SESSION 2
BIOLOGICAL ISSUES

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THE ECOLOGY OF BACULOVIRUSES: TOWARDS THE DESIGN OF VIRAL PEST CONTROL STRATEGIES

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ABSTRACT

Baculoviruses and other diseases of insects are becoming recognised as important agents for the control of insect pests. To exploit the full potential of such pathogens and to ensure their safe use, a fuller knowledge of the ecology of the pathogen (and of its hosts) is required. Recently, molecular and theoretical advances have been used as 'ecological tools' to investigate certain aspects of the host-pathogen interaction. This paper reviews some of those advances, and discusses how this ecological knowledge may aid the design of control strategies.

The pest-pathogen interaction is first placed in the context of a simple model framework. Those features that lead to the suppression of the pest are then discussed. By considering where simple models and the real world depart, more complex models have been developed with closer links to the key features of pest-baculovirus interactions. Future research should focus on parameterising, testing and extending these models.

INTRODUCTION

Pathogens of insects, and specifically baculoviruses, have been used to control pest populations throughout this century (Entwistle & Evans, 1985). In more recent years, their potential for genetic modification has opened up the possibility of increasing their efficiency and producing a 'designer baculovirus'. It is now widely appreciated that the safe and effective use (and design) of baculoviruses requires a greater knowledge of how these pathogens behave in natural populations of insects. Whilst the ecology of baculoviruses has been relatively unstudied, developments in two areas are providing us with greater insights: i) molecular techniques and ii) theoretical models. Molecular techniques have revealed that one infected insect will yield a number of virus strains that are closely related, yet significantly different in the way in which they interact with the insect. How is this variability maintained in the population? Which variants will be most useful in the control of insect pests? Theoretical models have progressed from a basic framework constructed as recently as the early 1980s, to models more specifically tailored to the baculovirus-insect interaction. This allows us to explore control strategies in a variety of 'what if' scenarios. This combination of molecular and theoretical work provides valuable information on the ecology of baculoviruses, yet much still remains to be studied, particularly the linking of empirical data with the new generation of mathematical models.

This paper will provide an overview of developments in this area, highlighting how a knowledge of the ecology of baculoviruses can be useful in pest control, using both natural and modified viruses.

The infection cycle

Baculoviruses are often highly specific - those isolated from Lepidoptera and Hymenoptera frequently infect only one or two species. This is a great advantage in considering their use in intergrated pest control programmes, as they will not infect other natural enemies of the pest, though specificity is clearly a disadvantage for commercial producers. The virus persists in the environment by means of occlusion bodies (OBs). These are packages of infectious units (virions) coated in a protein which provides protection from the degrading effects of UV light. As such, OBs can persist in an active state for several weeks (even several years), but in direct sunlight they can be inactivated in a matter of hours (Cory & Bishop, 1993). Larvae become infected when they consume a sufficient number of these particles; for example a lethal dose for susceptible hosts may be as few as 50 OBs, whilst for semi-permissive (less susceptible) hosts 5,000 OBs may be required (Cory unpublished data). The protein coat breaks down in the alkaline environment of the insect gut, and the virions pass through the gut lining into the body tissues, where replication occurs over a period of 4-10 days, until the larva becomes a bag of virus. After death, the body rapidly breaks down, and newly formed OBs are liberated over the surrounding vegetation.

It is the persistence and relative hardiness of the OBs that allows a baculovirus to be formulated as a spray. Additives to the formulation, such as UV protectants, stickers and spreaders can assist in effectiveness (Entwistle & Evans, 1985). In addition to the initial mortality of pests from ingesting sprayed OBs, the dying insects themselves become a further source of OBs, liberating them over the feeding areas of other susceptible insects. Thus, there is a second chance to hit the pest population after the first round of infection. This is a distinct advantage of using a pathogen over a conventional insecticide, and is referred to as secondary cycling (see also the discussion of cycling of fungal pathogens by Thomas in these proceedings). The alternative way to introduce a baculovirus into a target population is through the release of laboratory-infected insects. In these ways, baculoviruses can be used to control pests in a number of different scenarios:

1. True biopesticides: This is when immediate control is required, as often is the case with short term crops.
2. Biopesticides with longer term action: Immediate control needs to be followed up by longer term (within season) action provided by secondary cycling.
3. Classical biocontrol: Long term suppression of pests through the interaction between the baculovirus and host over several generations (and across seasons).

Examination of the way in which a baculovirus interacts with its host suggests that different attributes of the baculovirus-pest relationship will provide better control in these three different situations. To investigate the nature of the baculovirus-pest interaction, it will first be considered in a basic model framework.

A FRAMEWORK FOR BACULOVIRUS-PEST INTERACTIONS

Much of the theoretical work in this area has its roots in a basic model developed in the early 1980s (Anderson & May 1981). This model is illustrated below (Figure 1) and serves to define the virus-host interaction by describing the change in three dynamic variables: S, the susceptible pests; I, infected pests; and W the OBs persisting on plant surfaces. This model has

simplified the virus-host system, but it is nevertheless a useful starting point. For example, it allows the relationship to be broken down into four basic processes: i) transmission (v) when susceptibles (S) consume OBs (W) and become infecteds (I); ii) speed of kill once infected (α); iii) yield (λ) of OBs from an infected insect; and iv) inactivation (μ) of OBs while on plant surfaces. This model is particularly suitable for baculoviruses because the pathogen is modelled with infectious units external to the host, whereas previous models had assumed that susceptible individuals became infected through direct contact with infected individuals. It allows us to pose the question: what sort of baculovirus will cause long term suppression of a pest?

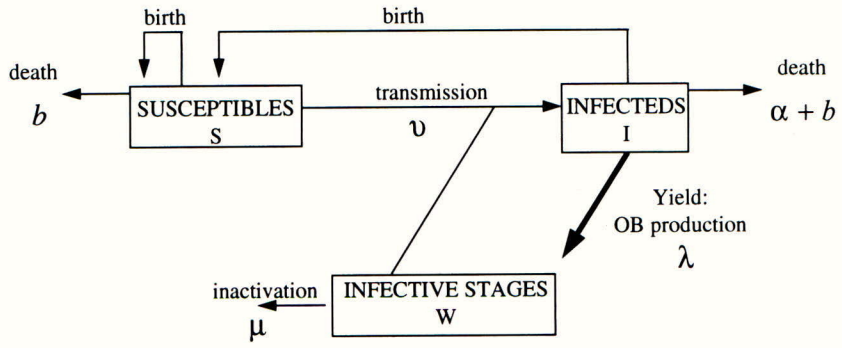

Figure 1: A basic model of a host - pathogen interaction.
S = density of susceptible pests; I = density of infected pests; W = density of active OBs persisting on the plant surface; b = death rate due to natural causes; α = death rate due to disease; v = transmission rate of the disease, transferring individuals from the S class to the I class; λ = rate of OBs being liberated from infected insects; μ = inactivation rate of OBs due to UV light. Taken from Anderson and May (1981).

This can be answered in two steps. Initially, the conditions under which a pathogen such as a baculovirus can cause suppression of the host population must be defined. Secondly, those features that would maximise this suppression can be determined. In answering the first question, it was discovered that this model exhibited four classes of behaviour (depending upon the relative sizes of the parameters describing the four processes).

1. The pathogen can fail to become established in the host population. This is most likely to occur when the yield (λ) is low, and especially when the time taken to kill the host is short (i.e. virulence, α, is high). These conditions compound each other, as baculoviruses that kill quickly do not have much chance to replicate inside the host.
2. The pathogen can become established, but fail to suppress the host. This is more likely to happen if the virulence of the pathogen (α) is less than the reproductive rate of the host. In other words the host population is growing faster than the pathogen is reducing it.
3. The pathogen regulates the host population, suppressing it to a stable level. This occurs with pathogens of intermediate virulence (α), especially if the OBs do not persist for very long in the external environment (i.e. μ is high).
4. The pathogen regulates the host population, but instead of a stable equilibrium, the host and pathogen oscillate in cycles of fixed amplitude. Whether or not this is desirable in a control context will depend upon the periods of these oscillations and whether they exceed an economic threshold. This sort of behaviour is most likely to occur with pathogens of high virulence (α) and high persistence (low μ).

If we wished to design the perfect baculovirus for long term control, it can be seen that a key parameter to manipulate will be the virulence (α), or speed with which the host is killed $\left(\frac{1}{\alpha}\right)$. This not only determines if the pathogen is capable of regulating the host (i.e. is in category 3 above), but also how low that stable equilibrium is (see Figure 2 below). As discussed above, if α is below the host rate of increase, the pathogen cannot regulate the pest. However, high virulence also leads to higher equilibrium populations because the pathogen does not have much chance to replicate inside the host. In contrast, the greater the yield of OBs from an infected cadaver, the lower the host equilibrium population density.

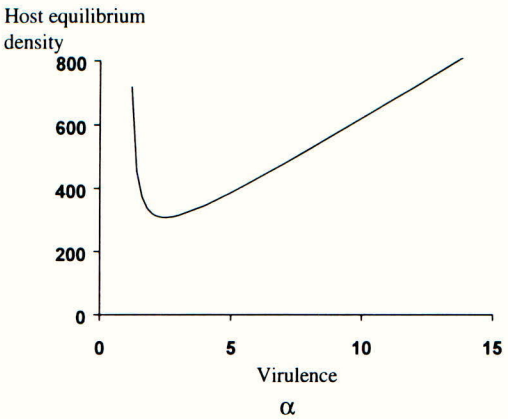

Figure 2. Suppression of a host at equilibrium as a function of the virulence of a pathogen (with all other parameters of the model kept constant).

It can be concluded from this that those baculoviruses which successfully suppress the host population to low levels (i.e. are most suitable for long term control) are not necessarily those which kill the host quickest (i.e. are most suitable for use as biopesticides). These two control strategies require very different 'designer pathogens'. This conclusion results in part from the trade-off between speed of kill and yield of virus from the cadaver. The next section focuses on the relationship between these two parameters.

THE CONSEQUENCES OF INCREASED SPEED OF ACTION

1. Variation in speed of kill in natural populations

The advent of more sophisticated molecular techniques has allowed natural populations of baculoviruses to be examined in more detail. This has revealed that there is considerable genetic variation to be found within one isolate (e.g. Maeda *et al.*, 1990) - an isolate being defined as baculovirus isolated from one insect or group of insects from one species at one time and location. More recently, attention has focused on the phenotypic consequences of these differences. Plaque-purified clones of gypsy moth, *Lymantria dispar* NPV have been shown to vary in a number of parameters including virulence (Lynn *et al.*, 1993). The

existence of such variation in natural populations raises a number of questions. What maintains this genetic diversity? How does it influence the baculovirus-host interaction?

These empirical observations suggest that the selective forces at work in natural populations are rather more complex than the simple virulence-yield trade-off described above. Theoretical work clearly needs to be extended beyond the one pathogen one host scenario of the Anderson and May model. Whilst theory has since been developed to look at two hosts and one pathogen (Bowers & Begon, 1991), and also to look at two pathogens which are directly transmitted in a host population (reviewed in Begon & Bowers, 1995), it has yet to tackle the difficult problem of competition between two pathogens that are transmitted by means of external stages. One reason for this is the complex problem of how the two pathogens compete inside an individual host. Such developments are necessary to investigate the ecological process behind the co-existence of such a range of variants.

This also highlights some important considerations for the use of natural baculoviruses in control programmes. Baculoviruses are particularly suitable for use in developing countries, as they can be produced locally. At the end of each season, infected insects may be collected from the field and re-passaged to produce sufficient virus for the next application. However, the field material will contain a mixture of wild type isolates, and it is conceivable that re-passaging in laboratory conditions may select for particular variants, e.g. those with highest yield. These are not necessarily the variants most suitable as biopesticides, where rapid speed of action is more desirable. This potential problem could, however, be turned to our advantage. It should be possible to determine and control the composition of an isolate (defining it here as a 'mixture of strains'), and so design an activity profile suited to a specific biocontrol programme. A mixture of maximum yield variants and rapid action variants could theoretically allow the practitioner to control the extent to which immediate control is achieved (control scenario 1 above) and longer term control is required (moving through to control scenarios 2 and 3). Thus there is great potential to produce 'designer biopesticides'.

2. Artificially increasing the speed of kill

One of the greatest drawbacks in using baculovirus biopesticides is that even if the most desirable strains are selected, there is a distinct time lag between ingestion of the OBs and death of the insect. This is particularly undesirable when considering pests that require immediate control (scenario 1). However, the genetic manipulation of baculoviruses has enabled the speed of action to be increased artificially. Comparison of an engineered virus with its wildtype clone also allows the yield-speed of kill relationship to be investigated more directly. One particular system has been studied in some detail: *Autographa californica* NPV (AcNPV) and its recombinant AcNPV-ST3, in which an insect-selective scorpion toxin gene has been inserted. Both laboratory and field data have confirmed that the modified virus does indeed kill faster: there was a 25% reduction in time to death in the laboratory and this results in a ten-fold reduction in yield (Stewart *et al.*, 1991). Whilst the results in the field were a little less dramatic there was a significant reduction in crop damage (Cory *et al.*, 1994).

More recently, the relationship between yield and speed of kill has been examined in more detail (Cory *et al.*, unpublished data), using a range of doses and both a permissive (highly susceptible) and semi-permissive (less susceptible) host (*Trichoplusia ni* and *Mamestra brassicae* respectively). There are a number of points that arise from these data. Firstly, the

generalisation that those insects that die earlier yield less virus holds true, both within and between virus comparisons (for example see Figure 3 a & b below). Secondly, the relationship is broadly concave for the engineered virus yet convex for the wildtype virus. This is a mystery, but it is likely to be a consequence of the action of the toxin compared to the baculovirus itself in killing the insect. The concave relationship of the engineered virus suggests that further reductions in time to death are likely to be matched by significant further reductions in yield. Whilst this may be considered a disadvantage for those situations in which secondary cycling is desirable, it is a positive feature from the point of view of risk assessment. Lower yields will put the modified virus at a disadvantage compared to the wild type, and will consequently be less likely to invade and establish in non-target populations.

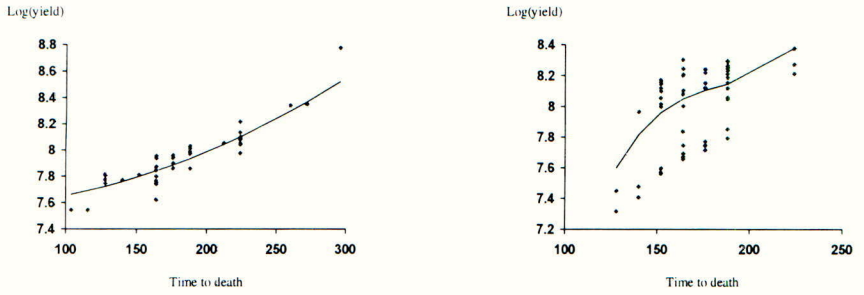

Figure 3: The relationship between log(yield) (predicted from weight) and time to death (hours) for a) AcNPV-ST3 in *M. brassicae*, at a concentration of 10^8 OBs per ml; b) AcNPV in *M. brassicae* at a concentration of 10^8 OBs per ml.

In summary, the trade-off between virulence (speed of kill) and yield (OBs produced per cadaver) can be manipulated both by selection of natural variants and by genetic modification, to control the nature of the interaction between the pest and the baculovirus.

REFUGES - SOURCES OR SINKS?

This section considers a third parameter of the basic Anderson and May model - persistence of the OBs in the external environment (embodied by μ, the inactivation rate of OBs). The life expectancy of the occlusion bodies may vary quite considerably, depending upon the microhabitat. Exposed to full sunlight, OBs can decay within a few hours (Cory & Bishop, 1993), but in more protected surroundings, such as the soil, OBs can persist from one season to the next - even up to several years (e.g. Jaques, 1974). However, the more protected surroundings tend to be those in which the insect hosts are not actively feeding, so whilst the virus may persist there, it is effectively removed from the cycle of infection.

Hochberg (1989) developed the basic Anderson and May model to examine the consequences of dividing the population of OBs (denoted W in Figure 1) into two parts: i) W - pathogen exposed on leaf surfaces, decaying moderately rapidly and available to be ingested by susceptible hosts; and ii) Q - pathogen in a refuge decaying rather more slowly, but unavailable to infect new hosts (Figure 4). The rate of translocation of pathogen between Q and W is crucial in determining the impact of the refuge on the host. If translocation from Q to W is high (ν is high), then time spent in the refuge is minimal and it is as if it doesn't exist.

Conversely, if translocation from Q to W is low (ν is low), then the refuge acts as a sink - effectively removing virus from the transmission cycle. At intermediate values, the refuge has maximum impact, acting as a buffer by re-introducing virus into the transmission cycle when pathogen densities are low, and storing pathogen when densities on the transmissible surfaces

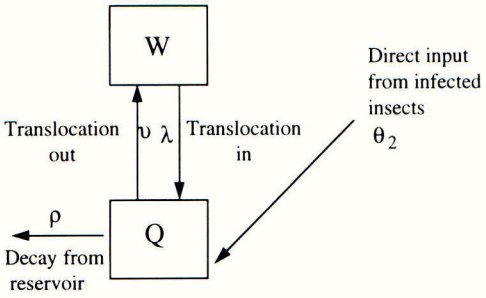

Figure 4: A model of a refuge (Q) and feeding surface (W), with the four parameters that influence the impact of the refuge. ν = rate of translocation from the refuge to the feeding surface; λ = rate of translocation from the feeding surface to the refuge; ρ = rate of decay of OBs in the refuge; θ_2 = input of OBs directly from infected insects on death.

are high. This buffering activity has an important stabilising influence on the pathogen-host interaction, and could also be responsible for the persistence of virus between seasons, and the initiation of epizootics when host densities build up.

This model makes an important strategic prediction. Increasing the flow of virus from established reservoirs to the habitat of the host should lead to lower and more constant populations. There are some observational data to support this - the best known example being the lepidopteran pasture pests *Wiseana spp.* (Crawford and Kalmakoff, 1977). The larvae succumb to an NPV, which accumulates in the upper layers of the soil. This is thought to form a reservoir, re-seeding the transmissible surfaces (grass swards) with OBs. If pastures are grazed then virus is transferred from refuge to feeding surface at a moderate rate, and pest densities are low and stable. However, there are frequently outbreaks after ploughing, or when grazing stops - which may be due to the cessation of translocation. This highlights another set of biological factors that should be measured and manipulated to assist in pest control. For example, it is possible that other invertebrates such as scavengers (e.g. Vasconcelos *et al.*, 1996) could act as passive vectors in moving virus.

Finally, this model also provides insights into those scenarios in which refuges of this nature are most likely to be influential. Larvae which succumb to the virus AcNPV-ST3 are typically paralysed before death, and consequently fall from the plant directly onto the soil surface. This effectively greatly raises the parameter θ_2 (input of OBs from infected insects directly into the reservoir), and consequently increases the range of values of ρ and ν which result in a buffering effect of the refuge. It is possible, therefore, for a soil refuge to be influential in the persistence and secondary cycling of such engineered viruses in crops. Management and manipulation of these reservoirs could be important in this context.

WHERE THE SIMPLE MODELS ARE WRONG

Both the Anderson and May (1981) and Hochberg (1989) models are simplistic in a number of respects. They are phrased in continuous time, and this results in a number of biological features being omitted: for example

- there is a time delay between infection and death of minimum duration
- the insect passes through a number of stages (eggs, instars, pupae) which differ in their susceptibility
- longer term dynamics may occur in a seasonal context, with generations being relatively synchronised within the season
- continuous time models frequently emphasise equilibrium conditions; but in the context of control, is equilibrium what we are really after?

It is instructive to incorporate such features of 'biological realism' into models to investigate how this alters the dynamics.

1. Stage structured and seasonal models

Insects are rarely susceptible to baculoviruses throughout their life cycle (as is assumed by placing all uninfected individuals in the S class in Figure 1). Therefore models have been developed in which the insect passes through a number of stages, some of which are susceptible and some resistant to infection. This was achieved using delay-differential equations, which allow specific time delays to be built in (Briggs & Godfray, 1995), including a time-delay between infection and death. The result is a model with rich and complex behaviour. In general, such models are less stable, and require additional features such as pathogen refuges to re-stabilise the dynamics.

The stage-structured model of Briggs & Godfray (1995) was then put into a seasonal context (Briggs & Godfray, 1996), with the host insect having one generation a year, and the pathogen going through one or two cycles of infection (depending upon the time taken to kill the host). The broad patterns produced are similar to those discussed above. The time lags are de-stabilising, and additional features are required to re-stabilise the interaction.

2. Non-equilibrium approaches

Perhaps the most relevant theoretical advances for biocontrol and biosafety have been in the variety of non-equilibrium models developed over the last few years. These include a number of spatial models. The division of the host population into a number of subpopulations loosely connected with each other was termed 'metapopulation dynamics'. The fate of the whole metapopulation is of greater interest than the fate of any individual subpopulations; if subpopulations were unstable, the whole metapopulation could still exhibit persistence. Local instability could therefore be turned into global stability (e.g. Hanski & Gilpin, 1991). Other models have then put these subpopulations into a spatial context, so that a subpopulation is only linked to those immediately around it. If the hosts and pathogens within a subpopulation follow a discrete time version of the Anderson and May model, and both hosts and pathogens disperse to adjacent patches between generations, the metapopulation exhibits a variety of patterns. Long term persistence of both host and pathogen can result, but White *et al.* (1996) found that chaotic spatial patterns could be produced. A related model by Wood and Thomas

(1996) predicted the persistence of virulent pathogens - which would have otherwise been driven to extinction in a spatially homogenous model.

Thus, these models predict the persistence of pathogens (and their hosts) without the necessity of a stable equilibrium. How can such conclusions contribute to the design of control strategies? The desired outcome of a control programme would be long term suppression or local extinction of a pest. The latter could be achieved by virulent (possibly modified) baculoviruses, but the corollary is that such virulent pathogens could indeed persist in other parts of the metapopulation. Whilst persistence is desirable from a control context (if the pest re-invades, the pathogen is ready and waiting), it is less desirable from a biosafety aspect (long term persistence of a modified virus could constitute a more serious threat to non-target populations). The development and parameterisation of such models will be a fertile area for future research - and will be vital for the most efficient and safe use of modified biopesticides.

CONCLUSIONS

The variation inherent in baculovirus populations, and the techniques to genetically modify these viruses, provide us with a number of potential control strategies. Theoretically it should be possible to manipulate the virus (either genetically or by the selection of variants) to control its position on the biopesticide - classical control continuum (Figure 5), depending upon the mixture of immediate and long term control required. It is largely true that theoretical ecologists have devoted more effort to the study of classical control, with its emphasis on stability and equilibrium, than on the dynamics of immediate control. This area has much to contribute to the investigation of the behaviour of natural and modified baculoviruses in non-target populations beyond the agricultural field. Little is known or understood about how baculoviruses persist in wild populations - yet clearly this area is vital for risk assessment.

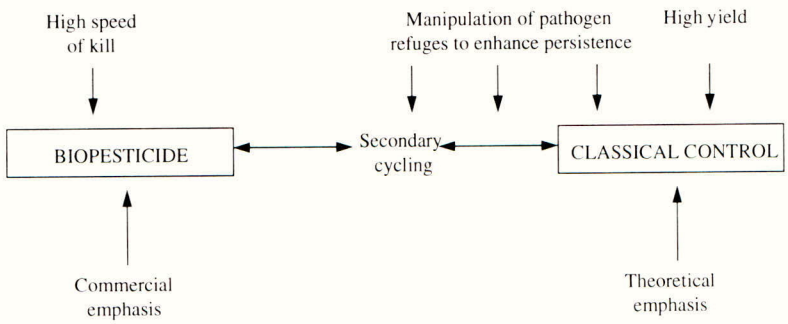

Figure 5: The biopesticide-classical control equilibrium

More recently, the theoretical emphasis has shifted towards non-equilibrium models. These provide a framework to investigate quite how long pathogens may persist in the environment (e.g. Godfray & Briggs, 1995) - even virulent ones, previously expected to 'burn out'. As is frequently the case in ecology, theoretical studies are far in advance of the empirical attempts to parameterise and test the models; with some notable exceptions (e.g. Thomas and Woods in this volume). This must be one area of future focus - both to realise the potential of baculoviruses and other pathogens in the control of insect pests, and to develop the adequate assessment of risk simultaneously.

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