Dynamics of pathogen population

Population dynamics is the study of how the size and structure of populations respond to the forces that act on them.

The measurement of populations

Before it is possible to study changes in populations, it is first necessary to decide what the individuals are that are being counted. A population is composed of individuals at various stages in their life-cycles. It seems sensible to start by discussing a relatively easy example, *Blumeria graminis* (cause of cereal powdery mildew) on a cereal crop. Three components of the population can be immediately distinguished. First, there is a population of suspended or recently deposited and ungerminated conidia, each of which is potentially capable of infection. Second, there is a population of physiologically independent mycelia growing on leaf surfaces. Some of these are too young to sporulate, others are sporulating, still others have exhausted the food available to them and have ceased to sporulate. Finally, there may be a population of cleistothecia. A measure of the population will have to include several numbers, describing the number of each type of individual per square metre (or per square metre of host). The conidia could be measured by various techniques and the sporulating mycelia counted visually. More sophisticated methods using staining and microscopy would be necessary to count the immature mycelia directly. There is still the problem that a count of visually distinct colonies is not necessarily a count of physiologically distinct pathogen individuals; one colony may contain several individuals. A final problem would be sampling cleistothecia, since dead host tissue will be difficult to survey adequately. An independent categorization could divide the population according to genotype or

virulence, or by infection with a hyperparasite. Similar sampling and estimation problems would arise.

Regardless of which stages are used, the ‘stage structure’ of the population can be described by specifying the proportion of it that exists in each stage. If the stages are simply age groups, then the stage structure corresponds to the age structure, or the proportion of the population in each of a series of age groups.

A more difficult problem is posed by a leaf blotching or spotting infection, *Phaeosphaeria nodorum* (cause of wheat glume blotch) or *Mycosphaerella graminicola* (cause of septoria tritici blotch), for example. The extent of visible damage to the leaf does not necessarily correspond in any simple way to the number of physiologically independent infections that have occurred, and non-sporulating mycelia are hidden within the leaf. It may be possible to measure the amount of mycelium, or of mycelium of a particular type immunologically or by DNA sequence-based assays but this still does not correspond in a simple way to a notion of population. Estimates of spore density would be possible based on washing leaves to remove spores released or available for release but not yet bound to a leaf surface; this has proved useful in practice.

It is still more difficult to define the population of a root-infecting fungus like *Gaeumannomyces graminis* (cause of take-all of cereals). Transmission from host to host is only very rarely by spores and substantial amounts of mycelium can exist in the soil as well as on the root surface of susceptible hosts. Single physiological individuals are hard to define, let alone count. In some soil-borne pathogens transitions between stages may be very fast, leading to very rapid changes in age-structure; if some stages are not easy to observe, because they do not infect test plants

or cannot be cultured, it may be very hard to obtain sensible estimates of population sizes.

In practice, it is usually necessary to fall back on measuring the pathogen population indirectly, by correlation with a measure of disease or a biochemical or DNA-based assay. Biochemical assays have the advantage of precision, but by their nature do not distinguish changes in numbers of individuals from changes in size of individuals; empirical relations between the assay and population need to be constructed for each pathogen, and supplementary information on the stage-structure may be needed. Here, it is only necessary to note that most crops can be subdivided into units which can be categorised as diseased or not. The proportion of diseased units in an area will often be a useful proxy for the true population density of the life-stages which are reproducing actively on the host tissue under study.

The size of the unit used will depend on both the time-scale and on the purpose of the measurement. For example, with a systemic virus disease such as cocoa swollen shoot virus, the obvious unit is the infected plant; for a foliar fungal disease, such as septoria tritici blotch of wheat, it may be more appropriate to consider infected leaves or portions of leaves. However, progression into or from dormant forms independent of a host may require a different definition of the sampled units. The relationship between incidence, pathogen biomass and number of physiological or genetic units of pathogen in an area will vary for almost every pathosystem. It will usually be neither simple nor independent of the environment and host.

Finally, it should be noted that the study of pathogen population dynamics will rarely be possible in isolation from the host population dynamics, since most pathogens affect growth and reproduction of their hosts.

Time-scales

A natural time-scale for changes in a pathogen population is set by the generation time of the pathogen, the time taken for one propagule to infect and give rise to another. However, for pathogens with complex life-cycles, there may be more than one natural time-scale, depending on which life stage is being considered. For example, in a heterocyclic rust, the generation time of asexually produced, physiologically independent, individuals in a uredinial cycle may be much shorter than the generation time of sexually produced genetically distinct individuals, say from telium to telium.

Often, this notion of generation time will broadly correspond to the latent period for a typical fungal pathogen. It differs in that it refers to the time when, on average, new infections are actually formed under the prevailing conditions, rather than to when infectious pathogen stages are produced. The latent period of *Mycosphaerella graminicola* on the flag leaf of wheat cv. Riband has been measured as about 300°C days above a base temperature of 2.4°C: this is, for example, 30 days at an average temperature of 8.6°C. If rainfall were rare, the generation time might be substantially longer than this. However, in a rainy period, the generation time would correspond closely to the latent period. For systemic virus diseases, the generation time may also be related to vector biology since, with a slow-moving vector, it may on average take a considerable extra time after a plant becomes infectious before it actually causes further infections.

'Short-term' may be used to refer to a few pathogen generations. For a rapidly reproducing pathogen such as *Phytophthora infestans* (cause of late blight of potato), this may mean a few weeks; for a forest pathogen like Cocoa swollen shoot badnavirus (CSSV), it may mean a few years. However, patterns of short-term

change may differ greatly depending on whether the host population is also changing. The term ‘pathocron’ has been introduced and defined as the ratio of the latent period to the ‘phyllocron’ or leaf emergence interval. Its usefulness in clarifying how host growth affected the expression of disease has been stressed. The case of an annually reproducing pathogen like *Sclerotinia sclerotiorum* (cause of pink rot of celery and other diseases of vegetables, and stem rot of sunflower) suggests that, in general, it will be useful to consider the *relative* timescales over which pathogen and host populations change. For example, although CSSV has a long ‘generation’ time, its host the cocoa tree (*Theobroma cacao)* has an even longer generation time and the host population will normally change little over one or a few pathogen generations. Similarly, a potato crop stops producing new leaves a few months before harvest, while the life cycle of *P. infestans* can be completed in a week or so. So, once the crop has stopped producing new leaves, it is possible to ask how the potato blight epidemic develops for the remainder of the season in an approximately *fixed* host population; after harvest, it is possible to ask how the population in the tubers changes during the off-season, without considering changes in the tuber population.

Many very interesting processes require the simultaneous study of host and pathogen populations because both are changing at comparable rates. In the example of *P. infestans*, it is not possible to study the transfer of fungus from a predominantly tuber-borne population in the off-season to a predominantly foliar population in the growing season without considering how both populations are changing. Similarly, a fungus like *Sclerotinia sclerotiorum* infects annual crops like oilseed rape and sunflower once a year, so the generation times of host and pathogen are similar and neither population can be considered fixed.

Changes in populations

Changes in population size may come from birth, death, immigration or emigration. Changes in the stage-structure of a population may arise in the same way: individuals may progress from one stage to the next (for example, cleistothecia → immature mycelia) or may enter the stage from a previous one in the life-cycle (for example, immature mycelium → sporulating mycelium). Immigration and emigration will usually only occur at certain stages.

If a population is described by specifying the number of individuals in each of a series of stages, matrices can be used to model the population and the ways in which it will change. Such models describe the change of the population in jumps, specifying the numbers and structure at one time and then attempting to specify how these will have changed at a definite time, one time-step, later. The time-step appropriate depends on the biology of the organism and the time-scale of interest; for some pathogens, changes from hour to hour may be appropriate, for others, changes from year to year may be useful to study.

The data describing the population can be set out in a vector or list of numbers in each stage. The changes to this list from one time-period to the next can be set out in a matrix or table showing two things: the proportion of each stage which will have advanced to the next stage (for example, from latent infection to sporulating infection, or from sporulation to over-seasoning stage), and the number of new-born individuals. The number of new-borns will be proportional, among other things, to the total number of propagules produced by all the infectious stages of the pathogen. Emigration will usually be implicit in such a representation, causing a reduction in the number of births, since a live pathogen individual is usually associated with a plant host that cannot move far.

Immigration can be included by adding to each stage in the population vector the number of individuals in the stage that immigrate in one time-step. Often, it is useful to consider a large area of host, so that almost all new infections come from parent infections or over-seasoning stages within the crop and emigration and immigration can be ignored. How large an area this is depends on how the pathogen propagules move around. Fungal spores moved by rain splash move only a few metres, so a field 50 m on each side would be a reasonably self-contained population. It is the minimum dimension of the area that matters in this context. A hectare of ground consisting of a 1 m wide strip 10 km long is unlikely to be a closed system. On the other hand, for a virus moved by whitefly, such as African Cassava Mosaic geminivirus or a wind-blown pathogen such as powdery mildew of cereals, an important, if small, fraction of infections may occur hundreds, thousands or tens of thousands of metres from the source plant. In such cases, areas measuring many kilometres in both dimensions are necessary in order to have an isolated system. For many purposes, this is not appropriate and immigration (especially) needs to be considered explicitly.

Density-dependent and density independent factors

The factors controlling the growth rate of a pathogen population may be completely uninfluenced by the population: temperature, rainfall, or developmental changes in the susceptibility of hosts are good examples. These factors are as likely to increase a population when it is small as when it is large. If controlled entirely by such factors, a pathogen population would change by a random walk, with no predictable long-term trend and the certainty of eventual extinction when a long run of bad seasons occurred, or when it exterminated (eliminated) its host. However, there are also many factors that influence population growth rates in ways which tend to *regulate* the population, that is, to return it to some central value. These operate over both

short and long time-scales. Over short time-scales, rare pathogens will not trigger secondary defences in their hosts, will not support large populations of hyperparasites or mycoviruses and will not compete with each other for infection sites or host tissue; common pathogens will do all three. Over longer time-scales, rare pathogens will not cause rapid evolution of resistance in their hosts and are not liable to reduce their host population density, while common pathogens will select strongly for resistance and may tend to reduce their host population, hence intensifying competition within the pathogen population. Examples of all types of factors will be given in subsequent sections.

Short-term change in a static host population

As discussed earlier in time scales section, the notion (idea) of short-term change encompasses a wide range of actual time-scales. A lettuce crop may be in the ground for only 12 weeks; a tree crop 150 years. Whether the host population can be considered constant over this time-scale depends on the nature of the pathogen: for a systemic virus, a single lettuce crop may represent a fixed population but for a pathogen whose host was leaf or root tissue, there would be considerable host turnover during the season.

It is useful to begin by considering a very simple setting in which there is negligible immigration and the host population does not change over the time to be considered. In this setting, a pathogen population may change in two ways. First, numbers overall may grow or decline. Second, the stage-distribution may alter. This in turn involves two types of change: the relative balance between active infectious phases of the pathogen, as when pathogen individuals advance from a latent to a sporulating relationship with their host, and alternate or resting stages that may increase or disappear as when over-seasoning or sexual stages develop. For example, consider

an apple orchard in which there is a population of *Venturia inaequalis* (cause of apple scab). At the start of the host-growing season, the population is small and exists as perithecia and inactive mycelium on twigs. Then as the season develops, these stages decline and disappear, while an asexual population increases. At the end of the season, the asexual population generates over-wintering forms once again.

It is useful to imagine a thought experiment in which a single young pathogen individual is placed in an otherwise healthy plant population. In the case of the example in the previous paragraph, this would be a newly infected apple leaf. Each subsequent unit infected by this initial one is immediately removed and replaced by a new healthy one, until no new infections are produced. The total count of new units infected (NUI) is known as R_0. Real variants of this experiment involve complications but can be done: for example, R_0 was measured for *Puccinia striiformis* (cause of yellow rust) growing on wheat in the Netherlands, between the start of stem extension and flowering, as 55 ± 16; for *Peronospora farinosa* (downy mildew) on spinach (*Spinacia oleracea*) in the Netherlands in the autumn the figure was 3 ± 2. It is obvious but important that a disease cannot increase in a crop unless R_0 is greater than 1: the 'threshold theorem'. This quantity is therefore of central importance in managing invasions of new disease, in predicting ranges, or in eliminating disease.

An individual will not be infectious until some time after it has infected a host. This interval is the latent period. Then typically, infectiousness (for example, spore production) will increase before declining as the pathogen ages or runs out of food. The exact timing of the different phases will vary from individual to individual and will depend on the environment. However, it is helpful to do some thought experiments to see what would happen over some time, if such outside factors stayed the same.

To begin with, useful results can be derived by assuming that diseased hosts remain uncommon (unusual), so that the proportion of propagules which infect does not change. In this case, the conclusions important for pathology are as follows. First, as time passes, the proportions of the pathogen population lying in each age class gradually stop changing. Second, these proportions are the same regardless of the initial age structure of the population. Third, the total population grows by a constant factor each day. Since the age structure is constant, the number of pathogen individuals at any given stage also grows exponentially at the same rate as the whole population. Because the age structure does not depend on the initial composition of the population, this exponential growth rate is characteristic of the host, pathogen and environmental circumstances but does not depend on how the epidemic started.

An important conclusion is that measuring a single stage of the population, which is often all that can be done in practice, is usually a reasonable relative measure of the entire population involved in the multiplication, although such a measure can give no information about life-stages which are not involved in the multiplication process. Thus, to return to the *Venturia inaequalis* example, the relative proportions of latent individuals, sporulating individuals and conidia will tend to become constant during a period of consistent weather. By contrast, the proportion of the population existing as perithecia – either from the previous or the current season – will bear no necessary relation to the other stages.

The initial *per capita* rate of multiplication of the population is usually given the symbol *r*. It can be estimated from a graph of log(disease) against time before disease becomes common (say, before about 10% of tissue is infected). However, it is useful to know how it is related to the basic reproductive rate R_0 and to the latent period (*P*). The exact relationship is not expressible in a single simple formula but a crude approximation is:

$$r = \frac{log_e\,(R_0)}{P}$$

This is a fairly good approximation provided *r* is not too large. The definition of the generation time to be used needs to be something like 'the average age of the lesion at which spores are produced'. This means that the rate at which disease increases in the host ('the rate of the epidemic', or the 'apparent infection rate', or the 'malthusian parameter' or the 'intrinsic rate of increase') increases only slowly with R_0 unless R_0 is close to 1, but decreases inversely with the latent period (Fig.).

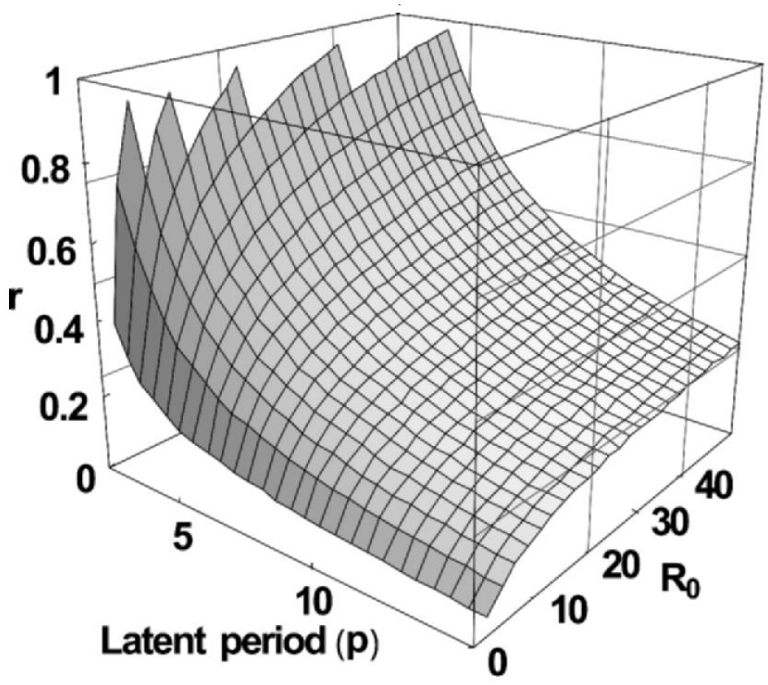

Figure: *The relationship between the intrinsic rate of increase of a disease,* r, *the basic reproductive rate* R_0, *and the generation time* p. *The time-units for* r *and* p *are the same. For a disease that reproduced slowly, they might be in years* $^{-1}$ *and years; for a faster disease, they might be in days*$^{-1}$ *and days. Control actions which reduce* R_0 *by a fixed proportion will work best if* R_0 *is already small; those which increase* p *by a fixed proportion will work best if* p *is small.*

The growth rate of a pathogen population will be affected by the environment and by crowding; the stage-structure of the population will alter at the same time. For example, the latent period may be decreased, or infection permitted or prevented for

a short time; or when host condition, or light, or temperature provides appropriate signals, individuals may change physiologically to over-seasoning or migratory life-stages.

This model can be used to explore, in a preliminary way, the way in which environmental and management factors affect populations. For example, if fungicide is to be used to control an epidemic, it must be applied long before disease becomes sufficiently severe to cause economic loss, because a large proportion of disease may be latent. The effect of fungicide on an epidemic depends on how the fungicide acts, on the latent period and on R_0. Fungicide action can conceptually be split into protectant and eradicant components. Some fungicides act only as protectants: examples are Bordeaux mixture, chlorothalanil and the dithiocarbamates; others have both protectant and eradicant activity to varying extents. Protectant fungicides act only at the point of infection and therefore alter only the infection rate of the pathogen. Eradicant activity, residing inside the plant and therefore associate with systemic fungicides only, may affect R_0 by killing entire individual mycelia, by reducing the spore production or the length of time for which spores are produced, or by lengthening the latent period. Protectant activity, no matter how effective, will allow latent infections to grow. For disease with high r but fairly long latency, with an incubation period similar to the latent period, this implies considerable increase in disease severity after the application of fungicide.

Systemic fungicides, such as the azole group or the phenylamides, may have both eradicant and protectant effects. They may kill a proportion of established and growing pathogen individuals, they may prevent a proportion of new infections by interfering with infection processes or germination and they may lengthen latent periods, in some cases very dramatically. This means that they may be able to work better on diseases with high rates but long latent periods, because the latent disease

will actually be eliminated to some extent. It will still be true that, if R_0 is large, changes in R_0 will have only slight effect on r. However, if latent periods of surviving pathogen individuals are lengthened, the control at any given actual time will be better than with the protectant, because fewer latent periods will have passed.

Affected host tissue and pathogen multiply at comparable rates

This is the common situation with foliar and root diseases in arable agricultural and annual horticultural crops. After harvest, the pathogen population usually decreases over the off-season. Then the crop starts to grow again, typically producing leaves and roots in a roughly exponential pattern until competition slows growth to a steady rate, before ceasing at maturity or flowering. This 'expolinear' pattern has been shown to apply to many crops, from *Vicia* beans to wheat, although the rate of growth will vary, particularly because of temperature variations. Nonetheless many crops, such as cereals, produce new leaves at the same time as older leaves are dying off. Net crop growth arises because the new leaves appearing are more numerous or bigger than the old ones dying. Typical crop doubling times at the start of the growing season are a week or less, corresponding to leaf area index increasing by around 10% per day.

The pathogen population dynamics in this case are determined by the relative rates of multiplication of host and pathogen and by the fate of the pathogen on dead host tissue. Because both host and pathogen are continually being born and dying, disease severity may be a very poor measure of the pathogen population. Furthermore, the deductions of the previous section no longer apply and many more patterns of growth or decline are possible. If the pathogen can exist and sporulate on dead tissue, it may be necessary only to allow for host growth. If the pathogen is multiplying

faster than the host tissue, disease severity and the pathogen population will increase; if the reverse occurs, the population will decline.

Host tissue born recently, within one latent period of the present, cannot be visibly diseased. If the pathogen did not affect the death rate of host tissue, the pathogen population would tend to increase so that all host tissue becomes infected, even if invisibly. However, most pathogens tend to accelerate the death of host tissue on which they are growing and more heavily infected tissue will tend to die faster than lightly infected tissue. If the pathogen also dies when the host dies, then an equilibrium may exist, with a steady but dynamic population density substantially less than the maximum possible. Furthermore, the effect of the host tissue dying is to reduce the standing population of pathogen substantially *more* than the population of the host. In effect, therefore, the host is regulating the pathogen population; this can actually be an evolutionary force leading to more rapid death of infected tissue.

There is evidence for temporary equilibria like this existing in some UK grasslands, where disease levels in perennial plants, principally the grass *Holcus lanatus*, were steady at about 6% of green tissue infected throughout the summer in several years. This may also be an informative way to look at cereal disease levels during the vegetative growth phase, although it is unlikely that an equilibrium would be reached because there is rapid net growth of the crop throughout the season.

Once the crop reaches maturity and tissue turnover ceases, disease will tend to increase according to the patterns discussed in the previous section. However, the lifetime of host tissue is often only one or a few latent periods long, so this epidemic phase will usually be short and will often start from a high initial inoculum level represented by the standing population during vegetative growth.