CROP PROTECTION PROGRAMME

Evolution within *Bemisia tabaci* and associated Begomoviruses: A strategic modelling approach to minimise threats to sustainable production systems in developing countries.

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Executive Summary

The evolution, on time scales measured in years, of *Bemisia tabaci* and associated begomoviruses into new adapted types severely hampers the development of sustainable agriculture. This project aimed to provide a strategic analysis to identify potential consequences of pest and disease management measures on evolution within *B. tabaci* and begomoviruses in Forest Agriculture and High Potential Cropping systems.

We have shown that it is possible to elucidate the key effects of disease management on the evolution of begomoviruses. We have, for example, shown that it is possible to distinguish components of crop resistance that do or do not put a selection pressure on the virus to evolve more harmful strains. The components of resistance that do not put such a selection pressure on the virus are good candidate disease management methods for the development of sustainable agricultural systems. It has become evident that with further studies it should be possible to develop methods for the selection of resistant cultivars and to select crop and disease management methods that do not provoke the virus to evolve into more harmful variants.

We have shown that the evolutionary dynamics of *Bemisia tabaci* whiteflies caused by crop and pest management depends to a large extend on the life-history parameters of the whitefly that can undergo rapid evolutionary change. Although some information exists in this respect for other insect species, notably for aphids, virtually nothing is known about this key aspect in *Bemisia tabaci*. Research into evolving life-history traits in *Bemisia tabaci* should therefore be a key future research area.

A review of the literature identified that begomovirus genetic diversity is extremely complex and that there is still much to be answered. It is very likely that further insight in the molecular genetics will be available in the near future without special additional DFID commitment. There is however very little research done to elucidate the links between the molecular genetics of begomoviruses and the epidemiological characteristics of the diseases caused by these viruses. Our modelling studies on the evolution of begomoviruses have shown that this information is key to the further development of disease management strategies that do not provoke the virus to evolve into more harmful types.

In several meetings with researchers of the Tropical Whitefly IPM Project we have discussed our findings and discussed the use of these findings in future research. Especially the methods to distinguish components of resistance and to base a breeding programme around selection for resistance that does not provoke the evolution of more harmful virus strains seems a promising route.

I. General background

The development of sustainable cropping systems is of paramount importance for the elimination of poverty in tropical countries. In the longer-term only sustainable cropping systems can eliminate food shortages and benefit poor people as both producers and consumers. The evolution, on short time scales measured in years, of pest and disease species into new adapted types severely hampers the development of sustainable agriculture. The short-term evolution of *Bemisia tabaci* and associated begomoviruses is an important constraint to production in Forest Agriculture and High Potential cropping systems.

Bemisia tabaci, the main whitefly pest species in the tropics, severely reduces yields by direct damage through feeding and indirectly through the transmission of viruses. For example, yield losses of cassava in Africa were estimated to be 15-24% of total production, which amounts to a yearly loss of 1.3-2.3 billion US dollars (Thresh *et al.*, 1998). Yearly cotton losses in Pakistan were estimated to be 1 billion US dollars between 1992 and 1997 (Briddon & Markham, 2000). Comparable losses for sweet potato, tomato, cucumber, melon, and beans have been reported (Oliveira *et al.*, 2001). In the last two decades the world-wide upsurges in *B. tabaci* populations have resulted in an increase in a range of tropical and sub-tropical *B. tabaci* transmitted diseases. The *B. tabaci*-transmitted begomoviruses have become recognised as emergent pathogens in a range of agro-ecosystems world-wide.

Bemisia tabaci adapts readily to new host plants and regions (Oliveira *et al.*, 2001). Over 600 host species are presently known. Populations of *B. tabaci* are now found on all continents except Antarctica. Several biotypes have been described, which are by some authors considered to be cryptic species (Oliveira *et al.*, 2001). Evidence suggests that *B.tabaci* is a highly dynamic species-complex that is presently undergoing evolutionary change (Oliveira *et al.*, 2001). Little is known about the effect of *B. tabaci* control measures on evolution within the species complex. Any *B. tabaci* control measure will exert selection on the species-complex and may induce evolutionary change. We can only develop sustainable production systems with control strategies that do not provoke the evolution of more damaging biotypes/species. In light of the growing awareness of the highly dynamic nature of the species complex, strategic insight is needed in its evolutionary change and resulting consequences for whitefly dynamics, whitefly-transmitted virus epidemiology and sustainable disease management.

Equally, it is increasingly clear that the begomovirus grouping is a dynamic species-complex from which 'new' viruses 'emerge' (Brown, 2000). Dramatic examples of the emergence of new types are the recent epidemic in West Africa of cassava mosaic disease that started in the 1980s in Uganda (Legg, 1999) and cotton leaf curl in Pakistan and India (Briddon & Markham, 2000). Evidence suggests that the number of tomato-infecting begomoviruses increased from 3 in 1970 to 17 in the 1990s (Polston & Anderson, 1997). The effect of selection imposed by begomovirus management on the evolution of new virus types/ species is presently unknown. In the light of the growing awareness of the highly dynamic nature of the begomovirus complex, strategic insight is needed in its evolutionary change and resulting consequences for sustainable disease management.

In the Crop Protection Programme (CPP) call from which the project reported here emerged, it was stated that 'Much of the whitefly and whitefly transmitted virus disease management work in recent years has focused on specific problems in restricted geographical regions, and less attention has been given to strategic concepts of management.' In response the project reported on here has strengthened the Tropical Whitefly IPM Project with additional cross-cutting strategic approaches to whitefly/whitefly transmitted disease management. Various published reports and papers show that the evolution, on time-scales of years, of new whitefly biotypes and begomovirus strains severely hampers the development of sustainable Forest Agricultural (cassava) and High Potential (legumes or vegetable-based) cropping systems. In the project reported here we have thus addressed strategic issues concerning whitefly and begomovirus evolution.

The research hypotheses of the project are:

1. Pest and disease management are driving factors in the adaptive evolution of *B. tabaci* and begomiviruses.
2. It is possible to categorise crop management strategies that do or do not provoke adaptive evolutionary change of *B. tabaci* and begomiviruses.
3. It is possible to develop pest and disease management strategies that do not provoke the adaptive evolution of *B. tabaci* and begomoviruses into a direction that is more harmful to crop production.

II. Project Purpose

The aim of the project was to provide a strategic analysis to identify potential consequences of pest and disease management measures on short term evolution of *B. tabaci* and begomovirus in cropping systems in the developing countries. This strategic analysis involved four steps:

1. Develop and analyse models that elucidate the possible effects of begomovirus management on evolutionary changes. For this project purpose we studied the evolutionary effects of virus transmission through cuttings, and the evolutionary consequences of the introduction of resistant and tolerant cultivars.

1a. Virus evolution under the influence of resistance: A model was developed to analyse the effects of introducing a resistant cultivar on the evolution of the virus titre a virus builds up in a plant. We have distinguished four types of resistance/tolerance mechanisms.

1b. Evolution of viral plant diseases with two routes of infection: A model was developed for crops that are multiplied through the use of propagation material (as in cassava, sweet potato, plantain, etc.). The evolving virus trait used in this work is the within plant virus titre build up. The model has been analysed using analytical techniques from adaptive dynamics.

2. Develop and analyse models that elucidate the possible effects of *B. tabaci* management on evolutionary changes. A model was formulated and analysed to study the evolution of *B. tabaci* biotypes under the influence of (a) the introduction of a new crop or new crop variety, (b) the use of pesticides, and (c) the structure of the cropping

system, comparing large scale monocultures with intercropping. Three life-history traits, that might undergo evolution, were studied: (a) the feeding rate of the whitefly on the crop species/varieties, (b) the efficiency of conversion of food into offspring, and (c) the death rate of the whitefly on each crop.

3. Develop an overview of existing knowledge and information on recent *Bemisia tabaci* and begomovirus evolutionary changes. An overview was prepared of the known changes in *B. tabaci* and begomivirus populations with special emphasis on changes that can be attributed to adaptive evolution of the species involved. This review also reveals the possible evolutionary preasure on *B.tabaci* and begomoviruses due to pest and disease management world-wide.

4. Transfer the knowledge generated, and further research needed, to research teams and CPP management. In several meetings with researchers of the Tropical Whitefly IPM Project we have discussed our findings and discussed the use of these findings in future research. During a visit of one of the team members to CIAT, Colombia, extensive discussions have taken place on the research findings. During the IX International Plant Virus Epidemiology Symposium to be held in Lima, Peru on April 4-7, 2005, a special session will be held to discuss the work further and develop future research plans.

III. Research Activities and Outputs

For the project purposes the research activities and outputs will be discussed in detail in this section. The purposes discussed are:

1a. Virus evolution under the influence of resistance
1b Evolution of viral plant diseases with two routes of infection
2. Models that elucidate the possible effects of B. tabaci management on evolutionary changes
3. An overview of existing knowledge.

To avoid confusion each of these output-sections, 1a, 1b, 2 and 3, start with equation and figure numberings from 1.

III. 1a. Virus evolution under the influence of resistance

Plant viruses are a major limiting factor in agricultural production (Thresh, 1980; Waterworth & Hadidi, 1998). Plant viruses are a major threat to agricultural production, especially in lesser developed countries, and seriously hamper the elimination of poverty (Rybicki & Pietersen, 1999; Thresh, 1983; Fereres *et al.*, 2000). Despite efforts to manage plant virus diseases, the problems with several virus groups are increasing, especially in the developing world (Rybicki & Pietersen, 1999). A well studied example is the diseases caused by the whitefly transmitted geminiviruses. Several, seemingly new, diseases caused by members of this virus group, and many new strains associated with previously known diseases have emerged over the past decades (Brown, 1990; Polston & Anderson, 1997; Padidam *et al.*, 1999; Mansoor *et al.*, 2003; Varma & Malathi, 2003). The reason for the emergence of new strains is thought to be the rapid evolutionary changes of the virus due to large

population sizes, the potential for rapid genetic change and short generation times (Padidam *et al.,* 1999; Varma & Malathi, 2003).

For most viral plant diseases there are very few direct means of control such as exist for many diseases caused by fungi and bacteria. The available disease management options include the organisation of agricultural practice to reduce disease, the use of cultural control such as sanitation programmes, the control of the vector population through the use of insecticides, and the breeding and growing of resistant crop cultivars. Of these methods vector population control has often been found to be difficult (Perring *et al*., 1999; Satapathy, 1998). Sanitation, in the form of roguing, and the use of resistant crop cultivars has in many cases been effective (Holt & Chancellor, 1996; Holt *et al.*, 1997, 1999; Jeger, 2004). Much effort has, for example, been put into various programmes to breed for resistance of crops to geminiviruses (Rubio *et al,*. 2003; Morales, 2001; Lapidot & Friedmann, 2002; Belliot & Arias, 2001; Thresh *et al.,* 1994). Any disease management effort will, however, put a selection pressure on the virus population to adapt to the new circumstances. Given the rapid evolutionary changes possible in virus species it is no surprise that disease control initially effective is sometimes quickly rendered ineffective due to adaptation of the virus. Well known examples are the evolutionary changes that overcome crop resistance.

Much research, both experimental and theoretical, is being done to aid the development of effective control programmes. Mathematical model are often used to study the effects of plant, virus and vector characteristics on the development of disease epidemics (Jeger *et al*., 1998, 2002; Madden *et al.*, 2000; Zhang *et al.*, 2000a, 200b; Chan & Jeger, 1994; Holt & Chancellor, 1996). Some of these models are of a generic nature targeted at the elucidation of general principles. For example Jeger *et al.* (1998) and Madden *et al.* (2000) study the effect of different modes of virus transmission by the vector on epidemic development, and Jeger *et al.* (2002) study the epidemiology of cropping systems with replanting form a plant nursery. On the other end of the scale we find several models developed to study the effect of disease management measures on the control of particular virus epidemics (Fargette & Vie, 1995; Holt & Chancellor, 1996; Holt *et al.*, 1997; Bertschinger, 1997; Jeger & Thresh, 1993). These models have provided valuable insight and have helped in the development of disease control programmes.

Despite their usefulness in the development of disease control programmes, one of the significant shortcomings of these mathematical models is that they all ignore the evolutionary dynamics of the virus, and, as described above, this evolutionary dynamics can have a major impact on disease control. The aim of this chapter therefore is to make a first approach to the development of models to study disease management methods for viral plant diseases that do take the evolutionary response of the virus to the management measures into account. Our approach will use the theory of Evolutionary Stable Strategies (ESS) as introduced by Maynard Smith in the mid-1970th (see Maynard Smith, 1982). ESS is a strategy such that if a resident population engages in an evolutionary stable strategy it cannot be invaded by a population with a different strategy. A further development of this idea is the continuous stable strategy (CSS), which is defined as an ESS that can be proven to be the endpoint of evolution under the circumstances studied (Eshel, 1983). This approach has been used in many

evolutionary studies and applied widely to study animal disease epidemiology (Dieckmann *et al*., 2002 and references therein).

In this chapter we will restrict our attention to the possible evolutionary consequences of the use of resistant and tolerant crop cultivars on the evolution of the virus; and conversely the effect of virus evolution on the epidemiology of the disease. A recurrent problem in the literature on breeding for resistance to virus diseases is the lack of standard terminology used by different researchers. This is most apparent in the difference in terminology between plant pathologists and plant breeders, but even within each group of researcher terminology might differ. In this chapter we will introduce three types of resistance and one of tolerance. We will define each at the level of the process parameters they affect, and our definitions should be seen as operational definitions for the purpose of this chapter.

We will restrict our attention to one evolving virus trait only, the multiplication rate of the virus within an infected cell. (It is good to note here immediately that our conclusions will thus only be valid for the evolution of this trait.) It is well known for begomoviruses that the within cell virus multiplication rate is a trait under evolution and therefore be a useful starting point.

The development and analysis will involve a number of steps that need to be combined. These are:

1. Virus-vector population model. A model for the epidemiology of the virus disease has to be formulated. We will use a simplified variant of a well studied plant-virus-vector model (Jeger *et al*., 1998; Madden *et al.,* 2000).

2. Within-plant virus dynamics. A model for the dynamics of the virus within the plant has to be formulated. We will use the model introduced by Nowak & Bangham (1996) and Bonhoeffer *et al.,* (1997) as it incorporates the essential features of virus dynamics, although no detailed account is given of the replication process.

3. Relation between within-plant dynamics and population dynamic parameters. The two models have to be combined in the sense that we have to describe how the parameters defined in the epidemic model depend on within-plant dynamics of the virus.

4. Resistance and tolerance of cultivars. The next step is to specify the nature of a resistant or tolerant crop. Resistance/tolerance of a crop will be expressed in terms of a change in specific parameter values. We will introduce four different types of resistance/tolerance, each changing a specific set of parameters.

5. Evolutionary stable strategy. The evolutionary dynamics is then described. To this end we will use existing knowledge on the calculation of evolutionary stable strategies (Maynard Smith, 1982). The ESS of the within-plant virus multiplication rate will be calculated for all four types of resistance/tolerance.

6. Results. We will compare the evolutionary stable virus multiplication rate, the associated titre the virus builds up in the plant and the density of healthy plants before and after the evolution of the virus has taken place.

Although our model is of a generic nature will relate our findings to the evolution of begomoviruses and in the discussion compare the types of resistance we define with the specific system of tomato yellow leaf curl (TYLC). This name was given to a complex of begomoviruses that cause a disease in tomato in the Mediterranean basin, tropical Africa, South-east Asia, North America, South America and the Caribbean region which can, if not controlled, have devastating consequences including total

crop loss (Moriones & Navas-Castillo, 2000). Both experimental and modelling approaches are being used to study the epidemiology of this disease and to aid the development of control programmes (e.g. Holt *et al*., 1999). One of the key findings of the theoretical study of Holt *et al.* (1999) was that 'varietal resistance can be an important component of disease management' (Jeger *et al.*, 2004). As discussed above this finding was based on a model that does not include the evolutionary consequences of use of the resistant variety. The breeding of resistant tomato cultivars is presently the subject of much research (Vidavsky & Czosnek, 1998; Lapidot *et al.*, 2001; Murathi *et al*., 2003a,b; Gomez *et al.*, 2004; Michelson *et al.*, 1994; Pietersen & Smith, 2002). Therefore this specific example forms a suitable illustration of the conclusions and will be discussed.

In the following the numbered sections **1** though **5** refer to the above steps in the development of the work; **(i)** through **(iv)** refer to the four types of resistance/tolerance studied.

The model and the evolutionary stable strategy

1. Virus-vector population model

A graphic representation of the model is given in Fig. 1.

The plant population: The density of healthy and infectious plants is denoted by $H(t)$ and $I(t)$, respectively. The density of healthy plants increases due to planting and decreases due to harvest and inoculation of the plants with the virus by the vector. The density of infectious plants increases due to inoculation of the healthy plants and decreases due to harvest and mortality due to the virus infection. This mortality due to the virus infection includes both mortality directly caused by the virus and mortality due to roguing of visibly infected plants. These assumptions lead to the system of differential equations

$$\begin{aligned} \frac{dH(t)}{dt} &= \text{planting rate} - \text{harvest rate}_H - \text{inoculation rate} \\ \frac{dI(t)}{dt} &= \begin{bmatrix}\text{rate of becomming}\\ \text{infectious}\end{bmatrix} - \text{harvest rate}_I - \text{death rate} \end{aligned} \tag{1}$$

The planting rate is assumed constant, σ, and the harvest rate is denoted by *h*, giving the terms

$$\text{planting rate} = \sigma, \quad \text{harvest rate}_H = hH(t), \quad \text{and harvest rate}_I = hI(t) \tag{2}$$

The planting and harvest processes can also be written as $\sigma(1-H/K)$, where $K=h/\sigma$, as used in Jeger *et al.* (1999) and Madden *et al.* (2000). We assume that there is a constant probability ϕ per time unit that an infectious plant is removed from the system due to the disease, either being rogued or dying from the disease, giving

$$\text{death rate} = \phi I(t) \tag{3}$$

There is a constant per capita inoculation rate α and the infection term takes the form

$$\text{inoculation rate} = \alpha H(t) Z(t) \tag{4}$$

where $Z(t)$ is the density of viruliferous vectors (i.e. those who have aquired the virus and can transmit the virus to other plants).

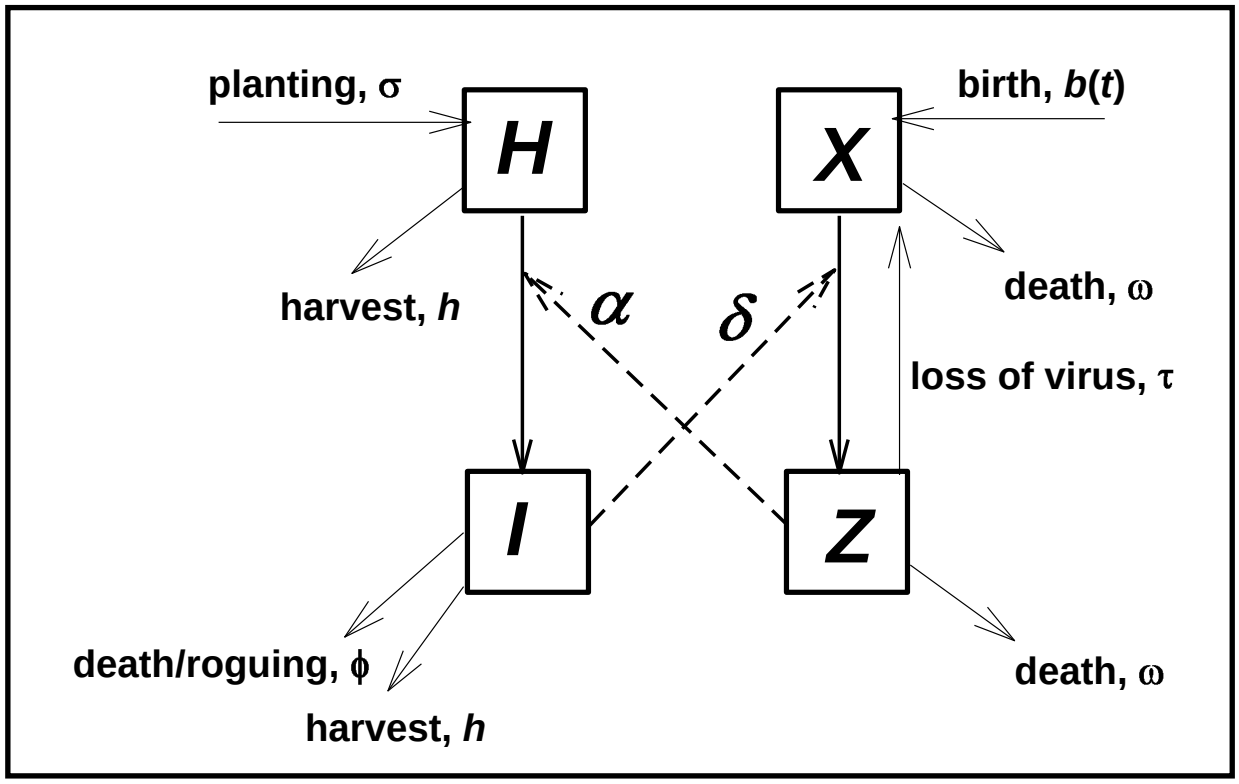


Figure 1: Graphical representation of the virus vector model. Boxes are the state variables, density of healthy plants, *H*, density of infectious plants, *I*, density of non-viruliferous vectors, *X*, density of viruliferous vectors, *Z*.

Substituting equations (2) to (4) into (1), the model describing the plant population takes the form

$$\begin{aligned} \frac{dH(t)}{dt} &= \sigma - hH(t) - \alpha H(t)Z(t) \\ \frac{dI(t)}{dt} &= \alpha H(t)Z(t) - hI(t) - \phi I(t) \end{aligned} \tag{5}$$

The vector population: The density of vectors increases due to birth of vectors and decreases due to vector mortality. Further, the density of non-viruliferous vectors, $H(t)$, decreases due to acquisition of the virus by individuals feeding on infectious plants and increases due to viruliferous vectors loosing the virus. The density of viruliferous vectors, $Z(t)$, increases due to non-viruliferous vectors acquiring the virus and decreases due to viruliferous vectors losing the virus. It is assumed here that there is no vertical transmission of the virus to vector progeny. This leads to

$$\begin{aligned} \frac{dX(t)}{dt} &= \text{birth} - \text{death}_X - \text{aquisition rate} + \text{loss rate} \\ \frac{dZ(t)}{dt} &= \text{aquisition rate} - \text{death}_Z - \text{loss rate} \end{aligned} \tag{7}$$

For now we denote the birth rate by the general function $b(t)$. Each insect vector has a probability ω per time unit to die. This gives us

$$\text{birth rate} = b(t), \quad \text{death rate}_X = \omega X(t), \text{ and } \quad \text{death rate}_Z = \omega Z(t) \tag{8}$$

The viruliferous vector has a probability τ per time unit to lose the virus, which gives

$$\text{loss rate} = \tau Z(t) \tag{9}$$

implying that the mean viruliferous period of the vector equals $1/\tau$. For the acquisition rate we use a similar term as for the rate at which plants become infected, which gives

$$\text{aquisition rate} = \delta X(t) I(t) \tag{10}$$

The model equations for the dynamics of the vector population thus are

$$\begin{aligned} \frac{dX(t)}{dt} &= b(t) - \omega X(t) - \delta X(t) I(t) + \tau Z(t) \\ \frac{dZ(t)}{dt} &= \delta X(t) I(t) - \omega Z(t) - \tau Z(t) \end{aligned} \tag{11}$$

We assume the total vector population to be of constant size, implying $X(t)+Z(t)=P$. Taking the derivative with respect to time yields

$$\frac{dX(t)}{dt} + \frac{dZ(t)}{dt} = 0 \tag{12}$$

Substituting the equations (11) into (12) we find that $b(t)=\omega X(t)+\omega Z(t)=\omega P$. This assumption leads to a simplifying consequence: one of the vector equations has become redundant because $X(t) = P-Z(t)$, and we only retain the equation for the density of viruliferous vectors as

$$\frac{dZ(t)}{dt} = \delta (P - Z(t)) I(t) - \omega Z(t) - \tau Z(t) \tag{13}$$

Equations (5) and (13) are a simplified version of the plant-virus-vector model introduced and analysed by Jeger *et al.* (1998, 2004) and further studied by Madden *et al*. (2000). We refer the reader to these papers for details on the analysis and the dynamics of the system. Here we only state, without proof, those results needed here.

When the basic reproductive number, R_0, is larger than unity the disease can invade the system of healthy plants. The basic reproductive number is given by

$$R_0 = \left(\frac{\alpha \hat{H}}{\omega + \tau} \right) \left(\frac{\delta P}{h + \phi} \right) \tag{14}$$

where $\hat{H} = \sigma/h$, is the density of plants in the absence of the disease. When $R_0>1$ the density of healthy plants, infectious plant and viruliferous vectors develops towards a stable steady state H^*, I^*, Z^*. This steady state is given by

$$H^* = \frac{\sigma}{h} - \frac{h+\phi}{h} I^*; \; Z^* = \frac{\delta P I^*}{\delta I^* + \omega + \tau}; \; I^* = \frac{\alpha \delta P \frac{\sigma}{h} - (h+\phi)(\omega+\tau)}{\alpha \delta P \frac{h+\phi}{h} + \delta (h+\phi)} \tag{15}$$

2. Within-plant virus dynamics

A graphic representation of the model is given in Fig. 2.

The density of uninfected cells, infected cells and free virus particles will be denoted by $U(t)$, $V(t)$ and $W(t)$, respectively. With 'free virus particles' we denote the viruses in the cytoplasm. Note that the density of free virus particles is equivalent to the virus titre in the plant. The density of uninfected cell increases due to a constant production rate λ and decreases due to mortality and infection by a free virus particle.

The density of infected cells increases due to infection of uninfected cells and decreases due to cell death. The number of free virus particles increases due to the production of new virus particles in infected cells. Further free virus particles are removed from the system at a constant rate. This leads to the system

$$\frac{dU(t)}{dt} = \text{cell production} - \text{cell death}_U - \text{infection}$$
$$\frac{dV(t)}{dt} = \text{infection} - \text{cell death}_V \qquad (16)$$
$$\frac{dW(t)}{dt} = \text{virus multiplication} - \text{removal}_W$$

Assuming a constant cell production rate, λ, and a constant per capita uninfected cell death rate, d, a constant per capita infected cell death rate, a, and constant per capita virus particle removal rate, u, we have

$$\text{cell production} = \lambda, \ \text{cell death}_U = dU, \ \text{cell death}_V = aV, \text{ and}$$
$$\text{removal}_W = uW \qquad (17)$$

The infection of uninfected cell with free virus particles follows a linear response with contact rate parameter β, which gives

$$\text{infection} = \beta UW \qquad (18)$$

Finally we assume a constant per infected cell virus production rate k, and have

$$\text{virus production} = kV \qquad (19)$$

Substituting equations (17), (18), and (19) into (16) yields as our model equations

$$\frac{dU(t)}{dt} = \lambda - dU - \beta UW$$
$$\frac{dV(t)}{dt} = \beta UW - aV \qquad (20)$$
$$\frac{dW(t)}{dt} = kV - uW$$

This model of within-plant virus dynamics is discussed in detail in Nowak & Bangham (1996) and Bonhoeffer *et al.*, (1997). We refer the reader to these publications for details and will state, without proof, the aspects of the dynamics of this model needed in this chapter.

When the within-plant basic reproductive number, $\beta\lambda k/adu$, is larger than unity the virus can develop a population in the plant after it has been introduced by a viruliferous vector. The densities of uninfected cells, infected cells and free virus particles develop towards a stable steady state value. In steady state the ratio of infected to healthy cells, V^*/U^*, is given by

$$\frac{V^*}{U^*} = \frac{\beta}{a} W^* \qquad (21)$$

For large within-plant basic reproductive number, the steady state free virus particle density is approximately

$$W^* = \frac{\lambda}{au} k \qquad (22)$$

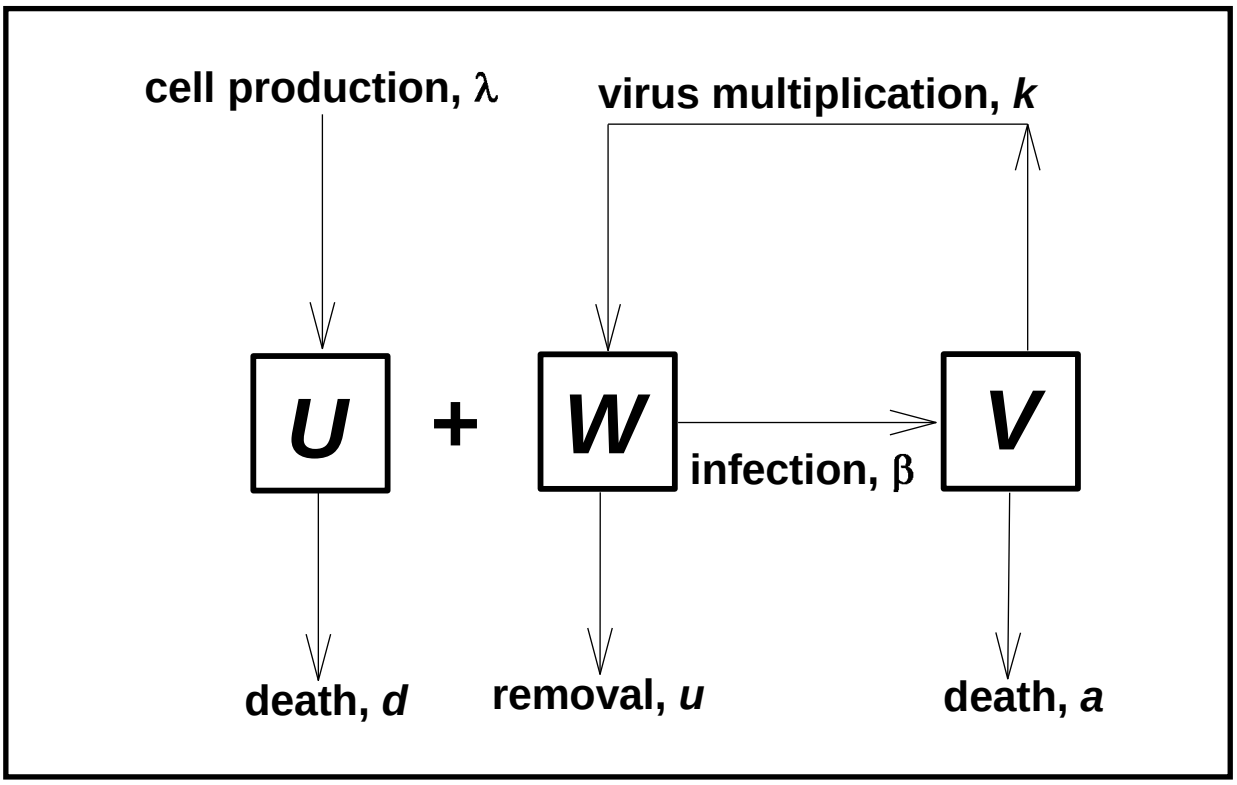


Figure 2: Schematic illustration of the within-plant virus dynamics. Boxes are state variables, *U* is the density of uninfected cells, *W* is the density of free virus particles, and *V* is the density of infected cells.

Note that equation (22) defines the relation between virus multiplication rate, *k*, and within-plant virus titre.

The evolving trait. As mentioned in the introduction we restrict our attention to the evolution of the within-plant virus multiplication rate *k*. In the discussion section we will discuss possible trade-offs, biological attainable maximum values for *k* and speculate about its effects on the results.

3. Relation between within-plant dynamics and population dynamic parameters

Crop planting rate, σ, harvest rate, *h*, insect density, *P*, insect mortality rate, ω, and the mean viruliferous period of a vector, $1/\tau$, are independent of the dynamics of the virus within the plant. We will discuss the relation between within-plant virus dynamics and the population level parameters acquisition rate, δ, inoculation rate, α, and the plant mortality rate, ϕ.

Insect vectors acquire the virus while feeding in the plant phloem. When the density of free virus particles in the plant is low the probability that the vector picks up the virus while feeding will be low, the probability to acquire the virus during a feeding period will increase with free virus particle density and level off at unity for very high virus titre. We thus model the relation between acquisition rate parameter δ and the virus titre as

$$\delta(k) = \theta \frac{\psi W^*}{1 + \psi W^*} = \theta \frac{\psi \frac{\lambda}{au} k}{1 + \psi \frac{\lambda}{au} k} := \theta \frac{Ak}{1 + Ak} \quad (23)$$

where ψ is the shape parameter and $A=\psi\lambda/au$.

We will assume here that the inoculation rate parameter, α, is a constant, independent of the density of free virus particles in the plant where the vector acquires the virus. For non-persistent (stylet borne) and circulative viruses this assumption amounts to assuming that the number of virus particles picked up by the vector is constant. This assumption is a reasonable first approach as most recent information suggests that the virus particles initially attach to specific binding sites on the stylet, making the number of particles to be limited to the number of binding sites. For persistently transmitted propagative viruses the number of virus particles available for inoculation depends, besides the number picked up, on the reproduction rate within the vector. Though these assumptions seem reasonable we will show in the discussion section that this assumption can be relaxed without a change in the qualitative results.

Plants with a high ratio of infected to healthy cells will have a larger probability to die due to the disease. Moreover symptom severity is related to virus titre (e.g. Rubio *et al*., 2003) and thus symptom severity will be higher in plants with a high V^*/U^* ratio, which makes them more prone to roguing sanitation. The infected plant mortality rate, ϕ, will thus increase with V^*/U^*. Due to lack of quantitative information on this aspect we take a linear relation, giving

$$\phi(k) = \eta \frac{\beta}{a} W^* = \eta \frac{\beta \lambda}{a^2 u} k := Bk \quad (24)$$

where $B=\eta\beta\lambda/a^2u$.

4. Resistant and tolerance of cultivars

Resistance and tolerance of a crop against a virus disease can be expressed in several of the parameters of the virus-vector population model and the within-plant virus dynamics model. We introduce four resistance parameters, ε, each relating to a specific mechanism. Each resistance parameter, ε, will be introduced such that for a value $\varepsilon=1$ the cultivar shows no resistance expressed through this particular mechanism and a value $\varepsilon=0$ implies the full extent of resistance. Note that we do not advocate here that a resistant cultivar will have a decreased value of one of these parameters only. Resistant cultivars can in theory have any combination of these resistance parameters. We will here, however, separate each of these mechanisms to be able to study the contribution of each mechanism alone, to the evolution of the virus, and subsequent effects on the effectiveness of the resistant cultivar.

(i) *Inoculation resistance*, relates to a reduction of the inoculation parameter, α, in the virus-vector population model. Introducing the resistance parameter we write

$$\alpha = \varepsilon_\alpha \tilde{\alpha} \quad (26)$$

(ii) *Acquisition resistance*, relates to a reduction of the acquisition rate parameter, δ, in the virus-vector population model. Introducing this resistance parameter we write

$$\delta = \varepsilon_\delta \theta \frac{Ak}{1 + Ak} \quad (25)$$

Note that this type of resistance does not give a smaller acquisition rate due to lower within-plant virus titre, but means that the virus, for equal virus titres, is acquired less easily from the resistant cultivar by the vector.

(iii) *Virus titre reducing resistance*. Some resistant cultivars are known to have reduced virus titre. The mechanisms by which the virus titre is reduced are largely unknown. We therefore model this type of resistance in the simplest way by assuming that the steady-state free virus particle density, W^*, is reduced by a factor ε_W. Substituting this assumption into equations (21) and (22) and subsequently in (23) and (24) we find

$$\delta = \theta \frac{\varepsilon_W A k}{1 + \varepsilon_W A k} \qquad (27)$$

$$\phi = \varepsilon_W B k$$

(iv) *Tolerance*. A cultivar which expresses tolerance is characterised by having the same virus titre as a non-tolerant cultivar, but with a lower symptom severity. This implies that acquisition and inoculation remain the same but the mortality/roguing rate, ϕ, is reduced by a factor ε_ϕ because roguing rates and plant mortality due to the virus infection will be smaller. We thus have

$$\phi = \varepsilon_\phi B k \qquad (28)$$

In the analysis of the evolution of the virus we use the most general form involving all four types of resistance and write

$$\alpha = \varepsilon_\alpha \tilde{\alpha}, \quad \delta = \varepsilon_\delta \theta \frac{\varepsilon_W A k}{1 + \varepsilon_W A k}, \text{ and } \phi = \varepsilon_\phi \varepsilon_W B k \qquad (29)$$

5. Evolutionary stable strategy and continuously stable strategy

An evolutionary stable strategy (ESS) is defined as a strategy such that when the entire population consists of individuals with that strategy, no other strategy can invade (Maynard Smith, 1974). In the model developed here this means that the ESS virus multiplication rate, k_{ESS}, is that value of k that, if the viruses in the population have k_{ESS}, it can not be invaded by viruses with another value of k. For models of the type we use in this chapter it has been shown that the ESS value of k is also the value of k for which the basic reproduction number (14) attains its maximum value (Claessen & de Roos, 1995; van Baalen & Sabelis, 1995). (Note: it is very well known that in many cases evolution does not maximise the basic reproductive number nor the exponential rate of population increase (Dieckmann *et al.*, 2002). That the basic reproduction number is maximised in the present model is convenient but does not apply more generally.)

We will here only briefly describe the reasoning behind this result and refer the interested reader to the extensive literature on evolutionary stable strategies (Dieckmann *et al.*, 2002 and references therein). Assume that the resident population has a virus multiplication rate k_{res}. According to equation (15) the steady state density of healthy plants then is given by

$$H(k_{res}) = \frac{\sigma}{h} - \frac{h + \phi(k_{res})}{h} \frac{\alpha \delta(k_{res}) P \frac{\sigma}{h} - [h + \phi(k_{res})][\mu + \tau]}{\alpha \delta(k_{res}) P \frac{h + \phi(k_{res})}{h} + \delta [h + \phi(k_{res})]} \qquad (30)$$

Assume that the invader has multiplication rate k_{inv}. The basic reproduction number, $R_0(k_{inv}, k_{res})$ of the invader in the resident population then is given by

$$R_0(k_{inv}, k_{res}) = \left(\frac{\alpha H(k_{res})}{\omega + \tau}\right)\left(\frac{\delta(k_{inv})P}{h + \phi(k_{inv})}\right) \tag{31}$$

because the density of healthy plants is set by the resident virus strain with a multiplication rate k_{res}. The invader will die out if $R_0(k_{inv}, k_{res})<1$ and will invade if $R_0(k_{inv}, k_{res})>1$, whenever $k_{inv}=k_{res}$ the $R_0(k_{inv}, k_{res})=1$. We are looking for a resident virus multiplication rate k_{res} such that no mutant invaders will invade, meaning $R_0(k_{inv}, k_{res})<1$ for all k_{inv} except for $k_{inv}=k_{res}$ where $R_0(k_{inv}, k_{res})=1$. This k value can only be a k value that maximises (31), and since the k value at which (31) attains its maximum is independent of k_{res} we find k_{ESS} as the maximum of the expression $\delta(k_{inv})P/(h+\phi(k_{inv}))$. Since the position of a maximum does not change when a function is multiplies with a constant, this implies that the evolutionary stable virus multiplication rate, k_{ESS}, can be found as the value of k that maximises

$$R_0(k) = \left(\frac{\alpha \tilde{H}}{\omega + \tau}\right)\left(\frac{\delta(k)P}{h + \phi(k)}\right) \tag{32}$$

where $H = \sigma/h$. Without further proof we note here that the k value that maximises the basic reproduction number (32) is also the continuously stable strategy in this model, ensuring that the virus population will evolve towards this k_{ESS} value.

Results

In this section we will first calculate the evolutionary stable virus multiplication rate and derive the first conclusions on the effect of planting resistant crops. We then turn to the effect of introducing a resistant crop and what it does to the density of healthy plants before the virus has had time to adapt to the new circumstances. Finally we will consider the situation that develops after the virus has evolved to the evolutionary stable state as set by the new circumstances.

Evolutionary stable strategy

Substituting (29) in (32) and calculating the maximum we find that the ESS value of k is given by

$$k_{ESS} = \frac{1}{\varepsilon_W}\frac{1}{\sqrt{\varepsilon_\phi}}\sqrt{\frac{\sigma\, a^3 u^2}{\eta \beta \lambda^2 \psi}} \tag{33}$$

Equation (33) leads to our first important results. This expression shows that the ESS virus multiplication rate is *independent* of the resistance parameters ε_α and ε_δ. This means that planting resistant cultivars with inoculation resistance and acquisition resistance will not put any selection pressure on the virus to evolve a different within cell multiplication rate, k. Virus titre reducing resistance, ε_W, and tolerance, ε_ϕ, clearly do put a selection pressure on the virus to increase its within cell multiplication rate.

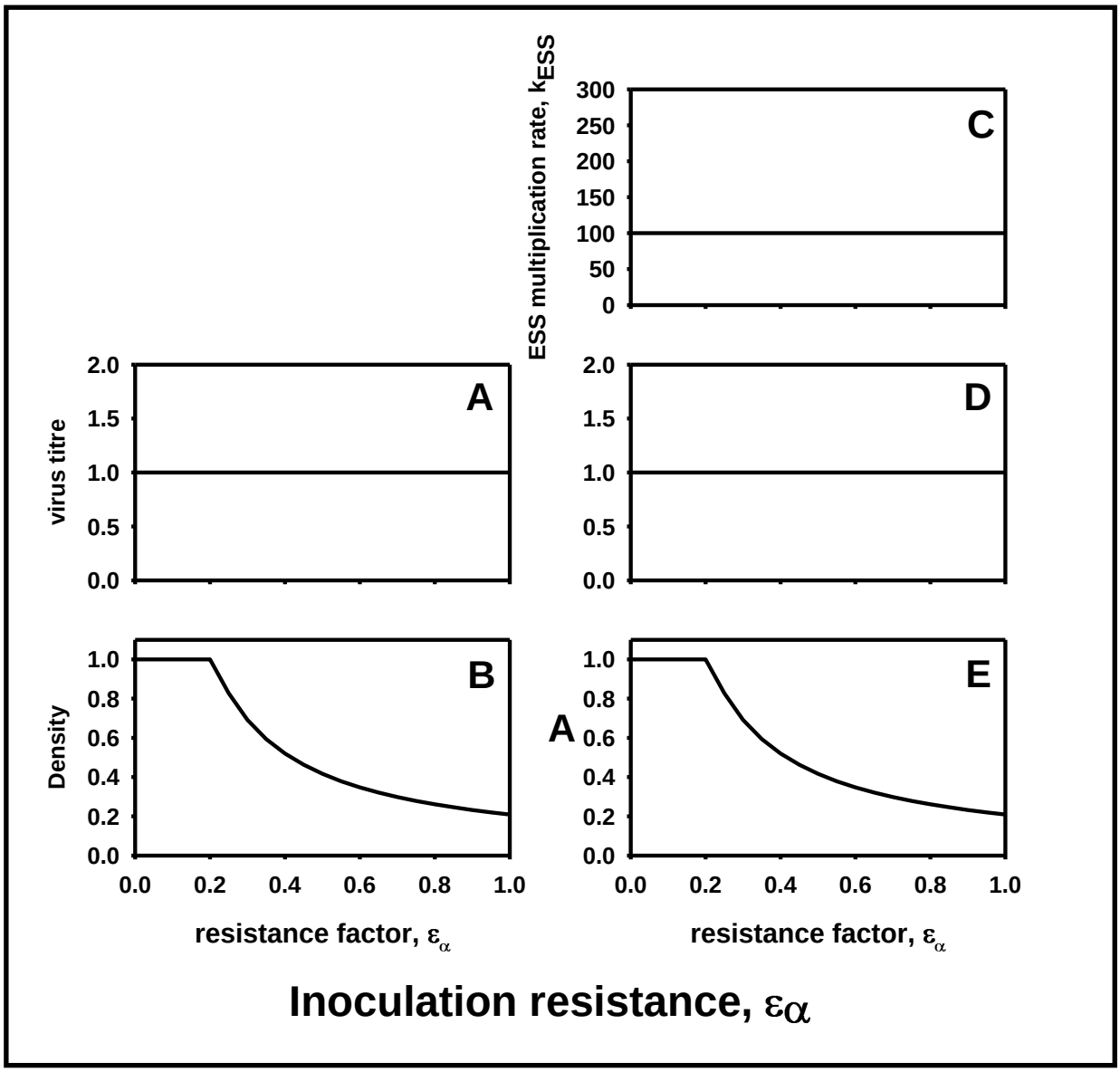


Figure 3: The effect of inoculation resistance on virus multiplication rate, graph C, within-plant virus titre, graphs A and D, and the density of healthy plants, graphs B and E. Default parameter valuesn used are α=0.008, σ=0.003, *h*=0.003, *P*=50, ω=0.12, τ=0.1, θ=0.016, A=3*10^{-5}, B=0.01.

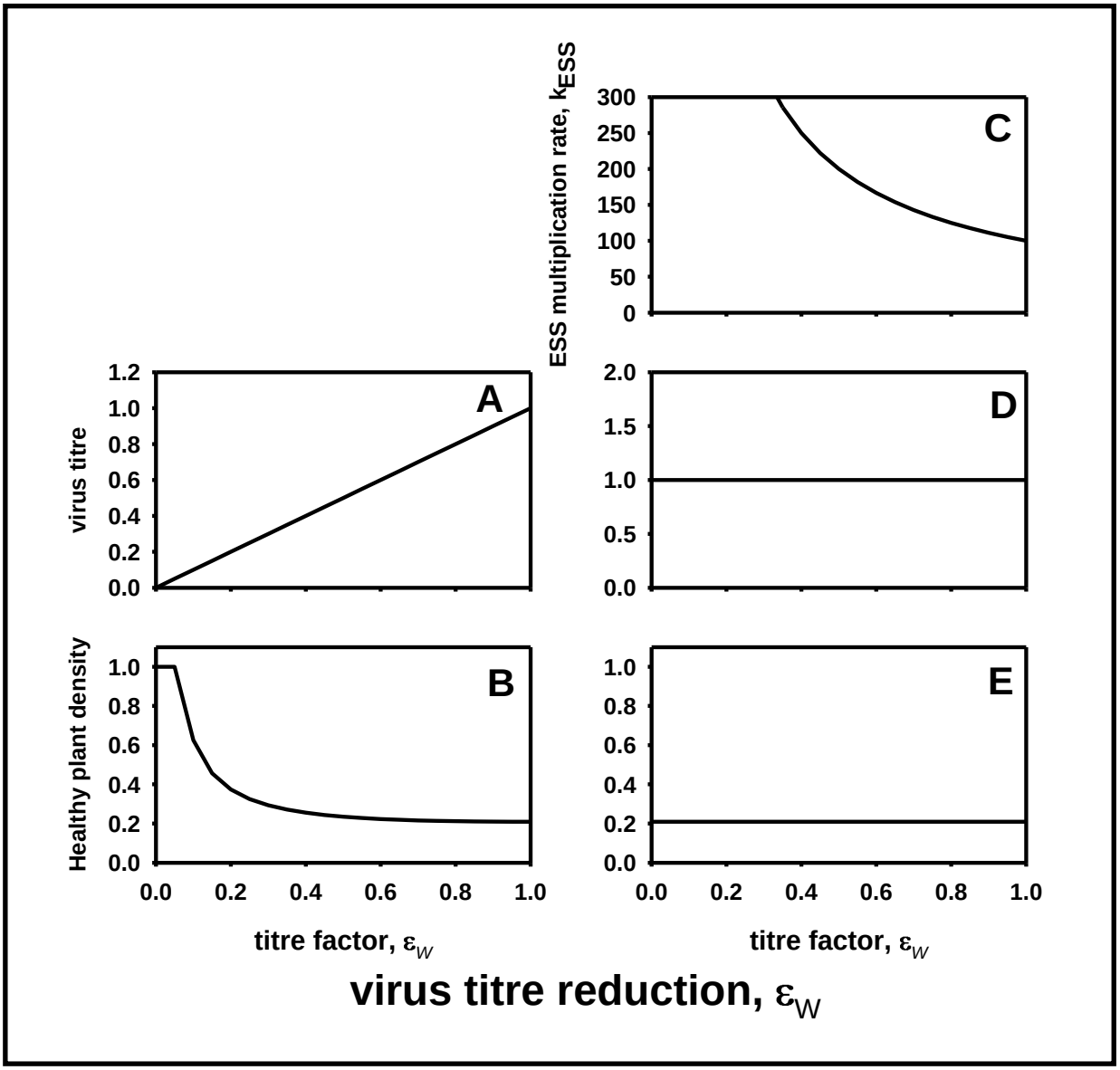


Figure 4: The effect of virus titre reducing resistance on virus multiplication rate, graph C, within-plant virus titre, graphs A and D, and the density of healthy plants, graphs B and E. Default parameter valuesn used are α=0.008, σ=0.003, h=0.003, P=50, ω=0.12, τ=0.1, θ=0.016, A=3*10^{-5}, B=0.01.

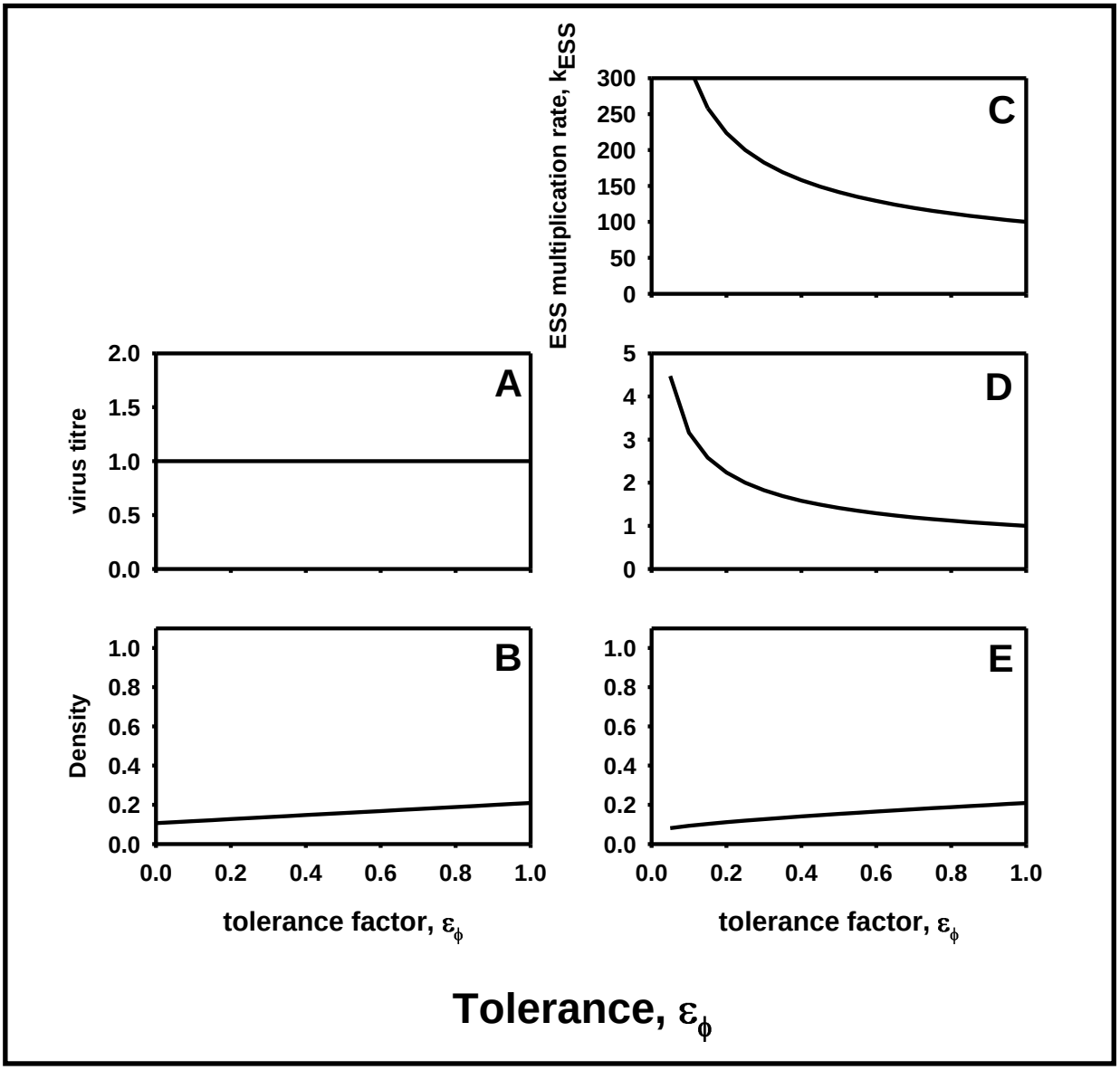


Figure 5: The effect of tolerance on virus multiplication rate, graph C, within-plant virus titre, graphs A and D, and the density of healthy plants, graphs B and E. Default parameter valuesn used are α=0.008, σ=0.003, *h*=0.003, *P*=50, ω=0.12, τ=0.1, θ=0.016, A=3*10^{-5}, B=0.01.

At introduction of the resistant cultivar

When the resistant cultivar is introduced and the virus has not yet responded to this change in its environment, the within cell virus multiplication rate is the default value. In this situation we can calculate the virus titre, equation (22), and the steady state density of healthy plants, equation (15), as related to the level of resistance as expressed in the resistance parameters ε. For the interpretation of the figures note again that the resistance parameters ε are defined such that ε=1 equals the non-resistant cultivar and ε=0 the maximally resistant cultivar. The virus titre as function of the resistance parameter ε is depicted in Fig. 3 through 6 A, and the density of healthy plants in this situation is given in Fig. 3 through 6 B.

(i) *Inoculation resistance*. From figure 3A we see that, as modelled, the virus titre in the plant is not affected by the value of the inoculation resistance parameter, ε_α. The more resistant the cultivar, the larger the density of healthy plants, Fig. 3B, which is biologically obvious. For small values of the resistant parameter, ε_α, the virus dies out and all plants are healthy.

(ii) *Acquisition resistance.* The results for acquisition resistant cultivars differ only in minor quantitative details from those of the inoculation resistance shown.

(iii) *Virus titre reducing resistance*. As modelled the virus titre decreases with increasing resistance, when the resistance parameter, ε_W, approaches zero (meaning absolute resistance) the virus titre decreases to zero. The density of healthy plants increases with increasing resistance. For very small values of the resistance parameter the virus dies out and all plants are healthy.

(iv) *Tolerance.* Tolerance does, as modelled, not affect the virus titre. As can be seen from figure 5B the density of healthy plants slightly decreases with increasing tolerance. This is due to the fact that tolerant plants have a smaller mortality/roguing rate as modelled in equation (28), and infected plants remain longer in the system. This implies that for a tolerant cultivar to be effective this decrease in the density of healthy plants has to be outweighed by the gain, in terms of additional yield, as compared to a non-tolerant cultivar, due to the tolerance of the crop to the disease.

After evolution of the virus

We now turn to the situation where the resistant cultivar has been introduced and the virus has responded to this change in its environment by evolving to the new evolutionary stable within cell virus multiplication rate, k_{ESS}. This value is calculated from equation (33), subsequently using this k_{ESS} the corresponding virus titre, (22), and steady state density of healthy plants, (15), is calculated (Fig. 3, 4 and 5 graphs C, D and E).

(i) *Inoculation resistance* (Fig.3) As shown in equation (33) the introduction of inoculation resistance does not put an evolutionary pressure on the virus to change its multiplication rate. Fig. 3C shows this again with the ESS value of k equal for any resistance parameter, ε_α, value. Corresponding to this the virus titre in the plant is not affected by plant resistance, nor does the density of healthy plants change as compared to the situation prior to evolution.

(ii) *Acquisition resistance*. Again the results are very similar to the ones for inoculation resistance and the same conclusions hold.

(iii) *Virus titre reduction* (Fig. 4) After the virus has fully evolved to the ESS set by introduction of the resistant cultivar, the within cell virus multiplication rate, k_{ESS}, has increased. The more resistant the cultivar is (the smaller ε_W) the larger the ESS

multiplication rate. As a consequence the virus titre evolves to the value it had before the resistant crop was introduced. The density of healthy plants decreases and in steady state it has reached the value it had before introduction of the resistant cultivar.
(iv) *Tolerance* (Fig. 5) As with the virus titre reducing cultivar the introduction of a tolerant cultivar puts an evolutionary pressure on the virus to increase it's within cell multiplication rate, *k*. The ESS value of *k* is larger for more resistant cultivars (smaller ε_ϕ). Once the ESS is approached the virus titre in the plant has increased. The virus titre is higher in more tolerant cultivars. The density of healthy plants has not changed much, though it is slightly reduced compared with the situation prior to evolution.

Discussion

In this chapter we have shown that it is possible to quantify the consequences of the use of resistant cultivars for the evolution of plant disease viruses. We used a modelling approach in combination with evolutionary stable strategy calculations as applied to animal and human diseases (Dieckmann, 2002). Inevitably in a first approach, we have restricted our work to a simple model, a specific set of four types of resistance/tolerance responses, and considered only one of the possible evolving virus life-history traits. In future research we will need to consider other virus traits that might undergo evolutionary change when a resistant cultivar is introduced, to develop a more general overview of the evolutionary consequences of use of resistance.

At the time a resistance cultivar is introduced the virus is not adapted to the new situation and our findings, Fig. 2, 4 and 5 graphs A and B, correspond to those found in other modelling studies (Fargette & Vie, 1995; Holt *et al.,* 1999; Holt & Chancellor, 1996; Jeger *et al.,* 2004). Inoculation resistance, acquisition resistance and virus titre reduction decrease the density of infected plants and increase the density of healthy plants, the magnitude of this effect depending on the effectiveness, expressed in the value of the parameters ε, of the resistance. The effect of tolerance on the dynamics of a viral plant disease has not previously been modelled. The density of healthy plants slightly decreases with increasing levels of tolerance (smaller ε_ϕ), which is understandable as the mortality/roguing rate decreases with increasing tolerance of the crop.

The results shown in Fig 3, 4, and 5 graphs A and B show that our model applied to the situation prior to evolution agrees with the common views on use of resistance. Results obtained when allowing the virus to evolve, Fig 3, 4, and 5 graphs C, D, and E, thus are not a result of a model specification that contradicts present consensus in the situation prior to evolution.

When the virus has had the time to adapt to the new environment set by the use of host resistance, two types of effects on the dynamics of the disease are found:

The first type, including (i) inoculation resistance and (ii) acquisition resistance, does not put a selection pressure on the virus to evolve towards a larger within cell virus multiplication rate. This implies that the virus titre in infected plants does not change, and, provided that yield is correlated with virus titre, the yield per infected plant will not change. Furthermore the density of healthy plants in the system does not change. This means that yield loss due to the disease will not change in any

way when inoculation resistant or acquisition resistant cultivars are deployed. These types of resistance are thus durable in a situation where within-plant virus multiplication rate is the evolving trait. These types of resistance thus are good candidate disease management methods in the development of sustainable agricultural systems. One element that needs to be taken into account however is the possible existence of a cost, in terms of reduced yield, that might be associated with host resistance.

The second type, including tolerance and virus tire reducing resistance, do put a selection pressure on the virus to evolve towards larger virus multiplication rates (Fig. 4C and 5C).

(iii) For tolerant cultivars this implies that, after the virus has adapted to the tolerant cultivar, the within-plant virus titre has increased, while the density of healthy plants has slightly decreased. Whether this has a negative effect on crop yield depends on the balance between the yield gain of the infected plants due to the tolerance of the plants, and the yield reduction due to the increased virus titre in the plants and the decreased density of healthy plants.

(iv) For plants expressing virus titre reducing resistance the evolutionary response of the virus causes the within-plant virus titre to evolve back to the titre of the non-resistant cultivar, Fig 4 difference between graph A and D. Also the density of healthy plants will evolve back towards the density it would have for a non-resistant cultivar. This implies that when the virus evolved to the ESS set by the use of such resistant cultivars the damage will have approached the level it had before the introduction of the resistant cultivar.

The cultivars from this second group are thus not durable in the sense that they put an evolutionary pressure on the virus that might reduce the effectiveness of the tolerance/resistance. Whether tolerant cultivars and virus titre reducing cultivars have a contribution to make is however not only dependent on the selection pressure put on the virus but also dependents on additional factors. The tolerant cultivar might still have a positive effect on yield even when the virus has evolved the new ESS simply because the tolerance provides a sufficient amount of addition damage reduction. Furthermore it can be that the ESS virus multiplication rate is outside the attainable range for the virus under consideration. Finally the time at takes the virus to evolve towards the new ESS virus multiplication rate is of importance here. Depending on the frequency with which genetic changes occur, the magnitude of the change in phenotype such genetic changes induce and the rate of invasion of a new genotype/phenotype it can be that the resistant cultivar can be used for a long time.

We are thus not advocating that tolerance and virus titre reducing cultivars will never have a contribution to the management of virus diseases but we have shown that there are potential problems with the use of such cultivars that need to be considered to maximise the effectiveness of use of resistant cultivars. The inoculation resistant and acquisition resistant cultivars do not express such potential problems with selection for higher virus multiplication rate.

Our model and the approach developed in this chapter are to be seen as a first step in this area. The model is simple and several model extensions have to be investigated before general conclusions can be drawn. Our model however does allow for several extensions without any change in the conclusions.

Figures 3, 4 and 5 were constructed using the parameter values discussed in the figure legends. We have however drawn figures for a wide variety of parameter values

around the values used (plus and minus 100%) and found that though quantitative difference will occur for different parameter values, the qualitative trends as shown in figure 3, 4 and 5 do not change.

The plant-virus-vector population model does not include a latent period for the plant nor a latent period for the virus in the vector. These model extensions are discussed in the papers by Jeger *et al.* (1998) and Madden *et al.* (2000), where the basic reproductive number is also calculated. It is easily seen that, given the relation between parameters in the population model and the parameters of the within-plant virus dynamics model, the ESS value of the virus multiplication rate, *k*, still is the value maximising the basic reproductive number. Moreover it is easily seen that the qualitative trends shown in figures 3, 4 and 5 will not change when introducing these model extensions.

We have assumed that the inoculation rate parameter α is independent of the titre the virus builds up in the plant and, because virus titre is related to virus multiplication rate, equation (22), to *k*. This is probably the least well motivated assumption about the relation between virus titre and population model parameters we have used. It is however possible to relax this assumption without any change in our results and conclusions. One might argue that the inoculation rate is related to virus titre as

$$\alpha(k) = \tilde{\alpha}\,\frac{\rho W^*}{1+\rho W^*} = \tilde{\alpha}\,\frac{Ck}{1+Ck}$$

and introducing the resistance parameters we have

$$\alpha(k) = \varepsilon_\alpha \tilde{\alpha}\,\frac{\rho \varepsilon_W W^*}{1+\rho \varepsilon_W W^*} = \varepsilon_\alpha\,\tilde{\alpha}\,\frac{\varepsilon_W C k}{1+\varepsilon_W C k}$$

Substituting this expression into the basic reproductive number and calculating the ESS value of the virus multiplication rate *k*, we find that k_{ESS} can be calculated from

$$2\sigma + \sigma\varepsilon_W \left(A + C\bar{k} + \varepsilon_\phi \varepsilon_W B\,\bar{k} - \varepsilon_\phi \varepsilon_W{}^3 ABC\,k^3\right) = 0$$

This equation can only be solved numerically. We have redrawn figure 3, 4 and 5 using this expression and found that there are no qualitative differences although quantitative differences do occur.

We have thus already investigated a number of model extensions but more remains to be done.

Breeding programmes to develop tomato cultivars resistant to tomato yellow leaf curl (TYLC) have been based on the introgression of resistance or tolerance from accessions of wild *Lycopersicon* species to cultivated tomato. Main used wild species are *L. peruvianum*, *L. chilense*, *L. pimpinellifolium* and *L. hirsutum*. Some of the types of resistance and tolerance we distinguished in this chapter can be found in the breeding lines of the tomato breeding programmes although it is not always easy to separate the various types of resistance and tolerance.

Gomez *et al.* (2004) developed four tomato lines introgressed from *L. chilense* (named LD3, LD4, LD5 and LD6). They showed that in these lines virus accumulation was very low (0.09 to 1.00 ng per plant 60 days after inoculation) in infected plants and no symptoms developed. These breeding lines seem a clear example of virus titre reducing resistance as defined in equation (27). They compared these lines with the commercial F_1 hybrids 'ARO 8479' and 'HA 3108'. These hybrids developed high virus titres (over 1000 ng viral DNA per plant 60 days after

inoculation). Symptom severity was very low in these hybrids. ‘ARO 8479’ and ‘HA 3108’ thus seem to be clear examples of tolerant lines as defined in equation (28). Vidavsky found similar results with plants from accessions LA1777 and LA386 of the wild species *L. hirsutum*. Their BC1F4 line (denominated 902) does not show any virus accumulation even upon extensive whitefly mediated inoculation, which is a clear example of virus titre reducing resistance. Their BC1F4 line (denominated 908) did support virus accumulation but no symptoms developed, another example of tolerance. Various other authors evaluated resistant/tolerant cultivars and found examples of virus tire reduction and tolerance, often in combination (Rubio *et al*., 2003; Maruthi *et al.,* 2003a,b; Michelson *et al.,* 1994; Pietersen & Smith 2002)

In the light of our findings about the durability of inoculation resistance and acquisition resistance it is remarkable how little research has been done on these aspects of the epidemiology of the disease in systems where resistant cultivars are deployed. A notable exception is the work of Lapidot *et al*. (2001). They measured acquisition and transmission of the virus by whiteflies for several resistant lines, and measured amounts of TYLCV DNA in inoculated plants and viruliferous whiteflies. Their analysis did not allow distinguishing a lower acquisition rate/amount by the whitefly vector due to low plant virus titre, as modelled in equation (27), from pure acquisition resistance as modelled in equation (25). Besides mechanisms operating on a biochemical or plant anatomical level that cause inoculation and/or acquisition resistance as can be detected using methods similar to those developed by Lapidot *et al.* (2001), plant architecture might also lead to inoculation and/or acquisition resistance. For example high densities of leaf trichomes or sticky leaf hears can hinder the movement of the whitefly vector and cause a reduction of acquisition and/or inoculation (Muigai *et al*., 2002; Snyder & Carter 1985).

As Rubio *et al.* (2003) state in their paper the lack of good methodologies for the evaluation of components of plant resistance/tolerance is a limiting factor in the breeding for resistance. Further, as Lapidot & (2002) state, the lack of clear standard terminology about resistance/tolerance might hinder to unravel resistance components in breeding programmes. We hope that our definitions, as expressed in equations (25) to (28) can help categorise resistance components and help develop methods to distinguish these.

In breeding programmes visual assessment of symptoms is often a means to sort the plants that are used for further breeding. This method will inevitably cause a bias towards the breeding of tolerant and virus titre reducing resistance crop lines. Plants expressing inoculation resistance or acquisition resistance will not be recognised because they will develop symptoms once infected. Further in the evaluation of the usefulness of cultivars for the management of viral plant diseases it is necessary to know which mixture of resistance/tolerance mechanisms is operating in the cultivar and to which extend. Only when it is known to what extend tolerance is operating in a cultivar we can evaluate whether the evolution of the virus towards higher virus multiplication rate will render the cultivar ineffective to prevent yield loss.

Rubio *et al*. (2003) provide the first steps towards a methodology that enables breeders to analyse the various types of resistance/tolerance operating. They concentrated on the unravelling of virus titre reduction and tolerance mechanisms. In combination with the methods use by Lapidot *et al.* (2001) to estimate acquisition resistance and inoculation resistance it should be possible to develop a methodology to separate the four types of resistance/tolerance discussed in this chapter.

Further work on the evolutionary consequences of the use of resistant cultivars on virus populations needs to concentrate on the robustness of our findings to changes in the modelled system and on the study of the evolution of other life-history traits of the virus species. We hope to return to these points in following publications.

III. 1b Evolution of viral plant diseases with two routes of infection

In this chapter we show how disease management affects the virulence of viral plant diseases transmitted by insect vectors and through cuttings used for crop propagation.

One of the major constraints to crop production, especially in tropical and sub-tropical regions, is the occurrence of diseases caused by viruses (Rybicki & Pietersen, 1999). Various studies have shown that the impact of viral plant diseases on crop production is increasing (Rybicki & Pietersen, 1999; Varma & Malathi, 2003; Boulton, 2003). Until the last decade, for example, epidemics caused by geminiviruses were relatively localised, but problems with geminiviruses have spread throughout much of the world (Boulton, 2003). Major efforts to control these diseases involve sanitation programmes, control of the vector and the introduction of new crop cultivars. Furthermore several organisations and governments are developing programmes for *in vitro* propagation of planting material which provides farmers with guaranteed virus free planting material (Robinson, 1996; Feng *et al*.,2000).

Viruses are notorious for their rapid evolutionary change to adapt to new circumstances. Of the viral plant diseases geminiviruses are particularly notorious, with severe epidemics caused by newly emerging geminiviruses (Varma & Malathi, 2003). Dramatic examples of the emergence of new virus strains are the recent epidemic in West Africa of cassava mosaic disease (Gibson *et al*.,1996; Legg, 1999) and the cotton leaf curl epidemic in Pakistan and India (Briddon & Markham, 2000).

Any virus disease management method will exert a selection pressures on the virus that might lead to the evolution of new and potentially more damaging strains. Little is known, however, about the relation between virus evolution and plant disease management (Dieckmann *et al*., 2002). The short-term evolutionary changes in plant disease viruses caused by disease management methods can severely constrain the development of sustainable agriculture. Insight into the effect of disease control on virus evolution is needed for future development of sustainable agriculture.

In this chapter we consider crops that are multiplied through cuttings. This important class of crops involves several providing staple food in tropical and sub-tropical regions, such as cassava, sweet potato and plantain. Virus diseases of such crops normally have two routes of transmission. Insect herbivores act as vectors of the virus, and cuttings from infected plants used as planting material may contain the virus giving rise to new infected plants. The management of viral diseases in these crops frequently involves interference with virus transmission routes or with the death-rate of infected plants. Virus transmission and infected plant longevity critically depend on the titre the virus builds up in the plant, and virus titre in its turn is one of the virus life-history characteristics that can undergo evolutionary change. This directly implies that disease management could lead to the evolution of virus strains

with a higher virus titre in the plant and, since virus titre is closely correlated with yield loss, to more damaging virus strains.

Previous work by Lipsitch *et al.* (1996) studied the effect of horizontal (vectored) and vertical (cutting) transmission on the evolution of virulence. There model shows how different trade-off relations among model parameters contributing to fitness (vertical transmission, horizontal transmission, host death) generate different evolutionary outcomes. The structure of their model however does not lend itself to study the effect of the management of viral plant diseases on the evolution of the titre the virus builds up in the plant. Here we advance their work by analysing underlying mechanisms and the extent to which these can be adjusted by practical plant disease management in farming systems. By identifying the mechanisms we show that it is possible to study the consequences of disease management on the evolution of the virus disease. Specifically we are interested in identifying those disease management strategies that do not provoke the evolution of virus strains that build up a high virus titre in the plant.

We will use the concept of Evolutionary Stable Strategies (ESS). An ESS is defined as a strategy, in our case a value of the titre the virus builds up in the plant that can not be invaded by any other strategy (Maynard Smith, 1982). We will refer to the titre the virus builds up in the plant by the word ‘’virulence’’, which corresponds to the use of this word in the animal disease literature, relating to pathogen induced host death rate, and plant pathology where it is used for the capacity of the pathogen to cause disease. Further we will adapt to the convention in plant pathology to use ‘healthy plants’ to denote not (yet) infected plants, to avoid confusion with susceptible as opposed to resistant crop cultivars.

2. THE MODEL

(a) Plant-virus dynamics

Plant viruses are systemic diseases, meaning that after inoculation and multiplication cycles the virus spreads throughout most of the plant. This implies that the plant individual is the natural unit for modelling virus diseases. We consider a model with state variables H, representing the density of healthy crop plants, and I, the density of infected crop plants. The model describes planting of crop plants both from cuttings and from planting material obtained from *in vitro* propagation programmes, as described in the introduction. Further the model describes plant harvest, rouging management and vectored virus transmission.

We assume a fixed planting rate σ, of which a fraction ϕ is planted from cuttings and a fraction $1-\phi$ from *in vitro* propagated planting material. Collecting cuttings from the crop a farmer might recognise a plant to be infected and not use it. The probability that an infected plant is recognised as being infected is denoted by p. This implies that from n plants visited by the farmer $n((1-p)I+H)/(I+H)$ will be used to take cuttings. The farmer takes m cuttings from each plant and to collect $\sigma\phi$ cuttings per time unit for planting she has to visit $(\sigma\phi/m)(I+H)/((1-p)I+H)$ plants per time unit. A fraction, r, of the cuttings taken from infected plants will not contain the virus. This phenomenon is known as reversion (Fargette & Vié, 1995; Gibson & Otim-Nape, 1997; Fondong *et al.*, 2000), and is due to virus diseases not developing systemically through the entire plant. The planting rate of healthy and infected plant from cuttings therefore is $\sigma\phi(r(1-p)I+H)/((1-p)I+H)$ and $\sigma\phi(1-r)(1-p)I/((1-p)I+H)$ respectively.

Plants are harvested after $1/\eta$ time units, implying a harvest rate of η for both healthy and infected plants. Sanitation management and naturally increased death rates of infected plants results in a probability ω per time unit that an infected plant is removed from the system. The transmission rate of the virus from infected to healthy plants by the vector is denoted by β.

Hence, the dynamics of the densities of healthy and infected plants is described by

$$\begin{aligned} \frac{dH}{dt} &= \sigma(1-\phi)H + \sigma\phi\frac{r(1-pI)+H}{(1-pI)+H} - \eta H - \beta IH \\ \frac{dI}{dt} &= \sigma\phi\frac{(1-r)(1-pI)}{(1-pI)+H} - \eta I - \omega I + \beta IH \end{aligned} \qquad (2.1)$$

Model equations (2.1) have a globally asymptotically stable internal steady state when the basic reproductive ratio of the disease, R_0, is larger than unity, with $R_0=(\phi(1-r)(1-p)\eta^2+\beta\sigma)/(\eta^2+\eta\omega)$ (summary of these calculations available on request).

(b) The evolving trade

We consider as the evolving trade the titre the virus builds up in the plant. Virus titre is known to be a trade that can evolve. The newly evolved strain of cassava mosaic virus disease presently invading West Africa, for example, has been reported to build up higher virus titres in the plant than the previously existing strain (Gibson *et al.*, 1996). As mentioned in the introduction the titre the virus builds up in the plant will be termed virulence, and is denoted by x. Several of the parameters in model equations (2.1) are related to the titre the virus builds up in the plant. These are

1. Transmission rate. The probability that a vector acquires the virus when feeding on a plant is larger when the virus titre is larger. This implies that the transmission rate is a function of virulence, $\beta(x)$. We will use $\beta(x)=\beta_s x$.
2. Death rate. The larger the virus titre in the plant the more damage the virus causes to the vital functions of the plant. Moreover, higher virus titre is associated with more symptom expression, implying that the infected plant will be recognised easier during sanitation inspection rounds by the farmer. We will use $\omega(x)=\omega_s x^2$, and the parameter ω can be interpreted as rouging rate parameter.
3. Cutting selection. Symptom severity is an increasing function of the titre the virus builds up in the plant. This implies that the probability that the farmer recognises a plant to be infected and not use it for cuttings increases with increasing virus titre. We will thus use $p(x)=1-\exp(-(\ln(2)/x_{half})x)$, where x_{half} is the virulence at which 50% of the infected plants is recognised as infected.
4. Reversion. The extent to which the virus becomes systemic throughout the whole plant is correlated with the average titre the virus builds up in the plant. This implies that the probability that a cutting from an infected plant is reversed (does not contain the virus) decreases with virus titre. We will use $r(x)=\exp(-r_s x)$.

Figure 1 shows the dependence of the parameters on the virulence, x.

(c) Invader fitness and evolutionary stable strategy

As mentioned model equations (2.1) have a globally asymptotically stable internal steady state when the basic reproductive ratio of the disease R_0>1. The success of a mutant invader thus can be determined from its fitness, defined as the natural rate of

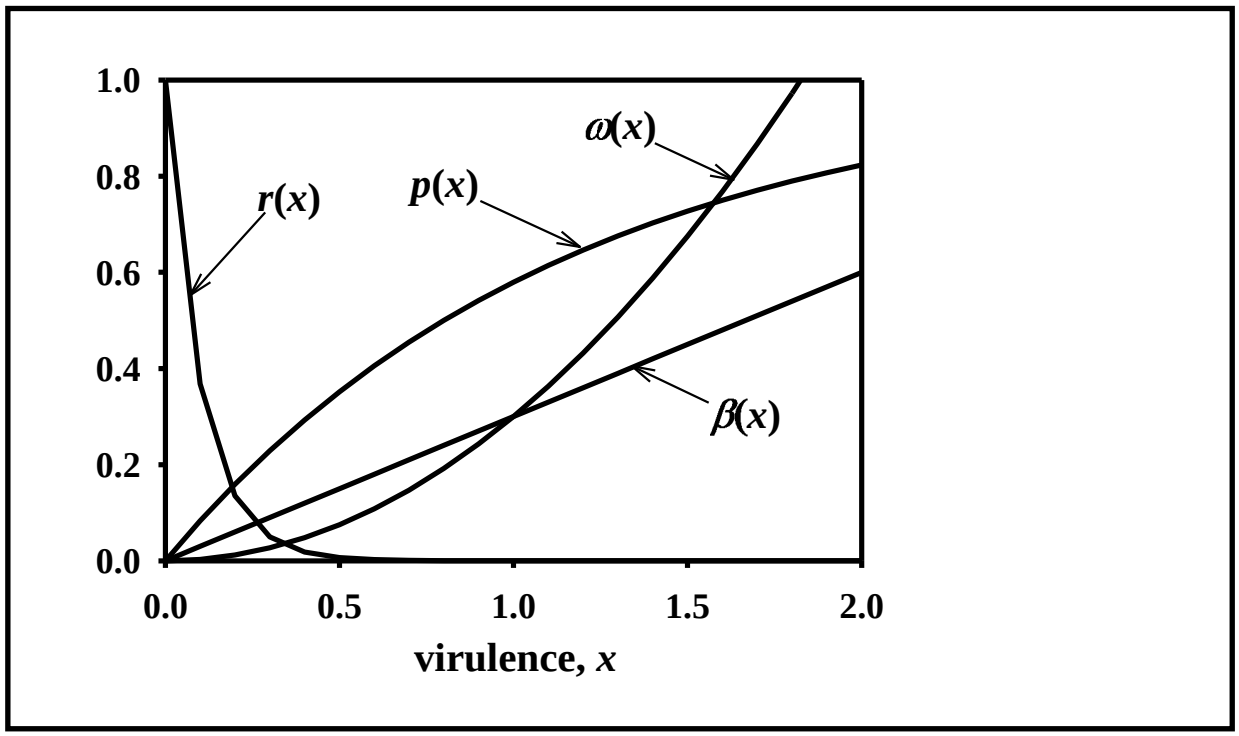


Figure 1: The effect of within-plant virus titre, virulence x, on the reversion rate, *r*(*x*), the probability to recoginse a cutting to be infected, *p*(*x*), the plant death rate, ω(*x*), and the transmission rate, β(*x*).

increase, in an environment where system (2.1) is in its internal steady state, ($H^\bullet$, $I^\bullet$), (see Rand *et al*., 1994; Geritz *et al*., 1998).

Assume that the resident population has virulence x^*, and is thus characterised by $\beta(x^*)$, $\omega(x^*)$, $p(x^*)$, and $r(x^*)$. The fitness of an invader with virulence x, $W(x, x^*)$, is given by

$$W(x,x^*)=\sigma\phi\frac{(1-r(x))(1-p(x))}{(1-p(x^*))I^\bullet+H^\bullet}-(\eta+\omega(x))+\beta(x)H^\bullet \qquad (2.2)$$

A mutant invader will spread when W>0 and will die out when W<0. A virulence value x^* is evolutionarily stable if no mutant invader with virulence x can spread, that is if $W(x, x^*)<W(x^*, x^*)$ for all $x\neq x^*$. In other words x^* is an ESS (Maynard Smith, 1974) when $W(x, x^*)$ has a maximum in x at $x=x^*$. The ESS virulence value will be denoted by x_{ESS}.

To ascertain that the virulence of the virus will evolve towards its ESS value (in small evolutionary steps) invaders with a virulence x larger than, but close to, the resident value x^* should invade when the virulence of the resident is smaller than the ESS virulence value, $x^*<x_{ESS}$, and when the virulence of the resident population is larger than the ESS virulence, $x^*>x_{ESS}$, invaders with a virulence smaller than but

close to the virulence of the resident should invade (Eshel, 1983). An ESS is attracting, is a Continuously Stable Strategy, CSS (Eshel, 1983), when $W(x, x^*)>0$ for $x\in(x^*, x_{ESS})$ when $x^*<x_{ESS}$, and $W(x, x^*)>0$ for $x\in(x_{ESS,} x^*)$ when $x^*>x_{ESS}$.

The criteria for ESS and CSS were evaluated numerically.

Table 1: Model parameters and their interpretation

parameter	description	default value
η	harvest rate	[1]0.002
σ	planting rate	[2]0.0015
ϕ	Fraction planted with cuttings	[3]1.0 – 0.0
x_{half}	Cutting selection parameter	[4]∞
r_s	Reversion parameter	[5]10.0
ω_s	Rouging rate parameter	[6]0.005
β_s	Transmission rate parameter	[7]0.025

[1] Holt *et al.* (1997) use 0.002-0.004 day^{-1} as default range for cassava. Sweet potato has a similar cropping period as cassava and η=0.002 is an appropriate value. The results presented here do however not change qualitatively for this change in parameter value.

[2] Planting density of cassava is 0.5 plants per m^2 (Holt *et al.,* 1997). The maximum density in the absence of disease is σ/η. Thus σ=0.0015. For sweet potato the planting density is 4 plants per m^2 (Gibson pers. comm.) giving σ=0.015. The results presented here do however not change qualitatively for this change in parameter value.

[3] African small holders replant cassava and sweet potato with cuttings from previous crop only. Commercial sweet potato growers use disease free material from *in vitro* propagation programmes (Feng *et al.,* 2000). This parameter thus varies between 0 and 1.

[4] African small holders do not apply cutting selection in cassava on a standard basis (Legg pers. comm.). For sweet potato some cutting selection takes place. Farmers recognise 10 to 50% of the plants that are infected (Gibson & Aritua, 2002; Aritua *et al.,* 1998). And around 40% of the farmers realise that the disease can be transmitted by cuttings (Aritua *et al.,* 1998). It is known that farmers who are aware of the disease transmission by cuttings do select their planting material. This would result in 5 to 20% of the infected cuttings being selected out.

[5] For the default parameter set, x_{ESS}-default=0.255, which implies that the probability for a cutting from an infected plant to be reversed is exp(-0.255*10)≈0.08. Fondong et al. (2000) measured 5 to 40% reversion depending on the susceptibility of the cultivar. For sweet potato reversion has not been described in the literature.

[6] No quantitative data available. Roguing is applied in some places in cassava and sweet potato. Our default parameter represents the situation without rouging. Legg (pers. comm.) observed small holder farmers in Africa pulling out clearly infected young plants. Further young plants that are infected are known to grow slowly, and therewith are overgrown by healthy plants (compensatory growth). For the parameters chosen, for x_{ESS}=0.255, about 10%, ($\omega_s * x_{ESS}^2/((\omega_s * x_{ESS}^2 + \eta))$, of the infected plants die or are removed before harvest.

[7] For x_{ESS}=0.255 we find β=0.0064. This is a realistic value for both cassava and sweet potato. Cassava: there are round 50 whitefly per plant, V, (Legg et al 1995), inoculation rate is 0.008, λ_1, (Holt *et al.,* 1997), acquisition rate is 0.008, λ_2, (Holt *et al.,* 1997), duration of viruliferous period is 4.5 days, δ(1/(rate of leaving viruliferous state (0.1, Dubern 1994)+ insect death rate (0.12, Holt *et al.,* 1997))). β is equal to V(σ/η), λ_1, λ_2,$\delta \approx 0.007$. Sweet potato: Experiments from Gibson *et al.* (2003) show a rate of increase, r, of 0.024 per day. In the model $dI/dt=\beta SI-\eta I$ which gives an initial rate of increase of $r=\beta S-\eta$, where $S=\sigma/\eta$. Substituting all other default parameter value yields β=0.0064.

(d) Parameter values

Parameter values are chosen to represent cassava and sweet potato like crops and two of their respective viral diseases, cassava mosaic virus and sweet potato chlorotic stunt crinivirus (SPCSV). The values, based on published data, is motivated in the subscript of Table 1.

(e) Disease management

We study the effect of disease management on the evolutionary stable virulence and the resulting densities of healthy and infected plants. Implementing a disease management measure amounts to changing the value of a model parameter. The disease management methods to be considered are

1. *In vitro* propagated planting material. Planting part of the crop fields with *in vitro* propagated guaranteed virus free planting material. This disease management strategy does decrease the numerical value of ϕ.
2. Selection of planting material. Improved cutting selection implies that the numerical value of the parameter x_{half} decreases.
3. Sanitation programmes. Increasing rouging rates increase the numerical value of the parameter ω_s.
4. Tolerant cultivars. Introducing a tolerant cultivar that shows less symptoms and less yield loss at the same virus titter in the plant implies that the infected plant death rate and/or the rouging rate decreases. This disease management strategy is reflected in a decreased numerical value of the parameter ω_s.
5. Resistant cultivars. Introducing a resistant cultivar will cause the acquisition of the virus by the vector and/or the inoculation of the virus into the plant by the vector to decrease. This is reflected in a decreased numerical value of the transmission rate parameter β_s.
6. Vector control. Reducing vector density, using insecticides, will decrease the transmission rate, which equals a decreased numerical value of the parameter β_s.

3. MODEL OUTCOMES AND PRACTICED DISEASE MANAGEMENT

The numerical exploration of the ESS and CSS criteria show that for each parameter set there is one CSS virulence value. Figures 2 and 3 show these CSS virulence values, for ranges of the parameters related to disease control strategies, together with the density of healthy and infected plants that will develop when the virus has evolved this CSS virulence.

The model outcomes as summarised in Figures 2 and 3 show how the evolutionary stable virulence depends on disease management methods. In this section we summarise these effects for each disease management method and describe to what extend these methods are used in practical management of viral diseases.

1. *In vitro* propagated planting material. Our results show that introducing *in vitro* propagated plantlets as planting material leads to a higher evolutionary stable virulence and at the same time a reduction in the density of infected plants (Figure 2). The balance between increased yield loss due to a higher virulence and increased yield gain due to an increased density of healthy plants determines whether the introduction of *in vitro* propagation programmes is worthwhile. An economic evaluation of such a

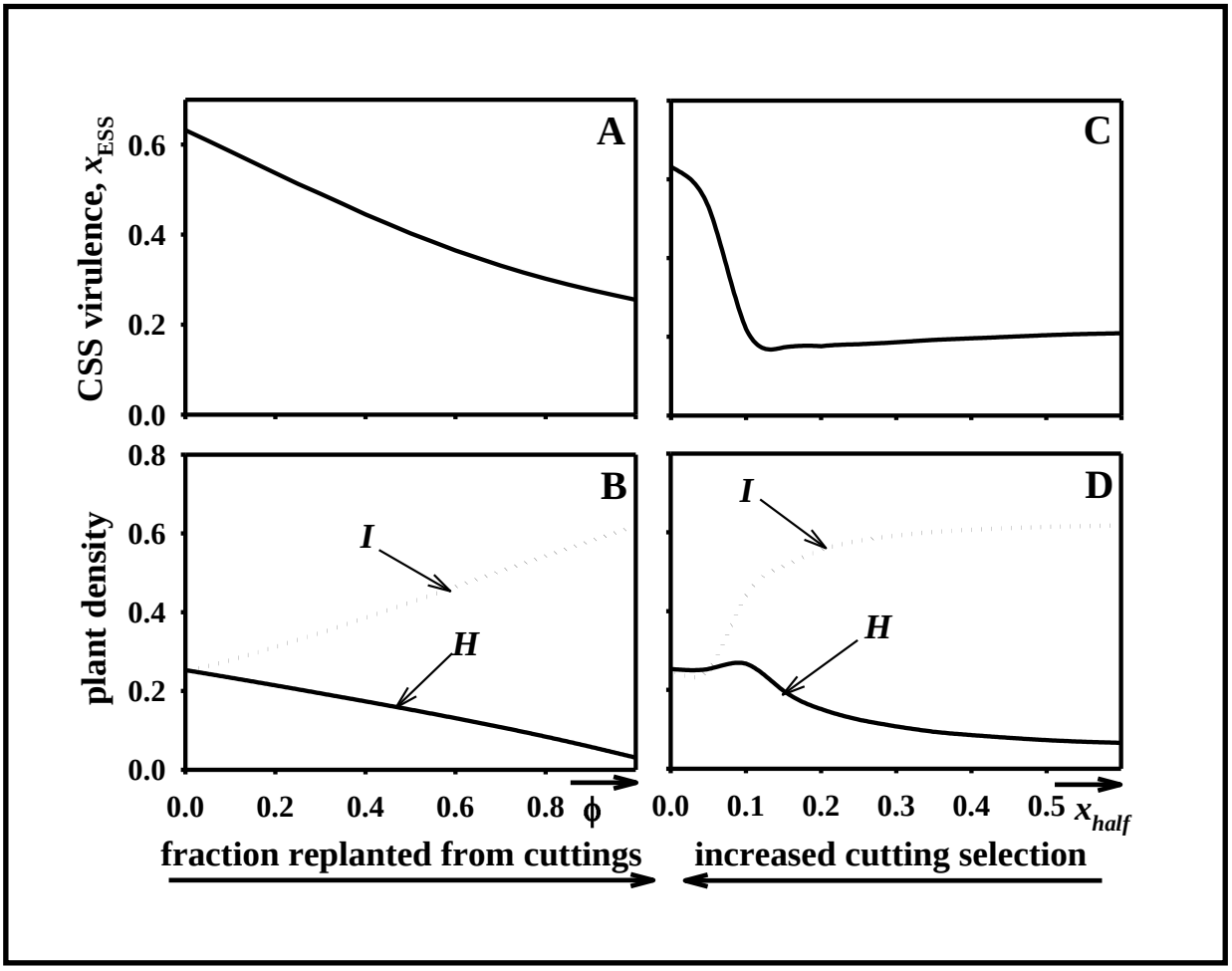


Figure 2: The effect of the fraction of plant planted from cuttings, graphs A and B, and the increase cutting selection, graphs C and D, on the ESS virulence, x_{ESS}, graphs A abd C, and the density of healthy, *H*, and infected, *I*, plants, graphs B and D.

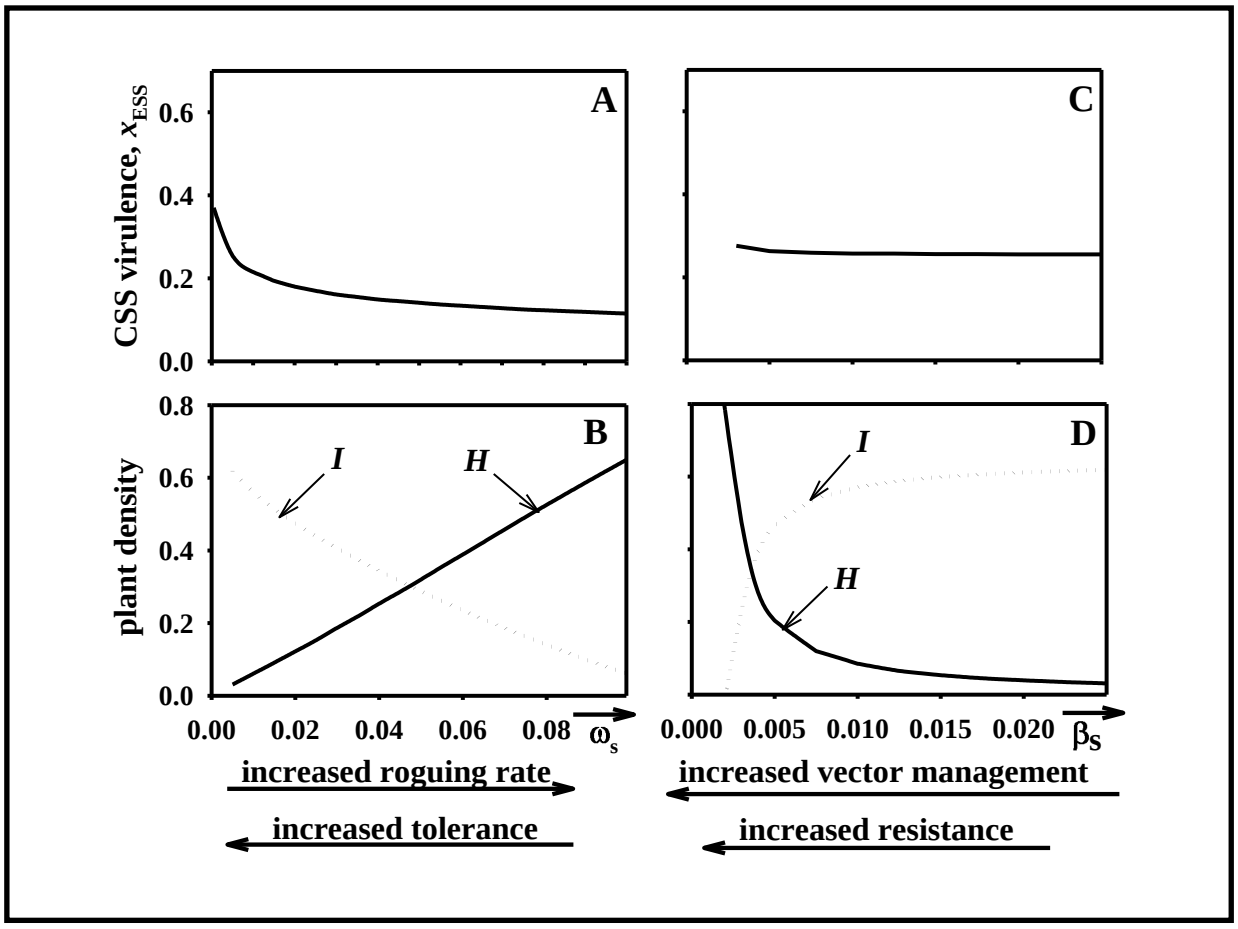


Figure 3: The effect of rouging rate, tolerance, graphs A and B, and vector management, resistance, graphs C and D, on the ESS virulence, x_{ESS}, graphs A and C, and the density of healthy, *H*, and infected, *I*, plants, graphs B and D.

programme should also recognise that the costs of *in vitro* propagated planting material are higher than the costs of cuttings the farmer can obtain from her own crop.

Presently there are two well established *in vitro* propagation programmes for banana (Robinson, 1996) and for sweet potato (Feng *et al.,* 2000). Some Mediterranean and subtropical countries have developed *in vitro* propagation programmes for banana, but other countries, e.g. the Latin American countries, have not adopted the technique. *In vitro* propagation of sweet potato is used in China, South Africa and Egypt. The main constraint, as discussed in the literature, seems to be the high costs of this technique. None of the authors discussing such programmes takes the possibility of evolutionary changes in the virus into consideration.

2. Selection of planting material. Our results show that increased cutting selection leads to changes in the evolutionary stable virulence. Increasing selection efficiency from $x_{half}>0.6$ to $x_{half} \approx 0.17$ results in a small decrease in the CSS virulence (figure 2C) and a small decrease in the density of infected plants (figure 2D). Further decreasing x_{half} the CSS virulence sharply increases and the density of infected plants sharply decreases. This means that a large shift in virulence occurs precisely in the parameter region where the cutting selection effectively reduces the infected plant density.

Selection of planting material is practiced routinely by farmers to control SPVD in sweet potato (Gibson & Aritua, 2002). It is not known whether this has led to an increase in the virulence of the virus. For cassava, training programmes are being developed to make farmers aware of the possibility of cutting selection and to show how to recognise infected planting material. Our results show that such newly developed methods have to be evaluated carefully for their effects on the virulence of the virus before implementation. As with the introduction of in vitro propagated planting material it depends on the balance between increased yield loss due to increased virulence and increased yield gain due to reduced disease incidence whether cutting selection is advisable.

3. Sanitation programmes. Our results show that increased rouging intensity decreases both the evolutionary stable virulence and the disease incidence (Figure 3A, B). Both experimental and theoretical studies have shown, without considering possible evolutionary consequences, that rouging can be an effective disease control method in many crops including cassava and sweet potato (see e.g. Fargette *et al.*, 1994; Jeger *et al.*, 2004). Our finding that rouging will also put a selection pressure on the virus to develop towards lower virulence shows that rouging management is a good candidate disease control method in the development of sustainable agricultural system.

4. Tolerant cultivars. The introduction of tolerant cultivars increases both the evolutionary stable virulence and the disease incidence (Figure 3A, B). Both these effects tend to increase disease related yield loss. The usefulness of tolerant cultivars for sustainable disease control thus strongly depends on the extent to which the cultivar is tolerant, and compensates for higher virulence and disease incidence.

Screening methods to select new cultivars often use visual assessments only to select material for further breeding (Aritua *et al.*, 1998; Bellotti & Arias, 2001). This method of selection has a high probability to select for tolerance in the breeding material and not aspects of crop resistance (see next paragraph). We conclude that in

breeding tolerant cultivars aspects of virus evolution and increased disease incidence have to be considered before introduction of such cultivars.

5. Resistant cultivars. The introduction of resistant cultivars that decrease the disease transmission rate have very little effect on the evolutionary stable virulence level and can contribute to a reduction of disease incidence (Figure 3C, D). In experimental and modelling studies that do not consider evolutionary aspects it has been shown that the introduction of resistant cultivars can be a useful means to control virus diseases in crops like cassava, sweet potato and plantain (Bellotti & Arias, 2001; Gibson & Aritua, 2002). Our finding that resistant cultivars do not put a selection pressure on the virus to develop higher virulence implies that this method of disease control is a good candidate in the development of sustainable agricultural systems.

6. Vector control. Vector control has the same effects on the evolutionary stable virulence and on disease incidence as the introduction of resistant cultivars, making it a potential candidate for sustainable disease control (Figure 3C, D). The management of vector populations in the field, however, is often not very effective or leads to the application of large quantities of insecticides. We conclude that when environmental and biological considerations do allow for the vector population to be controlled at low levels, this can be a useful component in the development of sustainable disease control methods.

4. DISCUSSION

There is a remarkable difference in the effects of disease management interfering with the horizontal transmission (vectored transmission) and the vertical transmission (cutting transmission). Interference with vertical transmission affects the CSS-virulence of the virus, and does so in a predictable way. One would expect that interference with the horizontal transmission also has a predictable, opposite, effect. Our results however show that interference with horizontal transmission has no effect of CSS-virulence.

When cuttings used for planting are selected the farmer interferes with the vertical transmission route of the virus. For intermediate effective cutting selection (x_{half} larger than approximately 0.2) decreasing x_{half} selects for virus strains that build up a lower virus titre in the plant because this decreases the probability that a farmer recognises the plant to be infected and not use it for cutting material. When the cutting selection is very effective however (x_{half} smaller than 0.2) vertical transmission rates become so small that the virus attains a higher fitness if it relies more on horizontal transmission. Horizontal transmission is more effective at higher virus titre and the ESS virulence jumps to much higher values. Similar reasoning explains the effect of introducing *in vitro* propagated planting material. Interference with the vertical transmission route results in considerable changes of the evolutionary stable virulence.

The consensus view in the literature is that vertical disease transmission (cutting transmission in the present model) promotes low virulence of the disease and horizontal (vectored) transmission promotes high virulence (Dieckmann *et al.*, 2002). Such effects of vertical transmission on virulence were also found by Lipsitch *et al.* (1996). The finding that interference with horizontal transmission does not change the CSS-virulence contradicts Lipsitch *et al.* (1996) findings but does agree with the

insensitivity of ESS-virulence with respect to horizontal transmission found in SI models (see van Baalen, 1995). The different responses to interference with transmission have implications for virulence management and therewith for the development of disease management programs that ensure the sustainability of agricultural production.

The management of pathogen virulence is a much investigated topic, with the recent publication by Dieckmann *et al.* (2002) giving an extensive overview of the state of the art. Very few of the theoretical and experimental studies relate to the management of plant pathogens, although some of the theoretical studies could be applied to plant pathology. In this chapter we studied virulence management of viral plant diseases, where we considered the evolution of the titre the virus builds up in the plant. Our conclusions thus only relate to the evolution of this trade of the virus. Other trades of the virus can also evolve, such as the ability to silence genes expressing plant resistance, and need to be considered before general conclusions can be drawn. Furthermore additional mechanisms, such as the much investigated effect of multiple infections, have to examined for their effect on the qualitative results presented here.

On basis of our results we can start to classify disease management methods that can help to develop sustainable agricultural production and methods. Generalising from the model outcomes we can distinguish two groups of disease control methods.

1. The first group, including rouging, vector management and cultivar resistance, are disease management methods that do not put a selection pressure on the virus to evolve towards higher virulence. Vector management and the use of resistant cultivars have no effect on the CSS-virulence and rouging even reduces the CSS-virulence. These methods thus are good candidate disease management methods in the development of sustainable agricultural systems.
2. The second group, including *in vitro* propagated planting material, cutting selection and the use of tolerant cultivars, do put a selection pressure on the virus to evolve towards higher virulence. This does not immediately imply that these disease control methods should not be implemented, however. It depends on the balance between loss, due to potential higher virulence, and gain, due to reduced disease incidence, whether these methods can contribute to the development of sustainable agricultural system. It does however show that evolutionary considerations have to be taken into account when developing disease control methods involving in vitro propagation, tolerant cultivars and cutting selection.

III. 2. Models that elucidate the possible effects of *Bemisia tabaci* management on evolutionary changes

Bemisia tabaci (Homoptera: Aleyrodidae) was first described as a pest of tobacco by Gennadius in 1889. Due to its ability to adapt quickly to new environments, it can now be found on over 600 host plant species and on all continents except Antarctica (Oliveira *et al.,* 2001). The occurrence and persistence of Bemisia tabaci in almost all regions of the world ranging from tropical to more temperate zones, has been facilitated by mankind (Byrne *et al.,* 1990). Mostly due to the transportation of whiteflies to new localities and changing of the existing environments through different practices that allow these insects to become more successful in surviving. Over the last two decades whitefly damage to crops, caused by feeding and geminivirus transmission, has increased leading to excessive reduction in harvestable products.

This increase in damage is said to be correlated with the occurrence of new biotypes of *Bemisia tabaci* of which 24 have been identified (Perring, 2001; Legg *et al.,* 2002). Biotypes are populations that lack morphological distinction, but that possess other characteristics which serve to separate them from other populations (Claridge *et al.,* 1997). Of these biotypes some are widespread e.g. the B-biotype and others are less common (Bedford *et al.,* 1994). Some are polyphagous whereas others are almost monophagous. Due to the occurrence of these biotypes it is suggested that *Bemisia tabaci* is undergoing evolutionary change (Brown *et al.*, 1995). The biotypes differ mostly in biological traits like fecundity, longevity, feeding rates, ovipositioning rates and developmental rates (Diehl & Bush, 1984; Costa & Brown, 1991; Bedford *et al.,* 1994). Their biology is highly influenced by their environment, which is determined by factors such as the crops that are available, the temperature, relative humidity and natural enemies (Powell &Bellows, 1992; Berlinger *et al.,* 1996; Drost *et al.,* 1998). Some of these factors are changed through crop and pest management practices. In this research we investigate the following three examples of management practices that are said to bring about changes in the population dynamics of *Bemisia tabaci*:

- **(I)** The introduction of a new crop or new crop variety into a cropping system (Morales & Anderson, 2000).
- **(II)** The use of pesticides (Rodriguez & Cardona, 2001)

There is some doubt to whether or not the above mentioned crop and pest management practices lead to *evolutionary* changes in *Bemisia tabaci*. In this paper we use Adaptive Dynamics approximations (Metz *et al.,* 1992; Geritz *et al.,* 1998) to investigate if the three crop and pest management practices mentioned above can lead to the evolutionary change observed in *Bemisia tabaci.*

Adaptive dynamics

To study the evolution of *Bemisia tabaci* we use the adaptive dynamics approach (Geritz *et al.,* 1998). Adaptive dynamics studies the population dynamics of rare mutants, and combines the occurrence of various types of mutants into evolutionary stability conditions. The assumption is made that reproduction is clonal and all variation is generated due to mutations. These approximations allow us to look at phenotypic evolution and more intricate ecological interactions. All mutant

phenotypes that occur are assumed to be random, similar to the resident phenotype, independent of the function they change and follow some probability distribution. The fate of rare mutants is determined by their fitness, also termed the Invasion fitness. Invasion fitness is defined as the long-term exponential growth rate (λ) of a population in an environment set by the resident population (Metz *et al.*, 1992). The fitness of the mutants, in an environment determined by the resident, is used in addition to natural selection to determine the course of evolution.

The analysis

Once the phenotypic trait (x_{res}) of the resident population that is likely to be undergoing evolution is selected, the evolution of such a monomorphic population can be studied. Mutants with trait or strategy x_{mut} can invade the resident population if they have a fitness advantage over the resident phenotype, a positive fitness $\lambda_{xres}(x_{mut})>0$. On the contrary mutants that have a fitness disadvantage, a negative fitness $\lambda_{xres}(x_{mut})>0$, will go extinct. Tools used to study the evolution are Pairwise Invasibility Plots. These plots contain the fitness of invader phenotypes for different values of the resident phenotype (Figure A1). The region with the minus (-) sign in the plots represents negative fitness values and the region with the plus sign (+) contains positive fitness values. The principal diagonal contains invaders that are equal to the resident, their fitness therefore is zero. It is assumed that the invaders that do emerge are very similar to the resident; therefore we focus on a small band around the

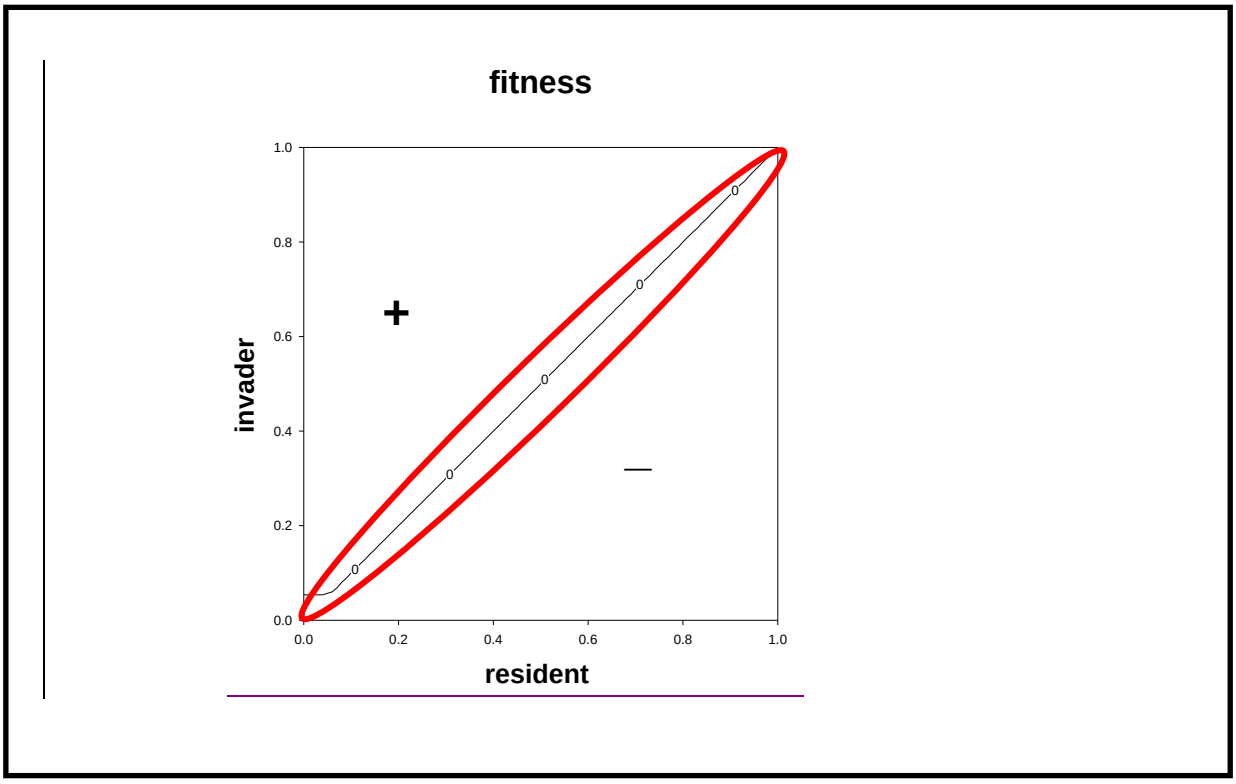


Figure 2.1. Example of Pairwise Invasibility Plot, showing the sign of the fitness calculated for numerous pairs of resident and invader phenotypes. On the x-axis the resident phenotype and on the y-axis the invader phenotype are denoted. The band around the diagonal is showing the boundary for possible invaders.

diagonal. To determine what invaders can spread we look along a vertical line through the resident phenotype. The parts of the line in the positive area indicate invaders that can spread and take over the resident population. The parts of the line in the negative region indicate invaders that will go extinct. In Figure 2.1 we see that no matter what the resident strategy is at start of evolution, mutants that have a larger phenotype can spread and take over. The end result of evolution is a resident with phenotype strategy equal to 1. The intersection of the diagonal and another line where the fitness equals zero is called a **Singular Strategy** (x^*). At this strategy directional selection does not take place. Singular strategies can be one of the following:

1). A continuously stable strategy (CSS). This singular point (x^*) is convergence stable and an evolutionary stable strategy (ESS) that cannot be invaded by any mutants (Figure 2.2). The outcome of evolution in this case is a population that is monomorphic, consisting of one biotype.

2). An Evolutionary Branching point.This singular strategy attracts evolution and once it has been reached can be invaded by mutant phenotypes that are larger; $x_{mut} > x^*$ and smaller $x_{mut} < x^*$ (Figure 2.3). The outcome of evolution in this case can be a population that is dimorphic, consisting of two biotypes.

3). An Evolutionary repeller (Figure 2.4). When the singular strategy is an evolutionary repeller a monomorphic population will evolve away from it.

Model description

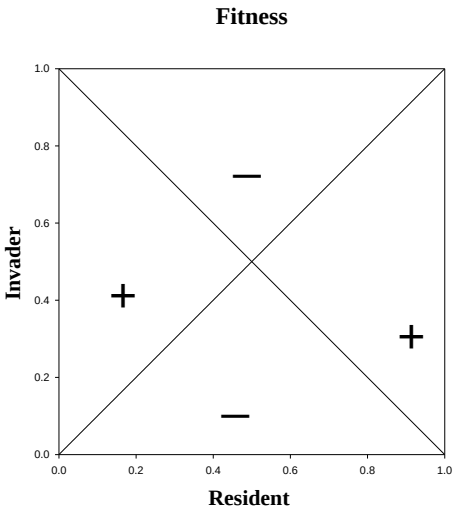
In order to apply Adaptive dynamics we need to construct a Population dynamic model that contains the ecological aspects of the population. To model the ecology of whiteflies we assume that there are two species of host plants modelled as two different crops (Figure 2.5). Each crop's rate of increase and decrease depends on the planting rate and harvesting rate and the natural mortality of the crop. Another cause of host plant decrease is pest-induced mortality, which occurs as a result of feeding by the pest. There is one population of whiteflies divided into two subpopulations over these two crops. Initially the whitefly population is monomorphic, consisting of one biotype. The total number of whiteflies on a host plant increases due to immigration and birth and decreases due to mortality and emigration. We assume that not all migrating whiteflies succeed in finding a host. Population Dynamics of host plants and Whitefly.

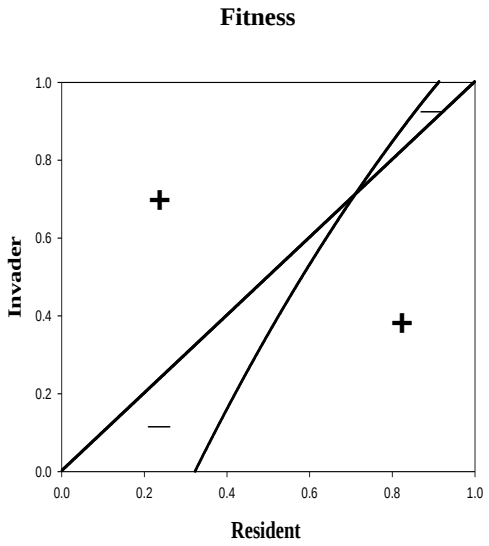
We construct a continuous time model with the assumptions made above. For each crop in the model the following equation can be constructed

$$\frac{dH_i}{dt} = \begin{bmatrix} increase\ in \\ hostplant\ biomass \end{bmatrix} - \begin{bmatrix} decrease\ due\ to \\ harvest\ and\ mortality \end{bmatrix} - \begin{bmatrix} decrease\ due\ to \\ feeding\ by\ whiteflies \end{bmatrix} \quad (1)$$

We assume that the increase in total biomass is density dependent and only influenced by planting and the effect of growth is negligible

$$\begin{bmatrix} increase\ in \\ hostplant\ biomass \end{bmatrix} = r_i\left(1 - \frac{H_i}{K_i}\right) \quad (2)$$





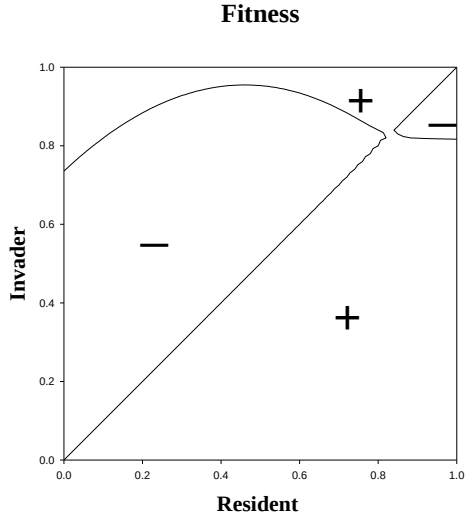


Figure 2.2. Example of a Pairwise Invasibility Plot showing an CSS. On the x-axis the resident phenotype is denoted and on the y-axis the invader phenotype.

Figure 2.3. Example of a Pairwise Invasibility Plot, showing a branching point. Axis as 2.2.

Figure 2.4. Example of a Pairwise Invasibility Plot showing an evolutionary repeller. Axis as 2.2.

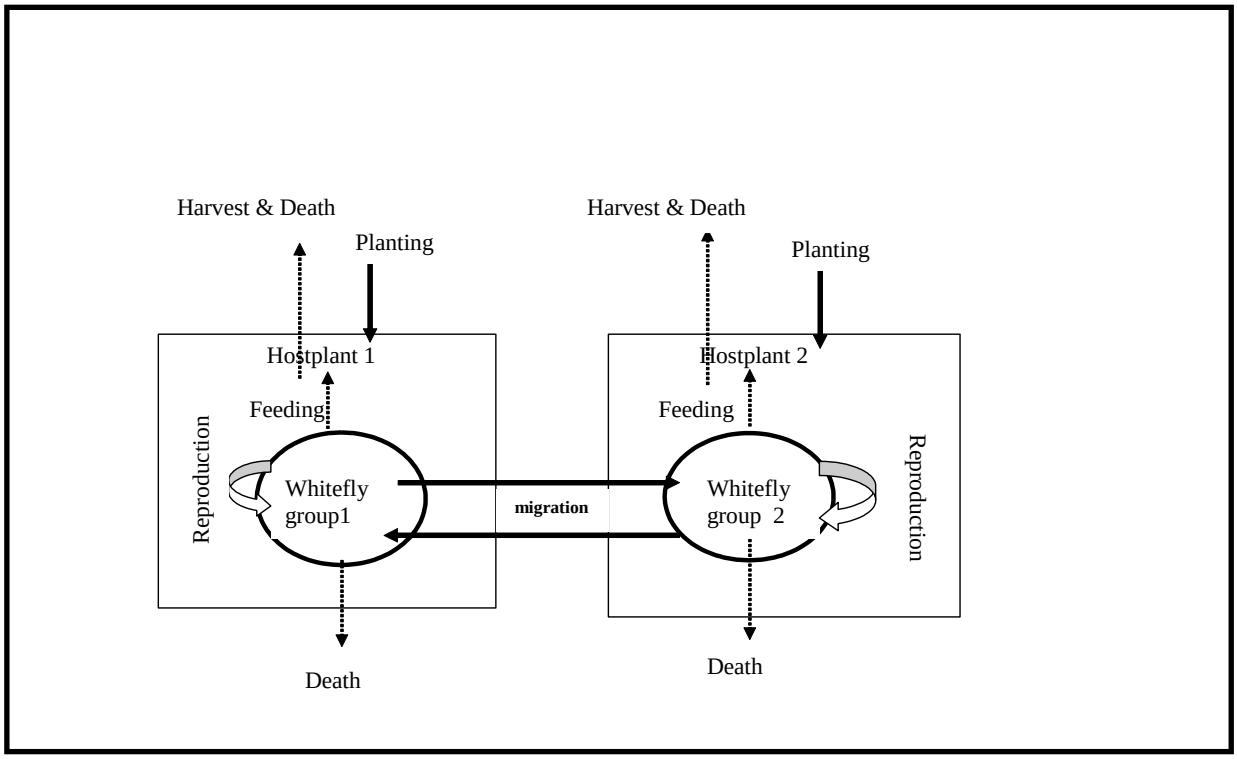


Figure 2.5. A schematic representation of the model. Showing the relationship between the host plants and the whiteflies.

Because feeding by whiteflies on host plants occurs only on leaves we assume that H_i represents the total host plant leaf biomass, r_i is the rate of increase of the host plants, and K_i is the plant density with i=1,2. We assume that harvesting and natural mortality are proportional to the amount of the crop in our system.

$$\begin{bmatrix} decrease\ due\ to \\ harvest\ and\ mortality \end{bmatrix} = \eta_i H_i \tag{3}$$

η_i is the rate of decrease due to harvesting and mortality. We assume that the amount of host plant consumed by the whiteflies on a host plant is proportional to the total number of whiteflies and the host plant biomass.

$$\begin{bmatrix} decrease\ due\ to \\ feeding\ by\ whiteflies \end{bmatrix} = \rho_i P_i H_i \tag{4}$$

ρ_i is the feeding rate per whitefly and P_i is the number of whiteflies on a host plant, with i=1,2.
The population dynamics for the whiteflies becomes

$$\frac{dP_i}{dt} = \begin{bmatrix} increase\ due\ to\ conversion \\ of\ \ food \text{ into } offspring \end{bmatrix} - \begin{bmatrix} decrease\ due \\ to\ death \end{bmatrix} - \begin{bmatrix} decrease\ due \\ to\ emigration \end{bmatrix} + \begin{bmatrix} increase\ due \\ to\ immigration \end{bmatrix} \quad (5)$$

We assume that the offspring is proportional to the amount of host plant biomass consumed

$$\begin{bmatrix} increase\ due\ to\ conversion \\ of\ \ food \text{ into } offspring \end{bmatrix} = e_i \cdot \text{[illegible]} \quad (6)$$

Here e_i the efficiency with which food is converted to offspring and can be described by the following equation

$$e_i = \frac{\tilde{e}}{\theta_i} \quad (7)$$

Where $\check{e}$ is the number of offspring that is produced per unit consumed host plant per whitefly and θ_i is the plant density.

$$\begin{bmatrix} decrease\ due \\ to\ death \end{bmatrix} = \mu_i P_i \quad (8)$$

μ_i is the vector mortality rate. The whiteflies can migrate freely between the two host plants and the migration rate is proportional to the number of whiteflies on the host plant.

$$\begin{bmatrix} decrease\ due \\ to\ emigration \end{bmatrix} = m_i P_i \quad (9)$$

We assume that migrating whiteflies have a survival rate that is constant and a constant chance of landing on either host plant during migration.

$$\begin{bmatrix} increase\ due \\ to\ immigration \end{bmatrix} = \psi\omega_{i,1} m_1 P_1 + \psi\omega_{i,2} m_2 P_2 \quad (10)$$

With m_i the migration rate, and ω_{ij} is the chance of finding a host plant of type i when migrating from host plant of type j, with i=1, 2 and j=1,2 and ψ is the percentage of whiteflies that survive during migration.

Trade-off relationship

It is suspected that for phytophagous insects natural selection is likely to favour moderate fecundity and viability on the different host plants and fitness is not maximised on any host.

We assume that there exists a trade-off in performance on the two host plants in the crop system. This implies that having a high fitness on one host comes at the expense of the fitness on the second host. For a phenotypic trait that is undergoing evolution and denoted as x we assume the following trade-off relationship

$$f(x_1) = B \cdot \frac{x_1 - 1}{x_1 - B} \quad (11)$$

Table1. Model parameters.

Parameter	***Description***	***Dimension***
H	*host plant leaf biomass*	grH/m^2
r	*planting rate*	grH/m^2day
η	*harvest rate and host mortality rate*	$1/day$
K	*plant density*	grH/m^2
P	*vector density*	$1/grH$
μ	*vector mortality rate*	$1/day$
ω	*chance of finding a host plant*	$1/day$
ρ	*feeding rate whitefly*	grH/day
m	*migration rate*	$1/day$
e	*conversion efficiency of food into offspring*	m^2/grH^2

The parameter values for the host plant and whitefly population dynamics where determined from literature to obtain realistic ranges. For the host plant dynamics parameter values were taken from cassava (*Manihot esculenta*), sweet potato (*Ipomoea batatas*) and cotton (*Gossypium hirsutum)* crops although no specific crop type is modelled. The plant density, *K*, can be highly variable and depends on the type of crop planted. For cotton we found it to be 345 grH/m^2 (Gutierrez *et al.*, 1975). For sweet potato the plant density is equal to 350 grH/m^2 (Agata, 1982) and for cassava it was found to be about 100 grH/m^2 (IITA, 1990). The parameter η is determined as the sum of the host plant mortality and the harvest rate. The mortality rate is deduced from the longevity of leaves. For cassava the average leaf lives about 60 to 120 days (IITA, 1990). The mortality of an individual leaf is between 0.008 and 0.016 day^{-1}. The average harvest rate varies with the crop and crop varieties. Cotton for example is harvested after 107 days or 122 days or 185 days after sowing (Harding, 1922). The harvest rate is 0.007 day^{-1}. For sweet potato the time of harvest largely depends on the variety and soil moisture content during the first month of plant development. However the actual commercial varieties used take different times to harvest. The early varieties take 3 to 4 months, the medium varieties take 4 to 6 months and the late varieties take more than 6 months with a maximum of 12 months. The growth cycle of any variety can be altered by an excess of moisture in the first 3 months after planting. This produces an intense foliar development and a late formation of tubers. Often in these cases the number of tubers is reduced. It can therefore be harvested any where between 3 to 6 months and beyond. The range of the harvest rate is from 0.001 to 0.003 day^{-1}. The harvest rate for cassava was in the range of 0.002 and 0.004 day^{-1}(Holt *et al.*, 1997). With these harvest rates and mortality rates the range for decrease due to harvest and mortality, η, becomes between 0.009 and 0.023 day^{-1}. The planting rate, *r*, is found to be equal to 0.05 plants per day which is equal to 2.5gram/dag (Holt *et al.*, 1997)

The only value found for the feeding rate of whiteflies was 0.02mg/mgDD given as the maximum demand rate by Mills & Gutierrez (1996). The maximum feeding rate per whitefly would then become 0.014x10^{-3} grH/day This gives us a range between 0 and 0.014x10^{-3} for the feeding rate. Because of the sensitivity of the model to the feeding rate we increased the maximum feeding rate to 1 grH/day and varied it across a range between 0 and 1 in order to better analyse the results. The migration rate, *m*, measured for *Bemisia tabaci* is between 0.68 and 0.94 (Byrne *et al.*, 1996).

We assume the chance of finding a host plant, ω equals 0. 5. The vector mortality rate is deduced from the longevity μ =1/(longevity). Longevity measured in literature varies from 1 day to about 38 days (Powell & Bellows, 1992; Enkegaard, 1993; Legg, 1996). The range for the mortality is between 0.026 and 1 day^{-1}. The efficiency of food conversion, e, is taken from Mills & Gutierrez (1996) is 0.5, but is across a range from 0 to 1.

Here x_1 is the value of the trait on host plant 1 and $f(x_1)$ is the value of the trait on host plant 2.

Literature shows that several traits are different between the biotypes and it could be that one of these traits is undergoing evolution (Lisha *et al*., 2003). For the analysis here we focus on the three following traits and for each trait we assume a trade-off.

1) The feeding rate. The feeding rates on host plant 1 and host plant 2 in equation (4) become ρ_1 and $f(\rho_1)$ respectively.
2) The efficiency of food conversion. The values in equation (6) will become for host plant 1 e_1 and for host plant 2 $f(e_1)$.

The trade-off functions we focus on in this paper are **(1)** concave where f"(x) < 0 with B=1.5 and g"(x) < 0 with A and C are 0.982 and 0.026 respectively and **(2)** convex where f(x) > 0 with B= -0.5 and g"(x) < 0 and C are 1.052 and 0.026 respectively.

They are of the following shape:

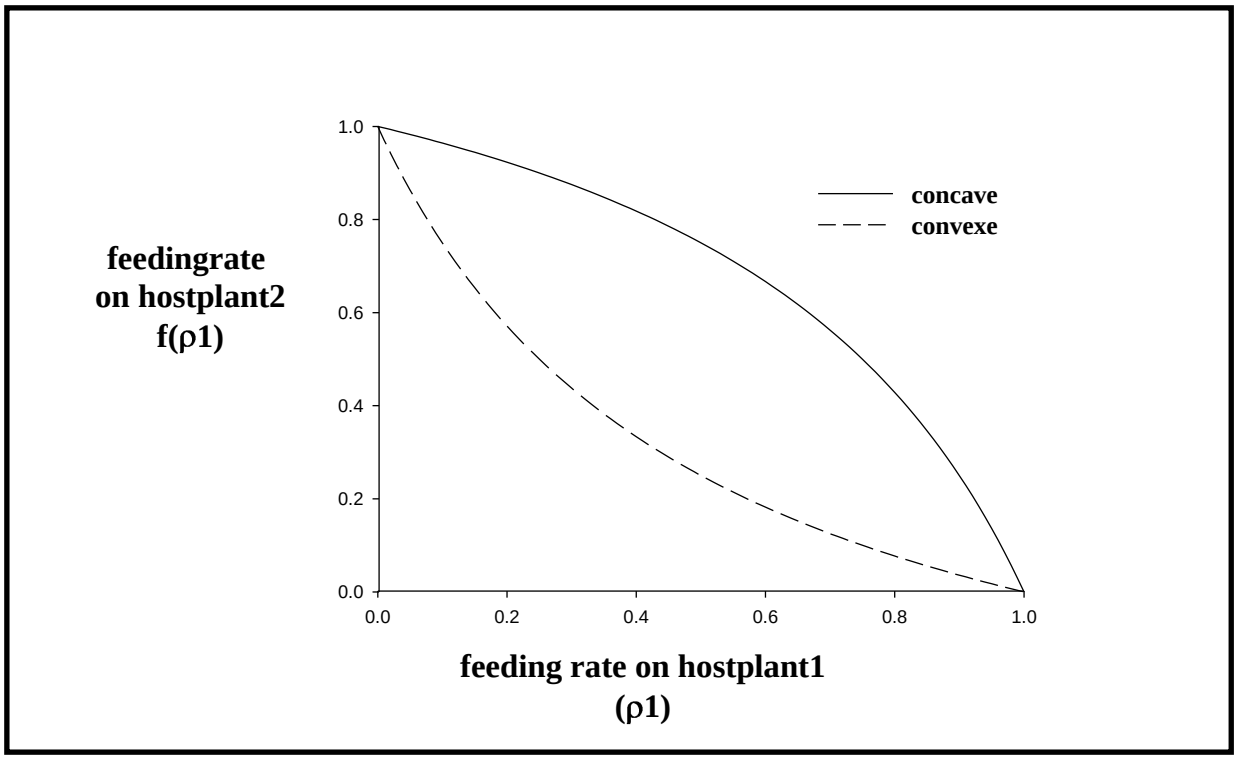


Figure 2.6: The graph shows the trade-off function for the feeding rate as the evolving trait. The function plotted is $f(x_1)=B(x_1-1)/(x_1-B)$ with B =1.5 for the concave shape and B=-0.5 for the convex shape. This graph also shows the trade-off relationship for when the evolving trait is the efficiency of food conversion

At x = 0 the biotype is completely specialised on host plant 2. At x = 1 the biotype is specialised on host plant 1.This implies that in the case of the feeding rate being the trait undergoing evolution that if the feeding rate is 0 on host plant 1 it will only feed on host plant 2 and the reverse is true if feeding rate1 equals 1. For the efficiency of food conversion the same holds true.

Methods

Stability of the population dynamics:
For each trait that we assume to be undergoing evolution we investigate the ecological dynamics of the population. We solve the set of equations of the population Dynamics to find the equilibrium densities of the host plants and Whiteflies. This is done numerically in Maple notebooks. To look at the adaptive dynamics of this model, we need to work with the parameter values that give positive stable population densities. Once the equilibrium densities are determined we look at their stability by applying the Routh-Hurwitz criteria (Edelstein-Keshet, 1988).

The Routh-Hurwitz criteria state that for the elements a_1, a_2, and a_3 of the characteristic equation determined from the Jacobian of the Population dynamics the following should be true:

$$a_1 > 0 \ ; \ a_3 > 0 \ ; \ a_4 > 0 \text{ and } \ a_1 * a_2 * a_3 > a_1^2 * a_4$$

Furthermore for each trait that we assume to be undergoing evolution we investigate the three crop and pest management practices by adjusting the model parameters as described below.

(I). Introducing a new crop to a cropping system
To look at the effect of introducing a second crop to a cropping system we start by just planting host plant 1 look at the results and look at how those change when we add host plant 2 to the crop system by increasing its planting rate.
We investigate two scenarios:

(1). there is an additional area used for the new crop, so adding the second host plant has no consequences for the capacity or planting rate of the crop that was already planted in our crop system.

(2). the total area used for both crops is constant thus adding a second crop would mean that the capacity and planting rate of the first crop has to be reduced to accommodate a second crop.

For the second scenario we assume that there is a maximum cropping capacity $K_{\max}$ and a maximum planting capacity r where

$$\begin{aligned} K_2 &= K_{\max} - K_1 \\ r_1 &= r - r_2 \\ K_1 &= \frac{r_1 K_{\max}}{r_1 + r_2} \end{aligned} \qquad (12)$$

(II) The effect of pesticide use
To look at the effect of pesticide use we increased the mortality rate (μ_i) for the whiteflies on the host on which pesticides are used. We investigate how evolution of whiteflies is influenced when pesticides are used on either host plant 1 or host plant 2.

The results for the feeding rate as the evolving trait.

The equilibrium values and stability analysis
The equilibrium densities $\hat{H}_1$, P_1, $\hat{H}_2$ and P_2 could only be solved numerically and this was done in Maple notebooks. I looked at the way feeding rate influences their

stability. For the range of the feeding rate the Routh–Hurwitz criteria were satisfied, this implies that the equilibrium densities are stable for this range. When the feeding rate is the evolving trait the equations for the mutant with feeding rate $\rho_{\max}$ become:

$$\begin{aligned}\frac{dP_{mut,1}}{dt} &= e_1 \cdot f(\rho_{mut})P_{mut,1}H_1 - \mu_1 P_{mut,1} - m_1 P_{mut,1} + \omega_1 m_1 P_{mut,1} + \omega_1 m_2 P_{mut,2} \\ \frac{dP_{mut,2}}{dt} &= e_2 \cdot f(\rho_{mut})P_{mut,2}H_2 - \mu_2 P_{mut,2} - m_2 P_{mut,2} + \omega_2 m_2 P_{mut,2} + \omega_2 m_1 P_{mut,1}\end{aligned} \quad (15)$$

The invasion fitness is determined as the dominant eigenvalue of the Jacobian of (15) and we assume that the resident system in (1) is at equilibrium.

$$Jacobian = \begin{bmatrix} e_1\rho_{mut}\hat{H}_1 - \mu_1 - m_1 + \psi\omega_{1,1}m_1 & \psi\omega_{1,2}m_2 \\ \psi\omega_{2,1}m_1 & e_2 f(\rho_{mut})\hat{H}_2 - \mu_2 - m_2 + \psi\omega_{2,2}m_2 \end{bmatrix} \quad (16)$$

Introducing a new crop to a cropping system.

In the case of a concave trade off relationship when we start out with a crop system where one crop is continuously planted the outcome of evolution is that the population will stay monomorphic (Figure 2.8). In the case of a convex trade-off relationship we get the same result as for a concave trade off relationship when there is only one host plant in our cropping system. No matter what the resident phenotype is any possible invader with a larger value has a fitness advantage. It will take over and replace the resident. A biotype can evolve that has a feeding rate equal to 1 on the crop planted, which means that it maximises its feeding rate on that host. This situation was simulated in order to verify if a host specialised biotype evolves (Fig 2.9)

Scenario 1: For the first scenario of replacing the monoculture by adding a second crop to the cropping system (See Methods) we get a PIP with a branching point (Figure 2.10). If the population of whiteflies had become specialised on host plant 1 in the monoculture, the population will evolve towards the branching point under the assumption of a convex trade- off relationship. At the branching point the population will split and two biotypes will evolve, Figure 2.11 It doesn't matter how high the planting rate of the second host plant is we still get branching. Under the convex trade–off relationship when the planting rate of the second host plant is increased so that r2=2, we see that if the population was specialised on the first host plant it will remain host specialised if not it will become host specialised (Fig 2.12). A slightly higher planting rate, r2=2.5, gives one Singular strategy (SS) (Fig 2.13). When the planting rate is r2=3 a PIP is generated with two SS's (Fig 2.11). If the population had specialised on the first crop the population will remain host specialised. The first SS is a Branching point, if this is reached the population split and become dimorphic. The second SS is a repeller. If the population had a very high feeding rate on host plant 1 (rho1>S.S) then the population will become specialised on that host plant. Even higher planting rates give the same results.

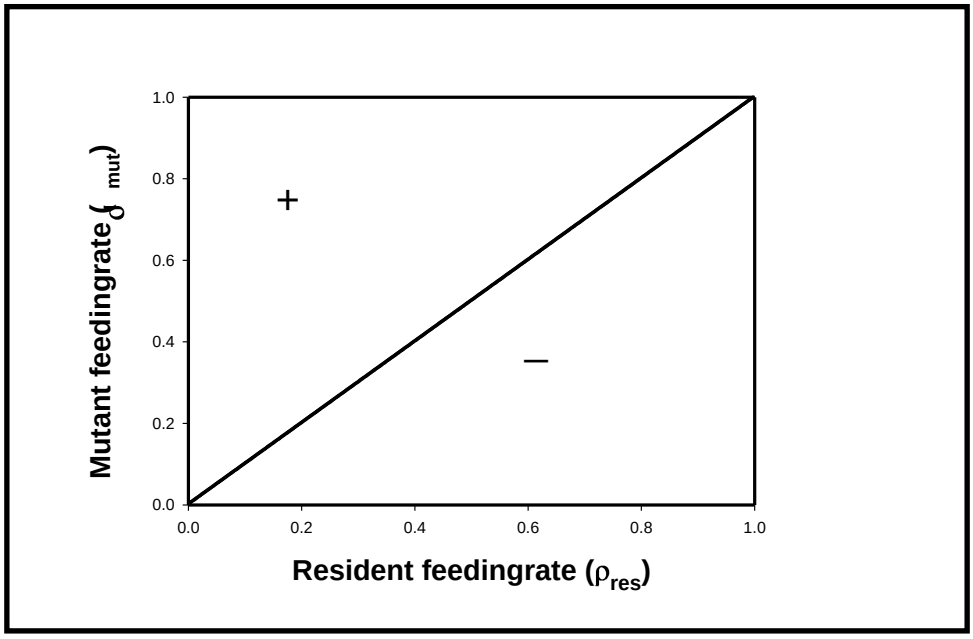


Figure 2.8 Shows the fitness for a convex trade off relationship for introducing a new crop. Here the planting capacity is not limited. Regions with positive fitness and negative fitness indicated by + and – respectively.The parameter values used are r_1=10, r_2=0, K_1=350, K_1=100, e_1=0.3, e_2=0.5, ψ=0.2, ω_{11}=0.5, ω_{12}=0.5, m_1=0.8, m_2=0.8, ω_{21}= 0.5, ω_{22}=0.5, η_1= 0.022, η_2= 0.014, μ_1= 0.07, μ_2=0.12.

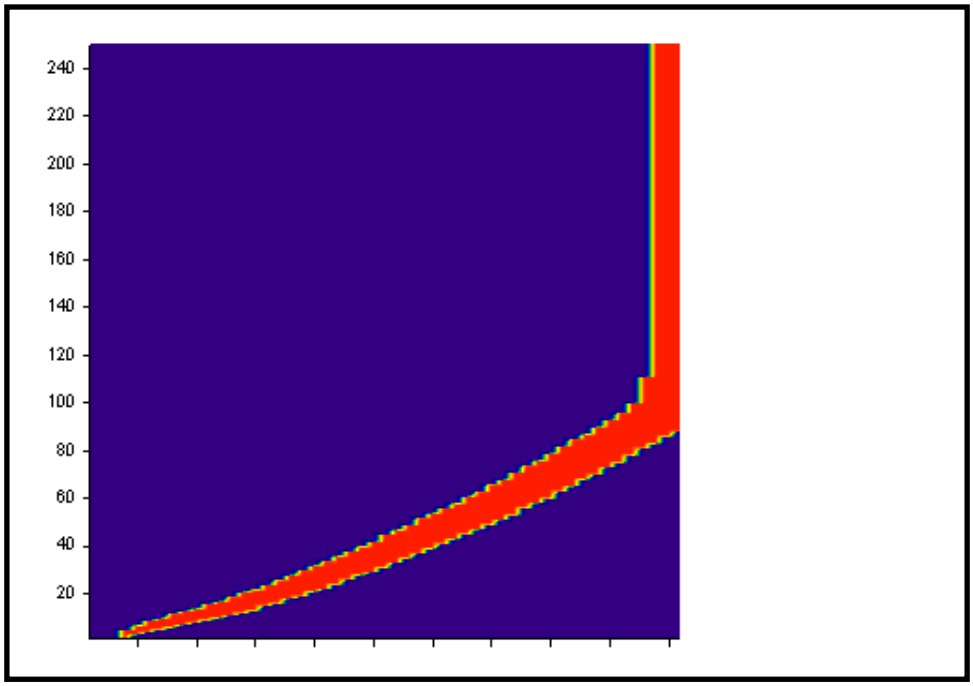


Figure 2.9. Shows the simulation of evolution for specialisation on host plant 1.

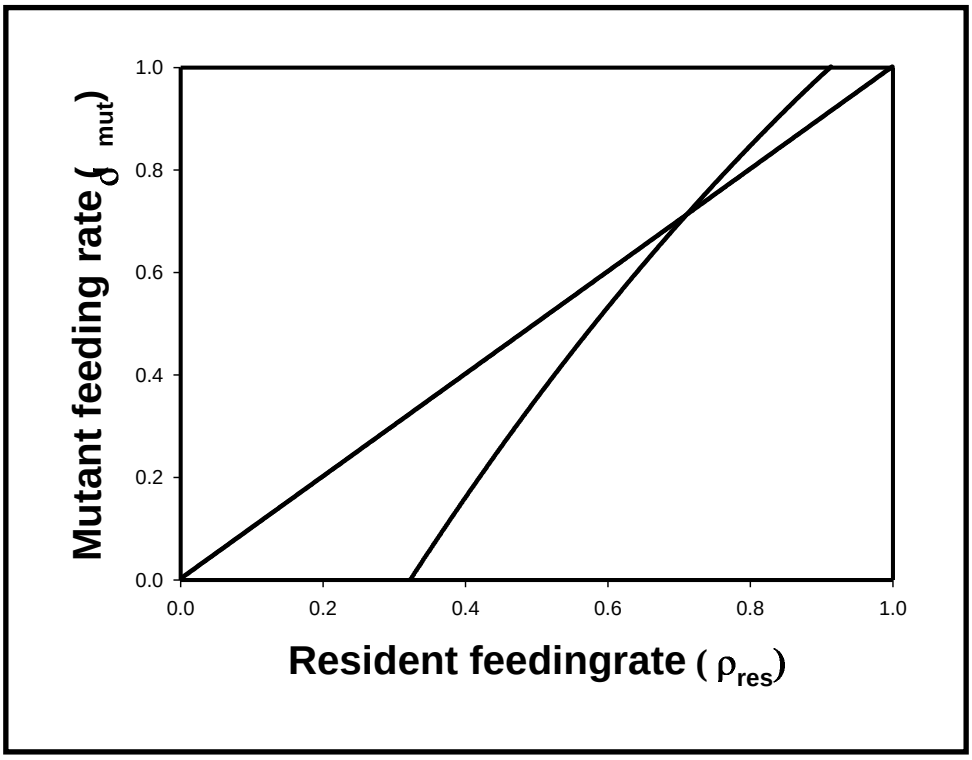


Figure 2.10 Shows the fitness for a convex trade off relationship for introducing a new crop. Here the planting capacity is not limited. Regions with positive fitness and negative fitness indicated by + and – respectively. The parameter values used are r_1=10, r_2=2, K_1=350, K_2=100, e_1=0.3, e_2=0.5, ψ=0.2, ω_{11}=0.5, ω_{12}=0.5, m_1=0.8, m_2=0.8, ω_{21}= 0.5, ω_{22}=0.5, η_1= 0.022, η_2= 0.014, μ_1= 0.07, μ_2=0.12.

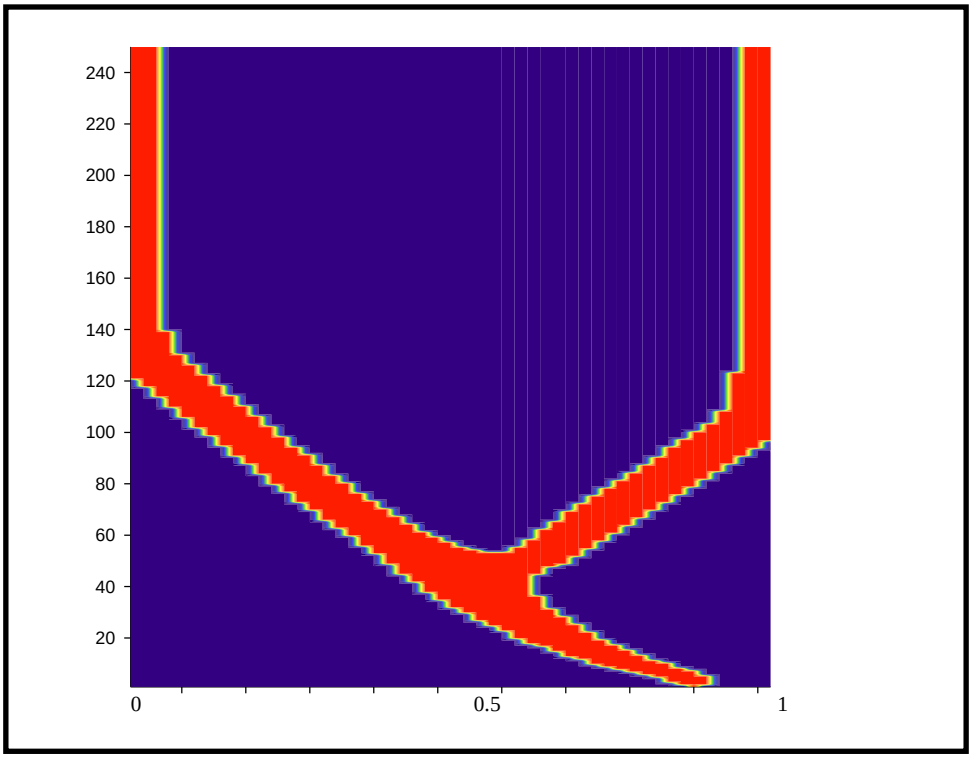


Figure 2.11. A monmorphic population splits and two biotypes evolve.

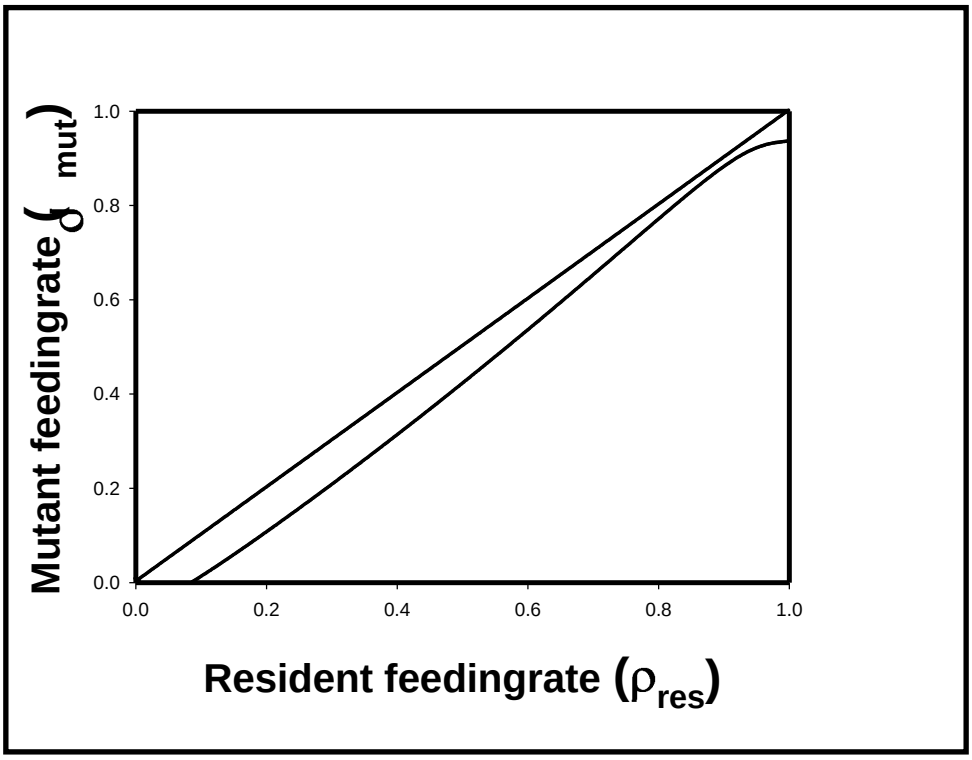


Figure 2.12. Shows the fitness for a convex trade off relationship for introducing a new crop. Here the planting capacity is not limited. Regions with positive fitness and negative fitness indicated by + and – respectively. The parameter values used are r_1=10, r_2=2, K_1=350, K_2=100, e_1=0.3, e_2=0.5, ψ=0.2, ω_{11}=0.5, ω_{12}=0.5, m_1=0.8, m_2=0.8, ω_{21}= 0.5, ω_{22}=0.5, η_1= 0.022, η_2= 0.014, μ_1= 0.07, μ_2=0.12.

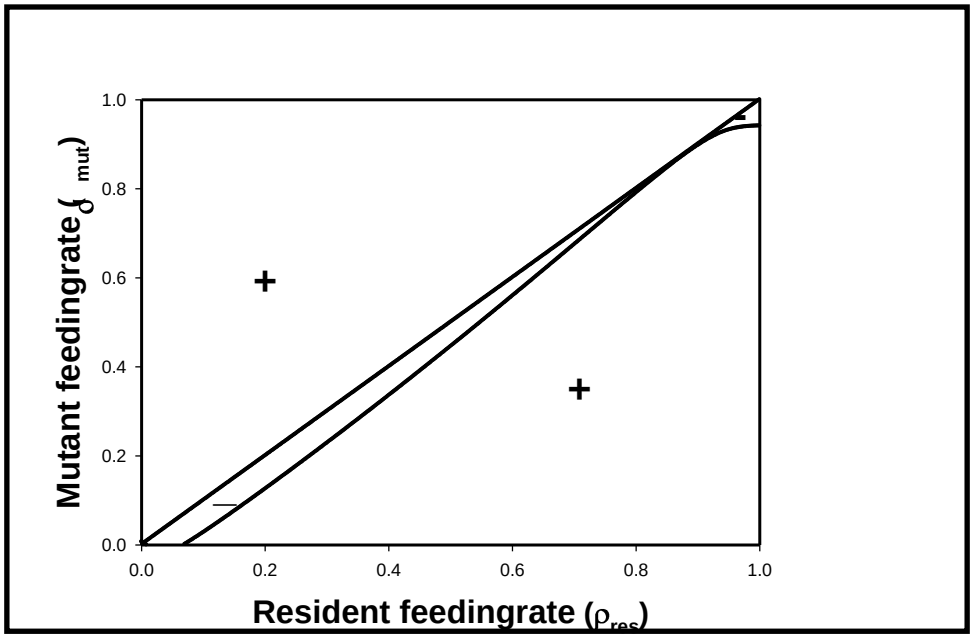


Figure 2.13. Shows the fitness for a convex trade off relationship for introducing a new crop. Here the planting capacity is not limited. Regions with positive fitness and negative fitness indicated by + and – respectively.

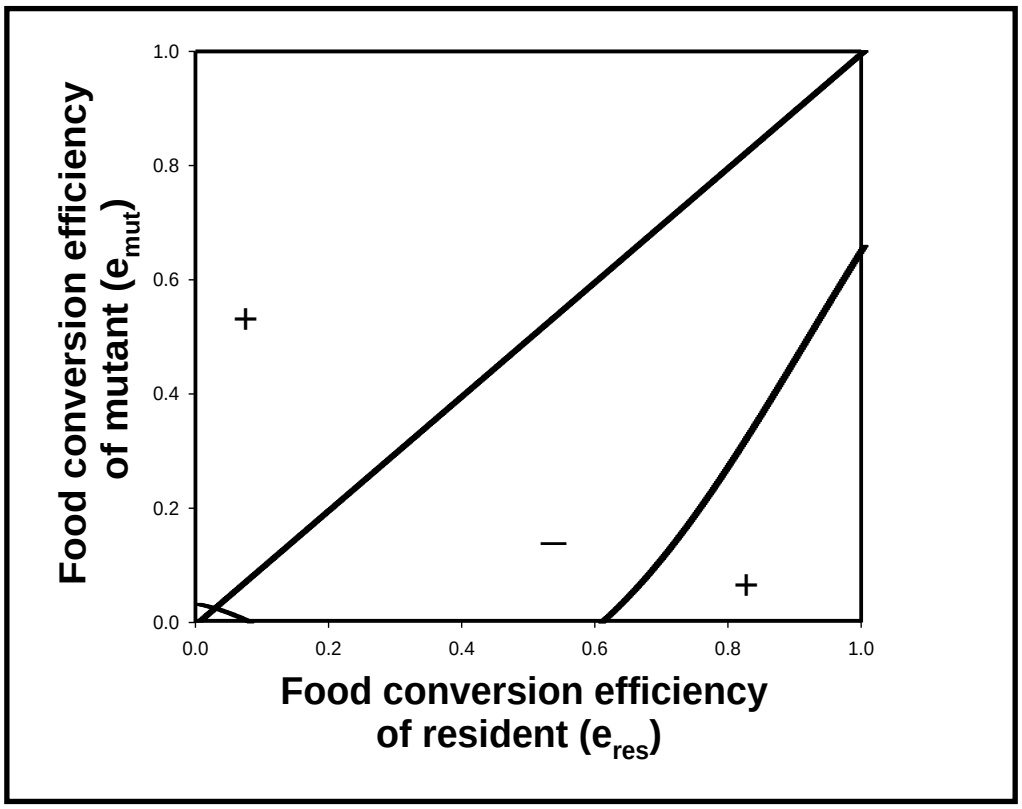


Figure 2.14. Shows the fitness for a concave trade off relationship for introducing a new crop. Regions with positive fitness and negative fitness indicated by + and – respectively. The parameter values used are r_1=10, r_2=2, K_1=350, K_2=100, e_1=0.3, e_2=0.5, ψ=0.2, ω_{11}=0.5, ω_{12}=0.5, m_1=0.8, m_2=0.8, ω_{21}= 0.5, ω_{22}=0.5, η_1= 0.022, η_2= 0.014, μ_1= 0.07, μ_2=0.12.

Scenario 2: We get similar results for introducing a second host plant at the cost of having to decrease the capacity and planting rate of the first host plant assuming a **concave** trade-off relationship. When the planting rate of the second crop is r2=2 we get branching point as described in Fig 2.10. Two biotypes will evolve with one specialised on host plant 1 and the other specialised on host plant 2 and this is the same for even higher planting rates for host plant 2. However in this case if the planting rate of host plant 2 becomes so large that planting of host plant 1 is very small, r2=9 and r1=1 a biotype will evolve that is specialised on host plant 2, the PIP generated has the same shape as that of figure 2.12.

These results remain the same for higher planting rates of host plant 2. If the planting rate is high enough, e.g. r2= 8, so relatively little of host plant 1 is planted, the population will evolve to become specialised on host plant 2 the graph generated is similar to that in Figure 2.10

The effects of pesticide use.

For the concave relationship if no pesticides are being used on either host plant 1 or host plant 2, the population will evolve towards the singular point (See Figure 2.10). At the singular point branching can occur; the population will then become dimorphic.

If we use pesticides on host plant 2 on low levels we get two SS's. The first is a branching point and the second a repeller. Depending on the resident strategy is the start of evolution we can either get specialisation on the first crop or evolution can be lead towards the branching point. In the latter case the population will become dimorphic. When we increase the level of pesticide use on host plant 2 the population evolves to become specialised on host plant 1. If pesticides were used host plant 1 instead starting with low levels of pesticide use , we get a branching point and if this is reached the population will become dimorphic (Fig 2.10). When pesticides are used on host plant 1 at high levels we get 2 SS's. One is a repeller and the other a Branching point. Depending on the strategy of the population at the start of evolution we can either get a biotype that is specialised on host plant 2 or the population can become dimorphic.

The results for efficiency of food conversion as the evolving trait.

The equilibrium values and stability analysis

Again the equilibrium densities could only be solved numerically, so we look at the way their stability was influence by different values of the efficiency for food conversion. The analysis done in Maple shows that the Routh-Hurwitz criteria were satisfied.

When the efficiency of food conversion is the evolving trait the equations for the mutant with efficiency e_2 become:

$$\begin{aligned}\frac{dP_{mut,1}}{dt} &= e_{mut}\cdot \wp_1 P_{mut,1}\bar{H}_1 - \mu_1 P_{mut,1} - m_1 P_{mut,1} + \omega_1 m_1 P_{mut,1} + \omega_1 m_2 P_{mut,2} \\ \frac{dP_{mut,2}}{dt} &= f(e_{mut})\cdot \wp_2 P_{mut,2}\bar{H}_2 - \mu_2 P_{mut,2} - m_2 P_{mut,2} + \omega_2 m_2 P_{mut,2} + \omega_2 m_1 P_{mut,1}\end{aligned} \quad (17)$$

The invasion fitness is determined as the dominant eigenvalue of the Jacobian of (17) and the resident system in (1) is at equilibrium.

$$Jacobian = \begin{bmatrix} e_{mut}\rho_1\hat{H}_1 - \mu_1 - m_1 + \psi\omega_{1,1}m_1 & \psi\omega_{1,2}m_2 \\ \psi\omega_{2,1}m_1 & f(e_{mut})\rho_2\hat{H}_2 - \mu_2 - m_2 + \psi\omega_{2,2}m_2 \end{bmatrix} \quad (18)$$

Introducing a new crop to a cropping system

Again when we start out with a crop system where one crop is continuously planted the outcome of evolution is that the population will stay monomorphic (Figure 2.8). No matter what the resident phenotype is any possible invader with a larger value has a fitness advantage. It will take over and replace the resident. A biotype will evolve that has an efficiency of food conversion equal to 1 on host plant 1.

Scenario 1: For the first scenario of replacing the monoculture by adding a second crop to the cropping system we get a SS that is a repeller at r2=2 (Fig. 2.14). We see that if the population had a high efficiency on host plant 2, it will remain specialised on host plant 2. But if the population had become specialised on host plant 1 when it

was planted as a monoculture, the population will stay specialised. Otherwise the population evolve towards being specialised on host plant1.
The situation doesn't change for when the planting rate is even higher, r2=15 we get the same PIP as for r2=2. In the case of a **convex** trade-off relationship we get the same result as for a concave trade-off relationship. When the planting rate of the second host plant is increased we see that the population will become specialised on host plant 2 if it already had a efficiency that was biased towards host plant2, otherwise the population will become specialised on host plant1. See fig 2.14. Increasing the planting rate of host plant 2 even more doesn't change the situation. One PIP is generated with one Repelling Singular strategy.

The effects of pesticide use.

For the concave relationship if no pesticides are being used on either host plant 1 or host plant 2 a PIP is generated that containes Repelling singular strategy like figure 2.14. The population will become specialised on host plant 2 if it was biased to host plant 2 already. Otherwise it will become specialised on host plant 1.

If we start using pesticides on the host plant 2 at low levels (μ_2=0.9) the population of whiteflies that has a feeding rate biased to feeding on host 2 will become specialised on that host. Otherwise the population will become specialised on host plant 1. The PIP again has the same shape as that of figure 2.14. The same is true for when pesticides are used at high levels on host plant2 (μ2=1.5). If pesticides are used on host plant 1 we get the same results as for the use of pesticides on host plant 2 for low and high levels of pesticide.

In the case of convex trade off relationship when no pesticides are used on either host we get the same results as in the case with concave trade-off relationship.
When pesticides are used on host plant 2 or host plant 1 the results are the same as is for concave relationship.

Discussion.

In this chapter we have constructed a model that investigates the effect of three management practices namely the introduction of a new crop, the use of pesticides and the crop structure on the evolution of different biotypes of *Bemisia* tabaci by using the Adaptive dynamics method. The traits we look at to investigate the evolution within whiteflies are the feeding rate on a host plant and the efficiency with which food is converted. The model describes the (trade-off) relationship between one population of whiteflies and two types of host plants. That relationship is assumed to be either concave or convex.

The effect of a monoculture on the evolution of biotypes

When we investigate the model with one type of host plant continuously planted in a monoculture we show that one biotype will evolve that is specialised on using that crop effectively irrespective of the type of trade-off or the trait that is undergoing evolution. If the trait that is undergoing evolution is the feeding rate the population will evolve to have a maximum feeding rate on the crop in the monoculture. Direct simulation of the evolutionary process confirms this When we assume that the trait

that is evolving is the efficiency of food conversion a biotype will evolve that has a high efficiency on the monocrop and when the mortality rate is undergoing evolution we see that one biotype will evolve that has a low mortality on that crop. Known examples of monocultures leading to host specialisation are mostly of countries where there is a long history of planting of food staple crops. Such systems are found in cassava and sweet potato cropping systems in Africa. Cassava and sweet potato are grown yearly in very large quantities and this has lead to the evolution of two biotypes of *Bemisia tabaci* that are revered to as the cassava strain and the sweet potato strain (Burban *et al*., 1992).

The effect of the introduction of a new crop on the evolution of biotypes

We looked at the introduction of a new crop for two scenarios. The first one assumes that there is a separate area available for planting the new crop and the second assumes that there is a constant total cropping area for both crops. We get differences in the outcome of evolution within traits between the concave and the convex trade-off relationship and differences between traits. For the feeding rate assuming a concave trade-off relationship when a new crop is introduced to the crop system branching occurs and two biotypes evolve, no matter what the feeding rate of the population is at the start of evolution. On the other hand under the assumption of a convex trade-off relationship the population will remain monomorphic if it had become specialised on the first crop or had a feeding rate biased towards feeding on that host plant at the start of evolution. One biotype will evolve that is specialised on that host. Otherwise the population will split and become dimorphic.

For the efficiency of food conversion and the mortality rate there is no difference in results between the concave and the convex trade-off relationship unlike with the feeding rate. The population will remain monomorphic but unlike with the feeding rate it can either specialise on host plant 1 or specialise on host plant 2 depending on its strategy at the start of evolution. The only difference between the two scenarios is that in the second scenario if crop 2 was introduced at a very high planting rate leading to a very low density of crop 1 the population will be forced on to host plant 2 and can become specialised on that host. There are no examples of introduction of new crops actually leading to the evolution of biotypes of *Bemisia tabaci*. Nevertheless a review by Morales & Anderson (2000) which gives an out line of the history of the whitefly along with the drastic changes in agriculture over the past decades in the Americas could serve as one. In this review examples are given of new crops being introduced and how the whiteflies seemed to adapt to these crops. This could in evolutionary terms eventually have lead to the evolution of more biotypes.

The effect of the use of pesticides on the evolution of biotypes

When pesticides aren't used and the trade-off relationship is concave the monomorphic population will become dimorphic, two biotypes will evolve in the case of the feeding rate. The biotypes that evolve are each specialised on of both hosts. For the efficiency of food conversion the population will remain monomorphic being specialised on host plant 1 or host plant 2. When pesticides are used on host plant 2 at a level that leads to a slightly higher mortality rate (μ_2=0.9) one biotype that is specialised on the first crop or two biotypes evolve depending on the phenotype of the population at the start of evolution. When pesticide use on host plant 2 is increased so much that the mortality rate (μ_2=1.5) is very high we get a biotype that is specialised

on the first crop. When pesticides are used on host plant 1 at low levels (μ_1=0.9) the population becomes dimorphic rather than just specialising on host plant 2 but when the level of pesticide use is increased even more (μ_1=1.5) we see that the population can either specialise on host plant 2 or become dimorphic depending on the strategy at the start of evolution. This is due to the fact that we model hostplant 1 to be a more attractive host for whiteflies in the first place. Only when the level of pesticide use is very high will the whitefly population specialising on the pesticide free crop. For the convex trade-off relationship we showed that for the feeding rate one host specialised biotype on host 1 will evolve when pesticides are not used. When low levels of pesticides are used on host plant 2 (μ_2=0.9) one biotype will evolve that is specialised on host plant 1 and this remains the same for at high levels of pesticide use. When pesticides are used on host 1 at low and high levels we either get a biotype specialised on host plant 2 or a biotype specialised on host plant 1.

For the efficiency of food conversion as the evolving trait there is no difference in the outcome of evolution between the concave and convex trade-off relationship. The host plant on which pesticides are used and the level of pesticide use seem to be of no consequence. One biotype will evolve that is either specialised on host plant 1 or host plant 2 depending on the phenotype of the population at the start of evolution.

III. 3. An overview of existing knowledge on begomovirus molecular genetics

Begomoviruses (Family *Geminiviridae*, Genus *Begomovirus*) have been in co-existence with their plant hosts for a very long time, with the earliest known record of a plant virus disease in 752 AD being considered to have been caused by a begomovirus (Saunders *et al.*, 2003). It has, however, only been in the past 20-30 years that begomoviruses have become particularly serious constraints to productivity of dicotyledonous crops, such as bean, cassava, cotton, cucurbits and tomato in tropical and subtropical regions (Brown, 1994; Polston & Anderson 1997; Briddon & Markham, 2000; Otim-Nape *et al.*, 2000; Morales & Anderson, 2001; Varma & Malathi, 2003). As a result, begomoviruses are currently one of the most important groups of emerging plant viruses, and agricultural intensification is often proposed as one of the main causes for their emergence (Varma & Malathi, 2003; Xie & Zhou, 2003). Other underlying factors have included the spread of more polyphagous and fecund vector populations, the evolution of more aggressive virus variants, movement of infected planting material and the introduction of more susceptible plant varieties (reviewed by Varma & Malathi, 2003).

The serious begomovirus epidemics reported since the 1980s in the Western Hemisphere and Europe have generally been associated with population increases of the whitefly vector *Bemisia tabaci* (Gennadius), as a result of the spread of a more fecund and polyphagous biotype, termed the B-biotype (also termed *B. argentifolii* by Perring *et al*. (1993). This biotype has, through its greater transmission efficiency and host range, increased the severity of begomovirus diseases and facilitated movement of begomoviruses, particularly between cultivated and weed hosts (Polston & Anderson, 1997; Varma & Malathi, 2003). This has led to new plant-virus combinations and new virus variants arising through recombination (Ribeiro *et al.*, 2003).

The B-biotype has, however, not been responsible for some of the most serious begomovirus epidemics that have occurred in the Old World. These have been associated more with the emergence of recombinant begomoviruses, although much elevated local vector biotypes populations have also been found to be a general phenomenon. The first field report of recombination in begomoviruses was that of Zhou *et al.* (1997) when a recombinant virus termed the *East Africa cassava mosaic virus*-Uganda variant (EACMV-[UG]) was found to be associated with the devastating cassava mosaic pandemic that has been spreading in East Africa since the late 1980s (Legg, 1999). This epidemic was causing annual estimated losses of around 1-2 billion dollars (Thresh *et al.*, 1998) and famine in Uganda. The EACMV-[UG] virus was found to be a recombinant virus of predominantly EACMV sequence but containing the middle part of the coat protein (CP) sequence of *African cassava mosaic virus* (ACMV) (Zhou *et al*., 1997). Soon after, the epidemic of cotton leaf curl disease (CLCuD) in Pakistan was reported also to be associated with recombinant begomovirus sequences (Zhou *et al.*, 1998). More recently it has been determined that the factor apparently driving the epidemic is association of these recombinant molecules with a particular satellite DNA molecule (Briddon *et al.*, 2003; Mansoor *et al.*, 2003b).

The main underlying factors leading to the emergence of begomovirus epidemics have been reviewed (Polston & Anderson, 1997; Varma & Malathi, 2003), but these reviews have not considered in detail the factors that may be leading to the selection of more virulent recombinants and genetic diversity in begomovirus populations in general. These are phenomena that are not yet understood fully, but due to increased research efforts in this field recently, the mechanisms shaping the evolution of begomoviruses are becoming clearer. The purpose of this review is to concentrate on the recent articles on begomovirus genetic diversity and virus evolution to determine whether patterns emerge that will assist in the development of more sustainable disease control and cropping practises through a better understanding of factors affecting the rate of begomovirus evolution. We consider first the sources of genetic variation in begomoviruses, for which a considerable literature exists, and the drivers of evolutionary change in begomoviruses, of which much less is known. Finally we consider the implications of evolutionary change for control of begomoviruses.

Sources of genetic variation in Begomoviruses

(i) Structure of begomovirus genome

The Begomovirus genus (family Geminiviridae) derives its name from *Bean golden mosaic virus* (van Regenmortel *et al.*, 1997), and is composed of viruses forming geminate quasi-icosahedral particles of 20 x 30 nm (Harrison, 1985), that contain circular single stranded (ss) DNA molecules. Begomovirus genomes described to date can be seen to fall into a number of genome categories, the original division being into monopartite or bipartite depending on whether they have one or two circular ssDNA components. Most of the described begomoviruses are bipartite containing DNA-A and DNA-B molecules each of these being approximately 2600-2800 nucleotides in size. The DNA-A molecule carries on its virion-sense strand gene AV1 encoding the coat protein and gene AV2 required for virus accumulation and symptom development (Padidam *et al.*, 1996), but lacking in New World begomoviruses (Harrison & Robinson, 2002). On the complement of this strand there are four genes termed AC1 to AC4. The first three of these (AC1 to AC3) encode the replication-associated protein (*Rep*), transcriptional activator protein (*TrAP*), and a replication enhancer protein (*REn*) respectively. AC4 is involved in host range determination, symptom severity and movement (Jupin *et al.*, 1994; Laufs *et al.*, 1995; Wartig *et al*., 1997). These virus genes are sufficient for virus replication and formation of virion particles, but usually require BV1 (nuclear shuttle protein) and BC1 (cell to cell movement protein) genes on DNA-B for inter- and intra-cellular movement (Hanley-Bowdoin *et al.*, 1999). However, some bipartite begomoviruses can infect plant hosts systemically with only DNA-A (Saunders *et al.*, 2002b; Galvao *et al.*, 2003; Maruthi *et al.*, 2004). As begomoviruses lack genes for DNA polymerases, their replication and transcription are dependent on host plant enzymes. Replication occurs in host plant cell nuclei by a rolling-circle mechanism "RCR" (Hanley-Bowdoin *et al.*, 1999) and by a more recently discovered recombination-dependent mechanism "RDM" (Jeske *et al.*, 2001; Preiss & Jeske, 2003).

DNA-A and –B components generally share a common region (CR) of around 200 nucleotides of high sequence identity (Harrison & Robinson, 1999), although

exceptions to this property are increasingly being found (Hill *et al.*, 1998; Pant *et al.*, 2001; Chakraborty *et al.*, 2003; Idris & Brown, 2004; Karthikeyan *et al.*, 2004). The CR contains motifs required for the control of gene expression and replication, including short specific reiterated motifs (iterons) to which *Rep* binds (Eagle *et al.*, 1994). This region also possesses the highly conserved nonanucleotide TAATATTAC containing the initiation site of rolling circle replication (Hanley-Bowdoin *et al.*, 1999) termed the origin of replication *ori*. The underlined A in this nonanucleotide is referred to as nucleotide 1, and begomovirus sequence numbering continues in the virion-sense orientation.

Begomoviruses that lack a DNA-B component are termed monopartite and many such begomoviruses have been reported outside the Americas. However, it is only for a relatively small fraction of these (e.g. tomato yellow leaf curl viruses (TYLCVs) and *Tomato leaf curl virus*-[Australia] that the DNA-A component alone has been shown to fulfill Koch's postulates (Kheyr-Pour *et al.*, 1991; Navot *et al.*, 1991; Dry *et al.*, 1993; Bananej *et al.*, 2004). For many other begomoviruses only a DNA-A has been found (Xie & Zhou, 2003), but until infectious clones are tested, it is not possible to conclude that such viruses only require a DNA-A component to cause wild type disease symptoms. The importance of using infectious clones was highlighted by cloned DNA-As of some monopartite viruses such as *Ageratum yellow vein virus* (AYVV), *Cotton leaf curl Multan virus* (CLCuMV) and *Tomato yellow leaf curl China virus* (TYLCCNV) being infectious in their respective hosts but unable to induce typical disease symptoms (Briddon *et al.*, 2000; Saunders *et al.*, 2000; Zhou *et al.*, 2003). These viruses have been found to form a third category of begomoviruses that require in addition to DNA-A, a ssDNA component termed DNA-β, to develop full disease symptoms (Saunders *et al*., 2000; Briddon *et al*., 2001; Jose & Usha, 2003). The DNA-β seems to be involved in either replication, systemic movement or the suppression of a host defence mechanism, as it is necessary for the accumulation of DNA-A to levels normally found in symptomatic plants (Saunders *et al.*, 2000).

DNA-β molecules are approximately 1350 nucleotides in length and have been found to be very widespread in the Old World (Briddon *et al.*, 2003; Bull *et al.*, 2004). These molecules are clearly playing an important epidemiological role; five diverse begomovirus species including *Papaya leaf curl virus* (PaLCV) have been found associated with CLCuD in Pakistan, but all require a single DNA-β molecule to cause CLCuD (Mansoor *et al.*, 2003b). Although CLCuD in India can be caused by distinct begomovirus species, these viruses are associated with similar DNA-β molecules (83-99% nucleotide identity) to the CLCuD DNA-β molecule found in Pakistan (Kirthi *et al.*, 2004; Chowda Reddy *et al.*, 2005b).

Monopartite begomoviruses with DNA-β molecules have been found to usually contain another group of ssDNA satellite, again about half the size of a DNA-A molecule (Mansoor et al., 1999; Saunders & Stanley, 1999; Sanz et al., 2000; Briddon et al., 2004). This type of satellite, termed DNA1 also requires the DNA-A helper virus for its replication, systemic movement and insect transmission, but differs in not being required for the proliferation of the begomovirus or disease symptom induction. The role of DNA1s in the disease process remains unclear, but their presence in nearly all monopartite viruses that contain DNA-β components suggest they do have a role (Briddon et al., 2004). A ~1.3-1.4 kb defective DNA component has been found to reduce accumulation of the monopartite AYVV in *Nicotiana benthamiana* (Saunders

et al., 2001), and they may function in a similar manner to defective DNA-B components in bipartite begomoviruses that compete during replication with full-size functional components (Stanley et al., 1990). Their role might therefore be to reduce disease severity, which could in some situations have ecological advantages for virus survival.

(ii) Sources of genetic variation

Begomoviruses replicate using plant host DNA polymerases in the nuclei of infected plant cells (Gutierrez, 1999; Gutierrez *et al.,* 2004). Genetic variation can arise in their ssDNA genomes though simple mutations or through more major processes such as recombination and pseudorecombination. Such processes are considered in more detail below.

Mutation: The rate at which mutation occurs, in the absence of selection, in small ssDNA plant viruses is not known (García-Arenal *et al.*, 2001; 2003). Post selection, intra-isolate mutations in Maize streak virus (Family *Geminiviridae*, Genus *Mastrevirus*) were reported to be distributed throughout the genome and have occurred at frequencies of around 10^{-4} to 10^{-5} (Isnard et al., 1998). High mutation frequencies for begomoviruses have also been found both in wild and cultivated hosts (Ooi *et al.,* 1997; Sanz *et al*., 1999). The mutation frequencies reported are often equivalent to that reported for RNA viruses, which originally came as a surprise as it was envisaged to be lower than for RNA viruses due to DNA polymerase proof reading activity. However, it appears that geminiviruses do not utilize the normal host mechanism for mismatch repair involving DNA methylation, possibly deliberately to enable mutations to be maintained (Roossinck, 1997)

Pseudorecombination (Reassortment): This describes the reassortment of DNA-A and DNA-B genomic segments, and has been found in begomoviruses present in both the Old and New World (Garrido-Ramirez *et al.*, 2000; Pita *et al*., 2001; Idris et al., 2003; Ramos et al., 2003). The feasibility of this process in begomoviruses was first shown in the 1980s (Stanley *et a*l., 1985). Recently, progress has been made in our understanding of the factors controlling the potential for pseudorecombination between two viruses. Ramos et al. (2003) found that *Tomato mottle Taino virus* (ToMoTV) pseudorecombines with *Potato yellow mosaic virus* (PYMV), but not with *Tomato mottle virus* (ToMoV) which has a higher Rep and REn similarity. Identity of the iterons was found critical for enabling pseudorecombination, with the first 10 amino acids of Rep controlling some key features of virus replication specificity (Ramos et al., 2003).

Single DNA-A components have also been found able to form associations with several different DNA-Bs and able to cause infection when co-inoculated with each of these (Karthikeyan *et al.,* 2004). Some monopartite begomoviruses have also been suggested to have acquired a DNA-B component permanently to become mono-bipartite (Saunders *et al.,* 2002b; Chakraborty *et al.,* 2003). Such genetic re-assortments challenge our understanding of the specificity between genome components in begomoviruses and offer the potential for rapid evolutionary change.

However pseudorecombinants will only be selected for where they have a major effect, with studies on other plant viruses showing that recombination is favoured over pseudorecombination with there being co-adaptation of genomic segments (García-Arenal *et al*., 2001; García-Arenal & McDonald, 2003).

Recombination: Recombination is the process by which segments from one nucleotide strand become incorporated into that of a different individual strand during the process of replication. This process can result in dramatic variation. Although the first reports of natural recombination in begomoviruses were relatively recent (Harrison *et al.,* 1997; Zhou *et al.,* 1997; Padidam *et al.,* 1999), it has become clear from a plethora of reports since then that recombination represents a normal rather than exceptional evolutionary mechanism of these ssDNA viruses. Recombination is common in the family Geminiviridae and footprints of recombination events can often be found (Padidam *et al.,* 1999). The frequent reports of recombination in begomoviruses can be explained by a recombination-dependent replication mechanism having been found to be common to a number of begomoviruses (Jeske *et al.*, 2001; Preiss & Jeske, 2003). The primary function of recombination could thus be to repair ssDNA defects that have arisen through mutation.

The first reports of natural recombination in begomoviruses were between DNA-A molecules of different characterised viruses (Zhou *et al.,* 1997; Sanz *et al*., 2000), and for these the approximate recombination junctions could be determined. The recombinant cassava mosaic virus associated with the cassava pandemic in Uganda, EACMV-[UG] was found to be a recombinant variant between ACMV and EACMV (Zhou *et al.,* 1997). Similarly Monci *et al.* (2002) reported a recombinant with known origins, i.e. between *Tomato yellow leaf curl Sardinia virus* (TYLCSV) and *Tomato yellow leaf curl virus* (TYLCV). There have been many other reports of sequences whose phylogenies suggest a recombinant origin, but for which the origin and recombination junctions remain unknown (Zhou et al., 1998; Lotrakul et al., 2000; Sanz *et al*., 2000; Berrie *et al*., 2001; Chatchawankanphanich & Maxwell, 2002; Galvao *et al*., 2003; Xie & Zhou, 2003). For example, phylogenetic analysis of the CP gene sequence of the *Tomato leaf curl Karnataka virus* (ToLCKV) groups it in a clade of five viruses, but based on the Rep (AC1) gene, it is no longer associated with any virus suggesting it is a recombinant virus (Chatchawankanphanich & Maxwell, 2002). High identities (91%-98%) with virus sequences from Pakistan (*Hibiscus rosa-sinensis* and tobacco) were found, but as complete sequences for these are not available, accurate phylogenies cannot be inferred. The parental types to cotton leaf curl viruses in Pakistan have also not been determined (Zhou *et al*., 1998; Sanz *et al.,* 2000). The rapidly increasing number of published sequences for begomoviruses, and improvements to software for predicting recombination junctions, may lead to evolutionary origins for the above viruses being clarified in the near future.

(iii) Mechanisms of genome rearrangements

Begomovirus are good model systems for studies of molecular evolution as infectious clones of their genomic components can be obtained with ease by PCR amplification (Briddon *et al*., 1993), and their genome structure and gene function have been studied in detail (e.g. Stanley, 1991; Lazarowitz *et al*., 1992; Tan *et al.,* 1995). Furthermore, studies to date have suggested various mechanisms involved in their evolution.

Evolution of begomoviruses and associated satellite molecules: Begomoviruses, like other members of the Family *Geminiviridae*, are suggested to have arisen from bacterial replicons (Rigden *et al.,* 1996), which became associated with plants through

integration facilitated by *Agrobacterium* or related bacterial species (Roossinck, 1997). Ancient integrated geminivirus sequences have been found in some *Nicotiana* species (Bejarano *et al*., 1996; Ashby *et al.*, 1997). Such integration has actually been estimated to have occurred at least twice during *Nicotiana* speciation (Bejarano *et al*., 1996; Murad *et al*., 2004) and this to have been approximately 25 million years ago (Czosnek *et al.*, 2002). Bipartite begomoviruses are considered to have evolved much more recently from monopartite begomoviruses by component duplication and the acquisition of novel genetic material (Harrison, 1985; Padidam *et al.*, 1996; Mansoor *et al*., 2003c). This theory is supported by homologies between genes on DNA-A and DNA-B (Kikuno *et al.* 1984). In a similar manner, DNA1s are thought to have evolved from nanovirus components ~1-1.1kb in size that through the addition of a region became an acceptable size to enable encapsidation in begomovirus CP (Rojas *et al*., 1998; Mansoor *et al.*, 1999; Stanley, 2004).

The origin of DNA-β molecules is less clear, but they might have adapted from being associated with a multi-component, but currently undiscovered, plant or even insect virus (Mansoor *et al*., 2003c). Viruses have been suggested to evolve from being plant viruses to animal viruses with animal ssDNA circoviruses thought to have originated from plant nanoviruses (Gibbs & Weiller, 1999). The highly diverse nature of DNA-β molecules suggests they are not a recent phenomenon and that they confer a distinct evolutionary advantage (Briddon *et al*., 2003; Zhou *et al.*, 2003; Bull *et al.*, 2004). Although the evolutionary timing of the acquisition of DNA-β components by Old World begomoviruses is unknown, they were probably around in Japan in 752 AD, more than a millennium before the recent intensification of agricultural practices, known to have assisted the spread and diversification of begomoviruses (Saunders *et al.*, 2003).

Associated DNA-A and -B components usually contain matching iterons that are specific to begomovirus species ensuring binding of their replication protein during the initiation of viral DNA replication (Eagle *et al.*, 1994). However, the CLCuD-Pakistan DNA-β particle has a loose association with five different monopartite begomoviruses, and although dependent on the DNA-A for their replication, no matching iterons in the DNA-β were found (Briddon *et al.*, 2003; Mansoor *et al.*, 2003b). The processes enabling association with these molecules are currently unknown, and they appear also to be able to increase disease severity of bipartite begomoviruses (Rouhibakhsh & Malathi, 2005). The ecological advantages supporting the association with DNA-βs may be to enable an additional way to infect new hosts. Support for this comes from the DNA-A molecule of the bipartite begomovirus *Sri Lankan cassava mosaic virus* (SLCMV) being able to infect ageratum when co-infected with an ageratum DNA-β (Saunders *et al.*, 2002).

DNA1s show a similar lack of specificity, with DNA1 from ageratum yellow vein plants being able to co-infect and be encapsidated with begomovirus ACMV components (Saunders & Stanley, 1999). Remarkably, it was also shown to be able to interact with the curtovirus *Beet curly top virus* resulting in its transmission by the leafhopper *Circulifer tenellus* (Saunders *et al.*, 2002a). Such co-infections illustrate how begomoviruses might be able to acquire genes from different virus genera by recombination.

Mechanisms controlling recombination: The precise mechanisms controlling recombination are unknown. Sanz *et al.* (2000) searched for regions of identity at

recombination junctions in CLCuVs, but failed to find even dinucleotide repeats around predicted recombination junctions, which had previously been suggested to possibly be present (Liu *et al.*, 1998). The explanation for the presence of recombination hot-spots (Stanley, 1995; Sanz *et al.*, 2000; Saunders *et al.*, 2001; Kirthi *et al.*, 2002) therefore remains uncertain. They could have arisen through particular regions being prone to recombination, or more probably are due to only certain regions resulting in variants that can have selective advantages, as reported for some RNA viruses (Moreno *et al.*, 2004; Bonnet *et al.*, 2005)

The greatest recombination hotspot appears to be around nucleotide 2600-140, encompassing the intergenic region containing *ori* (Navas-Castillo *et al.*, 2000; Sanz *et al*., 1999, 2000; Kirthi *et al.*, 2002; Bananej *et al.*, 2004; Jovel *et al.*, 2004). This hotspot may be due to DNA-A having been found to have a propensity to donate its origin of replication to other DNAs (Roberts and Stanley, 1994; Saunders *et al.*, 2001, 2002b; Jovel *et al.*, 2004), ensuring replicative compatibility between components, and facilitating the capture of foreign components. In addition, host plant family selection for viruses with recombinant IR sequences seems probable (Simon *et al.*, 2003) presumably due to the 5' end of IR being involved in binding *Rep*, and host plant components possibly being thought to be involved in evolution of the *Rep* region (Bradeen *et al.*, 1997; Harrison & Robinson, 1999).

In contrast, the main evolution of the CP gene is in response to insect vector specificity (Bradeen *et al.*,1997; Frohlich *et al*., 1999; Simon *et al.*, 2003). Very different evolutionary selection pressures are thus operating on some of the virus-sense genes (e.g. CP) versus complementary sense genes (e.g. *Rep*). This is likely to be the underlying reason for different evolutionary origins being proposed in most recombinant begomoviruses for the virus sense genes (AV2 and AV1) versus the complementary sense genes (AC1-AC4 and 5' end of the IR). Zhou *et al.*, (1998) first reported that it was only the virus sense genes of different EACMV that showed significant differences to each other and seemed to have been acquired by recombination with unidentified begomovirus(es). Selection of EACMV recombinants with different AV1 (CP) genes could have been favoured by different prevailing local vector populations or biotypes. In the absence of vector selection, the most variable selection pressure on the virus will be that to different hosts, and hence will be on genes AC1 to AC4 genes that interact predominantly with host factors. This would explain the very frequent reports of recombinants in the AC1-AC4 region for both monopartite (Navas-Castillo *et al.*, 2000; Xie & Zhou, 2003; Bananej *et al.*, 2004; Kirthi *et al*., 2004) and bipartite begomoviruses (Berrie *et al.*, 2001; Galvao *et al*., 2003; Idris & Brown, 2004).

Recombination hotspots appear to be centered around overlapping gene regions with hotspots reported around the AV2/AV1 junction, the AC3/AC2 junction, the AC2/AC1 junction, and the AC1/AC4 junction (Kirthi *et al*., 2002; Chakraborty *et al.*, 2003). Some of the bias towards gene junctions in recombinations reported will be due to studies often having compared the maximum nucleotide identities of total DNA-A versus those of the individual genes. However in some reports (Kirthi *et al*., 2002; Chakraborty *et al.*, 2003) this is not the case, and there appears to be a genuine link between predicted recombination junctions and the regions where genes overlap. The exchange of whole genes could be expected to be selected for as these genes already encode functional proteins that operate in the selective host or vector. A fitness cost of recombinant proteins has been suggested both in *Watermelon mosaic virus* and *Cucumber mosaic virus* (Moreno *et al.*, 2003; Bonnet *et al.*, 2005). As the

above viruses differ greatly in their genome structure and expression, the fitness cost of recombinant viral proteins might be a general phenomenon in plant viruses. For geminiviruses the presence of overlapping coding regions in different reading frames will further restrict the potential for viable recombinants. Overlapping genes were found to have a higher ratio on non-synonymous to synonymous base substitutions (Sanz *et al.*, 1999), with the evolution of AC4 and AV2 consequently being constrained by AC1 (*Rep*) and AV1 (CP) respectively.

The overlapping junction between the AC1 and AC2 virus genes for ACMV-[CM] has been found to be a target for host plant gene silencing (Chellapan *et al.*, 2004) and recombination may occur in response to such evolutionary pressures.

Gene silencing: Plant viruses can induce a host plant defense mechanism known as post-transcriptional gene silencing (PTGS), as well as being the target of it (Voinnet *et al*., 1999; Moissiard & Voinnet 2004). To date, three natural pathways of RNA silencing in plants have been revealed (Baulcombe, 2004). All pathways involve the cleavage of dsRNA formed during viral replication into short (21–26 nt) RNAs known as short interfering RNAs (siRNAs) and microRNAs. The siRNAs then guide the cleavage of highly homologous RNA molecules. PTGS in plants prevents virus accumulation and hence selection will favour viruses that have strategies to counteract this host plant defence mechanism. Both RNA and DNA plant viruses have been found to encode suppressor proteins of silencing and our knowledge of plant viral suppressors of RNA silencing is expanding at a rapid pace (Moissiard & Voinnet, 2004; Roth *et al.*, 2004). The ubiquitous nature of silencing suppressors suggests they are an absolute requirement for plant viruses to spread in plants (Moissiard & Voinnet, 2004). Plant viruses produce proteins that suppress PTGS at different stages, and the great diversity of these suppressor proteins suggest that they had pre-existing functions (Moissiard & Voinnet 2004). For example, some proteins with PTGS suppressor activity act by inhibiting adenosine kinase (ADK) thus possibly suppressing silencing by interfering with host plant DNA methylation (Wang *et al.*, 2003). Methylation of viral DNA interferes with viral replication (Brough *et al.*, 1992) and hence the inhibition by geminiviruses of ADK could have functioned originally to assist viral replication and have been selected for by this property in addition to its effect on the suppression of PTGS.

Despite geminiviruses not replicating through dsRNA intermediates, there have been a number of reports of PTGS in plants infected with these ssDNA viruses (Voinnet *et al.*, 1999; van Wezel *et al.*, 2002; Dong *et al.*, 2003; Lucioli *et al.*, 2003). Chellapan *et al.* (2004) postulate that dsRNA could be formed by virion-sense and complementary-sense transcripts annealing to each other due to overlaps at their 3' ends. This would form a short dsRNA that might after extension induce PTGS. Cassava mosaic begomoviruses trigger PTGS and produce virus-specific short interfering RNAs (siRNAs) of 21 to 26 nucleotides in both *Nicotiana benthamiana* and cassava (Chellapan *et al.*, 2004). Different targets for PTGS were reported for ACMV-[CM] versus *East African cassava mosaic Cameroon virus* (Chellapan *et al.*, 2004). Such differences in the targets therefore seem to create a selection pressure for recombinant viruses lacking the targeted genes, which might explain some recombination "hotspots" through the improved fitness PTGS-targeted regions post recombination.

The transcriptional activator protein TrAp encoded for by AC2 is involved in the suppression of PTGS in the host, as reported for both bipartite *African cassava mosaic virus*, and monopartite *Tomato yellow leaf curl China virus* (Voinnet *et al.*, 1999; Wezel *et al*., 2002; Dong *et al*., 2003). TrAP of ACMV, SqLCV, TYLCV and *Tomato golden mosaic virus* (TGMV) were shown to be able to complement a TGMV-AL2 mutant in tobacco protoplasts (Sunter *et al.*, 1994). Hence TrAP functions interchangeably among begomoviruses, and recombinant molecules containing a "foreign" TrAP may maintain fitness. The mechanisms by which PTGS is suppressed are not known but TrAP protein genes from monopartite and bipartite geminiviruses show a highly conserved motif termed a "zinc-finger" (Van Wezel *et al.*, 2002). Mutations in three conserved cysteine residues in this motif in TYLCV-China abolished zinc and ssDNA binding, C2-mediated pathogenesis and the suppression of PTGS (van Wezel *et al.*, 2003).

Another mechanism viruses may use to avoid PTGS may be to become associated with nucleic acids that are resistant to degradation (Baulcombe, 2004), as illustrated by some satellite and defective interfering RNAs (Szittya *et al.*, 2002; Wang *et al.*, 2004). The mechanisms by which these RNAs are resistant to degradation is not known, but their secondary structures may be protective, or they might be compartmentalized such that they are not exposed to the RNA silencing mechanism (Baulcombe, 2004). Satellite DNA-β molecules are reported suppressors of PTGS (Mansoor *et al.*, 2001), explaining how a diverse range of begomoviruses are able to infect cotton when associated with a single satellite molecule (Mansoor *et al.*, 2003b).

Successful infection of viruses will result from a tight balance between PTGS and viral counter-defence mechanisms. The ability of TYLCSV to spread in transgenic plant expressing siRNAs to the *Rep* gene was considered to be a fine balance between inducing and suppressing gene silencing and virus replication (van Wezel *et al*., 2002). Moissiard & Voinnet (2004) highlight examples of the co-evolution of hosts and viral PTGS-suppressors, and remark that some viruses may even down-regulate the production of silencing suppressors. Reasons for down-regulation could either be related to the correct expression of proteins with overlapping ORFs, or to maintain host metabolism suitable for viral accumulation (Pfeffer *et al.*, 2002; Moissiard & Voinnet 2004). This could provide an explanation for the almost ubiquitous presence of DNA1s in monopartite viruses containing DNA-β molecules (Briddon *et al.*, 2004), as DNA1s have been shown to reduce viral accumulation (Saunders *et al.*, 2001).

Despite questions remaining on the exact mechanisms governing recombination, it has become clear that such events occur frequently, even between viral and subviral DNA (Saunders *et al.*, 2001). Field studies have also shown that recombinants arise frequently. Cotton plants (*Gossypium hirsutum*) were found to contain CLCuV DNA-A components, as well as circular ssDNA molecules of approximately half the size of DNA-A and derived from it by various combinations of sequence deletions, inversions, duplications and rearrangements just nine months post inoculation (Sanz *et al.*, 2000). The ability to detect viral recombinants in such a short time frame illustrates the immense evolutionary potential of begomoviruses.

(iv) Molecular diversity of begomovirus populations

In addition to the ability of begomoviruses to form new genetic variants with ease, genetic diversity enables virus populations to adapt to different selection pressures.

The genetic structure of a population refers to the amount and distribution of genetic diversity within and among populations (McDonald & Linde, 2002). There are few reports on the molecular diversity of begomoviruses in wild hosts. Ooi *et al.* (1997) were first to demonstrate that wild plants have a high molecular diversity of begomovirus strains which can infect cultivated plants. They studied *Tobacco leaf curl virus* (TLCV) that infects tobacco, tomato, and *Eupatorium* and *Lonicera* spp. in the wild, and found that five ~0.9 kb clones sequenced from a single plant had only 78-90% nucleotide identity to each other. A high molecular diversity was also found in CLCuVs from both cultivated cotton and weeds in Pakistan (Sanz *et al*., 1999; Mansoor *et al.*, 2003b), with nucleotide diversity at synonymous positions in several genes exceeding many high values reported for RNA viruses. Even in European countries where diversity is low (Briddon, 2002), Sanchez-Campos *et al.* (1999a) found 30 types of IR genes of *Tomato yellow leaf curl Sardinia virus* (TYLCSV, six genetic types of C2 and seven of V2. The prevalence of these variants was found to differ, with one C2 or V2 genetic type representing >90% of isolates, in contrast to the IR, for which the most abundant type represented only 20% of isolates. The type of genetic structure observed approximates a gamma distribution and was also observed for CLCuVs in Pakistan (Sanz *et al.*, 1999).

Due to the high degree of variation in geminiviruses, they should be considered as being quasispecies (Roossinck, 1997). It is important to bear this in mind as many studies have dealt with infectious clones of one sequence. In the field, the biological function of the virus may depend on the interaction between a swarm of variant sequences upon which selection acts. Within the population, sequences will be centered around one or more fitness peaks, but such fitness peaks will change in response to the environment (Roossinck, 1997). It should also be borne in mind that the assignation to species is a matter of opinion and convenience as virus species are generally fuzzy sets (Van Regenmortel, 2003). For begomoviruses this is particularly true due to genome rearrangements causing great difficulties for devising useful taxonomic criteria, as highlighted in the latest published taxonomic guidelines (Fauquet *et al.*, 2003). The latter article contains an updated list of begomovirus species based currently on these sharing with other species <89% nucleotide identity in their complete DNA-A molecule sequences. This is an artificial cut-off value and it is probable that with more research a continuum of complete DNA-A sequence percentage identities will be found. Species assignation of some recombinant viruses will always remain problematic, and similar viruses may fall into different species, and *vice versa*. DNA-A alone may also become uninformative as exemplified by the CLCuD epidemic in Pakistan being associated with at least five different begomovirus species (Briddon *et al.*, 2003; Mansoor *et al.*, 2003b). A single DNA-β molecule appears to be the critical viral genome factor involved in the epidemic, and hence a situation arises where species assignation is not related to epidemiology. Acquisition of subviral DNAs, recombination and pseudorecombination between diverse begomoviral DNA components not only presents enormous challenges to devise meaningful taxonomic criteria, but more importantly presents enormous potential for generating virus diversity able to adapt rapidly to new hosts or other changes in cropping practises.

(v) Mixed infections

Mixed infection of host cell nuclei are a prerequisite for recombination to occur. Mixed infections in hosts have been reported frequently, particularly in some well-

studied crops such as tomato (Torres-Pacheco *et al*. 1996; Sanchez-Campos *et al*., 1999a, 1999b; Sanz et al., 2000; Accotto et al., 2003; Mendez-Lozano et al., 2003; Ribeiro et al., 2003; Xie & Zhou, 2003; Jovel et al., 2004; Chowda Reddy, 2005a). Mixed begomovirus infections and recombination are also rife in many other crop and weed hosts (Sanz et al., 2000). Morilla et al. (2004) showed that mixed infections of TYLCSV and TYLCV occur clearly in at least one fifth of infected nuclei of tomato plants. The true level may be much higher but not detected due to the methodology used.

The composition of mixed infection complexes is affected by growing season and location (Torres-Pacheco et al., 1996), and by the predominating vector biotype and presence of bridging crops (Sanchez-Campos et al., 1999a; 1999b). In the absence of selection pressures introduced by man, some mixed infections have been shown to coexist over long periods of time. Mosaic of *Sida micrantha Schr*., a common weed in Brazil, is associated with at least two begomoviruses, which have coexisted in these perennial weeds over decades (Jovel et al., 2004). Such mixed infections provide a pool of genes potentially able to offer ecological advantages once suitable recombination events have taken place. Prior to recombination events, mixed infections further assist virus diversity through enabling *trans*-complementation of viral proteins to take place allowing the replication of viruses unable to infect such plants systemically. AC2, AC3 and movement proteins have been shown to be able to trans-complement defects in others (Frischmuth *et al.*, 1993; Saunders & Stanley, 1995). *Rep* and movement proteins have even been shown to be able to trans-complement between species and genera (Briddon & Markham, 2001). The ability of "non-viable" plant viruses to coexist in mixed infections will depend on the balance between any synergistic effects and the effects such interactions have on vector transmission (Zhang *et al*., 2001).

Begomovirus evolution – drivers of evolutionary change

(i) Selection of variants

Although mutation, reassortment and particularly recombination are known as the mechanisms that generate variation in begomoviruses, little is known about the selection pressures that operate and drive *Begomovirus* evolution. In general viruses appear to be selected for according to Darwin's concept of survival of the fittest (Roossinck, 1997), but selection pressures will be different depending on plant host and vector population(s). The various selection pressures operating on plant viruses have been described (Roossinck, 1997; García Arenal *et al*., 2001; Harrison, 2002; McDonald & Linde 2002). Once variants/recombinants have been created in the virus population, their frequency in the populations will be influenced by population size, their relative fitness to the parental genotype(s), and genetic drift. The maintenance of more fit variants is favoured in begomoviruses as through their recombination ability they can be considered as having mixed ("asexual/sexual") reproduction systems. Pathogens with mixed reproduction systems present the greater opportunity for evolutionary change (McDonald & Linde, 2002), as new combinations of genes that are fit can be held together.

A mutant strain in a virus population needs to multiply within its host and spread to new hosts before being lost through genetic drift. The greater the population size allowed, the greater the chance of a mutant increasing in numbers before a bottleneck occurs. Fitness in small asexual populations with high mutation rates can be lost as genetic bottlenecks are encountered (termed Muller's ratchet). Moreover, as the initial occurrence of a more virulent virus variant is presumed to be a matter of chance, such an event is more likely to occur the greater the diversity and size of the virus population(s).

Bottlenecks occur in begomovirus epidemiology for each vector transmission between plants, and from one host to another. During each vector transmission, only a subset of the virus population present in the host plant will be transmitted, as only a finite amount of virus can be acquired by the insect. This has been estimated to be equivalent to 600 million viral genomes (Czosnek *et al.*, 2002). Vector feeding on different plants can be additive in a single whitefly transmitting viruses from multiple feeds. There are different accounts of whether mixed feeding displaces one virus, from no displacement observed between subsequential feeding from TYLCV to TYLCSV (Czosnek *et al.*, 2002) to displacement observed with cucurbit begomoviruses (Cohen *et al.*, 1989).

Selection by the vector: *B. tabaci* is the only known vector species for begomoviruses. Adult *B. tabaci* transmit in a persistent circulative fashion, which means that within a few hours of acquisition of the virus, they can transmit the virus and can continue to do so for their whole lifespan, although the efficiency of this declines with time (Cohen & Harpaz, 1964). One begomovirus, *Tomato yellow leaf curl virus* (TYLCV), has been reported to also be able to be transmitted transovarially and sexually in *B. tabaci* (Ghanim *et al.*, 1998; Ghanim & Czosnek, 2000). However, other researchers have failed to detect transmission of their TYLCV strains in this manner (Polston et al., 2001).

Morphologically indistinguishable *B. tabaci* populations can have different host plant feeding preferences and virus transmission properties (Bird, 1957; Bird & Maramorosch, 1978; Bedford *et al.*, 1994; Brown *et al.*, 1995; McGrath & Harrison, 1995), which led to such distinct populations being described as biotypes (Diehl & Bush, 1984). Host plant related races (biotypes) were first reported in *B. tabaci* (Bird, 1957) from Puerto Rico with the findings of a specific "Jatropha" race and a more polyphagous "Sida" race. Later in West Africa, a cassava biotype and a more polyphagous biotype not found on cassava were reported (Burban *et al.*, 1992). Begomovirus disease outbreaks in the States in the late 1980s were found to be associated with the arrival of an exceptionally polyphagous biotype termed the B-biotype. Due to its distinct properties and mating barriers with the indigenous A-biotype, this biotype was described, contentiously, as a new species *Bemisia argentifolii* (Perring *et al.*, 1993). To date biotypes A to T have been described, but experimental rigour in ensuring the distinctiveness of these biotypes has not always been performed (Paul De Barro, personal communication).

The prevailing *B. tabaci* biotypes present in a region will affect the begomoviruses present, as although most biotypes can transmit a range of begomoviruses, they do so with very differing efficiencies depending on both virus species and vector biotype (Bedford *et al.*, 1994; McGrath & Harrison 1995; Maruthi *et al.*, 2002). Even for the more fecund and polyphagous B-biotype, some viruses, such as *Lettuce infectious*

yellows virus (LIYV), have been reported to decrease after its introduction, due to the B-biotype being a less efficient vector than the indigenous biotype for this virus.

The biological diversity in *B. tabaci* populations is reflected by an even greater diversity in a range of different genetic markers (Costa & Brown, 1991; Perring *et al.*, 1993; Gawell & Bartlett, 1993; Brown *et al.*, 1995; Frohlich *et al.*, 1999; Legg *et al.*, 2002; Maruthi *et al.*, 2001, 2004; Simon *et al*., 2003), and there has been debate about whether or not this diversity is indicative of numerous different species or a species-complex. Currently the most favoured view seems to be that *B. tabaci* represents a species-complex (Brown *et al.*, 1995; Frohlich *et al.*, 1999; Perring, 2001; Maruthi *et al.*, 2004).

Sequence data of the mitochondrial cytochrome oxidase I gene (mtCOI) has revealed that worldwide *B. tabaci* populations are distributed in at least six lineages (Frohlich *et al.*, 1999; Brown, 2000). Analyses of internal transcribed spacer 1 (ITS1) rDNA sequences of *B. tabaci* populations produced a phylogeny consistent with that derived from mtCOI (Frohlich *et al.*, 1999). These whitefly phylogenetic clusters are mirrored by those of begomovirus CP (Padidam *et al*., 1995) and complete DNA-A (Brown *et al.*, 2001) sequences from around the world, which also cluster according to their geographical origins. Exceptions to geographical clustering do exist, with a high degree of heterogeneity existing not only between *B. tabaci* populations from different geographical locations, but in some instances from different hosts in the same location (Bedford *et al*., 1994; Maruthi *et al.*, 2001). Moreover for viruses such as TYLCV, this has like the B-biotype of *B. tabaci* been moved internationally from the Middle East to the Americas and Mediterranean (Czosnek & Laterrot, 1997; Polston & Anderson, 1997; Accotto *et al*., 2003) and hence geographical clusters do not apply. The recent report of a lineage of south Asian legume-infecting begomoviruses grouping separate from begomoviruses found in neighbouring crops is harder to explain (Hameed & Robinson, 2004), but presumably is due to host adaptation as found for cassava viruses (Maruthi *et al*., 2002).

Vector-virus co-adaptation is implied by the above, but evidence for this is currently only circumstantial. In Pakistan three partial mtCOI gene sequence clades have been described for *B. tabaci*, of which only one was present in Punjab where CLCuD occurs (Simon *et al.*, 2003). It appears that the distribution of CLCuVs, or associated satellite molecule(s), is governed predominantly by a specific vector population, rather than host plant or geographic origin for which no link was found (Sanz *et al.*, 1999). Legg *et al.* (2002) also found a particular *B. tabaci* genotype that might be associated with the Ugandan cassava epidemic, which in turn is correlated with the presence of EACMV-UG (Harrison *et al.*, 1997; Zhou *et al*., 1997). However these studies have not studied the diversity of the whiteflies and viruses present on the same individual host plants, which would help finer elucidation of the relationship between vector and virus population diversity. Such studies have been initiated in a number of countries such as Uganda and Cameroon (Peter Sseruwagi, Patrick Njukeng, personal communication). Nevertheless, it is clear that begomoviruses appear to have a more specific interaction with their vector than that with their hosts, with the host range of the *B. tabaci* population largely determining the host range of the virus (Power, 2000). This suggests that begomoviruses can adapt to new hosts fairly readily, and is supported by frequent reports of new strains of begomoviruses able to infect previously unaffected hosts.

Selection by the plant host: The number of eggs laid by *B. tabaci* females is highly variable, varying between populations, different hosts and under different environmental conditions (Gerling *et al*., 1986). The plant hosts or varieties grown, and timing thereof, are therefore factors that can select for particular vector populations and hence virus strains co-evolving with these populations. In India, the existence of a cassava and a sweet potato biotype has been reported (Lisha *et al*., 2003). The cassava whiteflies reproduced on cassava, eggplant, tomato and tobacco, but not on cotton or sweet potato, whereas the sweet potato whiteflies reproduced on sweet potato, cotton, eggplant, tomato and tobacco, but not on cassava.

The choice of crops will also affect directly the prevalence of different virus genotypes, as even strains of one begomovirus species can have different host plant specificities, as illustrated by TYLCSV and TYLCV (Sanchez-Campos *et al.*, 1999; Moriones & Navas-Castillo, 2000). A natural recombinant arose from TYLCV and TYLCSV in the field and was apparently selected by it having a wider host range than its parents (Monci *et al*., 2002). Different crops can also have different effects on interactions between viruses in mixed infections. For example *Pepper huasteco yellow vein virus* (PHYVV) and *Pepper golden mosaic virus* (PepGMV) show a synergistic interaction in tobacco and *N. benthamiana*, but an antagonistic interaction in pepper (Mendez-Lozano *et al.*, 2003). It was the process of trying to understand synergism that in the early 1990s provided the first hints of viruses encoding silencing suppressors (Moissiard & Voinnet 2004). Suppressors of PTGS not only function differently depending on hosts, but may also vary greatly between strains of the same virus affecting the outcome of the plant virus interaction. Cassava plants infected with some cassava mosaic begomoviruses (CMVs) can recover whereas others do not, and much higher siRNA levels have been found to be linked to recovery versus non-recovery CMVs (Chellapan *et al*., 2004).

Particular virus genes such as AC4, as well as the C1 gene of DNA-β satellite molecules, have been shown to be the targets of PTGS (Mansoor et al., 2001; Chellapan *et al.*, 2004) and not surprisingly also to be implicated in the host specificity of begomoviruses (Jupin *et al.*, 1994; Laufs *et al.*, 1995; Wartig *et al*., 1997). These genes, like BV1 (Idris & Brown 2004) are commonly either of recombinant origin or exchangeable between different virus species through reassortment, which assists understanding how begomoviruses are able to change their host specificity readily when conditions for increased vector and virus populations are met. The CLCuD epidemic that emerged in Pakistan on cotton in the 1990s was soon accompanied by new virus diseases occurring in neighbouring tomato, tobacco, chilli and papaya crops (Hussain *et al.*, 2003; Mansoor *et al.* 2003c). Similarly in northern India, severe leaf curl in potato has only been noted since 1999 due to potato crops having been planted earlier when higher whitefly numbers were present compared to previous cultivation during winter months when whitefly numbers were low. Determination of the complete DNA-A sequence has shown the virus to be a *Tomato leaf curl New Delhi virus* (ToLCNDV, Usharani *et al.*, 2004) but to have the altered properties of being sap-transmissible and able to infect potato. ToLCNDV variants, or recombinants thereof, have also been reported to cause new diseases on many other crops such as cucumber (Green *et al.*, 2003), sponge gourd (Sohrab *et al.*, 2003), pumpkin (Muniyappa *et al.*, 2003) cowpea (Chowda Reddy *et al.*, 2005a) and chayote (Mandal *et al.*, 2004)

Begomovirus genetic diversity is thus influenced by a complex interaction of selection pressures exerted by both the genetic diversity of the vector and by that of

plant hosts for both the vector and the virus. To gain more accurate insight on the relative effect of the host and specific cropping practises on the genetic diversity of field populations of begomoviruses is therefore difficult. Large scale sequencing of begomovirus genomes from crop and weed host plants in small geographical areas is required together with an understanding of the prevailing host and weed genotypes and vector populations.

(ii) Epidemics in relation to the selection of particular begomoviruses

Disease epidemics are usually associated with a build up in vector populations, with there being a positive correlation between the size of the *B. tabaci* population and emergence of begomovirus diseases (Cohen *et al.*, 1988; Fauquet & Fargette, 1990; Cohen *et al.*, 1992; Polston & Anderson, 1997; Legg & Ogwal 1998; Otim Nape *et al.*, 2000; Banks *et al.*, 2001; Varma & Malathi, 2003). The build up of vector populations favours their migration into surrounding crops, and the spread of associated begomoviruses. *B. tabaci* populations are affected dramatically by temperature, humidity and rainfall (Sastry *et al.* 1978; Singh & Butter, 1984; Morales & Jones, 2004) and are often at their peak when temperature is high and rainfall low. Recently a climate probability model was developed to predict regions prone to epidemics (Morales & Jones, 2004). It was discovered that high disease regions all shared a dry season of at least four months each with less than 80mm rain, and a mean temperature of the hottest month exceeding 21^{o}C. Some vector-prone regions may, however, go for decades without experiencing particular problems (e.g. South America), but their "epidemic-prone" status means that simple changes can dramatically alter the situation. In Colombia, an extended dry period in 2002 in a marginal area, The Cauca Valley, resulted in serious outbreaks of the B-biotype of *B. tabaci* on tomato and beans, with resulting begomovirus disease epidemics.

Increased vector populations may also be due to the emergence of more fecund vector biotypes (Bink-Moenen & Mound, 1990; Brown *et al.*, 1995; Legg *et al*., 2002). The most characterised *B. tabaci* population increases worldwide have been associated with the spread of the B-biotype through international trade in ornamentals and other hosts (Brown *et al.* 1995). Disease epidemics followed its introduction in mediterranean Europe, India, and throughout the Americas (Cohen *et al*., 1992; Perring *et al.*, 1993; Brown *et al.*, 1995; Polston & Anderson, 1997; Banks *et al*, 2001; Ribeiro *et al.*, 2003). In addition to climatic changes and the spread of more fecund vector biotypes, changes in agricultural practises have also increased vector populations (Brown *et al.*, 1995; Polston & Anderson, 1997). The increased acreage of soybean in Brazil caused vector population increases and subsequently associated begomovirus disease epidemics (Ribeiro *et al.*, 2003). The increase in monoculture practises together with the development of insecticide resistance have led to increases of indigeneous whitefly populations in Mexico and the southern United States (Torres-Pacheco *et al.*, 1996). This together with the use of genetically uniform crops will have assisted begomovirus infections; asexual populations of hosts can be more prone to begomovirus infection than sexual populations (Yahara *et al.*, 1998). The introduction of high yielding tomato varieties in India was accompanied by ToLCV infection (Muniyappa *et al.*, 2000).

Recombination and reassortment are known for many plant viruses to have been linked to increased virulence, altered host range, or overcoming host resistance factors

(García-Arenal & McDonald, 2003). Similarly, for begomoviruses, although high vector populations have been associated with most epidemics, some of the most serious outbreaks in recent times have been associated with the emergence of recombinant variants (Harrison *et al.*,1997; Zhou *et al*., 1997; Sanz *et al.* 1999). These have appeared in the Americas several years after the introduction of the B-biotype (Polston & Anderson, 1997, Ribeiro *et al*., 2003) and are thought to be the result of an increase in mixed virus infections having arisen due to the more polyphagous feeding behaviour of this biotype.

Recombinant begomoviruses have also been associated with the cotton leaf curl disease epidemic in Pakistan. A highly susceptible cotton variety (S12) was introduced in to the Punjab province of Pakistan in the early 1980s resulting in a dramatic increase in CLCuD (Briddon & Markham, 2000). The causal viruses characterised molecularly in the 1990s were recombinant viruses and are thought to have originated from the region (Zhou *et al*., 1998) as no complete DNA-A sequence of CLCuVs associated with the CLCuD epidemic has been found elsewhere, although chimaeric sequences including sections typical of these viruses have. Although recombination has clearly played a major role in the CLCuD epidemic, its relative role compared to other factors associated with the epidemic, such as the particular DNA-β molecule associated with the disease, still awaits clarification.

Similarly the exact cause of the Ugandan cassava mosaic disease (CMD) pandemic remains unknown. There are significantly increased vector population associated with this pandemic but the origin and maintenance of this population remains unclear (Colvin *et al.*, 2004). Although in 1997 an invader Bt COI genotype cluster was consistently associated with the pandemic (Legg *et al.*, 2002), no differences in vector fecundity, virus transmission abilities, or mating barriers from pre- and pandemic populations have been found (Maruthi *et al.*,2001; 2002; Colvin *et al.*, 2004).
Various strains of a recombinant virus species (Zhou *et al*., 1997; Pita *et al.*, 2000) termed EACMV-[UG] are also certainly playing a major role reaching increased titres in plants particularly when present in mixed infections with ACMV. However EACMV-[UG] has also been found in Mozambique where it is not associated with an epidemic. The origin of the EACMV-[UG] remains uncertain as the assumed "parental" EACMV has not been found in Uganda, and thus it is not clear whether it was introduced in planting material from the Congo, arose in an undiscovered weed host, or was present in discrete locations in Uganda for a long time before the severe epidemic of the 1980s (J. Legg, personal communication). As the epidemic started in an area of Uganda undergoing civil unrest, field data to assist elucidation of the origin of EACMV-[UG] are lacking.

Similarly the very high vector populations associated with the epidemic, even if due to a particular vector haplotype (Legg *et al*., 2002), are not reproduced in W. Africa where this haplotype has also been found (Legg, pers. comm.). It is therefore likely that the particularly severe disease symptoms and spread that characterised the Uganda cassava pandemic was an interaction between particular recombinant virus strains with specific vector populations and particular host genotypes rather than having been caused by one sole factor (Colvin *et al.*, 2004). A mutually beneficial relationship between EACMV-[UG] and African cassava *B. tabaci* alone could explain the rapid disease spread; EACMV-[UG] infection results in an increased

fecundity and hence density of whiteflies in the green areas of the mosaic, which are particularly limited due to the severity of the mosaic, and hence there is an increased emigration rate of infective adults and rapid spread of the epidemic (Zhang *et al.*, 2000; Omongo, 2003). Similar mutualistic interactions between virus infection and vector fecundity and feeding behaviour have been reported for other geminiviruses (Bosque-Pérez, 2000).

Implications for control of begomovirus diseases

The complexity of molecular interactions leading to the great potential for genetic diversity in begomovirus populations, and the drivers of evolutionary change arising from the host and vector, or both, have been considered in some detail. The emergence of begomoviruses is often linked to either changes in cropping practises or international movement of plant material (Polston & Anderson, 1997; Varma & Malathi, 2003). What is less clear due to a lack of research data, is the effect of cropping practices versus vector and environmental parameters, and which are key sustainable control practices that will minimise the likelihood of increased evolutionary change.

Studies on plant virus evolution do illustrate that any measure that reduces vector and/or virus populations should be beneficial in the control of begomovirus epidemics. A reduction in size of virus populations will assist control of begomovirus diseases by limiting the diversity of the virus populations (McDonald & Linde, 2002), as large virus populations have more evolutionary potential than small populations because more mutant alleles are present. Similarly older virus populations show a greater evolutionary potential than young populations as there has been more time for mutation to occur. Thus pathogens that maintain a high population year round can adapt faster to changing selection pressures, particularly pathogens like begomoviruses with mixed reproduction systems and in which there is high gene/genotype flow. As a result successful and sustained control of begomoviruses has only been achieved rarely and in general only in discontinuous cropping systems. ToMoV has been controlled in Florida through this virus not having any significant weed hosts and tomato production not being year-round (Polston & Anderson 1997). Production is synchronised and growers maintain a tomato-free period. Transplants are obtained from commercial areas distant from sources of infection. Such systems are not feasible for most tropical agroecosystems affected, as even with a knowledge of reservoirs of infection, their removal can be problematic. For example, in southern India, it is difficult to generate the presence of a whitefly host-free period with 173 plant species belonging to 31 plant families reported to be *B. tabaci* hosts (Saikia & Muniyappa 1989). Similarly in Pakistan, 229 *B. tabaci* host plant species belonging to 48 families are known (Attique *et al.*, 2003). Although not all hosts are present year round, the sheer number of hosts results in the ability for *B. tabaci* to be able to survive well all year round.

Further challenges faced in continuous cropping systems are that their associated climates are generally the same as those favoured by the vector *B. tabaci* (Morales & Jones 2004). Moreover, the ease at which begomoviruses can adapt to new hosts also contributes to the control of begomovirus diseases being most challenging in such

tropical climates as they will possess a greater diversity of potential plant hosts (Pimm & Brown 2004). A greater host plant diversity will potentially be able to support a greater virus population diversity, which in turn will favour more diverse recombinants, and increase the possibility of synergism between viruses (Fondong *et al.*, 2000; Pita *et al*., 2001) and adaptation of viruses to new hosts. Continuous cropping systems in the tropics are also characterised by the planting of crops rarely being synchronized, and hence crops are often infected at the vulnerable seedling stage from neighbouring older crops. Ramappa *et al.* (1998) found ToLCVD infection most rapid when the tomato crop was planted adjacent to older tomato crops. The incidence also increased more rapidly where tomato was grown continuously compared to where tomato was grown once a year.

In contrast, discontinuous cropping presents the opportunity for greater evolutionary bottlenecks than continuous cropping systems, as these are expected to occur on infection of a new host or following seasonal population dynamics of the virus' hosts and vectors. A reduction in population size will occur proportional to the size of the bottleneck, and will accentuate the founder effect events that take place. An increase in founder effects results in a smaller diversity within virus populations and in a greater diversity between populations (García-Arenal *et al.* 2001). A reduction in population size will also reduce the chances of recombination events occurring. Field data to support such bottlenecks affecting the molecular diversity of virus populations is provided by the studies of Kirthi *et al*. (2002). They reported greater intra-species diversity and predicted recombination events in tomato begomoviruses grown under continuous (southern India) versus discontinuous cropping systems (northern India). Sanchez-Campos *et al.* (1999b) found a tendency for genetic diversity of TYLCV populations in Spain to increase over time, except in the Murcia districts where there is more disruption of the cycle during winter for both host plant and vector.

Control of begomovirus diseases has for farmers with sufficient resources often involved insecticide treatments. Such treatments have often only had limited efficacy due to the development of insecticide resistance (Byrne et al., 1994) or through their effect on the target pest being counteracted by an even greater effect on its parasitoids. In some instances the use of insecticides has favoured selection of the more fecund B-biotype versus local biotypes. Moreover, even where insecticide treatments are effective, they sometimes are insufficient to reduce vector populations to a sufficiently low level to reduce virus transmission (Holt et al., 1999). As a result, the deployment of begomovirus resistant lines has often been viewed as the most desirable control option.

Host resistance: Plant hosts are termed resistant if they possess some characteristic that results in no virus infection. They are termed tolerant, when they support virus infections, but symptoms are milder than in other lines of the host. There is little true resistance to begomoviruses, or B. tabaci, available in most crops (Lapidot & Friedman, 2002). Plant material with tolerance should be implemented with caution as the tolerance can break down readily leading to high yield losses (Moriones & Navas-Castillo, 2000). Moreover, tolerant hosts still act as a reservoir of inoculum for other host cultivars. Epidemiological models also show that host tolerance is associated with selection of viruses with higher titres (Frank vandenBosch,

unpublished data). Nevertheless, there are many examples where tolerance as part of an IPM has brought epidemics under control, such as for the cassava mosaic epidemic in Madagascar in the 1930s (Ranomenjanahary et al., 2002).

Sseruwagi et al. (2004) reviewed current methods of control of cassava mosaic virus diseases in eastern Africa, and concluded that there was too much reliance on the use of resistance in cassava, and that in the future a more integrated disease control to complement host resistance was recommended. The mosaic-resistant cassava varieties currently deployed in east Africa support much increased whitefly populations. This presents a precarious situation, as should a resistance-breaking virus strain develop, its management will be much more difficult. Moreover, increased vector populations may have an impact on other whitefly-transmitted viruses. Preliminary transmission results of the Cassava brown streak (ipomo)virus with B. tabaci are of particular concern here as when the growing regions for these diseases overlap, there could be a rapid spread of CBSV through CMD-resistant cassava. The most desired resistance in cassava will be that to whitefly infection as this will reduce vector populations. It is postulated that a reduction in vector populations will change the selection pressures on the viruses such that they adapt to being milder viruses more able to persist through vegetative propagation (Frank vandenBosch, personal communication/unpublished data). Likewise, a higher density of vectors (aphids) has been found to favour the evolution of higher virulence in CMV (Escriu et al., 2003), in addition to its long established effects on virus epidemiology (Harrison, 1981).

The use of resistant varieties for control of begomoviruses thus needs to be considered carefully as the literature shows a clear effect of this approach on the selection of particular virus genotypes. Resistance needs to be incorporated as part of an integrated approach so that it remains as durable as possible. The many examples of host resistance rendered ineffective illustrate that selection does operate efficiently in cropping systems based on monoculture or with high genetic uniformity (McDonald & Linde, 2002). Resistance in cotton to cotton leaf curl viruses in Pakistan has recently broken down (Mansoor *et al.*, 2003a), and partially resistant cassava varieties in Uganda appear to select for more virulent viruses (Alicai, 2003).

Nevertheless, for resource-poor farmers genetic resistance will remain a favoured control strategy against plant viruses. Hence practises need to be put in place to minimise the evolution of resistance-breaking virus variants, as the durability of resistance is more linked to the nature of the pathogen population than the nature of the resistance genes involved (McDonald & Linde, 2002). The ability for recombination and genomic segment reassortment results in a vast evolutionary potential of begomoviruses making resistance to plant begomoviruses less effective than to other viruses (García-Arenal & McDonald, 2003). This applies to resistance genes introduced by breeding as well as those introduced by genetic modifications. A potential advantage of resistance introduced by transgenic technology is that it offers the ability to pyramid genes, and providing monocultures are avoided, may present a useful tool as part of an integrated control approach.

Quantitative resistance will be more effective against begomoviruses, as a breakdown in resistance is more likely to be in the gentler form of erosion (McDonald & Linde, 2002). The pest risk model of McDonald and Linde (2002) proposes that pathogen populations exposed to strong directional selection evolve faster than those exposed to weaker selection such as quantitative resistance, or to disruptive selection that can be introduced through spatial or temporal patterning of the selective force (McDonald & Linde, 2002). Gene rotation and mixtures need much more research

although initial research shows promise. Disease severity has been shown to be reduced in crops where the a number of different cultivars are grown or where the host is more genetically diverse naturally (Ooi *et al*., 1997; Sserubombwe *et al.*, 2001). Intercropping is also a well known solution to reducing the susceptibility of monocultured crops to disease (Zhu *et al*., 2000), but little progress has been made in incorporating it in a rational way in cropping systems to try to reduce virus disease incidence. Intercropping has been shown to be able to decrease whitefly numbers, and the spread of cassava mosaic disease at particular stages of plant development (Fondong *et al.*, 2002). Intercropping will also influence the feeding behaviour of vectors and the time spent on different hosts. Some hosts such as tomato are not preferred hosts for long term adult feeding, and as a result there is significant adult whitefly movement into and out of tomato fields (Ramappa *et al.* 1998). This is likely to encourage mixed infections, and subsequent recombinations, and this may contribute to the great diversity of begomoviruses found in tomato.

Quarantine: Quarantine measures should be reviewed to minimise the introduction of vector or new viral genotypes populations to the cropping system. International trade in plants has not only spread the more polyphagous *B. tabaci* B-biotype worldwide, but infected plants are also known to have spread TYLCVs from the Middle East to the Americas and Mediterranean (Czosnek & Laterrot, 1997; Polston & Anderson, 1997; Accotto et al., 2000), Japan (Kato et al., 1998), Reunion Island (Peterschmitt et al., 1999a), and northern Africa (Peterschmitt et al., 1999b; Fekih-Hassan et al., 2003). Plant movement has also been responsible for some of the increases in indigenous B. tabaci populations, as illustrated by the introduction of eggplant, okra and soybean into the New World, and cassava from South America to Africa (Brown & Bird, 1992). The exchange of germplasm has also led to the increase in begomovirus diseases through introduction of susceptible genotypes (see Varma & Malathi, 2003).

Symptomless tomato fruit has recently been reported to be able to act as a source of TYLCV for B. tabaci under experimental conditions (Delatte et al., 2003), this should be borne in mind as another possible, although inefficient, mechanism for long range dispersal of viruses. Such transmission does not only pose a potential threat to crops grown in tropical and subtropical regions, but also to those in more temperate countries which often import tomatoes and where *B. tabaci* has become a major pest in greenhouses. It should also be realised that produce may present a route for DNA-β/DNA1 containing disease complexes to reach the Americas in a similar manner to the previous introductions of both TYLCV and the B-biotype (Polston & Anderson, 1997). Such complexes could have a serious impact, even in a continent where bipartite begomoviruses predominate, in light of the recent finding that a beta molecule increased disease severity of the bipartite *Mungbean yellow mosaic virus* (Rouhibakhsh & Malathi, 2005).

Quarantine at borders of entry is thus paramount, particularly between geographically isolated regions. Models can be used to predict countries that need to pay particular attention to the import of viruses or vectors in vegetative planting material, or even produce. Morales and Jones (2004) highlight that Chile is a country that needs its quarantine to be particularly alert as its Central Valley has a climate ideal for B-biotype establishment.

Conclusions

The emergence of begomovirus epidemics have been postulated to have been a direct result of human activity and to relate to agricultural intensification (Brown 1994; Polston & Anderson 1997; Varma & Malathi 2003), as well as other changes to cropping systems (Briddon & Markham 200; Ribeiro et al., 2003). A study of the genetic diversity of members of the genus *Begomovirus* and factors affecting it illustrates that any cropping system employed will affect directly the genetic structure of both the vector and virus population. Changes in the genetic structure of vector populations will also affect the genetic structure of the virus population, and can on their own or combined with host-induced changes instigate the emergence of begomoviruses and new disease epidemics (Polston & Anderson, 1997; Brown, 2000; Legg *et al.*, 2002; Simon *et al.*, 2003). The complexity of factors involved make it clear that there is no single solution to controlling these epidemcs, and that man can only have an impact on plant virus evolution by exerting a limiting force on some of the changing selection pressures (Roossinck, 1997).

The potential of begomoviruses to evolve rapidly is immense and they can do so not only by acquiring genes from other begomoviruses but it appears also from other virus genera, such as nanoviruses. As a result crop management can only exert a limiting force on this rate of evolution by reducing the introduction of new viruses and by reducing viral population numbers and hence the frequency of more fit recombinant viruses arising. It is difficult to do this presently as field data on the effect of cropping practises on the evolution of any begomovirus sequence are lacking. There appears to be a real dearth of studies that have looked at the genetic stability of begomoviruses under field conditions and the effect of different cropping systems. There is some data available for TYLCVs in Spain (Sanchez-Campos *et al.*, 1999a, 1999b), and it would be interesting to carry out such studies under more complex tropical agro-ecosystems. More research is required on how particular crop mixtures can minimize the build up of virus populations, and the prevalence of mixed infections and hence virus recombination. Work on cassava intercropped with cowpea and maize shows promise (Fondong *et al.*, 2002).

A better understanding is needed of the feeding preferences of *B. tabaci* populations and the ability of such populations to interbreed. There may be a link between feeding preferences of whiteflies and the emergence of mixed infections and hence the potential for more aggressive virus recombinants. Resistance poses strong selection pressures, and begomoviruses can through their mixed ("asexual/sexual") reproduction systems be expected to break host resistance, unless this is managed effectively. Breeding efforts should target quantitative resistance that needs to be renewed regularly to stay ahead of the pathogen (McDonald & Linde 2002). Lessons should be sought from that gained in resistance management of GM crops, and the extended growing of monocultures should be avoided.

Epidemiological models aimed at gaining an improved understanding of factors influencing the selection of more virulent variants, and satellite DNA molecules, are needed. Begomoviruses appear to use defective particles to reduce their disease severity in particular situations and the benefits of the virus doing this require a better understanding. Patterns of recombination and the widespread nature of DNA

satellites are likely to be a result of these genome rearrangements enabling more efficient host gene silencing. Recent articles in related fields support the evasion of PTGS being the driving force for begomovirus recombination, and probably the acquisition of satellite molecules. Similar models are needed that investigate the life history traits in the whitefly population that lead to the emergence of biotypes with increased fecundity and/or host range that, combined with the more virulent virus strains, has led to the increased begomovirus disease problems worldwide.

IV. Publications and presentations

Publications:

Because this project developed a novel area of research and the project duration was only 2 years, the papers written and submitted have not yet been accepted for publication. These papers are:

- Seal, S., Van den Bosch, F., and Jeger, M.J. Begomovirus evolution – factors influencing the rate of evolution and global emergence of whitefly transmitted diseases and implications for sustainable control. Submitted to Critical Reviews in Plant Sciences.
- Demon, I., Jeger, M.J., and Van den Bosch, F. The effect of crop and pest management on the evolution of *Bemisia tabaci* biotypes. To be submitted to Journal of Applied Ecology.
- Van den Bosch, F., Jeger, M.J., and Gilligan, C.A. Disease management and the virulence of viral plant diseases. To be submitted to Journal of Applied Ecology.
- Van den Bosch, F, Akudibilah, G, Seal, S. and Jeger, M.J. Resistant cultivars and the evolutionary response of plant viruses. Submitted to Ecological Applications.

We have been invited to contribute two papers, based on our presentations at the IX International Plant Virus Epidemiology Symposium to be held in Lima, Peru on April 4-7, 2005, to an issue of Advances in Virus Research edited by Mike Thresh, to be published end 2005/early 2006.

- Jeger, M.J. and Van den Bosch, F. Evolutionary Epidemiology of Plant Viruses. To be submitted to Advances in Virus Research.
- Van den Bosch, F. and Jeger, M.J. The Effect of Cropping Practices on Begomovirus Evolution. To be submitted to Advances in Virus Research.

Presentations:

- Presenter: Frank van den Bosch. Title: Evolution within *Bemisia tabaci* and associated Begomoviruses. Presented at: meeting at East Malling with managers from the CPP. December 2003.
- Presenter: Frank van den Bosch. Title: Evolution within *Bemisia tabaci* and associated Begomoviruses. Presented at: meeting at NRI with representatives from the Tropical Whitefly IPM Project and the CPP. October 2003.
- Presenter: Inez Demon. Title: Effects of crop and pest management on the evolution of biotypes of *Bemisia tabaci*. Presented at: The 2003 National Meeting of the Royal Entomological Society, International Symposium on Insect Evolutionary Ecology. 28-31 July 2003, Reading.
- Presenter: Inez Demon. Title: The evolution of biotypes of *Bemisia tabaci*. Presented at: CIAT, International Centre for Tropical Agriculture. 12 December 2003, Colombia.
- Presenter: Gordon Akudibillah. Title: The effect of resistant cultivars on the evolution of whitefly transmitted diseases. Presented at: 1st International Symposium on Tomato Diseases. June 21-24. Orlando, Florida, USA.
- Presenter: Inez Demon. Title: The evolution of biotypes of *Bemisia tabaci*. Presented at: The Ministry of Agriculture, Animal Husbandry And Fisheries, 6 January 2004, Surinam.

- Presenter: . van den Bosch. Title: Disease management and plant pathogen evolution. Invitation to speak at the XVTH International Plant Protection Congress, 11-16 May 2004, Beijing.
- Presenters: M. Jeger and F. van den Bosch. Title: Evolutionary Epidemiology of Plant Viruses Invited key-note presentation at the IX International Plant Virus Epidemiology Symposium to be held in Lima, Peru on April 4-7, 2005.
- Presenters: F. van den Bosch and M. Jeger. Title: The Effect of Cropping Practices on Begomovirus Evolution. Invited talk in Special Topics Session at the IX International Plant Virus Epidemiology Symposium to be held in Lima, Peru on April 4-7, 2005.

V. Contribution of Outputs to developmental impact

The Tropical Whitefly IPM Project has made significant progress in the area of field-based ecological simulation modelling and in regional GIS modelling. Both types of models result in recommendations to prioritise pest and disease management options for effective control within a cropping season. However, the evolution, on time scales measured in years, of *Bemisia tabaci* and associated begomoviruses into new adapted types severely hampers the development of sustainable agriculture. The models developed in the Tropical Whitefly Project do not study this adaptive change of whitefly and begomoviruses. The project reported here therefore complements and strengthens the global whitefly project and provides a cross-cutting approach which will be of use in the further development of research programs.

The project aimed to provide a strategic analysis to identify potential consequences of pest and disease management measures on evolution of *B. tabaci* and begomoviruses in Forest Agriculture and High Potential Cropping systems. The project transfered the acquired strategic insight to the Tropical Whitefly IPM project, programme management of the CPP, and international organisations involved in the development of sustainable production systems.

The long-term goal of the project reported here is to contribute to the development of sustainable Forest Agriculture and High Potential cropping systems for benefit of poor people, in which pest and disease management methods do not provoke the adaptive evolution of pest and disease species into more harmful types. In the medium term this project will provide the strategic means to develop *B. tabaci* and begomovirus management strategies that do not provoke the short-term evolution of new types/species. This will help develop more sustainable cropping systems which is directly of benefit to poor people. This has been achieved through the project's short term goal (the project outputs) of a strategic analysis of potential consequences of pest and disease management measures on the adaptive evolution of *B. tabaci* and begomoviruses.

The project developed an awareness of the constraints that short-term evolutionary change in whitefly and begomoviruses have on the development of sustainable cropping systems. The participating institutes involved in the Tropical Whitefly IPM Project and collaborating in the project have shown interest to incorporate this awareness in the development of their future research strategies.

Follow-up indicated/planned:

1. We have shown that it is possible to elucidate the key effects of disease management on the evolution of begomoviruses. It has become evident that with further studies it should be possible to develop methods for the selection of resistant cultivars and to select crop and disease management methods that do not provoke the virus to evolve into more harmful variants. Future work needs to concentrate on development of experimental methodology to be able to dissect the contribution of various types of crop resistance in resistant cultivars, and develop selection programmes based on these methods.

2. We have shown that the evolutionary dynamics of *Bemisia tabaci* whiteflies caused by crop and pest management depends to a large extend on the life-history parameters of the whitefly that can undergo rapid evolutionary change. Although some information exists in this respect for other insect species, notably for aphids, virtually nothing is known about this key aspect in *Bemisia tabaci*. Research into evolving life-history traits in *Bemisia tabaci* should therefore be a key future research area.

3. The review of existing knowledge has shown that insight into the genetics and the possible evolutionary changes on the genome level of begomoviruses is rapidly accumulating. It is very likely that further and detailed insight will be available in the near future without special additional DFID commitment. There is however very little research developing to elucidate the links between molecular genetics of begomoviruses and the epidemiological characteristics of the diseases caused by these viruses. Our modelling studies on the evolution of begomoviruses have shown that this information is key to the further development of disease management strategies that do not provoke the virus to evolve into more harmful types. Investment into research elucidating the relation between molecular genetics and epidemiology of begomoviruses should be a priority area for DFID in further work.

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