borne diseases, as most processes are hidden in the soil and destructive sampling is time-consuming and makes it difficult to obtain enough observations of disease progress. Here we show how a combination of experimentation and modelling can be used in order to obtain parameters for primary and secondary infections for take-all disease of wheat. First, an experiment with one infected seedling and varying numbers of target seedlings allowed us to estimate the probability of secondary infection by growth of the mycelium through the soil and by growth via the crown of the plant. Several equations were tested for the contact term between susceptible and infectious roots. Secondly, an experiment with primary inoculum placed at different depths allowed us to estimate the probability of primary infection, taking into account secondary infections and the time needed for the roots to reach inoculum depth. In both experiments, the use of simple models was effective in isolating the desired effect from uncontrollable effects occurring in the soil. The probability of secondary infection through the crown was higher than the probability of infection through soil, and the contact term following the mass action or Reed-Frost equation gave a better fit to the data than the other equations tested. The probability of primary infection was higher when inoculum was placed just below the soil surface than when it was placed deeper in the soil.

1. Introduction

Epidemics can be considered either as "black boxes", transforming initial and environmental conditions into amounts of disease, or as systems including several interacting processes. The former approach might be sufficient when the modeller's goal is to describe epidemics or to extrapolate disease dynamics over time. But in order to predict the effect of combinations of several factors (e.g. different control methods), it is necessary to recognise the basic processes involved in epidemics and to model the effect of various factors and their interactions on the different components. The simplest representation of most epidemics divides host tissues into two categories: susceptible and infected, and includes two processes: primary (from inoculum to plant) and secondary (plant-to-plant) infections (Kermack and McKendrick, 1927). The rates of primary and secondary infection can be obtained by numerical fitting of curves from observed epidemics (top-down approach) but field experiments are lengthy and it is not always possible to control environmental conditions. Furthermore, the estimates of the parameters are often correlated, which reduces the accuracy of the estimation. Alternatively, it is possible to measure the epidemiological parameters individually in controlled conditions and then include them in epidemiological models (bottom-up approach). But since all processes almost always happen at the same time, it is difficult to design experiments that are able to disentangle the effects of the different processes.

These problems are particularly challenging in the case of soil-borne diseases, because a number of processes and variables are hidden in the soil. Thus, the top-down approach is hindered by the fact that sampling is usually destructive, preventing the monitoring of the dynamics of epidemics, except when symptoms are expressed above ground, as is the case of damping-off diseases (Otten et al., 2003). Clever experimental systems, involving transparent soil packs, transplantation of infected plants, or nylon meshes, have been used to study primary infections (Gilligan, 1980a), secondary infections (Suffert and Montfort, 2007) and

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infections across soil (Rekah et al., 1999; Suffert and Lucas, 2008), respectively. But it is not always possible to separate all processes, or to do so in a manner that will not alter the results quantitatively. Here, we hope to demonstrate how simple models can be used to overcome these difficulties, using take-all disease of wheat as an example.

Take-all disease of wheat, caused by *Gaeumannomyces graminis* var. *tritici* (Ggt) is one of the most common diseases of wheat worldwide (Garrett, 1981). It infects roots, disrupting water transport and assimilates translocation (Asher, 1972), killing the plant if it is infected early in its development or reducing both grain number and grain weight when infecting later (Schoeny et al., 2001). Primary infections arise from mycelium surviving on infected root and crown debris from previous crops (Hornby, 1981). Secondary (root-to-root) infection also occurs by mycelium growth, either along the roots or through soil, over short distances (about 10 mm, Pope and Jackson, 1973; Gilligan et al., 1994). Fungicides give only partial control of this disease (Schoeny and Lucas, 1999) so control measures include adaptation of cultural practices, mainly crop rotations involving non-susceptible break crops (Cook, 2003), but also other elements of the cropping system, such as soil cultivation, which affects the depth of inoculum (Colbach, 1994, 1995), or an ammonium form of nitrogen fertilization, which acts both directly through changes in rhizosphere pH (Smiley and Cook, 1973) and indirectly through stimulation of antagonistic microflora (Smiley, 1978; Sarniguet et al., 1992). Since no single technique is sufficiently effective, a combination of techniques must be used. It is thus critical to determine their effect on the different components of the epidemics. A spatio-temporal model simulating the spread of the disease over a few square meters was built in order to test the effect of combinations of cultural practices on take-all epidemics (Gosme M., Ph.D. thesis, ENSAR, Rennes). Some epidemiological parameters had already been measured for take-all: the rate of lesion extension along roots (Gilligan, 1980a,b), the size of the pathozone (the volume around a root where an inoculum propagule must be present if it is to have a chance of infecting the root) (Gilligan, 1980c, 1985), and the rates of primary and secondary infections (Bailey and Gilligan, 1999). However, not all of these parameters could be used directly for inclusion in our model, as they did not take into account the effect of depth of inoculum (to reflect the effect of soil cultivation) or the distinction between secondary infection through the soil or via the crown of the plant (which will affect the modelling of the effects of the sowing pattern).

The objective of this study was to combine experimentation with mathematical modelling to estimate the probability of primary infection at different depths of inoculum and secondary infection of root axes by mycelium growth through the soil or via the crown of the plant. To this end, two experiments were carried out: in the first one, only secondary infections occurred; in the second one, both types of infection occurred and the effect of secondary infection was taken into account by a simple model when estimating the probability of primary infection.

2. Material and methods

2.1. Biological material common to both experiments

2.1.1. Wheat seedlings

Wheat (*Triticum aestivum* L. cv. Talent) seeds were surface-sterilised by immersion for 5 min in 1% hypochlorite, rinsed three times for 5 min in sterilised distilled water and placed on wet filter paper in the dark at 20 °C for 5 days (target seedlings of experiment 1) or 3 days (all other seedlings).

2.1.2. Fungus

G. graminis (Sacc.) Arx & Olivier var. *tritici* Walker, strain IV-26, was isolated in 2000 in a naturally infested field in Brittany that had grown a continuous wheat crop for 4 years, it was kept on agar explants in 10% glycerol water in a refrigerator at 4 °C. It belongs to the G2 group (Lebreton et al., 2004). When needed, explants were transplanted twice for one week on PDA + antibiotics medium (Penicillin and streptomycin, 0.075 and 0.15 g l^{-1} respectively) in the dark at 20 °C. Explants from the growing edge of the colony were then either used to infect wheat roots (experiment 1) or oat seeds which were then dried and placed in soil to infect wheat seedlings which in turn were used as inoculum in experiment 2.

2.2. Fitting method

The parameters were estimated by Bayesian estimation, assuming that the observed number of infected roots per plant follows a binomial distribution with probability p_i, computed with the epidemiological model (see "theoretical incidence" for each experiment). The Gibbs sampling algorithm was used with uniform priors between 0 and 1. This algorithm uses a Markov chain Monte Carlo process (i.e. a random walk where the step only depends on the state of the variables at the current time). The algorithm follows the following steps iteratively: (i) one parameter is randomly chosen among the parameters to be estimated, (ii) a random normal deviate is added to the current value of the parameter, (iii) the likelihood of the new value of the parameter is computed using the epidemiological model and the data, (iv) the new value is accepted with a probability proportional to the ratio of the likelihoods of the old to the new parameter values (posterior likelihoods, but in our case the sample likelihood because we used flat priors) (v) if the new value is accepted, it becomes the current value and the process is repeated; if not, the current value remains unchanged and the process is repeated. Thus a series (chain) of parameter vectors is constructed. After a certain number of iterations during which the chain approaches equilibrium, the vectors represent a sample from the parameter's distribution. The first 1000 iterations were discarded and then the MCMC process was run for 200,000 iterations and recorded every 20 steps, resulting in 10,000 samples for the distribution of the parameters. The estimated values of the parameters are the means of the distributions. Two chains were run from different starting values in order to check for convergence (less than 5% difference between both estimates).

2.3. Experiment 1 (secondary infection through soil and crown)

2.3.1. Soil preparation

Ggt-free soil was collected in June from a plot that had never received any cereal crop and had been used for experiments on carrots and potatoes and so had been carefully protected against grass weeds. The top 15 cm was sampled, air-dried for one week and then sieved to keep the fraction smaller than 5 mm. A 1:1 (volume) mixture of soil and sand was placed in the pots two weeks prior to the start of the experiment and pots were allowed to stand in water until equilibrium was reached, after which they were transferred to a growth cabinet. Soil moisture was maintained at approximately 30% (w/w) throughout the experiment by watering the pots from below twice a week.

2.3.2. Seedling infection

The surface-sterilised wheat seeds were pre-germinated for only three days (at which time roots were 3–4 cm long). Seedlings were then inoculated by placing a 5 mm agar plug taken from the edge of the colony on one of the roots, the face bearing the fungus