Dokumentation and tutorial for:
Model for rust evolution and resistance durability as driven by selection through resistance gene use

Hans O. Pinnschmidt
University Medical Centre Hamburg-Eppendorf
Department of Medical Biometry and Epidemiology

March 2015

Summary

A model was adopted from Østergård & Hovmøller (1991)[a] and developed further to promote an understanding of the evolution of pathogen populations and the durability of disease resistance of crop cultivars as governed by selection pressure exerted by various types, sources and temporal deployment modes of host resistance, interplaying with virulence properties and aggressiveness of pathogen strains and the fitness costs associated with virulence features. The model addresses crop protection scientists, resistance breeders, extension workers, university teachers and their students, to stimulate ideas for deriving and testing hypotheses and concepts for sustainable disease resistance use and for exploring and developing resistance-based disease control scenarios and strategies that help to improve the durability of crop production systems, increase the market value of resistant varieties and the return on investment in resistance breeding, decrease disease-induced crop losses, protect genetic resources, reduce pesticide use in crop production and are compatible with organic farming practices.

The model of Østergård & Hovmøller (1991) depicts a system of three loci (*x, y, z*) of the haploid stage of a pathogen like powdery mildew on barley (*Blumeria graminis* f. sp. *hordei*), each with two alleles (*A* = avirulent, *V* = virulent) that interact with three loci of the corresponding major resistance (R) genes in the host (*Rx, Ry* and *Rz*, where the presence of an R gene confers resistance to the respective pathogen avirulence gene). The model could just as well be used to depict a system of three virulence factors (*x, y, z*) of the asexual stage of a pathogen, such as the dikaryotic uredo stage of wheat yellow rust (*Puccinia striiformis* f.sp. *tritici*, WYR), with each virulence factor conferring either avirulence (A) or virulence (V) to the corresponding R genes (*Rx, Ry, Rz*) in the host. Individual virulence/avirulence alleles and genotypes of the haploid mildew system would thus correspond to individual virulence/avirulence properties and phenotypes of the asexual dikaryotic rust system, respectively.

Frequency changes of virulence and avirulence alleles and genotypes (WYR asexual dikaryotic uredo stage: virulence and avirulence properties and phenotypes, respectively) of a pathogen population are computed by the model, as well as linkage disequilibria of alleles (WYR: linkage disequilibria of virulence / avirulence properties) and the mean relative fitness of the pathogen population over time. Following input data and parameters can be varied to explore the expected effects on pathogen populations: 1) the initial frequencies of individual pathogen genotypes (WYR: phenotypes), 2) the fractions of agricultural areas planted to host genotypes having particular disease resistance properties, 3) the resistance types (partial and/or complete) and sources (i. e. major genes for virulence-specific R and/or other genetic factors such as QTLs for virulence-non-specific R) possessed by individual crop cultivars, 4) the quantitative effects of R properties on pathogen fitness, 5) the fitness costs of individual virulence alleles (WYR: virulence properties) and 6) the relative aggressiveness of individual pathogen strains.

The model and some input data representing various scenarios for resistance gene deployment patterns and pathogen genotype (WYR: phenotype) frequencies in the initial pathogen population are on worksheets in an Excel file. Accompanying is a documentation and tutorial with step-wise exercises designed to explain how to use the model to design better strategies for durable resistance use, i.e. strategies that ensure low fitness of pathogen populations, combined with low selection pressure for virulence, - particularly multiple virulence -, resulting in low frequencies of virulent and, particularly, multiple-virulent pathogen genotypes (WYR: phenotypes) as well as high proportions of multiple-avirulent pathogen genotypes (WYR: phenotypes) over time.

[a] Østergård, H. & Hovmøller, M. S., 1991: Gametic disequilibria between virulence genes in barley powdery mildew populations in relation to selection and recombination. I. Models. Plant Pathology 40:166-177.

Acknowledgement: This work was part of the RUSTFIGHT project funded by the Danish Council for Strategic Research.

Introduction

Use of disease resistant crop cultivars is a key to environmentally friendly and economically viable disease control in modern crop production. It is put at risk by the evolutionary potential of pathogens to overcome the disease resistance of crop cultivars. Evolution of pathogen populations is driven by various evolutionary forces. One of them is selection of pathogen strains able to reproduce more efficiently than others on the crop cultivars they encounter in the field. This is governed by: 1) the virulence properties of the pathogen strain, 2) the fitness costs associated with virulence and 3) virulence-independent aggressiveness of individual pathogen strains. These three features interplay with virulence-specific and non-specific resistance properties of crop cultivars, such as major resistance genes providing complete protection against avirulent pathotypes and/or QTLs and other genetic factors providing incomplete protection (= ñpartial resistanceò) against any pathotype, respectively. Smart strategies for deploying disease resistance in crop cultivars aim at keeping the overall fitness of a pathogen population and the frequencies of virulent strains, particularly multiple-virulent ñsuper-racesò, as low as possible while promoting the occurrence of avirulence, particularly of multiple avirulence, in pathogen populations. This can significantly contribute to enhanced resistance durability and make entire growing systems more stable.

A model was developed to promote an understanding of the evolution of pathogen populations and the durability of disease resistance of crop cultivars as governed by selection pressure exerted by various types, sources and temporal deployment modes of host resistance, interplaying with virulence properties and aggressiveness of pathogen strains and the fitness costs associated with virulence features. It addresses crop protection scientists, resistance breeders and extension workers, as well as university teachers and their students, to stimulate ideas for deriving and testing hypotheses and concepts for sustainable disease resistance use and for exploring and developing resistance-based disease control scenarios and strategies that help to: a) improve the durability of crop production systems, the market value of resistant varieties and the return on investment in resistance breeding, b) decrease disease-induced crop losses, protect genetic resources and reduce pesticide use in crop production and that are compatible with organic farming practices.

Model description

The population genetic model described by Østergård & Hovmøller (1991) was adapted and modified. It depicts a system of three loci (*x, y, z*) of the haploid stage of a pathogen, such as summer conidia of cereal powdery mildews (CPM; *Blumeria graminis*), each with two alleles (*A* = avirulent, *V* = virulent), that interact with three loci of the corresponding major resistance (R) genes in the host (*Rx*, *Ry* and *Rz*). The presence of an R gene confers complete resistance to the respective pathogen avirulence gene. The model computes frequency changes of virulence and avirulence alleles and genotypes of a pathogen population, as well as linkage disequilibria of alleles and the mean relative fitness of the pathogen population over time as driven by the initial frequencies of individual pathogen genotypes and the fractions of agricultural areas planted to host genotypes carrying various major genes for virulence-specific resistance. This genetic model can be adopted for diploid pathogen life cycle stages, as long as there is no sexual recombination, such as in the case of the dikaryotic uredo stage of wheat yellow rust (WYR, *Puccinia striiformis* f. sp. *tritici*). The model is then representing the phenotypic rather than the genetic level and we may speak of 1) ñvirulence factorsò, 2) ñvirulence propertiesò and 3)

ñphenotypesò or ñstrainsò rather than of @1) ñgenesò or ñlociò, @2) ñvirulence allelesò and @3)ñgenotypesò, respectively.

The relative frequencies of pathogen phenotypes (CPM: genotypes) in (aerial) pathogen populations at the beginning of a season are computed as:

$$f_{ij} = f_i \cdot u_{ij} \; / \; w_j \qquad (1)$$

where f_{ij} = relative frequency of pathogen propagules (spores) of phenotype *i* (CPM: genotype *i*) establishing colonies on host variety *j* with $i = 1, ..., n^2$ where *n* = no. of virulence factors (CPM genetic model: loci). The subscript ñ ^{2} ñ denotes that two virulence properties (CPM genetic model: alleles) are possible: virulent and avirulent. For the system at hand with three virulence factors, the following eight pathogen phenotypes (CPM: genotypes) are thus possible: *AxAyAz* (triple avirulent), *VxAyAz* (virulent to *Rx*), *AxVyAz* (virulent to *Ry*), *AxAyVz* (virulent to *Rz*), *VxVyAz* (virulent to *Rx* and *Ry*), *VxAyVz* (virulent to *Rx* and *Rz*), *AxVyVz* (virulent to *Ry* and *Rz*) and *VxVyVz* (triple virulent = ñsuper raceò). Further, $j = 1, ..., m;$ where *m* = no. host varieties; f_i = relative frequency of spores of phenotype *i* (CPM: genotype *i*) in the aerial population being dispersed at the beginning of the season; u_{ij} = relative probability that spores of phenotype *i* (CPM: genotype *i*) are established on host variety *j*, depending on how well phenotype *i* (CPM: genotype *i*) can reproduce on host *j* (i. e. u_{ij} ***å*** *relative fitness* of f_i on variety *j*); w_j = normalising factor = $\sum_i f_i \cdot u_{ij}$ *with* $\sum_i f_{ij} = 1$ (i. e. w_j å average fitness of aerial spores on variety *j* relative to the fitness of a virulent spore). The phenotypic (CPM: genotypic) frequencies in aerial populations at the end of a season (f_i***ô***) are computed as:

$$f_i\hat{\boldsymbol{o}} = \sum_j f_{ij} \cdot w_j \cdot s_j \; / \; w\hat{\boldsymbol{o}} = f_i \cdot [\, \sum_j u_{ij} \cdot s_j \,] \; / \; w\hat{\boldsymbol{o}} \qquad (2)$$

where f_i***ô***= frequency of spores of phenotype *i* (CPM: genotype *i*) in the aerial population being dispersed at the end of the season, s_j = relative area of variety *j* within the considered area and *w****ô*** = normalising factor (average relative fitness) = $\sum_j w_j \cdot s_j$.

The above model was extended for the quantitative effects of virulence non-specific partial resistance as well as virulence-specific partial resistance, fitness costs associated with the presence of individual virulences and the relative aggressiveness of individual pathogen phenotypes. All these affect u_{ij} in a multiplicative manner not further explained here. It was assumed that the pathogen reproduces asexually only, selection is the only evolutionary force, spore dispersal is proportional to the respective relative host areas and the relative spore production potential is identical across pathogen phenotypes *i* and host varieties *j*. As described below, most of the driving parameters and variables of the model as well as cultivar deployment patterns can be modified to assess a broad range of scenarios graphically and numerically.

Getting acquainted with the model

Copy the Excel workbook file ñModel for rust evolution and resistance durability as driven by selection through resistance gene useò (.xls or .xlsx, depending on your Excel version) to your preferred working directory. It consists of three data sheets: 1) input & output, 2) f_i scenarios and 3) R scenarios. In sheet ñinput & outputò, you can modify input data and parameter settings to explore various modelling scenarios and you can view the corresponding results. Sheet ñf_i scenariosò contains a couple of different pre-defined scenarios for initial pathogen phenotype frequencies that you may want to use as input for model runs, as explained in exercise 10 below.

The sheet ñR scenariosò contains some pre-defined scenarios pertaining to different patterns of deploying cultivars carrying various resistance attributes over time. How to use these pre-defined R deployment scenarios in model runs is explained in exercise 11.

Now, please spend some time looking at figure 1 showing worksheet ñinput & outputò for a sample scenario. Carefully read through the explanations provided in the figure captions to understand the main elements.

1. Open the Excel-file containing the model. Press < no > when asked whether you want to open the file as read-only. By default, you will be in worksheet ñinput & outputò that shows a sample input-output scenario.

2. Identify the sections corresponding to those in figures 1 and 2. The dynamics of virulences and pathogen phenotypes are driven by the initial relative frequencies (f_i) of individual pathogen phenotypes *i* (light-pinkish section in figure 1 labelled ñ10ò), the host genotype deployment pattern over time (green section labelled ñ7ò) and the fitness parameter matrix u_{ij} (yellow section labelled ñ(12)ò).

3. The values of u_{ij} depend on: a) whether the virulence properties on the loci for virulence factors x, y and z of individual pathogen strains *i* code for virulence (1) or avirulence (0) against host R genes *Rx*, *Ry* and *Rz* (section ñ(2)ò, b) the associated total fitness costs (section ñ(5)ò) expressing the combined effect of fitness costs for individual virulences (section ñ6ò), c) the aggressiveness of individual pathogen strains (section ñ4ò) and d) interactions of the features in a) ï c) with the resistance properties of the individual host cultivars *j* (section ñ1ò), i.e. presence of virulence-specific R genes (*Rx*, *Ry*, *Rz*) and quantitative effects of these (1.0 = complete protection; < 1.0 = non-complete protection; 0.0 = absent) as well as presence and quantitative effects of virulence non-specific ñpartialò resistance (*pR*) ranging from 0.0 (absent, no protective effect) to 1.0 (full protection).

4. The graphical display of the model output corresponding with the sample scenario shows that: a) the double-virulent pathogen phenotype ñ*VxVyAz*ò becomes dominating with time (figure 2A), b) the virulence property *Vx*, followed by *Vy*, becomes the most prevalent one (figure 2B), c) strong selection for the double-virulence *VxVy* occurs, as indicated by a high linkage disequilibrium (*Dxy*) with a maximum at season 14 and d) the mean relative fitness of the pathogen population (figure 2A, dashed black curve indicated by the red arrow) remains below 0.2 throughout the seasons, indicating an overall good disease controlling effect of the resistance deployment strategy used in this sample scenario. Summary statistics of these outputs are presented in the blue section labelled ñ(11)ò in figure 1. Note: the numerical single-season model output used for the graphical display is ñhiddenò behind the graph panels. In case you want to access it, you have to push the graph panels aside and copy / paste the data behind them.

In the following exercises, we will, in a step-wise manner, explore ways to arrive at even better strategies for durable resistance use, i.e. strategies that ensure:

a) a low fitness of the pathogen population, combined with

b) a low selection pressure for virulence, - particularly multiple virulence -, resulting in

c) low frequencies of virulent and, particularly, multiple-virulent pathogen phenotypes as well as

d) high proportions of multiple-avirulent pathogen phenotypes over time.

Note that you can only make changes on input parameter values or data in the sections of data sheet ñinput & outputò that are labelled in figure 1 as ñ1ò (matrix defining presence and relative

quantitative effects of three genes for virulence-specific resistance), ñ4ò (non-specific relative aggressiveness of pathogen phenotypes), ñ6ò (relative fitness cost associated with the presence of individual virulence properties), ñ7ò (matrix indicating the fraction of area *s* planted to individual host cultivars *j* over consecutive seasons) and/or ñ10ò (initial relative frequencies f_i of individual pathogen phenotypes *i*). See caption of figure 1 for more explanations on these sections. The other sections, which are labelled in figure 1 with numbers in parentheses, are protected. Note also that after you have made changes in the above mentioned sections (1, 4, 6, 7 and/or 10), you have to press < F9 > in order to implement and have the model execute them. Note finally that the sum of the initial relative pathogen phenotype frequencies (section labelled ñ10ò in figure 1) as well as the sum of the relative areas planted to individual host genotypes in a given season (section labelled ñ7ò in figure 1) must equal 1.0! You can quickly check that in the control fields (figure 1: sections ñ(8)ò respectively ñ(13)ò) that will turn red if the control sum exceeds 1.0.

Completion of all exercises may take about three hours.

Exercises

Exercise 1: A scenario where no disease resistance is deployed over 25 seasons and fitness costs for virulence are zero.

1. Open the Excel workbook and use the default ñinput & outputò worksheet.
2. Set all relative fitness costs of virulence (section ñ6ò in figure 1) to 0.0.
3. Set the relative area *s* planted to host cultivar 1 (*j* = 1) to 1.0 and the areas for all other host cultivars (*j* = 2, é , 6) to 0.0 over all 25 seasons (section ñ7ò, figure 1).
4. Save the file as ñExercise 1ò.
5. Note changes in the relative fitness values of the u_{ij} matrix (yellow section ñ(12)ò in figure 1), compared to the u_{ij} matrix of the default scenario.
6. Key results: The pathogen population is highly fit throughout all seasons (mean fitness = 0.5) and, since virulence is not associated with any fitness costs and no selection pressure is exerted by any resistance factors, all virulences and pathogen phenotypes stably maintain their initial frequencies, even the complex phenotypes carrying multiple virulences (*VxVyAz*, *VxAyVz*, *AxVyVz*, *VxVyVz*; see graphical display in the lower right section of the worksheet).

Exercise 2: A scenario like in exercise 1 but fitness costs are 25% for the presence of each virulence.

1. Use the worksheet from exercise 1.
2. Set all relative fitness costs of virulence (section ñ6ò in figure 1) to 0.25.
3. Save the file as ñExercise 2ò.
4. Note that the relative fitness of pathogen phenotypes (see values of u_{ij} matrix), compared to exercise 1, decreases, the more virulences a phenotype possesses.
5. Key results: When no selection pressure for virulence is exerted because the grown cultivar is fully susceptible, the fitness costs associated with virulence quickly let the triple-avirulent pathogen phenotype *AxAyAz* dominate the population while phenotypes carrying virulences quickly vanish.

Exercise 3: A scenario like in exercise 2 but 75% of the host area are continuously grown to a cultivar with resistance gene *Rx* and 25% of the area to a fully susceptible cultivar.

1. Use the worksheet from exercise 2.
2. Set the relative area planted to host host cultivar 1 ($j = 1$) to 0.25 and the area for host cultivar 2 ($j = 2$) to 0.75 over all 25 seasons.
3. Save the file as ñExercise 3ò.
4. Key results: Using a single major R gene (*Rx*) quickly causes selection for the corresponding virulence (*Vx*) and phenotype (*VxAyAz*). Fitness of the pathogen population is initially greatly reduced, but this effect fades as *VxAyAz* is selected for over time. Over all 25 seasons, the mean fitness remains considerably lower (below 0.4) than in the scenario of exercise 2.

Exercise 4: A scenario like in exercise 3 but a cultivar with R gene *Rx* and a cultivar with Ry are used in yearly alternation (ñgene rotationò).

1. Use the worksheet from exercise 3.
2. Leave the cultivar deployment pattern of season 1 as it is (cultivar 2: 0.75, cultivar 3: 0.0).
3. For season 2, set the relative area planted to host cultivar 2 to 0.0 and the area planted to cultivar 3 to 0.75.
4. Fill up the remaining seasons with this alternating yearly deployment pattern of cultivars 2 and 3.
5. Save the file as ñExercise 4ò.
6. Key results: Rotating 2 major R genes over time has some additional fitness reducing effect on the pathogen population (mean fitness remains below 0.3 over all seasons) as compared to the scenario of exercise 3, but a double-virulent complex pathogen phenotype (*VxVyAz*) will quickly build up and dominate the pathogen population while other pathogen phenotypes vanish. Note that there is no intra-seasonal selection pressure for *VxVy* double-virulence, as the respective linkage disequilibrium (*Dxy*) remains zero throughout the seasons.

Exercise 5: A scenario like in exercise 4 but a single cultivar possessing both R genes, *Rx* and *Ry* (ñgene pyramidingò), is continuously grown.

1. Use the worksheet from exercise 4.
2. Set the effect value for R gene *Rz* of cultivar 5 to 0.0.
3. Set the relative area planted to host cultivar 2 and 3 to 0.0 over all seasons.
4. Set the relative area planted to host cultivar 5 to 0.75 over all seasons.
5. Save the file as ñExercise 5ò.
6. Key results: With respect to mean population fitness, the building up of the double-virulent pathogen phenotype *VxVyAz* and the vanishing of other phenotypes, the consequences of deploying pyramided R genes are quite the same as when the respective genes are rotated over time as in exercise 4. However, the linkage disequilibrium *Dxy* indicates strong selection pressure for double-virulence *VxVy*, especially during season 3.

Exercise 6: Gene rotation as in exercise 4 but yearly alternation of 3 cultivars (instead of 2) with R genes *Rx*, *Ry* and *Rz*.

1. Open file ñExercise 4ò, worksheet ñinput & outputò.
2. Set the effect value for R gene *Rz* of cultivar 4 to 1.0.
3. Leave the cultivar deployment patterns of season 1 and 2 as they are.
4. For season 3, set the relative area planted to host cultivar 2 and 3 to 0.0 and the area planted to cultivar 4 to 0.75.
5. Fill up the remaining seasons with this alternating yearly deployment pattern for cultivars 2, 3 and 4 so that the area planted to a cultivar is 0.75 every 3rd year and 0.0 in the years in between.
6. Save the file as ñExercise 6ò.
7. Key results: The effects of rotating three R genes resemble those of rotating two R genes (exercise 4) but there is a considerable additional fitness-reducing effect. The mean fitness of the pathogen population thus reaches a maximum of only about 0.2 across the seasons. However, a triple-virulent ñsuper raceò steadily evolves and dominates the population towards the end of the period. As in exercise 4, no intra-seasonal selection pressure for complex virulence (*VxVyVz*) occurs and the respective linkage disequilibrium (*Dxyz*) remains zero throughout all seasons.

Exercise 7: Gene pyramiding as in exercise 5 but with a continuously grown cultivar possessing 3 (instead of 2) pyramided R genes *Rx*, *Ry* and *Rz*.

1. Open file ñExercise 5ò, worksheet ñinput & outputò.
2. Set the effect value for R gene *Rz* of cultivar 5 to 1.0.
3. Save the file as ñExercise 7ò.
4. Key results: As regards mean pathogen population fitness, building up of the triple-virulent phenotype *VxVyVz* and vanishing of other pathogen phenotypes, the consequences of deploying 3 pyramided R genes resemble those of rotating these genes (see exercise 6). However, the linkage disequilibria indicate an enormous selection pressure, especially at around season 6 and 7, towards triple-virulence (see *Dxyz*) and, to a lesser extend, double-virulence (see *Dxz* and *Dzy*).

Exercise 8: Introducing the effects of partial resistance. The scenario resembles the one of exercise 7 but only 50% of the host area are continuously grown to a cultivar with the 3 pyramided R genes *Rx*, *Ry* and *Rz*, 25% of the area are grown to a cultivar with 50% non-specific partial resistance and 25% to a fully susceptible cultivar.

1. Use the worksheet from exercise 7.
2. Set the relative host area planted to cultivar 5 to 0.50 for all 25 seasons.
3. Set the relative host area planted to cultivar 6 to 0.25 for all 25 seasons.
4. Save the file as ñExercise 8ò.
5. Key results: Introducing a partially resistant cultivar with intermediate virulence-non-specific effect (50% resistant to any pathogen phenotype) into the R gene pyramiding scenario, even

on just 25% of the cropping area, durably inhibits the evolution of complex virulent phenotypes and ñsuper racesò (*VxVyVz*), durably promotes the evolution of avirulent pathogen phenotypes (*AxAyAz*) and helps to keep the mean fitness of the pathogen population at a low level.

Exercise 9: Introducing variation in aggressiveness of pathogen strains. The scenario resembles the one of exercise 8 but we arbitrarily assume that two of the pathogen phenotypes, the double-virulent *AxVyVz* and the single-virulent *VxAyAz*, have a virulence-independent increased relative aggressiveness of 1 respectively 0.75, compared to 0.5 of all other pathogen genotypes.

1. Use the worksheet from exercise 8.
2. Set the aggressiveness (green section 4 according to figure 1) of pathogen genotype *AxVyVz* to 1.0 and of *VxAyAz* to 0.75.
3. Save the file as ñExercise 9ò.
4. Key results: Increased aggressiveness increases the total fitness of the respective pathogen strain (check the corresponding values in the u_{ij} matrix of the yellow section and compare them with those of exercise 8), allowing them to outcompete others. Their frequencies therefore increase over time. In the current scenario, increased aggressiveness did not affect the mean relative fitness of the pathogen population much, as compared to the scenario of exercise 8.

Exercise 10: Introducing variation in initial phenotype frequencies of pathogen populations. For initial pathogen populations, we may assume that avirulences are much more common than virulences and that the relative frequency of individual pathogen phenotypes decreases, the more virulences they possess. In the previous exercises, we have used initial pathogen phenotype frequencies (f_i; section ñ10ò according to figure 1) representing f_i scenario #3 in sheet ñf_i scenariosò that accounts for these assumptions. Letôs try other initial f_i scenarios and see whether this matters.

1. Use the worksheet from exercise 7 (initial f_i scenario used here: #3 from sheet ñf_i scenariosò). Letôs try f_i scenario #1 which assumes that all pathogen strains are initially equally frequent (relative frequency = 1/8 for each pathogen phenotype).
2. Go into sheet ñf_i scenariosò, highlight all eight pathogen phenotype frequency values of initial f_i scenario #1, copy them to the clipboard, go back into sheet ñinput & outputò, choose ñpaste specialò and ñpaste valuesò onto the initial $f_i$ôs there, starting at the value for phenotype *VxVyVz*.
3. Save the file as ñExercise 10aò.
4. Key results: The triple virulent pathogen phenotype *VxVyVz* becomes dominating more rapidly than in exercise 7.
5. Now letôs try f_i scenario #4 which assumes an even rarer occurrence of higher order virulence than f_i scenario #3.
6. Go into sheet ñf_i scenariosò, highlight all eight pathogen phenotype frequency values of initial *fi* scenario #4, copy them to the clipboard, go back into sheet ñinput & outputò, choose ñpaste specialò and ñpaste valuesò onto the initial $f_i$ôs there, starting at the value for genotype *VxVyVz*.

7. Save the file as ñExercise 10bò.
8. Key results: The triple avirulent pathogen phenotype *AxAyAz* can initially reach much higher frequencies while the triple virulent phenotype *VxVyVz* develops considerably later than in f_i scenario #1 used in exercise 10a.

Exercise 11: Free play: explore scenarios of your own choice. For example, modify pathogen-related properties and features such as aggressiveness, virulence fitness costs and initial phenotype frequencies to ñmimickò different pathogen populations. Modify resistance-related parameters and deployment patterns (relative host cultivar areas over time; - find some inspiration on data sheet ñR scenariosò) to find sustainable resistance use strategies that ensure durable use of resistance sources over time. A ñgoodò strategy will: 1) keep the mean fitness of the pathogen population at a low level, 2) inhibit the evolution of complex (multiple-virulent) phenotypes and òsuper racesò and 3) promote the evolution of avirulent (*AxAyAz*) pathogen phenotypes. Look and describe what happens, take notes and/or save the resulting graphs, for example on a PowerPoint file. You may want to save the summary statistics table (blue section of the ñinput & outputò sheet) of each run on a separate Excel sheet by using ñcopyò and then ñpaste specialò and ñvaluesò.

Important note: the model accounts only for selection as driving evolutionary force. Thus, if a pathogen phenotype becomes extinct, perhaps because there was no susceptible host cultivar area in a particular year on which it could survive, it can never re-appear. Therefore, always ensure that there is some, even very small, host area in every year planted to a susceptible or partially resistant cultivar not possessing any specific R gene providing complete resistance. This will serve as a ñsurvival reservoirò for any pathogen phenotype under selection pressure.

Evaluation: tasks and solutions

Task 1) Describe the scenario in figure 1 with respect to pathogen parameter settings in the sections labelled ñ4ò and ñ6ò.

Task 2) Describe the scenario in figure 1 with respect to the initial pathogen phenotype frequencies in the section labelled ñ10ò.

Task 3) Describe the scenario in figure 1 with respect to host parameter settings in section ñ1ò.

Task 4) Describe the scenario in figure 1 with respect to the host cultivar deployment pattern over time (section ñ7ò in the figure).

Task 5) Identify the correct statements: a) the higher the virulence fitness costs, the easier it is for multiple-virulent phenotypes to develop, b) the higher the virulence fitness costs, the harder it is for multiple-virulent phenotypes to develop, c) virulence fitness costs do not affect the fitness of a pathogen phenotype possessing one or more virulence properties.

Task 6) Identify the correct statements: continuous use of a cultivar carrying a gene for virulence-specific disease resistance a) durably suppresses the development of the pathogen phenotype having the corresponding virulence, b) promotes the development of the phenotype having the corresponding virulence, c) durably suppresses the development of the pathogen phenotype having the corresponding avirulence.

Task 7) Identify the wrong statements: a) it is always a good idea to plant as much as possible of an area to cultivars possessing multiple genes for virulence-specific complete R because this

durably inhibits the evolution of pathogen phenotypes possessing the corresponding multiple virulences, b) planting a large fraction of an area to cultivars possessing multiple genes for virulence-specific R promotes the development of pathogen phenotypes possessing the corresponding multiple virulences, c) it is always a good idea to plant a relative large fraction of an area to cultivars possessing partial R because this exerts less selection pressure for virulence on the pathogen population than planting large area fractions to cultivars with virulence-specific complete R.

Task 8) Identify the correct statements: A cultivar possessing virulence-specific R with partial effect (e.g. *Rz* with 50% effect) will provide: a) partial resistance to infection by any pathogen strain, b) partial resistance to infection by the pathogen phenotype carrying the corresponding virulence (i.e. *Vz*) and complete resistance to any other pathogen strain, c) no resistance to pathogen phenotypes carrying the corresponding virulence (i.e. *Vz*) but partial resistance to infection by any other phenotype.

Task 9a) Identify the ñbestò scenario in exercises 1 to 10b, i.e. which is the best with respect to ñcontrollingò the pathogen population by keeping the maximum mean fitness of the pathogen population and the maximum relative frequency of the ñsuper raceò *VxVyVz* at as low as possible levels over all 25 seasons. Judge this based on the respective summary statistics of each scenario in the blue section of data sheet ñinput & outputò.

Task 9b) State what characterises this ñbestò resistance deployment scenario mentioned in Task 9a.

Task 10) Explain why in exercise 3, only the single-virulent pathogen phenotype *VxAyAz* is selected for over time while the complex phenotype s*VxVyAz*, *VxAyVz* and *VxVyVz*, that all carry *Vx*, disappear?

Task 11) Answer the following question. What do you think are the main causes for the steady increase of the double-virulent pathotype *VxVyAz* in figure 2A and the concurrent steady decline of virulence *Vz* in figure 2B? Why does the triple-virulent phenotype *VxVyVz* not prevail? Come up with some in-depth interpretation and convincing arguments!

Solution 1) Figure 1, section 4: all eight pathogen phenotypes (i = 1, é , 8 ≙ *VxVyVz*, é , *AxAyAz*) have the same relative aggressiveness of 0.5; section 6: all three virulence properties are associated with the same virulence fitness cost of 0.25 (25%).

Solution 2) Figure 1, section 11: the triple-virulent pathogen phenotype *VxVyVz* is present at an initial relative frequency (f_i) of 0.019 (1.9%), all three double-virulent phenotypes (*VxVyAz*, *VxAyVz*, *AxVyVz*) at f_i = 0.053 (5.3%), all three single-virulent phenotypes (*VxAyAz*, *AxVyAz*, *AxAyVz*) at f_i = 0.144 (14%) and the triple-avirulent *AxAyAz* at f_i = 0.391 (39.1%).

Solution 3) Figure 1, section 1: six cultivars (j) are defined: j = 1 which is fully susceptible (*Rx* = 0, *Ry* = 0, *Rx* = 0, *pR* = 0), j = 2 which carries the virulence-specific R gene *Rx* with complete effect (*Rx* = 1), j = 3 carrying *Ry* with complete effect (*Ry* = 1), j = 4 that carries the virulence-specific *Rz* with partial (50%) effect (*Rz* = 0.5), j = 5 with multiple specific R genes (*Rx* = 1, *Ry* = 1, *Rz* = 0.5) and j = 6 having virulence non-specific R with 50% partial effect (*pR* = 0.5).

Solution 4) The six cultivars are deployed over all 25 seasons at the following constant relative areas: cultivar 1: 11% (0.11), cultivar 2: 13% (0.13), cultivar 3: 15% (0.15), cultivar 4: 17% (0.17), cultivar 5: 19% (0.19) and cultivar 6: 25% (0.25).

Solution 5) Statement b) is correct.

Solution 6) Statements b) and c) are correct.

Solution 7) Statement a) is wrong.

Solution 8) Statement c) is correct.

Solution 9a) The scenarios of exercises 1 and 9 both achieve an equally good control in terms of keeping the maximum mean fitness of the pathogen population as low as 0.19. Of these two, the scenario of exercise 9 is the best because it achieves the lowest relative frequency (0.04) of ñsuper raceò *VxVyVz*.

Solution 9b) The ñbestò resistance deployment scenario is characterised by continuous deployment of a cultivar with triple specific R (*Rx* and *Ry* with 100% effect and *Rz* with 50% effect) on 50% of the host area, a fully susceptible cultivar on 25% of the host area and a 25% partially resistant cultivar on 25% of the host area.

Solution 10) Since only one R gene (*Rx*) is used, virulence property *Vx* is the only one needed for the pathogen to overcome this resistance. The fitness costs for virulence reduce the fitness and thus represent a selection disadvantage for phenotypes having ñunnecessaryò virulences other than *Vx*. Hence, there is selection against them and their relative frequencies thus decline.

Solution 11) Cultivars carrying the R genes *Rx*, *Ry* and *Rz*, singly as well as in combination, are continuously deployed in all years (figure 1, section 7). This causes selection for the corresponding virulence factor. However, since the effect of *Rz* is only partial (figure 1, section 1), presence of *Vz* is not essential and the selection pressure for *Vz* is thus not as strong as for *Vx* and *Vy*, causing the latter two virulences to dominate over time while *Vz* becomes nearly extinguished. The double-virulent *VxVyAz* has a selection advantage over the single virulent *VxAyAz* and *AxVyAz* because it can infect any cultivar used in the scenario. It has also an advantage over the double-virulent *VxAyVz* and *AxVyVz* and the triple-virulent *VxVyVz* that carry the ñunnecessaryò virulence *Vz* but have the same or, in the case of *VxVyVz*, even higher virulence fitness costs than *VxVyAz*.

References

Hovmøller, M. S., Østergård, H., Munk, L., 1997: Modelling virulence dynamics of airborne plant pathogens in relationto selection by host resistance. In I. R. Crute, E. Holub, J. J. Burdon (eds.) The gene-for-gene relationship in plantï parasite interactions, pp. 173ï 190. Wallingford, UK: CABI International.

Pinnschmidt, H. O., 2014: Host resistance gene deployment and selection-driven evolution of pathogen populations. In: Stevenson, K. L. & Jeger, M. J. (eds.): Exercises in plant disease epidemiology, 2nd edition. APS press, St. Paul, Minnesota, USA, ISBN 978-0-89054-440-2.

Further reading

Gandon, S. & Michalakis, Y., 2000: Evolution of parasite virulence against qualitative or quantitative host resistance. Proc. R. Soc. Lond. B 267:985-990.

Gilligan, C. A., 2008: Sustainable agriculture and plant diseases: an epidemiological perspective. Phil. Trans. R. Soc. B 363:741ï 759.

Leach, J. E., Vera Cruz, C. M., Bai, J. & Leung, H., 2001: Pathogen fitness penalty as a predictor of durability of disease resistance genes. Annu. Rev. Phytopathol. 39:187ï 224.

Leonard, K. J., 1997: Modelling gene frequency dynamics. In I. R. Crute, E. Holub, J. J. Burdon (eds.) The gene-for-gene relationship in plant*ï* parasite interactions, pp. 211ï 230. Wallingford, UK: CABI International.

Østergård, H. & Hovmøller, M. S., 1991: Gametic disequilibria between virulence genes in barley powdery mildew populations in relation to selection and recombination. I. Models. Plant Pathology 40:166-177.

Pariaud, B., Ravigné, V, Halkett, F., Goyeau, H., Carlier, J., & Lannou, C., 2009: Aggressiveness and its role in the adaptation of plant pathogens. Plant Pathology 58(3):409ï 424.

Pietravalle, S., Lemarie, S. & van den Bosch, F., 2006: Durability of resistance and cost of virulence. European Journal of Plant Pathology, 114:107ï 116.

Pink, D. A. C., 2002: Strategies using genes for non-durable disease resistance. Euphytica 124:227ï 236.

Robinson, W. P., Asmussen, M. A. & Thornson, G., 1991: Three-locus systems impose additional constraints on pairwise disequilibria. Genetics 129:925-930.

Sapoukhina, N., Durel, C. E. & Le Cam, B., 2009: Spatial deployment of gene-for-gene resistance governs evolution and spread of pathogen populations. Theor. Ecol. 2:229-238.

Acknowledgement

This work was part of the RUSTFIGHT project funded by the Danish Council for Strategic Research.

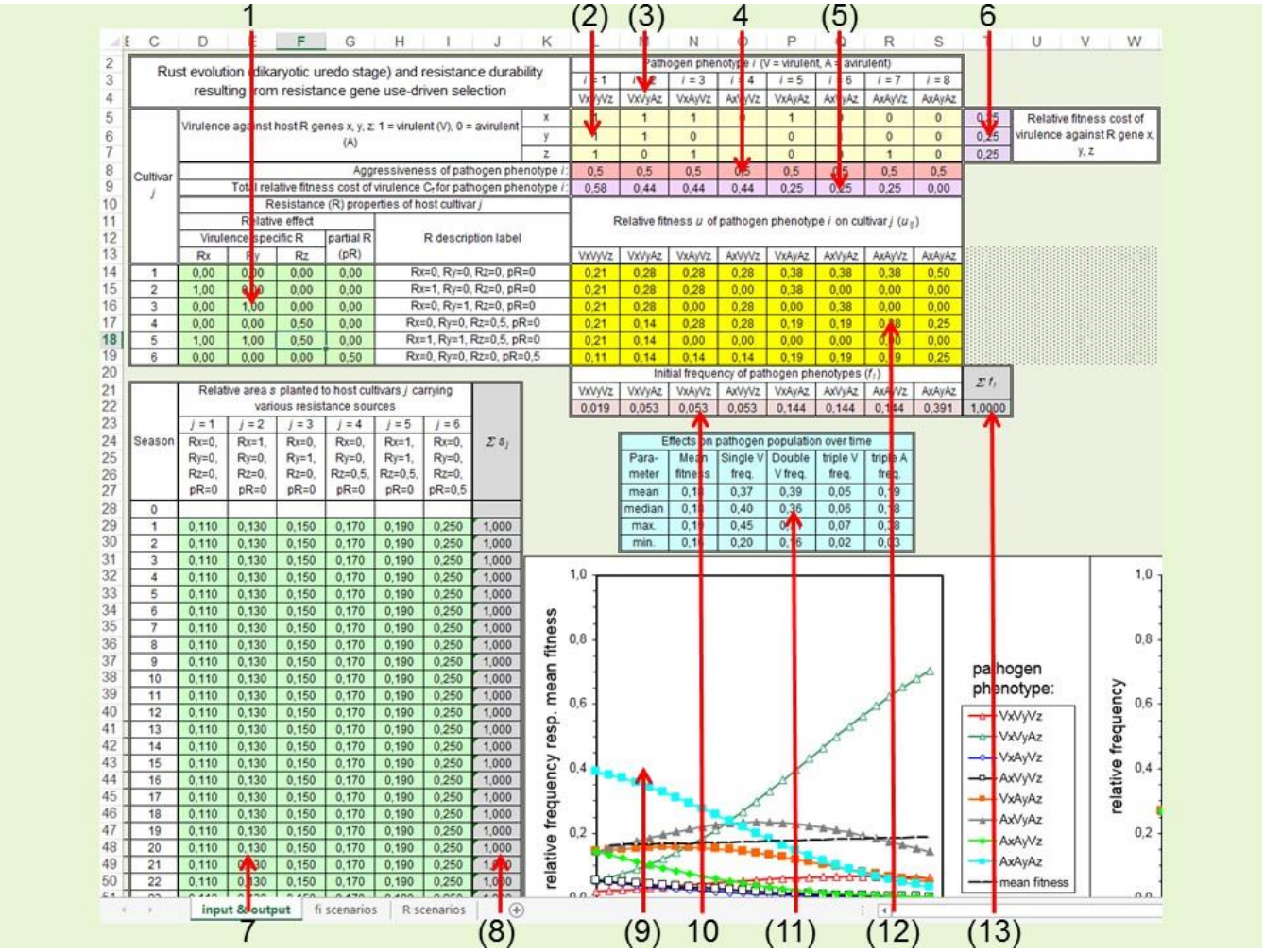

Figure 1. Sample screenshot of the upper section of the sheet ñinput & outputò. Numbers and/or parameters in the green cells can be modified by the user to create a broad range of individual scenarios pertaining to host and pathogen characteristics and to host genotype deployment areas. Labelling numbers (numbers 1 ï 6 above and 7 ï 13 below the figure, respectively; note that labelling numbers in parentheses denote sections that are locked and canôt be changed). See explanations in the text.

1 - Matrix defining presence and relative quantitative effects (0.00 = not present or not effective, é , 1.00 = present and 100% effective) of three genes for virulence-specific resistance (*Rx*, *Ry*, *Rz*) as well as virulence non-specific partial resistance (*pR*) in six host cultivars (*j* = 1, é , 6).

(2) - Non-editable matrix defining the presence (1) or absence (0) of factors conferring virulence against host resistance genes *Rx*, *Ry* and *Rz* in the respective eight pathogen phenotypes (*i* = 1, é 8).

(3) - Notation of pathogen phenotypes according to the presence of virulence (*V*) or avirulence (*A*) against host resistance genes *Rx*, *Ry* and *Rz* (examples: pathogen phenotype *VxVyVz* has triple virulence and can infect host cultivars bearing *Rx*, *Ry* or *Rz* in any combination; phenotype *VxAyAz* is virulent only on host cultivars bearing either no R gene or only *Rx*; pathogen phenotype *AxAyAz* has triple avirulence and can only infect host cultivars bearing none of the R genes *Rx*, *Ry* or *Rz*; non-editable).

4 - Non-specific relative aggressiveness of pathogen phenotype *i*.

(5) - Total relative fitness cost resulting from presence of virulence in pathogen phenotype *i* (non-editable).

6 - Relative fitness cost associated with the presence of individual virulences.

7 - Matrix indicating the fraction of area *s* planted to individual host cultivars *j* over consecutive seasons. Note: other cultivar deployment patterns can be copied and pasted from sheet ñR scenariosò).

(8) - Non-editable control variable allowing a quick check as to whether the sum of relative areas planted to individual host cultivars per season ($\sum s_j$) = 1.

(9) - Upper left part of the graphical display of model results (non-editable; see Fig. 2 for an example of the complete graphical display).

10 - Initial relative frequencies (f_i) of individual pathogen phenotypes *i*. Note: other f_i patterns can be copied and pasted from sheet ñf_i scenariosò.

(11) - Summary model outputs of current scenario: mean, median, maximum and minimum across seasons of mean relative fitness of pathogen population and relative frequencies of pathogen phenotypes with single, double and triple virulence and with triple avirulence (non-editable).

(12) - Matrix denoting relative fitness *u* of pathogen phenotype *i* on host cultivars *j* (u_{ij}; non-editable).

(13) - Non-editable control variable allowing a quick check as to whether $\sum f_i = 1$.

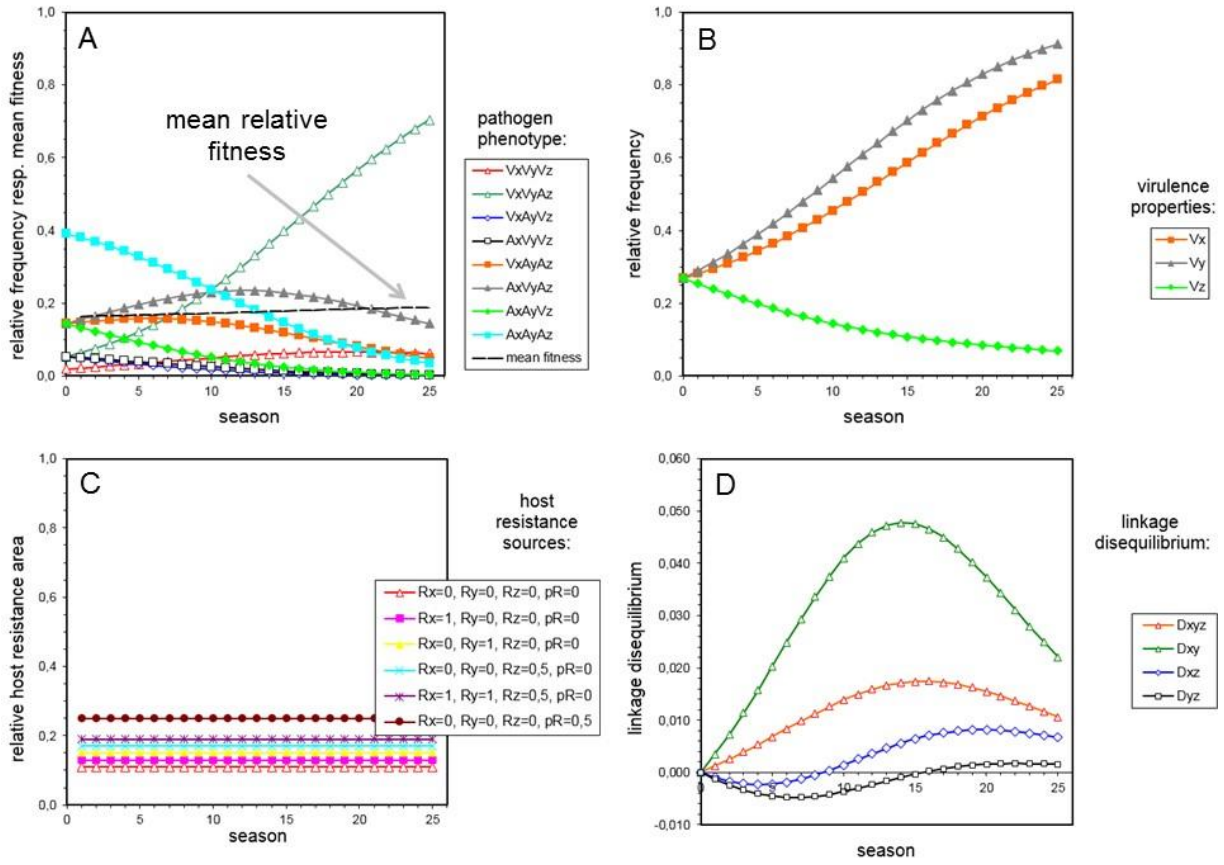

Figure 2. Graphical display of time series model outputs (lower right section of sheet ñinput & outputò) resulting from parameter settings and cultivar deployment patterns shown in Fig. 1: A) Relative frequencies of individual pathogen phenotypes (curves with plotting symbols) and mean relative fitness of pathogen population (dashed curve), B) Relative frequencies of virulence factors, C) Relative host genotype areas, D) Linkage disequilibria of virulence factors.

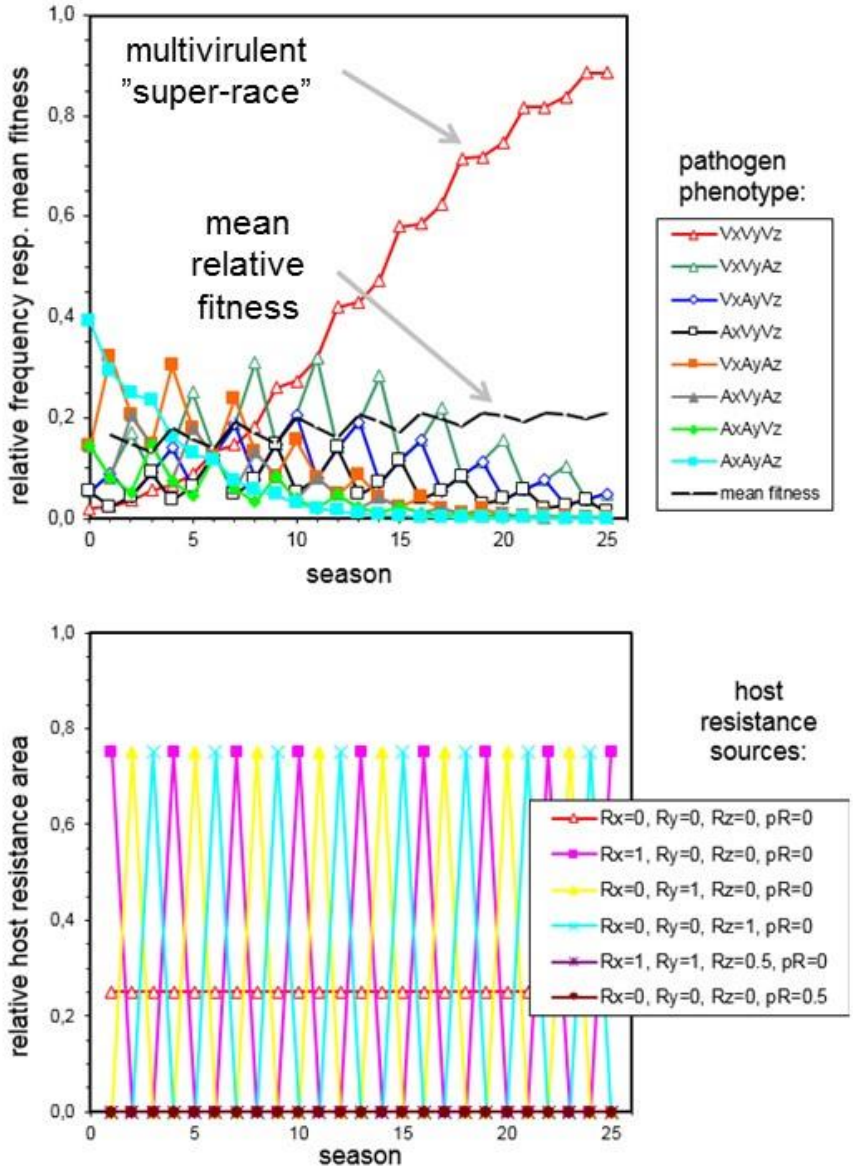

Figure 3. Graphical display of time series model outputs (lower right section of sheet ñinput & outputò) resulting from parameter settings and cultivar deployment patterns described in exercise 6, representing a scenario where 75% of the cropping area are planted in yearly alternation to a cultivar possessing either R gene *Rx*, *Ry* or *Rz* (ñgene rotationò, lower part of figure), the remaining 25% of the cropping area are planted to a fully susceptible cultivar, fitness costs are 25% for the presence of each virulence and the relative aggressiveness of any pathogen phenotype is 50%.