

RUSTWATCH



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Abstract

The Wheat Rust Toolbox (WRTB; https://web05.agro.au.dk/WheatRustToolbox) makes essential data and information obtained from RustWatch activities and other sources accessible and displayable in various forms, offering valuable tools for rust risk management at a spatio-temporal scale. Additional tools, procedures and concepts for wheat rust risk management were developed and are presented here. Data from RustWatch WP1, WP2 and WP3 and other sources were analyzed to these ends. The main thrust was on yellow rust but the tools, procedures and concepts presented are mostly examplary for the other rust species, too. Main emphasis was on characterization of rust reaction patterns of cultivars under diverse environmental conditions, decision aides for cultivar selection with regards to their rust vulnerability and similarity/dissimilarity of rust reaction, characterization of rust environments, examining the spread and evolution of rust genetic groups and races and exploring effects of host- and pathogen parameters and host resistance deployment patterns on rust population evolution and resistance stability via simulation modeling. Appendix D4.7 provides further materials, figures, tables, R code for conducting genotype-byenvironment and cluster analyses, R code for producing animated-, convex hull- and other plots for examining spatio-temporal spread of rust genetic groups and races, equations for estimating the speed of spread and/or virulence complexity changes of genetic groups and/or races as well as documentation, examplary input files and shiny R code for an app of a simulation model to explore rust population evolution and resistance stability. These materials are intended to be available to authorized users via WRTB.

Available information on the Wheat Rust Toolbox for rust risk management

In the context of developing risk management tools for RustWatch, the Wheat Rust Toolbox (WRTB; https://agro.au.dk/forskning/projekter/rustwatch/wheat-rust-early-warning/the-wheat-rust-toolbox/) provides data and information about:

1. Wheat rust surveys. From these, survey maps of severity levels of the three wheat rust diseases (leaf rust: LR, stem rust: SR, yellow rust: YR) in different countries and years can be obtained and the spatio-temporal importance of different rusts can be judged.

2. Genetic groups and races of rust diseases detected in leaf samples. Maps of occurrence and frequencies of genetic groups and races can be obtained from these data for different countries and years. Data exports moreover allow the user to determine on which cultivars the individual genetic groups and races were found.

3. Trap plant nurseries. The trap plant nursery data yield maps and tables displaying rust severity levels on yellow rust differential cultivars and susceptible check cultivars for all rust species in different countries, sites and years. From the susceptible check data, the rust-proneness of country-by-site-by-year environments can be judged for the three rusts. For yellow rust, indications of specific rust population patterns can be obtained.

4. Field nurseries. The field nursery data represent information on disease scores of individual wheat cultivars tested for the three rusts under a range of environmental (country-by-site-by-year) conditions. Tables and charts are available providing statistical key figures summarizing the main susceptibility features of the cultivars with respect to the three rusts. Data exports can then be used to look into the performance patterns of individual cultivars in more detail.

All above mentioned data and types of information represent valuable components for risk management tools in their own rights, even if considered in isolation. Other useful sources of information for risk management tools include, e.g., the RustWatch deliverable D2.2 "Evaluation of off-season testing for early detection of susceptibility of wheat lines to new races of yellow rust and leaf rust" which allows to judge the susceptibility of the tested RustWatch wheat cultivars to new rust races.

For deliverable D4.7, the focus was on developing additional tools and procedures that could make the Wheat Rust Toolbox even more versatile and useful for wheat rust risk management. Data from



RustWatch field nursery trials, rust sampling activities and trap plant nursery trials were analyzed for this purpose and a modeling approach was developed for exploring host-pathogen scenarios towards examining resistance deployment strategies for rust risk management. The main thrust was on yellow rust that may serve as an example for the other rust species.

New risk management tools

1. Tools for host resistance characterisation & selection of cultivars

1.1. Tool for susceptibility profiling of wheat cultivars based on field nursery data

1.1.1. An overall picture of the susceptibility to the three rust species of the wheat cultivars tested in the RustWatch field nurseries can be obtained from a variety of tables and graphs of the rust WRTB (see Wheat Rust Toolbox \rightarrow Trials \rightarrow Field nurseries \rightarrow Field nursery output \rightarrow etc.).

The actual WRTB features allow to evaluate the susceptibility of wheat cultivars to the three rusts, based on simple statistical measures, such as the mean, median, environmental standard deviation, median absolute deviation and other parameters.

1.1.2. For indepth pattern analyses, R code was developed to conduct GGE- (Genotype, Genotype x Environment) and AMMI (Additive Main effect, Multiplicative Interactive effect) analyses and to produce the associated biplots and related outputs, using the R package "statgenGxE" (van Rossum, 2021; https://biometris.github.io/statgenGxE/articles/statgenGxE.html). R code for producing heatmaps and conducting biclustering (i.e., simultaneously clustering cultivars and environments; Kolde, 2018; https://cran.r-project.org/web/packages/pheatmap/pheatmap.pdf) was also developed from the R package "pheatmap" (see App. A1.1.2.1 and A1.1.2.7 for R source codes). For now, the focus was on yellow rust alone as well as on all rusts analyzed together. The statgenGxE package can address additional features concerning genotype stability and mega-environments but we down-prioritized them for now. GGE biplots (App. A1.1.2.2 and A1.1.2.8, see Fig. 1.1.2a as an example) yield a picture of the most important rust reaction patterns of the cultivars when environmental main effects are adjusted for. AMMI biplots (App. A1.1.2.3 & A1.1.2.4 and App. A1.1.2.9 & A1.1.2.10) reveal environmental specificity in the rust reaction patterns of the cultivars, representing cultivar x environment interactions that can be due to the resistance properties of the individual cultivars interplaying with the virulence profiles of the rust races present in specific environments. AMMI results moreover yield the (environment-non-specific) cultivar main effects. The R code developed with the pheatmap package allows to present heatmaps of the actual cultivar-by-environment disease scores which are then arranged by a bi-custering algorithm to group cultivars according to the similarity of their disease reaction in the various environments and to simultaneously group environments with respect to similarity of the disease levels on the various cultivars (App. A1.1.2.5 & A1.1.2.6 and App. A1.1.2.11 & A1.1.2.12, see Fig. 1.1.2b as an example). The R code developed from the statgenGxE and pheatmap packages is useful for synoptically characterizing complex multi-environment disease patterns of cultivars at a reduced level of dimensionality. This pinpoints at the presence and absence of resistance factors in cultivars, thus facilitating the judgement of resistance properties of cultivars and the grouping of cultivars according to the similarity of their reaction patterns. This can be used in cultivar selection tools for crop production and breeding. However, missing data may pose a problem for the reliability of the results.

1.2. Susceptibility profiling of wheat cultivars based on RustWatch D2.2 data

The results from inoculated trials reported in RustWatch D2.2 were standardized so the values in A1.2.1 indicate the relative degree of susceptibility (\in [0,1]) that a cultivar displayed with respect to a particular rust race, depending on experimental conditions (e. g. seedling vs adult plant inoculation) and measured disease characteristic (e. g. infection type, IT vs % diseased leaf area, dla). Stacked bars (A1.2.2) provide a quick overview of these results. Judging from corresponding scatter plot matrices (A1.2.2), seedling and adult plant reactions for yellow rust do not seem to correlate well; the relationships are mostly L-shaped,



indicating that high seedling susceptibility is rarely corresponding to high adult plant susceptibility. In contrast, high adult plant susceptibility for stem rust may occur in cultivars even if their seedling susceptibility is low.

Consequences for tactical risk management: for cultivar selection in crop production, one may want to attach more importance to adult plant performance than to seedling performance when yellow rust is concerned while both types of resistance might be weighted more evenly with respect to stem rust.

1.3. Race x cultivar compatibility matrix based on leaf sample data

The matrix in A1.3 shows which genetic groups and races of yellow rust have been isolated on which wheat cultivars.

Consequences for tactical risk management: the "compatibility matrix" A1.3 indicates which wheat cultivars, including cultivars sharing the same cluster with them according to cluster analysis results reported under 1.1.2. (see App. A1.1.2.12), are potentially vulnerable to which yellow rust genetic groups and races. They are therefore at risk when the respective compatible yellow rust genetic groups or races have been detected in a wheat growing region. Different cultivars might then rather be used.

1.4. Proposed tools for aiding selection of rust resistant wheat cultivars

The tools are intended for two main goals: (1) recommendation of suitable varieties (advisory services) but also for (2) selection of resistant breeding lines and development of new varieties (breeding companies).

1.4.1 Complement the WRTB output of the field nursery trials (see 1.1.1) for each cultivar with results from the analyses decribed in 1.1.2, i. e., for GGE analyses: with PC1 and PC2 scores, for AMMI analyses: with main effects, PC1 and PC2 scores and for cluster analyses: with cluster memberships for different cluster number solutions.

1.4.2 Complement the WRTB further for each cultivar with the race-specific susceptibility profiles obtained from D2.2 data.

1.4.3 Enable WRTB to: a) select any of the cultivar parameters to be displayed, b) sort cultivars on multiple parameters, c) scatter plot any two cultivar parameters versus each other, d) use cluster membership numbers of cultivars as plotting symbols and cultivar names as data labels in scatter plots, e) select a range of individual cultivars and display selected multiple cultivar parameters as "star plots".

The above mentioned add-ons enable users to synoptic multi-criteria comparisons of cultivars to select the most promising ones or, if these are not agronomically suited for a particular environment, the nearest cluster co-member that is agronomically suited.

1.4.4 The race x cultivar compatibility matrix obtained from the rust sampling activities (see 1.3 and A1.3) should be made displayable so the user can check whether a preferred cultivar, that shares a cluster with a cultivar in the compatibility matrix, is at risk in a particular environment where a compatible rust race or genetic group is expected to be present. Information on the latter aspect can be obtained from the WRTB (Wheat Rust Toolbox \rightarrow Wheat rust samples \rightarrow Output)

1.4.5 Implement a cultivar selection algorithm:

a) Standardize susceptibility parameters p_j (such as mean, environmental SD, upper confidence limit of the mean, others, $j = 1 \dots m$) of cultivar *i* in relation to the "worst" cultivar so that all standardized susceptibility parameters $\in [0,1]$:

$$x_{ij} = \rho_{ij} / \rho_{jmax} \tag{1}$$

where p_{ij} = susceptibility parameter *j* of cultivar *i* (*i* = 1 ... *n*), x_{ij} = standardized parameter p_{ij} of cultivar *i*, p_{jmax} = maximum susceptibility parameter across all *i* for parameter *j*.

b1) Apply the following simple rule for picking the best performing cultivar:

$$y_i = max(x_{i1}, x_{i2}, ..., x_{im})$$
 (2)



where y_i = a multiple-criteria parameter representing the worst performance with respect to all chosen x_{ij} parameters. The best performing cultivar is then the one with the smallest y_i . This rule is somewhat rigid, however, since it bases a selection decision solely on the worst performance parameter per cultivar. b2) Alternatively, apply the following rule for picking the best performing cultivar:

$$y_i = 1 - (1 - x_{i1}) \cdot (1 - x_{i2}) \cdot \dots (1 - x_{im})$$

(3)

Here too, the best performing cultivar is the one with the smallest y_i . However, this rule combines all desired susceptibility parameters in a multiplicative, less-than-additive manner.

Tools serving as decision aides for cultivar selection with regards to their rust performance are already present in the output options for field nursery trials of the WRTB. Additions are proposed here, primarily consisting of parameters and affiliations resulting from cultivar-by-environment analyses and cluster analyses, as well as additional sorting, selection and graphical capabilities. Comparing and judging the rust performance of cultivars synoptically can thus be facilitated. Two exemplary algorithms for multi-criteria-selection of cultivars are presented although the actual selection criteria to be chosen need to be discussed. They should relate to race-specific varietal resistance, such as AMMI- and GGE-PC scores and environmental standard deviation of cultivars, as well as to race-non-specific ("partial") resistance, such as AMMI main effects and the upper confidence interval of the varietal mean. A challenge for future work consists in solving the question of how to integrate the characterisation of rust resistance with other important variety traits to get an all-round impression of the value of a variety.

2. Tools for examining spatio-temporal spread and evolution of rust races, genetic groups & pathogen populations – example yellow rust

2.1. Graphical tools for visualizing spatio-temporal spread of genetic groups & races

The WRTB graphical tools already allow to map the occurrence and frequency of genetic groups and races of rust species in various ways. Additional graphical methods were explored and are proposed such as "convex hull" plots (App. A2.1, see Fig.2.1a – Fig.2.1e as examples) as well as animated and kernel density plots (see App. A2.1b for examples). R source code and documentation is attached in App. A2. The examples in Fig.2.1a – Fig.2.1e and in the animated and kernel density plots illustrate the dynamics of how genetic groups and races typically appear locally, spread across regions or even continents and gradually "disappear" with time.

The proposed graphical tools can improve the comprehension of the spatio-temporal dynamics of spread of rust genetic groups and races, thus helping to appreciate emerging rust threats.

2.2. Simple models for estimating the dynamics of spread of genetic groups & races

The average distance (km/year) from the first geographical point of detection of individual yellow rust genetic groups and races was computed per year for each genetic group and race, based on WRTB rust sample data (Fig. A2.2). A general linear model was fit to these data, using race, no. years since first race detection (years since t_0) and the interaction term race \cdot years since t_0 as predictor variables:

 $In(distance/year+10) = b_0 + b_1 \cdot (years since t_0) + b_2 \cdot (race) + b_3 \cdot (years since t_0 \cdot race)$ (4) where b_0 : constant, b_1 : coefficient for years since t_0, b_2 : coefficient for race, b_3 : coefficient for interaction (years since $t_0 \cdot race$).

The corresponding general linear model for genetic groups looks as follows:

In(distance/year+100) =

 $b_0 + b_1 \cdot (\text{years since } t_0) + b_2 \cdot (\text{genetic group}) + b_3 \cdot (\text{years since } t_0 \cdot \text{genetic group})$ (5)

where b_0 : constant, b_1 : coefficient for years since t_0 , b_2 : coefficient for genetic group, b_3 : coefficient for interaction (years since $t_0 \cdot$ genetic group).

The models explained about 50% of the variation in the dependent variable (km/year from the first geographical point of detection). They indicated that, after one year, the distance from the first point of detection may on average vary between about 90 km (PstS4) and 1200 km (PstS14), depending on the



genetic group. The highest average distance from the first point of detection after one year was estimated for race PstS2,v27 (3228 km). See also App. A2.2.

The above mentioned models for estimating the dynamics of spread of genetic groups & races can help to improve our judgement regarding emerging rust strains, perhaps in connection with results from 2.3.

2.3. Simple model for estimating the virulence complexity of genetic groups and races by geographical sector over time

The virulence complexity of genetic groups and races was computed as the mean relative virulence frequency across the virulences Yr1, Yr2, Yr3, Yr4, Yr6, Yr7, Yr8, Yr9, Yr10, Yr17, Yr24, Yr25, Yr27,Yr32, YrSp, YrAvS and YrAmb for all yellow rust samples available in the WRTB database. A general linear model was fit to these data, using year and geographical sector as predictor variables:

Virulence complexity = $b0 + b1 \cdot \text{year} + b2 \cdot \text{geographic sector}$ (6)

where *b0* : constant, *b1* : coefficient for year, *b2* : coefficient for geographic sector.

The model explained almost 40% of the variation in virulence complexity found in the yellow rust samples and indicated significant increases in virulence complexity over time as well as significant differences between geographical sectors (Fig. 2.3a, Fig. 2.3b; see also App. A2.3).

Together with results from 2.2., the model for estimating time- and location-dependent variations in virulence complexity of rust strains can help to improve the preparedness of relevant stake holders with respect to emerging rust problems and future trends in virulence complexity. See also 2.4 for possible relations between race occurrence and cultivar use.

2.4. Relations between cultivar use and the occurrence of genetic groups and races – example Denmark

Cultivar use in Denmark over time is subject to pronounced changes as cultivars are only for few years in the top range of use in wheat production (Fig. 2.4a). Simultaneously, cultivar diversity has continuously gone up during the past 10 years (Fig. 2.4b), accompanied by marked changes in the yellow rust population structure (Fig. 2.4.c; see App. 2.4 for additional figures). Linking information on regional cultivar deployment patterns with information on the regional presence of rust races and with information on the resistance properties of the deployed cultivars might help to explain those structural changes in rust populations, possibly resulting in a "vulnerability mapping tool" to indicate which cultivars are at risk in which regions.

Data from Denmark indicate relations between cultivar diversity and yellow rust population changes. As a next step, cultivar deployment data need to be complemented and coupled with data reflecting cultivar diversity regarding resistance properties. Limitations to most rust sample related risk tools appear to be inconsistent sampling procedures, selection bias when collecting the samples and possibly also detection bias when analyzing the samples. Also, data availability and time for data assembly was a limiting factor for this task at the time of writing this document.

3. Modeling tool for exploring host resistance deployment effects on rust evolution & resistance stability

The REvoRS (**R**ust population **Evo**lution and **R**esistance **S**tability) model was programmed as a Shiny R application for exploring scenarios of host resistance deployment interacting with assumed characteristics of rust populations (see App. 3 for documentation, R source code and exemplary input files). It provides many options for modifying pathogen and host parameters and examining the resulting presumed effects on the evolution of rust populations and the durability resp. stability of host resistance over time. See also Hovmøller et al. (1993) for a basic modeling approach and Pinnschmidt (2015) for a more comprehensive documentation of an earlier model version.

How to run: A) Make sure the source code files "server.R" and "ui.R" are in the same subdirectory. B) Open "server.R" in RStudio and click on the green play button (Run app) on top. C) Move to the explorer window "RustWatch Model" which opens and click on "File Input" at the top of the window (note:



currently, the model runs correctly only if input files are used!). D) Choose "Upload from PC", then browse for .xlsx input files. Examples of input file are provided in App. 4, named Scenario 1 through Scenario 15. The file names represent short descriptions of what kind of scenario is contained on them.

E) Input file description:

Sheet "scenario description" contains a more detailed description of the scenario contained on the file. Note: when you create your own scenario files, you can write much more detailed descriptions than shown in these examples!

Sheet "parameters": the 1st line contains the names of the parameters: t = no. seasons to be simulated, m = max. no. cultivars involved in the scenario, n = number of virulence factors (or loci) to be considered (note: no. of phenotypes or races pertaining to a scenario = 2ⁿ), the 2nd line contains the numbers corresponding with line 1.

Sheet "cv_vector" contains a "cost of virulence" vector, i. e. information on the relative fitness cost of virulence (\in [0,1]) pertaining to the respective virulence factor.

Sheet "ai_vector": contains an "aggressiveness" vector, i. e. information on the relative aggressiveness $(\in [0,1])$ of each individual rust race *i*.

Sheet "jr_matrix": contains a matrix quantifying the degree of virulence-specific resistance (\in [0,1]) of host genotype *j* (row-wise) to the respective virulence factors (column-wise).

Sheet "pj_vector": contains a vector quantifying the degree of partial (virulence-non-specific) resistance $(\in [0,1])$ of each host genotype *j*.

Sheet "sjxt_matrix": contains a matrix quantifying the fraction of area (\in [0,1]) planted to individual host genotypes *j* (column-wise) in the consecutive seasons (row-wise). Note: the sum of all host genotype areas within a season must amount to 1!

Important note: always run the model through input files as described above since the interactive part of the user interface is still under development! Also, when creating your own input files, make sure that parameters match! For example, when the number of virulence factors "n" in sheet "parameters" is set to 5, this results in 2⁵ = 32 rust phenotypes resp. races. Hence, in sheet "ai_vector", you have to enter 32 lines specifying the relative degree of aggressiveness corresponding to the individual rust races. Also, you will have to specify the "cost of virulence" attributed to each of the virulence factors. If numbers don't match, the model will not run but produce error messages. Lastly, it is advisable to dedicate a small host area fraction to a fully susceptible host genotype as this area serves as a "sanctuary" for survival of any race that otherwise might be wiped out if certain specific resistances are used on 100% of the host area.

Currently, there is graphical output available for each scenario: 1) Cultivar area fraction and mean fitness of the pathogen population over seasons, 2) Relative frequencies of specific virulences and mean fitness of the pathogen population over seasons and 3) Relative pathogen race frequencies and mean pathogen population fitness (Fig. 3.1).

The REvoRS model can help to assess how host resistance deployment strategies affects pathogen population evolution and host resistance durability and stability, depending on various host- and pathogen factors. Options for strategic risk management may thus be explored and decision aides derived. For example, from the exemplary scenarios 1 to 15 provided in appendix 3, one may conclude: a) use of complete, specific R promotes the development of adapted races possessing matching virulences, b) shifting over time between cultivars possessing complete specific resistance (R gene rotation) or augmenting complete specific resistances in cultivars (R gene pyramiding) promotes the evolution of multiple-virulent races, depending on the fitness costs that a pathogen race has to pay for its virulences, c) in the presence of specific resistance, deploying cultivars possessing non-specific "partial" resistance does not stop the evolution of races possessing specific virulences; however, it reduces selection pressure and diminishes the fitness of races, thus lowering the probability of new infections and disease spread.

4. Concepts for characterizing environments with respect to their rust risks



Yellow rust trap plant nursery data from 2018 & 2019 were subjected to nonlinear categorical principal components analysis (Linting & van der Kooij, 2012). This was done for the actual disease scores as well as for their residuals after adjusting the disease scores for cultivar- and environment main effects by a general linear model. Component loadings were estimated based on a two-dimensional solution, using a vector model for the differential cultivars (black vector lines in Fig. 4) and a centroid model for the environments (red dots in Fig. 4). While results obtained for the actual disease scores are indicative for the actual disease pressure exerted on the differential cultivars in different environments (upper panel of Fig. 4), the results obtained for the cultivar- and environment adjusted residuals rather reflect specific interactions between the resistance properties of the differential cultivars and virulences and races present in the local rust populations (lower panel of Fig. 4). Linking these results to the cultivar clusters obtained in 1.1.2, one can obtain indications with respect to which cultivars may be at risk in which environment or region. Additionally, results from 1.1.2 can be used for characterizing environmental rust risks, e.g. based on the estimated environmental vectors and clusters.

Trap plant nursery data are useful for hinting at environmental rust risks, indicating environmental disease pressure and the presence or absence of specific virulences and races in local rust populations. Field nursery data (see 1.1.2) might likewise be used to these ends and are perhaps more robust than trap plant data in identifying similar environments, because they are based on more cultivars. However, they cover fewer environments. Limitations are imposed by missing data, by a limited no. cultivars used in trap plant nurseries and by a limited no. environments available in the field nursery data.

References

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Figures

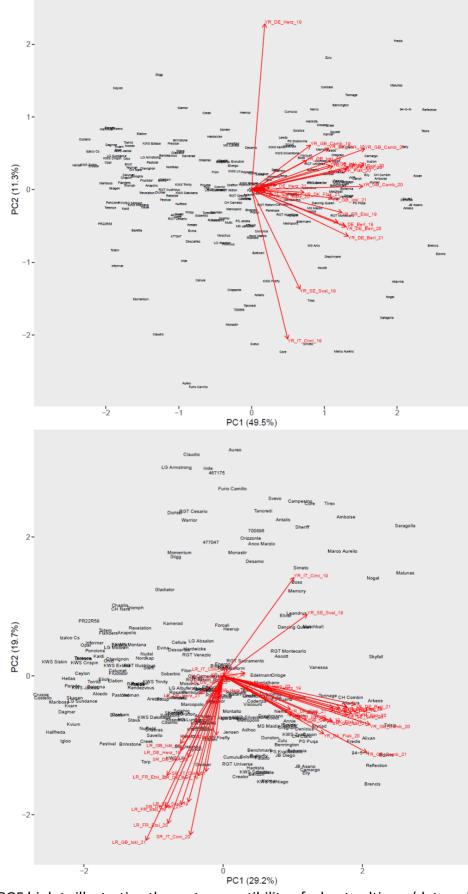


Figure 1.1.2a: GGE biplots illustrating the rust susceptibility of wheat cultivars (data point labels) in different environments (red vectors; characters represent rust species, country, site and year), adjusted for environmental main effects. Upper panel: data analyzed for yellow rust only; lower panel: data analyzed for all rust species together. See App. A1 for more detailed results and higher resolution plots.



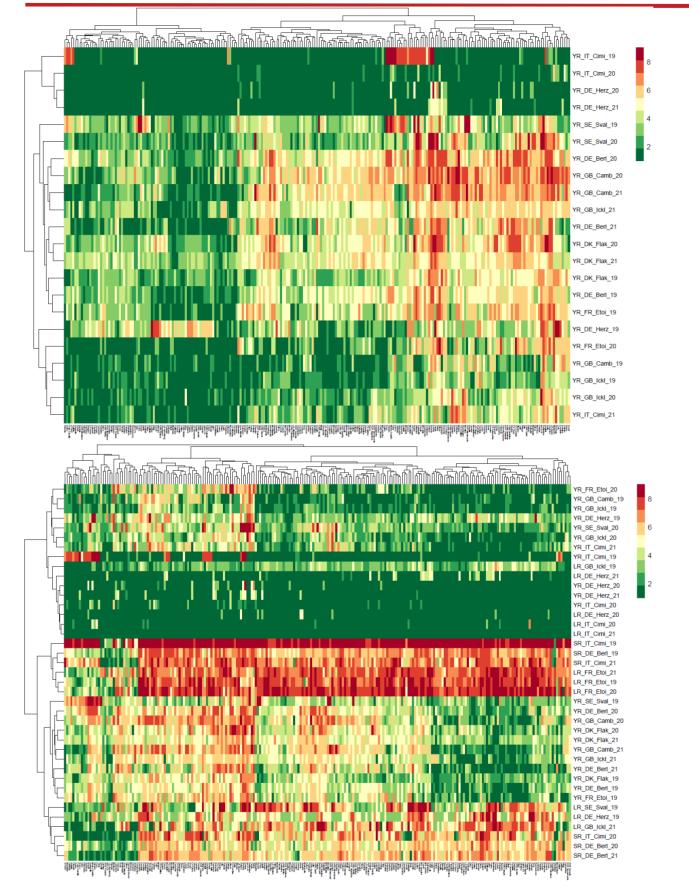
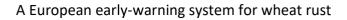


Figure 1.1.2b: Heatmaps with biclustering dendrograms illustrating similarities and dissimilarities of wheat cultivars (in colums) and environments (in rows; letters represent rust species, country, site and year) with respect to rust susceptibility scores. Upper panel: data analyzed for yellow rust only; lower panel: data analyzed for all rust species together. See App. A1 for more detailed results and higher resolution plots.



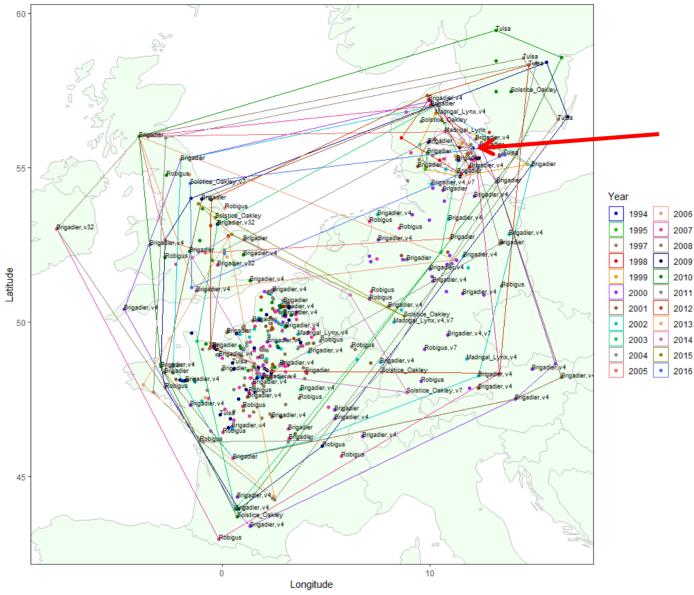


Figure 2.1a: Convex hulls showing spatio-temporal detection patterns of PstS0 yellow rust races (red arrow: 1st occurrence 1994 as race "Madrigal_Lynx,v4"). Overlapping data point labels were suppressed.



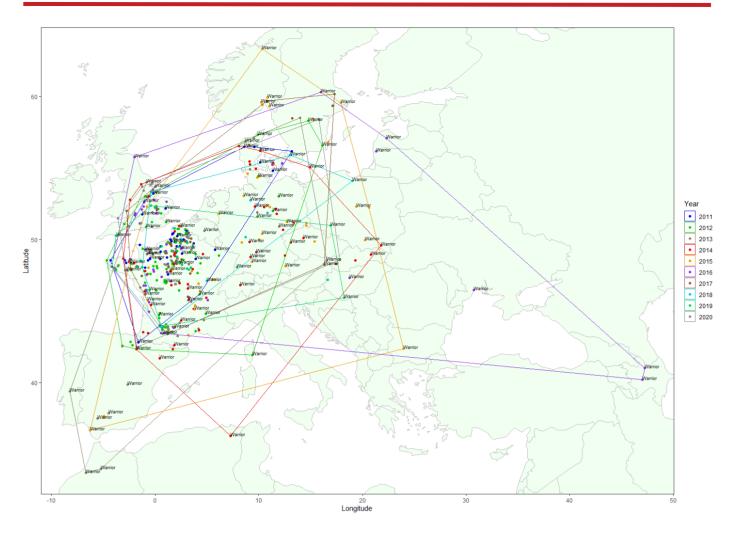


Figure 2.1b: Convex hulls showing spatio-temporal detection patterns of PstS7 yellow rust races. Overlapping data point labels were suppressed.



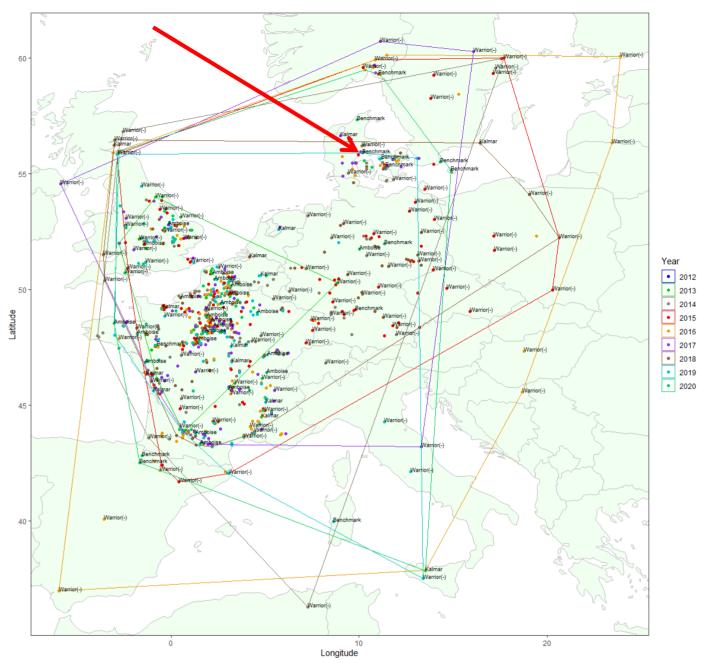


Figure 2.1c: Convex hulls showing spatio-temporal detection patterns of PstS10 yellow rust races (red arrow: 1st occurrence 2012 as race "Warrior(-)"). Overlapping data point labels were suppressed.



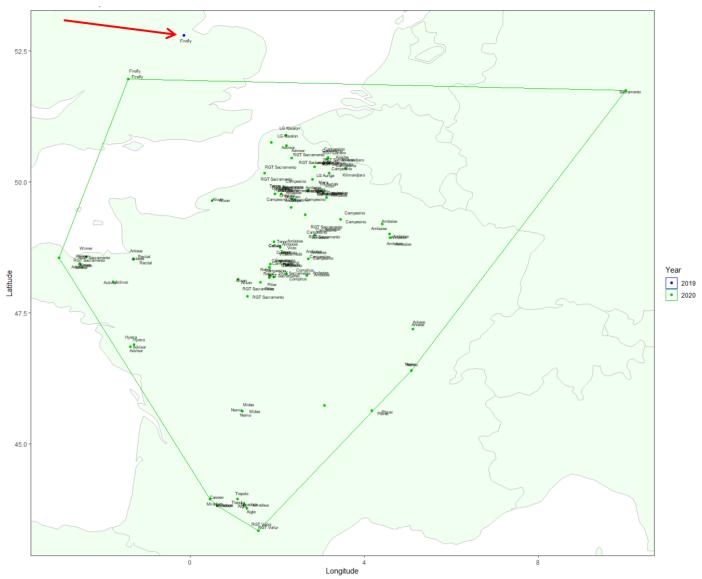


Figure 2.1d: Convex hulls showing spatio-temporal detection patterns of PstS10 yellow rust race "Amboise" on different cultivars in 2020 (red arrow: 1st occurrence 2019 on cultivar "Firefly"). Overlapping data point labels were suppressed.



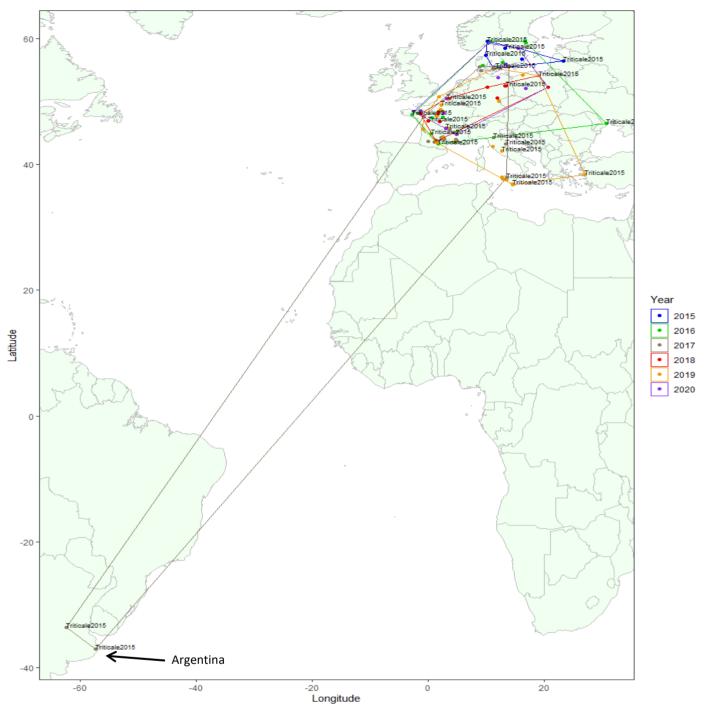


Figure 2.1e: Convex hulls showing spatio-temporal detection patterns of PstS13 yellow rust races. Overlapping data point labels were suppressed.



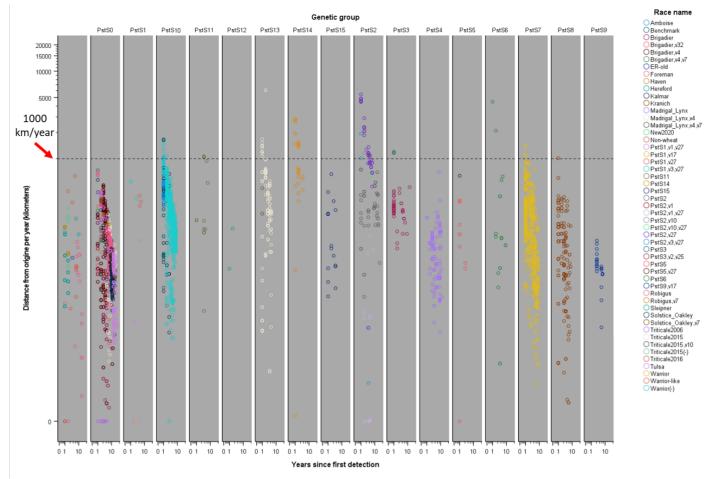


Figure 2.2: Distance per year (in km) from location of 1st detection of yellow rust races by genetic group.



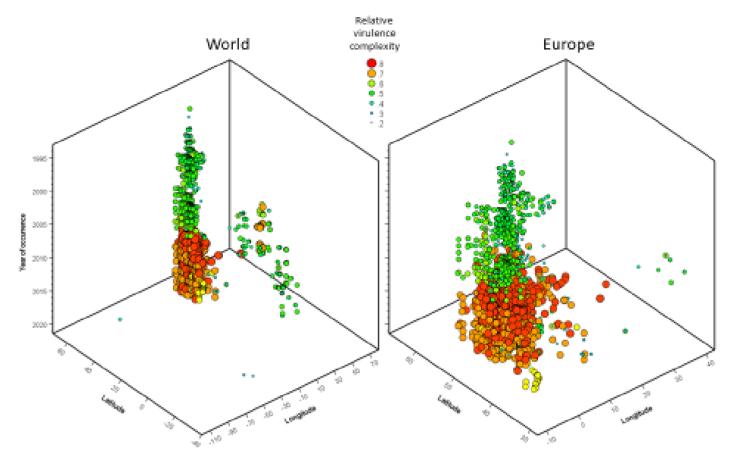


Figure 2.3a: Spatio-temporal changes of virulence complexity of yellow rust races.

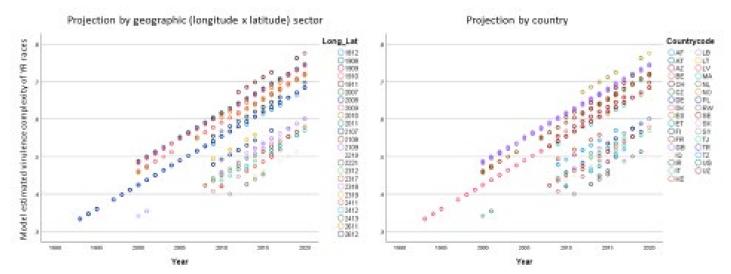


Figure 2.3b: Model-estimated virulence complexity of yellow rust races, projected by geographic sector as well as by country over time.



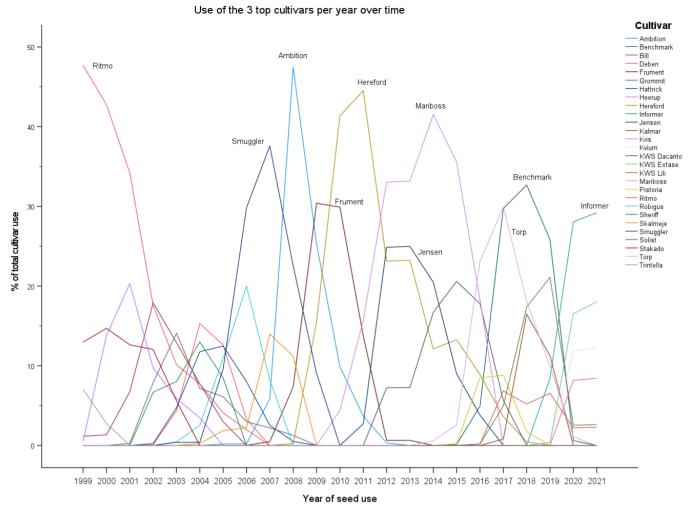


Figure 2.4a: Wheat cultivar use in Denmark over time. Data represent seed production per year of cultivars that belonged to the top three cultivars in any year, as a percentage of the total yearly seed production.



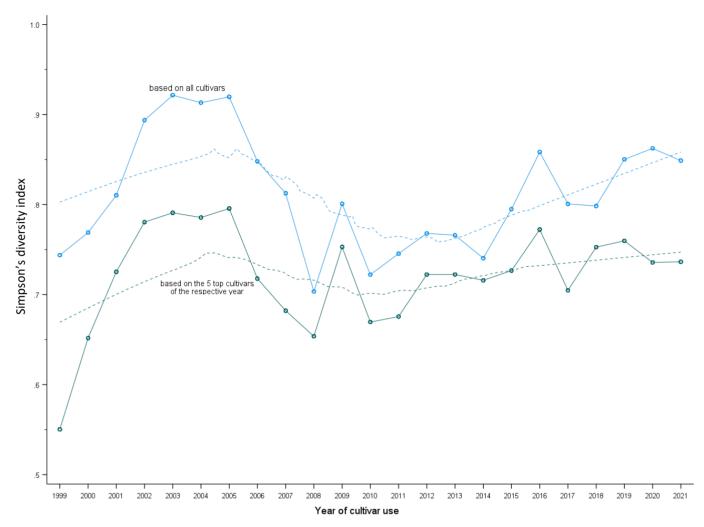
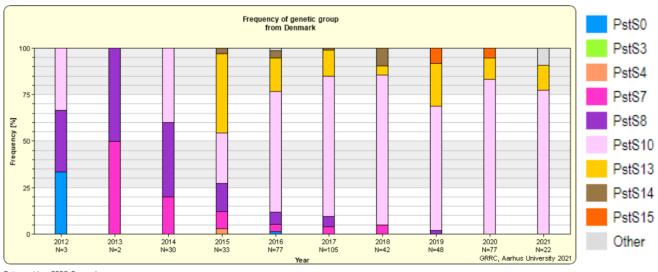


Figure 2.4b: Simpson's diversity index of winter wheat cultivar use in Denmark from 1999 to 2021.



Data provider : GRRC, Denmark.

Figure 2.4c: Frequency of yellow rust genetic groups in Denmark from 2012 to 2021 (source: Wheat Rust Toolbox).



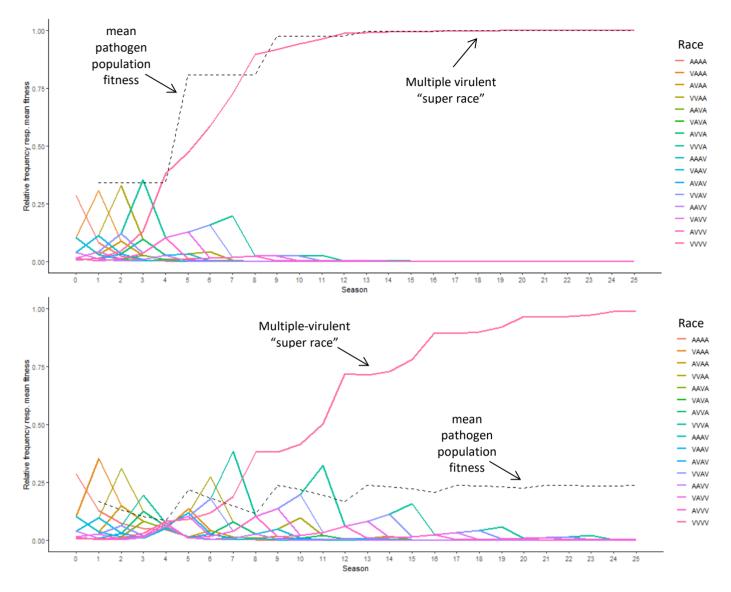


Figure 3.1: Simulated mean relative fitness of the pathogen population and frequencies of pathogen phenotypes (races) over 25 consecutive seasons. Example of REvoRS model output when four factors or loci for specific virulence (V1 – V4) are present in a pathogen population and four cultivars possessing a single gene for complete specific resistance (R1 – R4; V1 – V4 correspond to these!) are deployed in rotation at 90% of the host area; 10% of the host area is planted to a completely susceptible cultivar serving as a "survival refuge". Upper panel (= Scenario 8 in App. A3): no fitness costs for virulence to be "paid" by the pathogen and no partial resistance present in the cultivars; lower panel (= Scenario 11 in App. A3): like scenario in upper panel but fitness costs for virulence = 25% for each virulence and each cultivar possesses 25% partial (non-specific) resistance in addition to its specific resistance. Broken line: mean relative fitness of the pathogen population, colored lines: relative frequencies of individual races ("A" denotes avirulence and "V" denotes virulence for the respective virulence factor; AAAA = multiple avirulent race, ..., VVVV = multiple virulent "super race".)



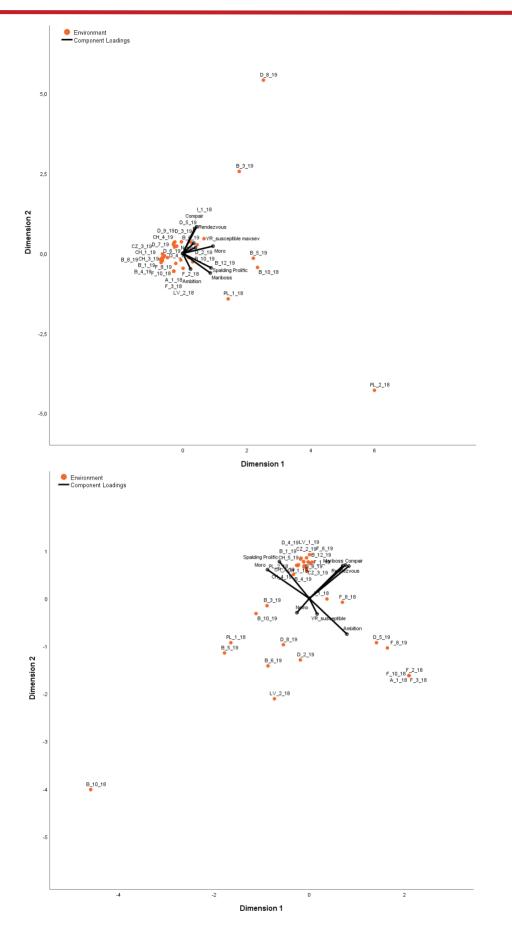


Figure 4: Component loadings of differential cultivars (black vector lines) and environmental centroids (red dots) estimated by nonlinear categorical principal components analysis, based on actual disease scores (upper panel) and their cultivar- and environment adjusted residuals (lower panel) for the 2018-2019 data. See App. A4 for higher resolution plots.