# IV BRAZILIAN
# WORKSHOP OF
# PLANT
# DISEASE
# EPIDEMIOLOGY

## Annals of IV WPDE

Curitiba - Brazil
December 2-4, 2013
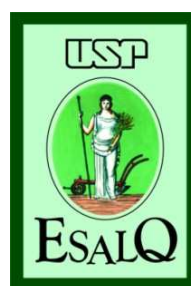
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# IV Brazilian Workshop of Plant Disease Epidemiology

Curitiba - Brazil, December 2-4, 2013

## Committee and Organizers

| Name | Function | Institution |
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# IV Brazilian Workshop of Plant Disease Epidemiology

Curitiba - Brazil, December 2-4, 2013

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## Timetable

### Monday, December 2nd, 2013 - Morning

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|---|---|
| 08h00 | **Registration** |
| 09h00 | **Welcome Remark**<br><br>Profa. Dra. Louise Larissa May De Mio (UFPR, Curitiba - PR, Brazil) |
| 09h10 | **Opening lecture**<br><br>**Spatial, temporal and population aspects of epidemics in fruit crops**<br>Dr. Harald Scherm (University of Georgia, Athens - GA, USA) |
| 10h10 | **Discussion**<br><br>Chair: Profa. Dra. Lilian Amorim (ESALQ-USP, Piracicaba - SP, Brazil) |
| 10h30 | Coffee Break |
| 11h00 | **Sampling optimisation for early detection of invading plant pathogens**<br>Dr. Stephen Parnell (Rothamsted Research, Harpender - Hertfordshire, UK) |
| 12h00 | **Discussion**<br><br>Chair: Dr. Francisco Ferraz Laranjeira Barbosa (EMBRAPA Mandioca e Fruticultura, Cruz das Almas - BA, Brazil) |
| 12h30 | Lunch Break |

## Monday, December 2nd, 2013 - Afternoon

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|---|---|
| 14h00-<br>15h40 | **Oral Session Part I**<br>Chair: Prof. Dr. Laercio Zambolin (UFV, Viçosa - MG, Brazil |
| 14h00 | **Integrating remote sensing, GPS, and GIS technologies for plant disease forensics: where to look for evidence in a 300-hectare crop**<br>Forrest W. NUTTER, Jr |
| 14h20 | **Optimizing intra-orchard sampling for early detection of citrus Huanglongbing**<br>Francisco F. LARANJEIRA, Renato B. BASSANEZI, Tim R. GOTTWALD, Frank van den BOSCH & Stephen PARNELL |
| 14h40 | **Incidence-severity relationship and spatial analysis of Fusarium Head Blight epidemics in southern Brazil**<br>Piérri SPOLTI, Denis A. SHAH, José Maurício C. FERNANDES, Gary C. BERGSTROM & Emerson M. DEL PONTE |
| 15h00 | **Sexual reproduction plays a significant contribution to *Moniliophthora roreri* populations from Ecuador**<br>Karina P. GRAMACHO, Lahyre I. S. GOMES, Bruna L. de BARROS , Jorge T. de SOUZA, Karina. H. SOLIS & Carmen SUÀREZ-CAPELLO |
| 15h20 | **Comparative epidemiology of *Lasiodiplodia* species prevalent in papaya orchards in the northeastearn brazilian**<br>Federico M. SACCHINI, Kamila C. CORREIA, Marcondes A. SILVA, Eliane M. INOKUTI, Marcos P.S. CÂMARA, Laura MUGNAI & Sami J. MICHEREFF |
| 15h40 | Coffee Break |
| 16h10-<br>17h30 | **Oral Session Part II**<br>Chair: Profa. Dra. Louise Larissa May De Mio (UFPR, Curitiba - PR, Brazil |
| 16h10 | **Evaluation of an infection risk alert as a component of a Late Blight decision support system**<br>Ian SMALL, Hilary MAYTON, Laura JOSEPH & William FRY |
| 16h30 | **Molecular epidemiology of the Tomato Chlorosis Virus in Brazil**<br>Júlio C. BARBOSA, Jorge A. M. REZENDE, Helen A. CALAÇA, Helcio Costa, Filipe JACQUES & Armando BERGAMIN FILHO |
| 16h50 | **Monocycle and fungicide sensitivity of *Phakopsora pachyrhizi* populations from organic and conventional production systems**<br>Ana Claudia KLOSOWSKI, Louise Larissa MAY DE MIO & Walmes Marques ZEVIANI |
| 17h10 | **Dispersal of *Colletotrichum acutatum* among citrus plants by honeybees in screenhouse**<br>Maria Cândida de G. GASPAROTO, Sílvia de A. LOURENÇO, Pedro G. de ASSIS, Lívia C. ESCATOLIN, Marcel B. SPÓSITO & Lilian AMORIM |

# Tuesday, December 3rd, 2013 - Morning

| | |
|---|---|
| 08h00 | **Contribution of epidemiology to plant disease management**<br>Prof. Dr. Armando Bergamin Filho (ESALQ-USP, Piracicaba - SP, Brazil) |
| 08h50 | **Simulation modeling in botanical epidemiology and crop loss analyses**<br>Drs. Serge Savary and Laetitia Willocquet (INRA Toulouse, France) |
| 09h50 | **Discussion**<br>Profa. Emerson Medeiro Del Ponte (UFRGS, Porto Alegre-RS) |
| 10h10 | Coffee Break |
| 10h40 | **Applied epidemiology to plant disease risk analyses**<br>Dr. Gustavo Mora-Aguilera (Colegio de Postgraduados do México, Texcoco - Estado de México, México) |
| 11h20 | **Advances in the epidemiological studies of tomato begomoviruses in Brazil**<br>Dra. Alice Kazuko Inoue Nagata (Embrapa Hortaliças, Gama - DF, Brazil) |
| 12h00 | **Discussion**<br>Chair: Prof. Dr Adalberto Corrêa Café Filho (UnB, Brasília - DF, Brazil) |
| 12h20 | Lunch Break |

## Tuesday, December 3rd, 2013 - Afternoon

| | |
|---|---|
| 14h00 | **Epidemiology for benefit-cost analysis of disease control practices**<br>Dr. Renato Beozzo Bassanezi (FUNDECITRUS, Araraquara - SP, Brazil) |
| 14h30 | **Effects of climate changing on the development of bean and rice diseases**<br>Dr. Murillo Lobo Junior (Embrapa Arroz e Feijão, Santo Antônio de Goiás - GO, Brazil) |
| 15h00 | **Molecular epidemiology to study diseases**<br>Dr. Braz Tavares da Hora Júnior (UFV, Viçosa - MG, Brazil) |
| 15h30 | **Discussion**<br>Chairs: Prof. Dr. Sami Jorge Michereff (UFPE, Recife - PE, Brazil) |
| 15h45 | Coffee Break |
| 16h15-17h05 | **Short Oral Poster Presentation (5 minutes)** |
| 16h15 | **Effect of temperature and wetness period on guava stylar end-rot caused by *Neofusicoccum parvum***<br>Antônio Fernandes NOGUEIRA JÚNIOR, Ivan Herman FISCHER & Lilian AMORIM |
| 16h20 | **Light intensity affects monocyclic parameters of *Mycosphaerella musicola – Musa spp*. pathosystem**<br>Francisco F. LARANJEIRA, Djalma M. SANTANA FILHO, Milene C. de SILVA, Zilton J.M. CORDEIRO & Hermínio S. ROCHA |
| 16h25 | **Effect of the leaf wetness on *Diplodia macrospora* infection in corn**<br>João Américo WORDELL FILHO & Cristiano Nunes NESI |
| 16h30 | ***Bacillus* spp*. and *Pseudomonas* sp*. as inhibitors of *Colletotrichum* group *acutatum in vitro* and interference in monocycle components of Glomerella Leaf Spot**<br>Rafaele Regina MOREIRA; Cristiano Nunes NESI & Louise Larissa May DE MIO |
| 16h35 | **Effect of tree exposition on coffee rust progress curve** |

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| | Adriano A. P. CUSTÓDIO, Edson A. POZZA, Gabriel B. VASCO & Leone S. D. SANTOS |
| 16h40 | **Development of risk analysis system for detection of pathogenic activity of *Rhizoctonia solani* in soil**<br>Eliane M. INOKUTI, Ana P.O. BARROS, Marcondes A. SILVA & Sami J. MICHEREFF |
| 16h45 | **Climatic conditions for development of European Canker of Apple in southern Brazil**<br>Ana B. C. CZERMAINSKI, Silvio A. M. ALVES |
| 16h50 | **The role of seed-borne inoculum in Brown Spot epidemics and effects on crop stand and yield of irrigated rice**<br>André A. SCHWANCK; Priscila R. MENEZES; Cândida R. J. de FARIAS; Daniel dos S. GROHS; Gustavo R. D. FUNCK; Aline de H. N. MAIA & Emerson M. DEL PONTE |
| 16h55 | **Monitoring of inoculum quantity and dispersal of *Colletotrichum spp.* associated with Glomerella Leaf Spot on apple**<br>Natasha A. HAMADA & Louise L. MAY DE MIO |
| 17h00 | **Composition of the *Fusarium graminearum* species complex populations in wheat cropping environments in southern Brazil**<br>Emerson M. DEL PONTE, Todd J. WARD, Piérri SPOLTI, Larissa B. GOMES, Camila P. NICOLLI, Paulo R. KUHNEM, Cleiltan N. SILVA & Dauri J. TESSMANN |
| 17h05-18h05 | **Poster session** |
| 01 | **Effect of temperature and wetness period on guava stylar end-rot caused by *Neofusicoccum parvum***<br>Antônio Fernandes NOGUEIRA JÚNIOR, Ivan Herman FISCHER & Lilian AMORIM |
| 02 | **Light intensity affects monocyclic parameters of *Mycosphaerella musicola* – *Musa spp.* pathosystem**<br>Francisco F. LARANJEIRA, Djalma M. SANTANA FILHO, Milene C. de SILVA, Zilton J.M. CORDEIRO & Hermínio S. ROCHA |
| 03 | **Effect of the leaf wetness on *Diplodia macrospora* infection in corn**<br>João Américo WORDELL FILHO & Cristiano Nunes NESI |

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| 04 | **Bacillus spp. and Pseudomonas sp. as inhibitors of Colletotrichum group acutatum in vitro and interference in monocycle components of Glomerella Leaf Spot**<br>Rafaele Regina MOREIRA; Cristiano Nunes NESI & Louise Larissa May DE MIO |
| 05 | **Effect of tree exposition on coffee rust progress curve**<br>Adriano A. P. CUSTÓDIO, Edson A. POZZA, Gabriel B. VASCO & Leone S. D. SANTOS |
| 06 | **Development of risk analysis system for detection of pathogenic activity of Rhizoctonia solani in soil**<br>Eliane M. INOKUTI, Ana P.O. BARROS, Marcondes A. SILVA & Sami J. MICHEREFF |
| 07 | **Climatic conditions for development of European Canker of Apple in southern Brazil**<br>Ana B. C. CZERMAINSKI, Silvio A. M. ALVES |
| 08 | **The role of seed-borne inoculum in Brown Spot epidemics and effects on crop stand and yield of irrigated rice**<br>André A. SCHWANCK; Priscila R. MENEZES; Cândida R. J. de FARIAS; Daniel dos S. GROHS; Gustavo R. D. FUNCK; Aline de H. N. MAIA & Emerson M. DEL PONTE |
| 09 | **Monitoring of inoculum quantity and dispersal of Colletotrichum spp. associated with Glomerella Leaf Spot on apple**<br>Natasha A. HAMADA & Louise L. MAY DE MIO |
| 10 | **Composition of the Fusarium graminearum species complex populations in wheat cropping environments in southern Brazil**<br>Emerson M. DEL PONTE, Todd J. WARD, Piérri SPOLTI, Larissa B. GOMES, Camila P. NICOLLI, Paulo R. KUHNEM, Cleiltan N. SILVA & Dauri J. TESSMANN |
| 11 | **In vitro evaluation of the sensitivity of Phomopsis amygdali to temperatures and photoperiods**<br>Tamires C. de ARAÚJO, Maristella DALLA PRIA & Louise Larissa MAY DE MIO |
| 12 | **In vitro sensitivity of Phomopsis amygdalli to essential oils**<br>Tamires C. de ARAÚJO, Maristella DALLA PRIA & Louise Larissa MAY DE MIO |

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| 13 | **Increase of Citrus Freckle Spot and Hard Spot on fruit with citrus black spot (*Guignardia citricarpa*) under different post-harvest treatments**<br>Paula S. PANOSSO, Sílvia de Afonseca LOURENÇO & Lilian AMORIM |
| 14 | **Temporal progress of Leaf Blight and Downy Mildew on *Vitis labrusca* grapes**<br>Gabriel Martins FERREIRA, Cristiano Nunes NESI, Luiz Antonio BIASI & Louise Larissa MAY DE MIO |
| 15 | **Anthracnose incidence in different rustic grape cultivars in Campo Largo, PR, Brazil**<br>Luciane Bertoletti BARROS, Gislâine MARGOTI, Louise Larissa MAY DE MIO & Luiz Antonio BIASI |
| 16 | **Attributes of lesions and spatial patterns of Rice Brown Spot field epidemics at the leaf scale**<br>André A. SCHWANCK & Emerson M. DEL PONTE |
| 17 | **Infectious period of Citrus Black Spot on sweet orange**<br>Silvia de A. LOURENÇO, Maria Cândida de G. GASPAROTO, Marcel B. SPÓSITO, Lilian AMORIM & Tim R. GOTTWALD |
| 21h00 | **Social dinner (by membership)** |

## Wednesday, December 4th, 2013 - Morning

| 08h30 | **Multivariate methods with aplications to plant epidemiology**<br>Prof. Dr. Carlos Tadeu dos Santos Dias (ESALQ-USP, Piracicaba - SP, Brazil) and Dr. Cristiano Nunes Nesi (EPAGRI, Chapecó - SC, Brazil) |
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| 09h20 | **Bayesian models to epidemic studies**<br>Dr. Jose Mauricio Fernandes (EMBRAPA, Passo Fundo - RS, Brazil) |
| 10h00 | **Discussion**<br>Chair Dr. Ana Beatriz Costa Czermainski (EMBRAPA Uva e Vinho, Bento Golçalves - RS, Brazil) |
| 10h20 | Coffee Break |
| 10h50 | **R tutorial and case studies for understanding plant disease epidemiology using R software**<br>Prof. Dr. Walmes Marques Zeviani (UFPR, Curitiba - PR, Brazil) |
| 12h00 | **Discussion**<br>Chair: Prof. Dr. Paulo Justiniano Ribeiro Jr (UFPR, Curitiba - PR, Brazil) |
| 12h20 | **Host definition for the next event**<br>**Closing Cerimony** |

# Abstracts

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# Lectures

## (Listed in chronological order)

# SPATIAL, TEMPORAL AND POPULATION ASPECTS OF EPIDEMICS IN FRUIT TREE CANOPIES

Harald SCHERM[1] & Sydney E. EVERHART[2]

[1]Department of Plant Pathology, University of Georgia, Athens, GA, USA (scherm@uga.edu); [2]Current address of second author: Department of Botany and Plant Pathology, Oregon State University, Corvallis, OR (USA)

Spatial patterns of plant disease can provide insights into key epidemiological processes such as sources of inoculum, mechanisms of propagule dissemination, and reproductive strategies of the pathogen population. In this study we characterized spatial patterns of brown rot (caused by *Monilinia fructicola*) and of the associated fungal genotypes in individual peach tree canopies. An electromagnetic digitizer was used to georeference and map symptoms within individual canopies, and three-dimensional methods of spatial statistics were applied to analyze the resultant data points for aggregation and association patterns. The approach was applied in a 3-year study to characterize spatio-temporal development of blossom blight and pre-harvest brown rot in 13 trees of different fruit maturity classes. Within-canopy disease aggregation correlated negatively with disease incidence ($r = -0.653$, $P < 0.0001$), showing that trees with higher brown rot levels had lower aggregation of affected fruit. Significant disease aggregation was most pronounced for early-maturing cultivars and/or early in the epidemic. This is consistent with a greater importance of localized, within-tree sources of inoculum at the beginning of the epidemic. Four of five trees having >10 blossom blight symptoms per tree early in the season showed a significant positive spatial association of pre-harvestfruit rot to blossom blight within the same canopy, suggesting that local inoculum produced following bloom infections contributed significantly to infection of the ripening fruit in each tree later in the season.

To complement these results with genotypic information about the associated pathogen isolates, 13 microsatellite markers were developed and applied to examine the fine-scale genetic structure of *M. fructicola* populations in six of the 13 trees, whereby isolates from every brown rot symptom in each tree were obtained and genotyped ($n = 113$ and 581 for blossom blight and fruit rot, respectively). Each canopy population had between 65 and 173 isolates and showed high genetic diversity (average $uh = 0.529$) and high genotypic diversity (average $D = 0.928$) within canopies. There were between 29 and 71 multilocus haplotypes per tree for a total of 264 haplotypes across the six trees, with about two-thirds of haplotypes being unique within each tree, i.e., represented only once. The percentage of unique multilocus haplotypes within trees was greater for blossom blight isolates (average 78.2%) than for fruit rot isolates (average 51.3%), indicating a greater

contribution of clonal reproduction during the pre-harvest epidemic. For fruit rot isolates, between 54.2 and 81.7% of isolates were contained within one to four dominant clonal genotypes per tree having at least 10 members. When spatial genetic autocorrelation analysis was applied to quantify fine-scale genetic structure of the pathogen population, all six fruit rot populations showed positive and significant genetic autocorrelation for distance classes between 0.37 and 1.48 m. We conclude that despite high levels of within-tree pathogen diversity, the contribution of locally available inoculum combined with short-distance dispersal is likely the main factor generating clonal population foci and associated spatial genetic clustering within trees.

# SAMPLING OPTIMISATION FOR EARLY DETECTION OF INVADING PLANT PATHOGENS

Stephen PARNELL[1], Tim R. GOTTWALD[2], Francisco F. LARANJEIRA[3] & Frank van den BOSCH[2]

[1]Rothamsted Research, Harpenden, AL5 2JQ, United Kingdom (stephen.parnell@bbsrc.ac.uk); [2]USDA-ARS, Fort Pierce, Florida 34945, USA; [3]Embrapa Cassava & Fruits, Cruz das Almas, Bahia, Brazil

There has been a significant increase in the number of invading plant pathogens associated with increases in global trade and travel in recent times. This is particularly evident in citrus production where numerous pest and pathogen invasions currently threaten citrus industries across the Americas. The citrus canker and citrus greening (syn. Huanglongbing, HLB) epidemics are notable and have caused major economic losses where they occur. To manage or eradicate an invading pathogen it is crucial that it is detected whilst it is still at low incidence. This requires intensive sampling and surveillance efforts at substantial cost. Large-scale surveillance surveys are expensive, often covering large geographic regions and stretching fiscal and manpower resources. However, despite this, how the widespread deployment of survey resources relates to the probability to detect an invading population remains poorly understood. There is a need for quantitative methods and information to support the design of sampling strategies for early detection of invading plant pathogens. To quantify the probability of early detection necessitates a model-based approach which can relate the spatial and temporal dynamics of invading pathogen populations with the dynamics of a surveillance system. We demonstrate how the incidence an epidemic has reached when it is first detected (detection-incidence, $q^*$) can be calculated using a relatively simple approximation (Parnell et al, 2012). That is, the mean detection-incidence is given by:

$$E(q^*) = \frac{r\Delta}{N} = \frac{rate\ of\ epidemic\ spread}{rate\ of\ sampling},$$

where $r$ is the initial rate of epidemic spread, $\Delta$ is the time-interval between rounds of sampling and $N$ is the sample size. This rule-of-thumb provides an insight into what determines the success of a surveillance program and provides a simple way to guide survey resource allocation in practice. We demonstrate the accuracy of this rule-of-thumb, using a stochastic spatially-explicit simulation model for HLB which captures the spatial and temporal complexities of observed HLB epidemics, and show how it can be adjusted for certain specific cases to improve accuracy further. This provides a method to determine sampling resources to ensure enough resource is committed to have an acceptable chance to achieve early-detection, but not more than is necessary. We also bring together state of the art epidemiological modeling and stochastic optimization techniques to show how the optimal

spatial pattern of sampling deployment across a landscape can be determined. We show that the optimal pattern of sampling resource deployment is often counter-intuitive; for example simply targeting the highest risk locations is rarely the optimal course of action and in many circumstances it is most effective to spread resources evenly across an area. By finding the optimal sampling patterns we can make comparisons with standard sampling procedures such as regular or stratified-random sampling and identify which of these standard survey designs are most effective.

Parnell, S., Gottwald, T.R., Gilks, W.R., van den Bosch, F. 2012. Estimating the incidence of an epidemic when it is first discovered and the design of early detection monitoring. Journal of Theoretical Biology 305, 30-36.

# GENERALIZED LINEAR MIXED MODEL THEORY AND USE IN PLANT PATHOLOGY

Alissa B. KRISS[1]

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(alissa.kriss@ars.usda.gov)

Linear mixed models (LMM), generalized linear models (GLM), and (general) linear models (LM) are all special cases of generalized linear mixed models (GLMM). The "generalized" component allows for non-Gaussian observations and the "mixed" component allows for both fixed and random effects. In recent years, major advances in understanding and implementation of GLMMs in software programs like SAS (SAS Institute Inc., Cary, NC, USA) and R (open source) have made them more accessible to a broader range of individuals, such as plant pathology graduate students and other professionals whose research involves these types of models.

Observations from studies in plant pathology are often from non normal continuous (e.g. exponential, gamma) or discrete (e.g., binomial, Poisson) distributions. For instance, we collect incidence data at a site (i.e., presence or absence of a pathogen) and make counts of lesions on leaves. Prior to the advent of generalized linear model theory, one may have assumed (perhaps not correctly) the observations were "well approximated" by the normal distribution, and proceeded with using a general linear model under the assumption that "the general linear model is robust" to departures from normality. While the robustness of the general linear model is at times satisfactory, one will have more power (ability to detect a true difference in treatment means) with the use of an appropriate generalized linear model.

The experimental design, whether planned or observational, sets the basis for the model construction (i.e., for the choice of linear predictor). The link function is a mechanism used to relate the linear predictor with the expected mean of the distribution chosen for the observations. The concepts and terms of factor, level, experimental unit, blocking, clustering, nested or cross-classification are used in defining the linear predictor. Determining whether variables should be defined as random or fixed effects (i.e., a mixed model) is also part of building the linear predictor. Random effects may be included in models to account for variation among blocks that are replicated across locations or sampling times or correlation among individuals. Historically, inference was primarily focused on the main effects and their interactions, and considering random effects as ancillary and not greatly explored. However, as will be discussed, recent studies (Kriss et al. 2012) used the estimated variance of the random effects to quantify the spatial heterogeneity of disease incidence.

## (G)LM(M)

**Generalized Linear Mixed Model (GLMM)**
Observations: $\boldsymbol{y}|\boldsymbol{b} \sim Dist(\boldsymbol{\mu}, \boldsymbol{R})$
Link: $\boldsymbol{\eta} = \mathscr{g}(\boldsymbol{\mu}|\boldsymbol{b})$
Linear Predictor: $\boldsymbol{X\beta} + \boldsymbol{Zb}$   $\boldsymbol{b} \sim N(\boldsymbol{0}, \boldsymbol{G})$
Estimated Mean: $\widehat{\boldsymbol{\mu}} = \mathscr{g}^{-1}(\widehat{\boldsymbol{\eta}}) = \mathscr{g}^{-1}(\boldsymbol{X\widehat{\beta}} + \boldsymbol{Z\widehat{b}})$

**Linear Mixed Model (LMM)**
Observations: $\boldsymbol{y}|\boldsymbol{b} \sim N(\boldsymbol{\mu}, \boldsymbol{R})$
Link: $\boldsymbol{\eta} = \boldsymbol{\mu}$ (Identity)
Linear Predictor: $\boldsymbol{X\beta} + \boldsymbol{Zb}$   $\boldsymbol{b} \sim N(\boldsymbol{0}, \boldsymbol{G})$
Estimated Mean: $\widehat{\boldsymbol{\mu}} = \widehat{\boldsymbol{\eta}} = \boldsymbol{X\widehat{\beta}} + \boldsymbol{Z\widehat{b}}$

**Generalized Linear Model (GLM)**
Observations: $\boldsymbol{y} \sim Dist(\boldsymbol{\mu}, \boldsymbol{R})$
Link: $\boldsymbol{\eta} = \mathscr{g}(\boldsymbol{\mu})$
Linear Predictor: $\boldsymbol{X\beta}$
Estimated Mean: $\widehat{\boldsymbol{\mu}} = \mathscr{g}^{-1}(\widehat{\boldsymbol{\eta}}) = \mathscr{g}^{-1}(\boldsymbol{X\widehat{\beta}})$

**Linear Model (LM)**
Observations: $\boldsymbol{y} \sim N(\boldsymbol{\mu}, \boldsymbol{R})$
Link: $\boldsymbol{\eta} = \boldsymbol{\mu}$ (Identity)
Linear Predictor: $\boldsymbol{X\beta}$
Estimated Mean: $\widehat{\boldsymbol{\mu}} = \widehat{\boldsymbol{\eta}} = \boldsymbol{X\widehat{\beta}}$

Kriss, A. B., Paul, P. A., and Madden, L. V. 2012. Characterizing heterogeneity of disease incidence in a spatial hierarchy: A case study from a decade of observations of Fusarium head blight of wheat. Phytopathology 102:867-877.

# CONTRIBUTION OF EPIDEMIOLOGY TO PLANT DISEASE MANAGEMENT: THE CASE OF CITRUS HUANGLONGBING

Armando BERGAMIN FILHO[1]

[1]Departamento de Fitopatologia e Nematologia ESA Luiz de Queiroz – Universidade de São Paulo (abergami@usp.br)

## BASIC CONCEPTS

Plant disease epidemics are classified into two basic groups, depending on the source of the inoculum that encounters the host over the course of disease development. In the first group, inoculum that causes infections is produced during the epidemic in or on individuals that had been previously infected during the current epidemic. Epidemics of this group are polycyclic in structure, and diseases that cause them are called polycyclic diseases or compound interest diseases (Vanderplank, 1963; Madden et al., 2007). In the second group, inoculum that causes infection is not produced by the pathogen in or on individuals that had been infected during the current epidemic in the considered crop, but in the soil, on secondary hosts, or in infected crop plants of the same host in another fields. Epidemics of this group are monocyclic in structure, and diseases that cause them are called monocyclic diseases or simple interest diseases (Vanderplank, 1963; Madden et al., 2007).

For both groups the inoculum that starts the epidemic is called primary inoculum and the infection caused by it is called primary infection. This process is called primary spread. Primary inoculum, of course, is not produced in the current epidemic, but in the soil, on secondary hosts, or in infected crop plants of the same host in another fields. On the other hand, secondary inoculum and secondary infection occur only in the group of polycyclic diseases. Secondary inoculum results from primary infections or from secondary infections that take place during the current epidemic. Secondary infections originate from secondary inoculum. This process is called secondary spread. As noted by Madden et al. (2007), the primary infection process that starts a polycyclic epidemic is analogous to the process that occurs throughout a monocyclic epidemic, and thus one can think of monocyclic epidemics as consisting of only primary infections.

Plant pathology textbooks in general consider that the epidemiological role of primary spread is to introduce the pathogen in areas where it is absent; the subsequent development of the epidemic is governed by the secondary spread (international textbooks: Gäumann, 1950; Agrios, 1988; 1997; 2005; Brazilian textbooks: Galli et al., 1968; 1978; Bergamin Filho et al., 1995; Amorim et al., 2011; Zambolim et al., 2012). The same view is predominant in modeling plant disease epidemics, in which most epidemics start by introducing few lesions

or infected individuals (primary inoculum) instantaneously at time t=0 (Vanderplank, 1963; 1965; Zadoks and Schein, 1979; Bergamin Filho and Amorim, 1996; Kranz, 1996; 2003; Segarra et al., 2001; Vale et al., 2004; Madden et al., 2007). The reasoning is that, with sufficient time, the secondary infections overshadow the initial events. In this view primary spread is relegated to a subservient role of the carryover of inoculum from the previous crop to initiate the first infections and is assumed to be of negligible importance relative to the dominating influence of secondary, plant to plant spread (Gilligan, 1994).

As discussed by Madden et al. (2007), however, "it may not be realistic in some cases to assume an instantaneous start of the epidemic. It is possible, for instance, that the primary infections occur over an extended period of time, possibly concurrently with the new (secondary) infections occurring due to spread from individual to individual." A theoretical approach for epidemics in which primary spread occurs over an extended period of time is proposed by Brasset and Gilligan (1988), Gilligan and Kleczkowski (1997), Gilligan (2002), and Madden et al. (2007). Disease progress curves in these cases are less clear cut compared to the results obtained for strictly polycyclic or monocyclic epidemics (Gilligan, 2002; Madden et al., 2007). Examples of epidemics in which primary spread occurs over an extended period of time are given by Thresh (1983); Camann et al. (1995); Bergamin Filho and Amorim (1996), Holt et al. (1999), Perring et al. (1999); Otten et al. (2003); Coutts et al. (2004); Bailey et al. (2005); Madden et al. (2007); Gottwald (2010); and Motisi et al. (2013).


**EPIDEMIOLOGY AND MANAGEMENT OF CITRUS HUANGLONGBING**

Huanglongbing of citrus (HLB), caused by *Candidatus* Liberibacter spp. and vectored by the psyllid *Diaphorina citri*, is an excellent example of all these underestimated aspects of plant disease epidemiology (Bové, 2006; Gottwald et al., 2007; Gottwald, 2010). This pathosystem has the following peculiar epidemiological characteristics that greatly influence disease management:

- in unmanaged groves, primary spread and secondary spread are present, although not necessarily simultaneously; epidemic is a mixture of monocyclic (primary) and polycyclic (secondary) processes (Gottwald et al. 2008; 2010);
- in well managed groves (with eradication of symptomatic trees and chemical vector control), secondary spread is not relevant; epidemic is a monocyclic (or primary) process;
- in managed and unmanaged groves, citrus is continually subject to infestation by immigrating bacteriliferous adults of *D. citri* and there is not any time during the year that a citrus grower could be assured dispersal will not occur (Yamamoto et al., 2001; Hall and Hentz, 2011; Hall et al., 2013); primary inoculum usually does not decay as

is the case with soil-borne pathogens; the main epidemiological role of primary spread is not related to introducing the pathogen in a field, but to sustain the rate of epidemic progress;

- even in well managed groves, primary spread from unmanaged groves is enough to cause infection in almost 100% of trees in two to five years (Belasque et al., 2010; Gatineau et al., 2010; Bassanezi et al., 2013b; Hall et al., 2013);

- *D. citri* moves bi-directionally between managed and unmanaged groves with a greater number of adult insects moving from unmanaged into managed groves than from managed into unmanaged groves (Boina et al., 2009); in most cases, the immigration of bacteriliferous vectors (in excess of those required for disease saturation) makes disease incidence insensitive to the mortality of vectors within the managed citrus groves (Belasque et al., 2010; Bassanezi et al., 2013a);

- local measures (eradication of symptomatic trees and chemical vector control, aimed mainly against secondary spread) are not sufficient to effectively manage the disease; area-wide management (aimed mainly against primary spread) is, at present, the best way to keep disease incidence at an acceptable level (Bassanezi et al., 2013b).

As a direct effect of the epidemiological study of Bassanezi et al. (2013b), the concept of area-wide (regional) management has become the mainstay of HLB control programs in the United States and Brazil. In the United States, the study has motivated citrus producers to come together to form citrus health management areas to attempt to control this disease more effectively on a regional basis. In Brazil, voluntary groups of citrus growers have been formed to control *D. citri* population with three to four coordinated area-wide insecticide applications in defined short periods (one to two weeks). Other Brazilian growers, with the agreement of their neighbors, are supporting the management of *D. citri* populations and infected trees in their neighboring groves to prevent HLB primary spread in their own groves.

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# SIMULATION MODELLING IN BOTANICAL EPIDEMIOLOGY AND CROP LOSS ANALYSES

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Simulation modelling is a critical approach for plant disease epidemiology. This is because simulation modelling can serve very different purposes, including: (1) synthesizing available data on epidemiological processes, (2) predicting epidemiological patterns, (3) developing a conceptual framework that captures available data, (4) organizing epidemiological knowledge to identify knowledge gaps, or (5) designing experiments aiming at testing a theory. We argue that very few, if any, other approaches available to (plant disease) epidemiologists offer so many applications. The downside of this advantage is that simulation modelling may loose its meaning if used, as it has indeed been, for several and possibly contradicting objectives; modelling must have a purpose. In this presentation, we shall endeavour to highlight some of the above applications of simulation modelling through examples.

Simulation modelling is a powerful approach to address dynamic processes, since it allows analysing the effects of the components of a system on its behaviour (e.g., the dynamics of an epidemic). In particular, simulation models enable exploring the behaviour of plant-pathogen systems, and exploring "futures", i.e., analysing how the considered system might behave under yet-undocumented conditions. Mechanistic simulation also allows bridging the gap between 'observers' and 'modellers'. Softwares matching international standards of quality such as STELLA® provide a visual support to modelling, involve as little calculus as possible, and so allow the user to focus on system's structure and properties, instead of programming syntax. Thus, simulation models are good educational tools: they can provide an intuitive hands-on experience in analyzing (plant-pathogen) systems.

One important application of simulation modelling in botanical epidemiology is the simulation of plant disease epidemics, considering an epidemic as a process resulting from the concatenation of sub-processes. The main state variables considered are numbers of healthy, latent, infectious, and removed sites, and the model simulates flows between sites as epidemics develop. Such a structure allows integrating the effects of various factors on plant disease epidemics, e.g.: climate, host dynamics, host plant resistance, pathogen population including genotypes with varying fitness, and disease aggregation. Simulation models thus enable to assess weather scenarios over relatively short periods of time, and address the question of superimposed, confounding weather effects influencing distinct

epidemiological sub-processes. Similarly, simulation modelling is a powerful approach to compare components of partial resistance in their overall effects on field resistance, making simulation modelling an integral tool of phenotyping host plant resistances.

Biology, and therefore plant pathology, deals with hierarchies. Simulation models allow accounting explicitly for such hierarchies (e.g., epidemics and their sub-processes; or, crop losses and their mechanisms). Epidemiological hierarchies, for instance, can be addressed through the auto- and allo-infection concepts, considering a range of stacked levels of plant hierarchy: from individual sites, to plant organs, to plants, to a crop, to a landscape. The effects of variation in auto- / allo-infection ratios, combined with varying sizes of levels of plant hierarchy, enable one to explore different spread strategies of the pathogens depending on the physical structure of the host population.

Another important research area is the simulation of yield losses caused by pests (pathogens, insects, and weeds). Damage mechanisms are incorporated into an agrophysiological model, allowing simulating pest effects on the physiological processes of crop growth, and on the resulting crop yield. Simple, universal, agrophysiological models incorporating generic damage mechanisms have been developed for rice and wheat using a single common structure. New models are being considered for other major crops.

Much of the simulation modelling work is derived, by necessity, from field observations. For instance, the generic yield loss simulation modelling above is grounded on extensive survey work in farmers' fields. Of course, the resulting model does not account for idiosyncrasies that make each field a unique individual. Yet, the overall structure of the model is a reflection of information gathered over many years on many individual cases. A principal challenge is to ensure that scientific questions can be addressed in a formal, testable manner. This can only be done on simple, not complicated, modelling structures. Achieving simplicity coincides with the need to clearly identify the objectives of modelling.

Many statistical modelling approaches can be used to analyse epidemiological data; the choice of an approach depends on both the objective and available data. Statistical modelling leads to deductions from observations. Unlike statistical modelling, however, simulation modelling does not depend on the available observational data. Instead, simulation modelling implies inductions, in the form of hypotheses forwarded on processes underpinning observed phenomena, leading to the identification of knowledge gaps and to experiments specifically designed to fill them. As a result, the development of a simulation model can be seen as a very powerful aid to implement a research strategy, as well as a research aim of its own.

Simple, parameter-sparse models can more easily be verified, assessed, shared, and used. Similarly to any other scientific hypothesis, a model may be considered acceptable until it
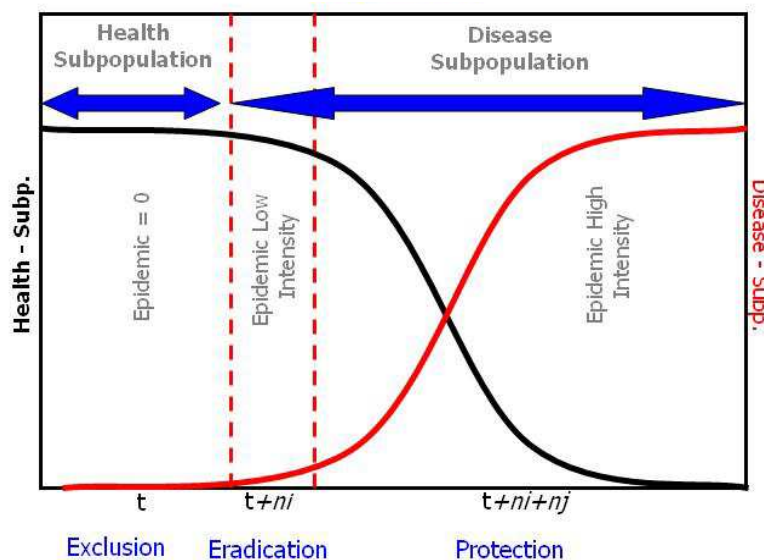
may be rejected as new evidence becomes available. The critical question of model evaluation is very briefly addressed.

# APPLIED EPIDEMIOLOGY TO PLANT DISEASE RISK ANALYSES

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Classical epidemiology focus on plant disease ***protection***. This view emphasizes disease measurements (*Yi*) on time in restricted space (the production unit). This approach has been the main epidemiology stream since Vanderplank`s seminal epidemiology book and strongly relay on the infection (*i.e.* the pathogenicity cycles) rather than on the contagious principle required for an effective development of our science. The main epidemiological consequence of the infection principle is the study of the infected population and/or the inoculum population resulting on a reactive epidemiology, either as descriptive studies centering on temporal rates (i.e., $r_G$, $r_L$, $r_M$, 1/b,) and spatial indexes (Morisita, Lloyd, etc.), or as inferential analyses predicting disease levels ($\hat{Y}_i$) or disease occurrence ($\hat{Y}o$). Has this classical view been effective for disease management on the ground of new, reemerging or increasing endemicity threshold of disease epidemics worldwide?. Shall we continue facing disease challenges armed with descriptive or inferential models fitted to heal but not to prevent?. It is imperative to recognize that we has been putting our efforts on subpopulations, either of the infected plant or the infectious pathogen and overlooking the healthy plant as the main population target to understand productive balance before being obsessed with the damage (Figure 1).



**Figure 1.** Subpopulation addressed on epidemiological studies for a correct application of the prevention and protection principles in plant disease management.

Recognizing these limitations liberates new epidemiological frontiers refreshing or discipline with new concepts, principles and methods in order to address the ***prevention***, long recognized principle (applied through exclusion and eradication) on disease management, but equally overlooked and relegated to the regulatory qualitative PRA (pest risk assessment) approach strongly oriented to trade commodities. Prevention place the subsystem plant on the center of the epidemiological system and the remaining components (clime, pathogen, etc.) are subordinated to that. Contagious now is truly relevant on the effort to estimate risk of infection and pathogen movement and establishment. The goal of epidemiology risk analysis (**ERA**) is the prevention of undesirable epidemic impacts. The objective is to emphasize studies on dispersion and survival (**D-S**) over the pathogenicity cycles. **ERA** relay on the following assumptions: 1). Real or potential *Yo* on specific dimensional space (focus) and time is fundamental for risk estimations. 2). The *region* become the risk framework to estimate epidemic inductivity recognizing a *source region* and an *exclusion region* as components of the **D-S** system. 3). *Pest load* (as extension of Garret`s *inoculum potential*) and the *epidemic force* at the *source region* should be estimated to determine risk weights. 4). The epidemiological system is the rational scheme to account for risk factors and the key is to maximize the measurement of regional variability (univariate- and multivariate-$\sigma^2$). Under these assumptions **ERA** apply both, for regulatory and endemic pests and become fundamental in epidemiological surveillance for planning and sustainable production at regional revel. Monitoring, early warning, epidemic impact, pest management and risk communication are main **ERA** components. In support to official phytosanitary Mexican programs, **ERA** have implied the development of stochastic and determinist models, often in combination with Monte Carlo`s simulations, for transect and bidimensional monitoring of *Elsinoe australis* and other citrus pests; integration of spatial dispersion at state, local and field level onto temporal flexible models to estimate the impact of *Candidatus* Liberibacter asiaticus (*C*Las) on citrus production (Salcedo et al., 2010. IICA); the calculation of multivariate-factor index to estimate regional risk of *C*Las establishment (Figure 2); the application of empirical and experimental disease gradients to estimate ARCOS (regional areas for control of *Diaphorina citri*, *C*Las`s vector) (*www.infito.org.mx/arcos*); and early warning for coffee rust (*Hemileia vastatrix*) management using weighted sampling and spatial analysis (*www.infito.org.mx/royacafe*), a recent example of a reemerging epidemic in Central America. Some of these developments will be presented and discussed. At the end, epidemiology is not anymore the study of epidemics but the study of the epidemiological system. The plant health is the real issue.
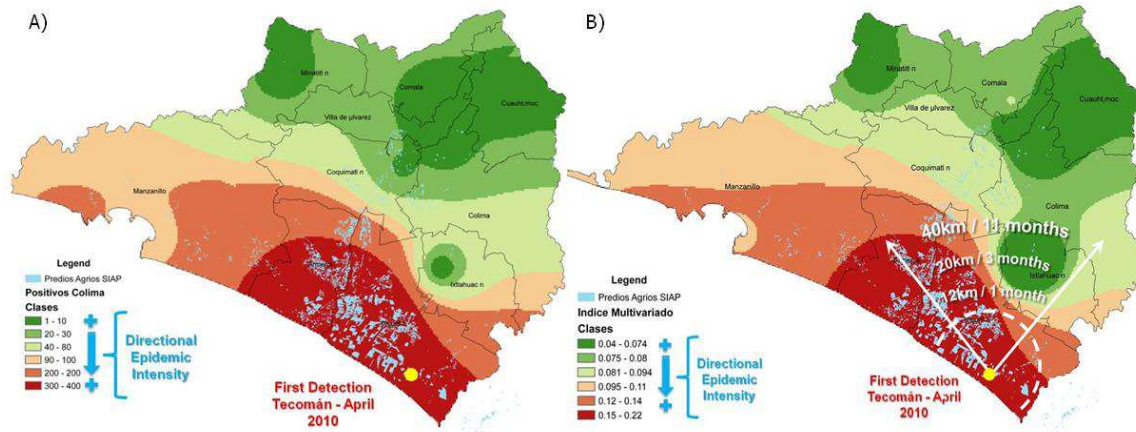
**Figure 2.** Application of multivariate factor index to estimate risk establishment of *Candidatus* Liberibacter asiaticus in Colima state, Mexico. **A)** Establishment projections with real *C*Las data. **B)** Risk *C*Las establishment.

# ADVANCES IN THE EPIDEMIOLOGICAL STUDIES OF TOMATO BEGOMOVIRUSES IN BRAZIL

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Begomoviruses (genus *Begomovirus*, family *Geminiviridae*) are one of the major problems in tomatoes in Brazil due to their high incidence throughout the country. It is believed that it occurred due to the introduction and spread of their vectors, the whitefly *Bemisia tabaci* B biotype, at the 1990's. Infected plants show typical begomovirus symptoms such as interveinal chlorosis, mosaic, leaf roll, leaf distortion, and stunting, resulting in low yield. Due to the high impact of the disease, a series of studies were performed to assist the development of a disease management strategy. Fields across the country were visited and sampled. Identification of begomoviruses is based on PCR with specific primers and by genome analysis. At least 16 begomoviruses were found in tomatoes, all unique to Brazil or South America. From these, two species appear to be the predominant ones, *Tomato severe rugose virus* (ToSRV) in central to south of the country and Tomato mottle leaf curl virus (ToMoLCV) in the north-east part. In transmission tests, an isolate of ToSRV was demonstrated to be more efficiently transmitted by whitelies than an isolate of Tomato golden vein virus (TGVV), another virus found in the central part of Brazil. This higher transmission efficiency may explain the prevalence of ToSRV isolates. A tomato-free period is mandatory in the main processing tomato growing region. The incidence of begomoviruses is being monitored in this region to evaluate the efficiency of this method for the control of the disease incidence. In the field, tomato and *Nicandra physaloides* (apple-of-Peru) are the two major ToSRV sources for tomatoes, but other weeds and crops, such as bean, soybean and cotton, planted neighboring the tomato fields, may also play a role as the virus reservoir. High yield losses may occur when plants are early infected. Therefore, vector control measures at the initial weeks after transplanting are critical to reduce the losses. Tolerant commercial hybrids are available mostly of fresh market types, while for processing a few options are available. The hybrid BRS Sena, for processing and tolerant to a begomovirus infection, was recently released by our Institute and seeds will be commercially available from 2014 on. The use of tolerant varieties is imperative in some growing areas, such as those in Goiás. Recently, a crinivirus (*Tomato chlorosis virus* – ToCV), also transmitted by whiteflies, was reported and became widespread in Brazil. Occurrence of both virus groups

in the same plants is frequent in the field, hence the study their interactions with the plant and vector became extremely important. We are currently working on both virus groups.

# EPIDEMIOLOGY FOR BENEFIT-COST ANALYSIS OF DISEASE CONTROL PRACTICES

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Plant disease management involves many technical decisions based on the efficiency of each control practice to avoid crop damage, but also it involves economics decisions based on the benefit-cost analysis of the disease control practices. Everyday growers face the dilemma: Would be worth removing inoculum sources and/or spraying against pathogens and vectors? What will be the benefit of these practices in terms of yield and profitability? To help answering the first part of this question it is needed to use the knowledge on plant epidemiology such as the temporal progresses of disease incidence and severity and their relationships with crop yield or damage in different scenarios of disease management. To illustrate that, an approach that has been developed and used in Sao Paulo State for citrus huanglongbing (HLB) management decision taking is described below. HLB is a serious disease of citrus worldwide because there are no resistant commercial varieties, no cure and grove's yield and life span are greatly compromised where the disease becomes endemic and no control measures are accomplished. The recommended HLB management is based on planting healthy citrus trees, inoculum removal and reduction of insect vector population. To characterize the future impact of the absence or presence of any control practice on expected yield of affected groves, a model has been developed (Bassanezi & Bassanezi, 2008). This model takes into accounting the initial disease incidence ($y_0$) and severity ($s_0$) in the grove (depend on the age of trees at first symptom onset), annual disease incidence and severity progress rates (depend on the age of trees at first symptom onset and the effect of each control practices), and disease severity-fruit yield relationship. Based on the literature and field observations it was assumed that in the absence of disease management the disease incidence and severity progress rates in younger groves were higher than in older ones, as well as the initial disease severity. For a temporal horizon n (years), the grove disease severity in the affected citrus block ($S_n$) is calculated every year by a discrete equation $S_n = \sum^{j=n} (y_j - y_{j-1})\ s_{n-j}$ , where $y_j$ is the estimated incidence of symptomatic trees at year j, $y_{j-1}$ is the estimated disease incidence in the previous year, and $s_{n-j}$ is the estimated disease severity in the trees after n-j years after the symptoms onset. After that, the estimated $S_n$ is used to estimate the relative yield of the affected citrus grove compared to yield of healthy citrus grove ($RY$) by the equation $RY = \exp(-1.85\ S_n)$ (Bassanezi et al., 2011). Then, a matrix with the distribution of number of trees in each age category is created

to simulate the grove aging during the temporal horizon taking into account annual rate of tree loss by any cause (including or not HLB, respectivelly for scenarios with HLB affected-trees eradication and without eradication) in each age category and the annual rate of trees resets. With historic data of expected yield for healthy trees in each age category it is calculated the expected yield in the timeframe for scenario of healthy grove and scenario with HLB affected-trees eradication. For scenarios without diseased trees eradication (no control practices or only vector control) the matrix of expected yield for the scenario of healthy grove is multiplied by the matrix of expected *RY* to calculate expected yield of grove with diseased trees. It has been considered that eradication of HLB-affected trees does not reduce disease incidence and severity progress rates especially in small or medium size farms (Bassanezi et al., 2013), but it does not allow trees resets because the high risk of secondary infections. Vector control reduces the disease incidence progress rate according to the stringency it has been applied and has allowed trees resets. Therefore, four basic scenarios can be run: a) Scenario of healthy grove, b) Scenario without HLB management, c) Scenario with only vector control, and d) Scenario with both vector control and inoculum eradication. The avoided yield loss by the accomplishment of only one or both control practices, calculated by the difference between expected cumulative yields of two different scenarios, represents the benefit of such practice. Predicted fruit prices multiplied by expected yield and the predicted cost values for each control practice for the temporal horizon are used to calculate the benefit-cost ratio. This methodology has been used to estimate the potential impacts of the introduction of HLB disease in citrus areas and to analyze the benefit-cost of governmental phytosanitary programs in order to control HLB spreading in the territory (Miranda et al., 2012), and to simulate the impact of HLB according to the situation of initial disease incidence and progress at grove level for growers decision taking about what disease control practice to use in short, medium and long terms (Neves, 2013).

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# EFFECTS OF CLIMATE CHANGING ON THE DEVELOPMENT OF BEAN AND RICE DISEASES

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According to the 4th report of the Intergovernmental Panel on Climate Change (IPCC), released in 2007, the increase in temperature, the higher incidence of UV-B radiation due to the destruction of the ozone layer and the more frequent extreme weather events are unequivocal signs of global climate change. Such changes threaten agricultural production and form new challenges for common bean (*Phaseolus vulgaris* L.) and rice (*Oryza sativa* L.), two crops that are extensively grown in Brazil. To estimate the risks and possible impacts of climate change on diseases of these crops, current activities of an ongoing project investigate the influence of weather variables on the development of both common bean and rice disease epidemics, in different Brazilian states. The project includes the recovery of historical records of severity and yield from dozens of field trials carried out since the 80's, by Embrapa's common bean and rice breeding programs. These time series were organized in a database. The historical data has being compiled and gathered to climate data. Climate data, by its turn, consists of maximum and minimum temperatures, precipitation and number of rainy days provided by INMET and includes the identification of years with the ENSO (El Niño - Southern Oscillation) phenomenon. Local and broad-scale estimates of disease and climate impacts on crop yield were achieved with descriptive and multivariate statistics. Furthermore, maps of current and future climate scenarios for Brazil for the 2020s, 2050s, and 2080s were prepared for the diseases targeted in this study, based on models defined by the IPCC. These maps show different shifts on arrange of diseases through the country, with huge consequences on cropping systems, yield expectations, breeding programs and challenges on integrated disease management.

# MOLECULAR EPIDEMIOLOGY TO STUDY DISEASE

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Over the past two decades, molecular biology has revolutionized epidemiology research. During that time, methods for genetically characterizing populations have become routine, and have provided us novel data and new insights into the epidemiology of plant disease. Although the term molecular epidemiology was coined in the early 1970's, many different definitions of molecular epidemiology have been published (Foxman & Riley, 2001). What distinguishes molecular epidemiology is both the "molecular," the use of the techniques of molecular biology to characterize nucleic acid or amino acid-based content, and the "epidemiology," the study of the distribution in time and place and determinants of disease transmission, manifestation, and progression (Foxman & Riley, 2001; Milgroom, 2011). By definition, molecular epidemiology training requires practical application of both the laboratory and epidemiologic techniques to address a real-world infectious disease problem and to valuable advances in our understanding of plant diseases and their management. Exploiting genetic variation of populations in a context interdisciplinary allow integrates concepts of evolution, epidemiology (ecology), and genetics. The intersection of these three fields defines molecular epidemiology as population biology of plant pathogens (Milgroom, 2011).

The use of genetic markers in epidemiological analyses allows researchers to make inferences about the role of evolutionary mechanisms: natural selection, mutation, gene flow, recombination, and random genetic drift, in shaping the genetic variation of populations. Estimating parameters related to these mechanisms is the first step for studying the population biology and the information on population genetics of a causal agent of disease can also reveal the mode and history of spread of the pathogen, the diversity and spatial patterns of genotypes, host and tissue specialization and evolution of virulence (aggressiveness) in pathogen populations (McDonald, 1997; Milgroom & Peever, 2003). The genetic diversity information of the pathogen population can be useful for better understanding disease outbreaks, predicting future disease development, accessing key information on the ecology of the pathogen and its evolutionary potential, and for developing effective management strategies (Milgroom & Fry, 1997; Meeûs et al., 2007; McDonald & Linde, 2002).

Advances of molecular epidemiology of *Microcyclus ulei*, the causal agent of South American leaf blight (SALB), in commercial plantations of rubber trees in large scale (Central and South America) and the influence of the deployment of resistant and susceptible *Hevea*

clones in a local scale will be discussed in detail.

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# MULTIVARIATE METHODS WITH APLICATIONS TO PLANT EPIDEMIOLOGY

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In phytopathological research, a simultaneous handling of several variables is required, and entails the use of multivariate statistics such as principal component analysis, cluster analysis, factor analysis, canonical correlation analysis and models AMMI (Additive Main Effects and Multiplicative Interaction). Principal component analysis (PCA) is a mathematical procedure that uses orthogonal transformation to convert a set of observations of possibly correlated variables into a set of values of linearly uncorrelated variables called principal components. The number of principal components is less than or equal to the number of original variables. This transformation is defined in such a way that the first principal component has the largest possible variance (that is, accounts for as much of the variability in the data as possible), and each succeeding component in turn has the highest variance possible under the constraint that it be orthogonal to (i.e., uncorrelated with) the preceding components. The method is mostly used as a tool in exploratory data analysis and for making predictive models. PCA can be done by Eigen value decomposition of a data covariance (or correlation) matrix or singular value decomposition of a data matrix, usually after mean centering (and normalizing or using Z-scores) the data matrix for each attribute. The results of a PCA are usually discussed in terms of component scores, sometimes called factor scores (the transformed variable values corresponding to a particular data point), and loadings (the weight by which each standardized original variable should be multiplied to get the component score). A biplot is often used with a principal components analysis to assist in interpreting the analysis. A biplot plots on the same plane the principal component scores and vectors representing the contribution of each of the original variables to these components. In the context of principal component analysis, it plots the first two components and the original variables, the latter as direction vectors, with the direction indicating the relatioship between the principal components and the original variables. PCA is closely related to factor analysis. Factor analysis typically incorporates more domain specific assumptions about the underlying structure and solves eigenvectors of a slightly different matrix. PCA is also related to canonical correlation analysis (CCA). CCA defines coordinate systems that optimally describe the cross-covariance between two datasets while PCA defines a new orthogonal coordinate system that optimally describes variance in a single dataset. Cluster analysis or clustering is the task of grouping a set of objects in such a way that objects in the same group (called a cluster) are more similar (in some sense or another)

to each other than to those in other groups (clusters). It is a main task of exploratory data mining, and a common technique for statistical data analysis, used in many areas. Cluster analysis itself is not one specific algorithm, but the general task to be solved. It can be achieved by various algorithms that differ significantly in their notion of what constitutes a cluster and how to efficiently find them. Clustering can therefore be formulated as a multi-objective optimization problem. The appropriate clustering algorithm and parameter settings, including values such as the distance function to use, a density threshold or the number of expected clusters, depend on the individual data set and intended use of the results. The AMMI model is a hybrid analysis that incorporates both the additive and multiplicative components of the two-way data structure. The AMMI biplot analysis is considered to be an effective tool to diagnose the genotype-by-environment interaction (GEI) patterns graphically. In AMMI, the additive portion is separated from interaction by analysis of variance (ANOVA). Then the PCA, which provides a multiplicative model, is applied to analyze the interaction effect from the additive ANOVA model. The biplot display of PCA scores plotted against each other provides visual inspection and interpretation of the GEI components. Integrating biplot display and genotypic stability statistics enables genotypes to be grouped based on the similarity of performance across diverse environments. A succinct overview of some these tools is presented with examples applied to peach cultivars considering its to yield and phenological characteristics, and its behavior to brown rot and leaf rust for an adaptability study.

# BAYESIAN MODELING: FUSARIUM HEAD BLIGHT OF WHEAT

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Wheat is an important crop in Southern Brazil where a sub-tropical climate is conducive to fungal diseases. Among them, Fusarium head blight (FHB) is of increasing concern to both yield and grain quality. Modeling outbreaks risk can be useful in disease management and new data and statistical approaches can improve disease understanding and predictions. Ultimately, management decisions need to be based on the outbreak probability. Currently, most approaches for risk assessment rely upon deterministic weather-driven models based on regression techniques.

Although, Bayesian models are becoming increasingly prominent across a broad spectrum of sciences they are seldom used in plant pathology.  At its core, Bayes' theorem is a device for accounting uncertainty. Accounting for uncertainty is critical whenever data limitations exists that lead to imprecise inference about disease outbreaks, for example. While Bayesian analysis is itself conceptually simple, it can be difficult to implement. Widespread use of Bayesian methodology could be possible because development of computing power.

This work demonstrates a Bayesian approach to estimate the FHB risk in a given year. The system is based on a Hierarchical Bayesian Model (HBM) to estimate the probability of FHB outbreaks. A hierarchical model is one that is written modularly, or in terms of sub-models, when combined with Bayes' theorem could be used to integrate the pieces together and account for all the uncertainty that is present. Prior distributions of the estimates for each sub-model were derived from previous studies using MCMC (Markov Chain Monte Carlo) algorithms. A sample-based approach combines inference from different crop/disease cycle processes: spore dispersal, wheat heading and flowering, infection risk and host susceptibility (Figure 1).
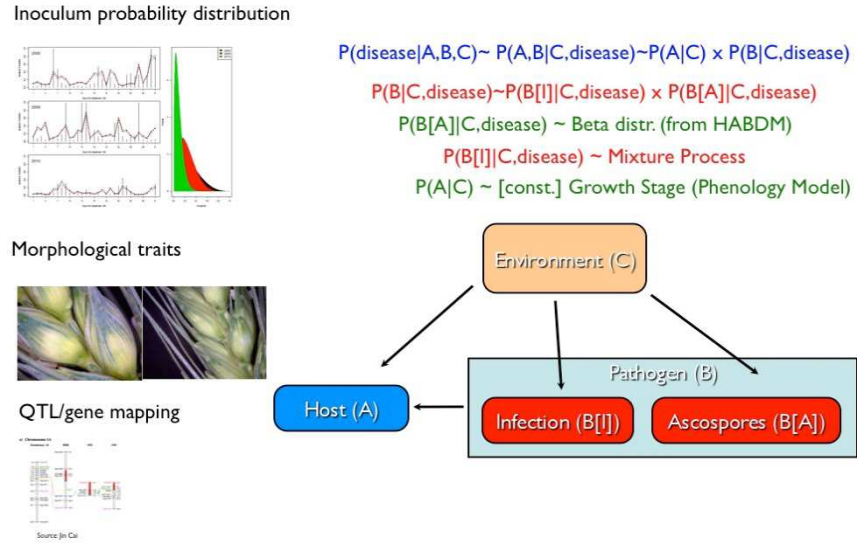
**Figure 1.** Schematic representation of the Hierarchical Bayesian Model.

A datasets comprising of FHB incidence, daily minimum and maximum air temperature, solar radiation and rainfall, in Passo Fundo (latitude 28°15'00"S and longitude 52°25'12"W) recorded during the wheat growing seasons of 2000 to 2006 was used for training the HBM. A similar dataset recorded in the period of 2009 to 2011 was used for validation.

The HBM successfully identified days with high risk of infection (Figure 2). This study contributes with novel statistical approaches to predict FHB risk than can be integrated to decision support systems to assist on FHB management. Hierarchical Bayesian modeling was demonstrated as both an useful analytical tool for FHB risk management and a natural investigative paradigm for developing and focusing on the release of new wheat cultivars.
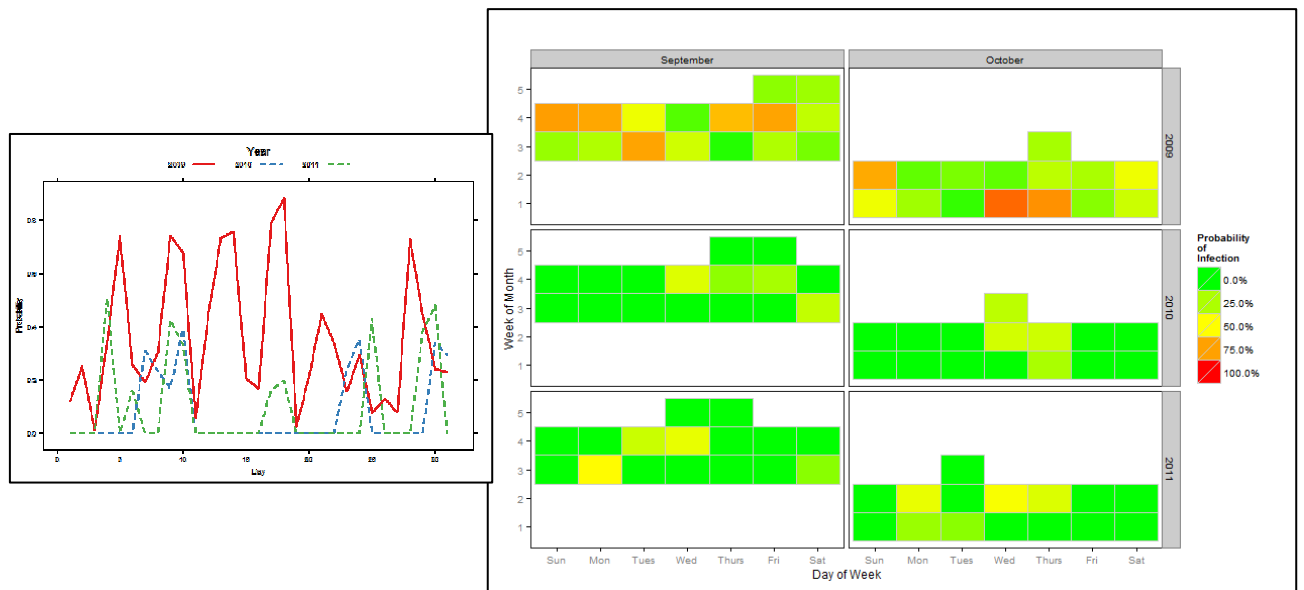
**Figure 2.** Fusarium Head Blight risk probability in Passo Fundo, RS, during 2009-2011 wheat susceptible window.

# R TUTORIAL ON STATISTICAL METHODS FOR PLANT DISEASE EPIDEMIOLOGY

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Increasing capabilities of data acquisition and computational resources for data analysis, as well as the tackling of more complex problems and data structures asks for revision and enhancements of statistical methods and practices applied to plant pathology studies. This presentation illustrates the usage of statistical methods which better explore information in the data using as references the analysis of three case studies. We aim to contrast the adopted methods with conventional methodologies highlighting the differences and impacts on the results. The case studies are also used to illustrate how the analysis can benefit from the usage of a flexible computational environment for the implementation of the statistical models and procedures.

The first case study explores different parameterizations of the logistic model when applied to the fitting of disease progress curves. A discussion explores computational advantages, statistical properties and precision, inferential methods, interpretation of the model parameters and, eventually if can be transformed for a convenient scale.

The second illustrates the modeling of the severity disease progress curve. The data comes from marked leaves monitored over time. We show that neglecting frailties related to the individual trees and ignoring the probable relation between severity and fall of the leaves can severely affect the inferences. The final analysis adopts a non-linear mixed effect model. The model fitting and the results are discussed.

The third case considers the influence of the temperature on the growth of some isolates of the fungi. The frequently adopted generalized Beta non-linear regression model is assessed with emphasis on estimative and assumptions. An alternative model based on response transformation and parameterization of polynomial functions is proposed.

The usage of the R environment for statistical analysis is illustrated throughout the discussion of the case studies. Emphasis is placed on data handling, graphical displays, modeling resources and flexibility given by the programming environment.

# Abstracts

■ ■ ■

## Oral Presentation

## (Listed in chronological order)

# INTEGRATING REMOTE SENSING, GPS, AND GIS TECHNOLOGIES FOR PLANT DISEASE FORENSICS: WHERE TO LOOK FOR EVIDENCE IN A 300-HECTARE CROP

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The detection of invasive biotic plant pathogens, deliberate or otherwise, remains a key challenge in forensics epidemiology. The integration of remote sensing, Global Positioning Systems (GPS), and Geographic Information Systems (GIS) technologies, coupled with geostatistical analyses, can provide valid, science-based evidence concerning the presence and geospatial distribution, of invasive plant pathogens (Nutter et al. 2010). Using the soybean rust pathosystem as a model, we have successfully extracted pathogen-specific spatial and temporal patterns from aerial, satellite, and ground-based sensors that can be used to detect, and accurately differentiate soybean rust, from other soybean diseases (with close to 100% accuracy). Using a GIS script that we developed called Gradient Finder, pathogen-specific spatial and temporal patterns can now be used to detect, identify, and map within-field anomalies caused by plant diseases. Using this approach, soybean rust disease foci can now be easily distinguished from disease patches caused by sudden death syndrome. Geospatial analyses can then be used to determine if spatial patterns are indicative of a natural or a deliberate introduction (i.e., a crime scene). With regards to the attribution of deliberately-introduced plant pathogens, remote sensing/GPS/GIS technologies can be used to locate the epicenters of primary disease foci (patches) within 1.5 m of the actual point(s) of introduction. We have developed three GPS/GIS tools for locating plant pathogen epicenters: contour, kriging, and transect-based detection methods. Thus, the integration of remote sensing, GPS, and GIS technologies can be used to deliver precise GPS coordinates as to where investigators on the ground should obtain pathogen isolates (and other evidence) for genetic analyses concerning the population structure of pathogen isolates.

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# OPTIMIZING INTRA-ORCHARD SAMPLING FOR EARLY DETECTION OF CITRUS HUANGLONGBING

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Huanglongbing (HLB) is the most serious citrus disease. It is caused by phloem-limited bacteria (*Candidatus* Liberibacter spp.) which are transmitted by insect psyllids. In Brazil it was reported in São Paulo, Paraná and Minas Gerais, but the vector is widespread in the country. It is of the utmost importance to develop and improve sampling procedures in order to maximize the probability of early detection in regions where the disease is still unreported. Our objectives were (i) to find an efficient spatial sampling scheme to detect the disease as soon as possible, and (ii) to know how many times one should scout a given orchard in order to find 100% of symptomatic plants. For both objectives an excess of 650 observed field maps were used as baseline. Objective (i) was accomplished by simulating 21 intra-orchard sampling schemes, including patterns in X, W, Double W, Diagonals, in clusters, per planting rows and edge focused patterns. In each simulation the plants were selected according to the sampling pattern and 'evaluated' considering the reported efficiency in Brazil (47.6%). In all cases a monomolecular function was fitted describing the relationship between HLB incidence and probability of detection. The best sampling procedures were always those which included the evaluation of plants on the orchard's edges ("Edge", "Double Edge", "Florida" or "Mexico") or systematically assessing a fraction of the planting rows. As the results consider only the detection probability, issues such as efficiency per assessed plant, sampling costs per plant and total sampling time should be further evaluated. Objective (ii) was accomplished by simulated scouting of 100% of the plants in an orchard, assuming a given detection efficiency. As the detection efficiency increases, a steep decrease in required re-inspections occurs, stabilizing after 50% of detection efficiency. Considering the reported detection efficiency in Brazil, an average of three re-inspections is needed to find 100% of the symptomatic plants.

# INCIDENCE-SEVERITY RELATIONSHIP AND SPATIAL ANALYSIS OF FUSARIUM HEAD BLIGHT EPIDEMICS IN SOUTHERN BRAZIL

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Studies on the relationship between Fusarium head blight (FHB) incidence and severity and on the spatial patterns of the disease have been conducted only in temperate winter wheat production regions. This study analyzed survey data on FHB incidence ($I$) and severity ($S$) in no-till commercial spring wheat fields in southern Brazil. A total of 160 fields in northern Rio Grande do Sul State, Brazil, were assessed in 2009 ($n = 51$), 2010 ($n = 49$), and 2011 ($n = 60$). In each field, 20 1m-row quadrats were randomly selected, from each of which 10 spikes from contiguous main tillers were sampled and rated visually in the laboratory for FHB incidence (spike is symptomatic or not) and severity (proportion of diseased spikelets per spike). Other field data recorded were the latitude and longitude coordinates, the grain developmental stage at sampling and the previous summer crop (corn or soybean) based on visible crop residue. Disease incidence data from each field were summarized using the dispersion index (D) and the C ($\alpha$) test. Data from all fields were summarized using the binary form of Taylor's power law (BPL) assuming a binomial distribution variance for a completely random pattern. A model based on complementary log-log transformation of incidence and severity described well this relationship, which was consistent among years, grain stage and previous crop, with similar slopes. Hence, a single model was fit giving an intercept of -2.53 and slope of 1.17. Heterogeneity analysis, based on D and C($\alpha$) test, showed that over dispersion (indicative of aggregation) was present in only 29 fields (18%), and these fields succeeded either corn or soybean. The fitted BPL equation suggested that, on average, the observed variance equaled the binomial variance, indicative of random patterns of diseased spikes in FHB-affected fields. Our results adds to the evidence that $S$ may be estimated from $I$ and confirms that FHB incidence is distributed predominantly at random in no-tilled fields of subtropical regions, which is in agreement with reports from temperate winter wheat regions.

# SEXUAL REPRODUCTION PLAYS A SIGNIFICANT CONTRIBUTION TO *Moniliophthora roreri* POPULATIONS FROM ECUADOR

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*Moniliophthora roreri,* a basidiomycete fungus causing frosty pod disease of cacao*,* infects only pods of *Theobroma* and related genera as *Herrania,* causing internal and external pod damage that result in devastating effects on cacao yield causing severe economic losses. Initially classified as an anamorphic fungus, recent publications showed that conidia were in fact basidiospores, but its mode of reproduction remains obscure. The objective of this study was to determine the importance of recombination in four *M. roreri* populations (south, north, central and east) from Ecuador using six microsatellite loci on 145 isolates. In all analysis it was assume that *M. roreri* is a functional diploid, i.e. it is a dikaryon. All data were consistent with this assumption. The associations among loci were evaluated applying tests for gametic disequilibrium and multilocus association. Linkage disequilibrium among loci was tested using Fisher's exact test based on an MCMC approach, implemented in ARLEQUIN. The index of association ($I_A$) and $r_D$, index less sensitive to the number loci were measured with 1000 randomizations using MULTILOCUS v 1.3 The exact test for linkage disequilibrium was not significant for most pairs of loci in all population, except for population from east, in which 40% of loci were in significant disequilibrium. The null hypothesis of random mating was accepted for all populations by Index of Association ($I_A$ and $r_D$), except to the population from east that exhibited significant gametic disequilibrium ($P < 0,001$). Our overall interpretation is that populations of *M. roreri* from cacao in Ecuador have a mixed reproductive mode, which includes a considerable sexual reproductive component and a significant degree of inbreeding.

# COMPARATIVE EPIDEMIOLOGY OF *Lasiodiplodia* SPECIES PREVALENT IN PAPAYA ORCHARDS IN THE NORTHEASTEARN BRAZILIAN

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Stem-end rot is an important postharvest disease in papaya in Brazil and worldwide. Historically, this disease was attributed exclusively to *L. theobromae*, but recent study using molecular methods revealed the presence of more species of *Lasiodiplodia* causing stem-end rot in papaya. The objectives of this study were to determine the influence of temperature and humidity on the disease severity in papaya fruits induced by five prevalent species of *Lasiodiplodia* in papaya orchards in northeastern Brazil (*L. brasiliense*- LB*, L. hormozganensis*- LH*, L. marypalme*- LM*, L. pseudotheobromae* – LP and *L. theobromae*- LT), evaluate the virulence of this species for different hosts, determine the effects of hot-water treatments *in vitro* (thermotherapy) and fungicides on the mycelial growth of the species. Five single-spore *Lasiodiplodia* isolates of each species were used for all experiments. *Lasiodiplodia* species not induced symptoms in papaya at $10^{o}$C, and only LPno induced symptoms at $15^{o}$C. All *Lasiodiplodia* species induced symptoms at temperatures of 20 to $35^{o}$C with increased disease severity with increasing temperature. Comparing the disease progress curves as a function of temperature, LT had the lowest final severity, lower disease progress rate and lower area under the disease progress curve, differing ($P \leq 0.05$) from the other species. *Lasiodiplodia* species did not induce symptoms without the use of moist chamber, and with 3 hours of use. With 6, 12 and 24 of moist chamber all species induced symptoms, especially LB and LT as the most virulent. *Lasiodiplodia* species were pathogenic in a range of inoculated hosts (avocado, banana, guava, mango, melon and papaya), but levels of virulence varied according to the host. *Lasiodiplodia* species demonstrated differential sensitivity to thermotherapy ($48^{o}$C for 5 min) and fungicides. A low sensitivity to thermotherapy was evidenced by LB and LH, while LP presented greater sensitivity. Regarding to fungicides, LT showed less sensitivity to thiophanate-methyl, while LB, LM and LP showed higher sensitivity. In relation to difenoconazole and azoxystrobin, LM and LM showed high sensitivity, differing LP that showed less sensitivity to both fungicides. The *Lasiodiplodia* species showed significant differences in epidemiology and sensitivity to control measures, indicating the need to consider the most prevalent species in the producing regions to the adequacy of the disease management strategies.

# EVALUATION OF AN INFECTION RISK ALERTAS A COMPONENT OF A LATE BLIGHT DECISION SUPPORT SYSTEM

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A dispersal-risk algorithm for potato or tomato late-blight has been developed using data obtained experimentally and from the published literature. The algorithm includes favorability of weather conditions for sporulation and release at the source, survival of sporangia in transit, and subsequent infection of host tissue at the target site. The weather data used include: temperature, relative humidity, wind speed and direction, and solar radiation. Historic (observed) data as well as forecast data are used. For each potential risk period independent indices are calculated for sporulation, dispersal and survival of sporangia, and for subsequent infection of target host tissue. Additionally, proximity of the target to an inoculum source is used. These indices are then integrated to provide an overall risk index. Because the algorithm uses future weather as well as historical weather, it enables users to take precautionary measures. Preliminary experiments suggest that forecast "high risk" periods could be used to enhance the efficiency of disease management practices.

# MOLECULAR EPIDEMIOLOGY OF THE *Tomato chlorosis virus* IN BRAZIL

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*Tomato chlorosis virus* (ToCV) is a crinivirus, family *Closteroviridae,* with long flexuous particles, and transmitted by whiteflies. The first report of ToCV in Brazil was verified in crops in the region of Sumaré, State of São Paulo in 2006. Since the first report of ToCV in Brazil studies have been realized. So, some advances in regard to occurrence, genetic diversity of ToCV, as well epidemiological aspects of ToCV in Brazil will be discussed as follows. During the period from 2006 to 2013 ToCV was detected in tomato plants from the following states in the country: Bahia, Espírito Santo, Goiás, Minas Gerais Rio de Janeiro, São Paulo e Parana.  The possible unnoticed occurrence of this virus in those areas may be associated with the fact that symptomatic plants are similar to those related to nutritional disorder caused by magnesium deficiency and injuries caused by mites. By comparing the partial nucleotide sequence soft heat shock protein HSP70 homologue gene, we assessed the genetic diversity of Brazilian tomato isolates of ToCV, as well as their relationship with other ToCV isolates found worldwide. The Brazilian ToCV isolates hared 99.9-100% nucleotide identity, which indicates low genetic diversity and a close revolutionary relationship to those from Mediterranean countries. Considering the recent detection of ToCV in Brazil, it is possible that a rapid spread of the virus throughout tomato-producing states of the country might account for this low genetic diversity. Such low genetic diversity also suggests that all Brazilian ToCV isolates may have originated from a single source, via a single introduction into the country from Mediterranean countries, in a yet unidentified manner. During the period from 2009 to 2011 surveys on the incidence of disease in seven crops in the municipality of Sumaré, SP allowed characterizing the temporal and spatial distribution of the disease. Progress curves of the disease showed a linear growth pattern, which implies a continuous ingress of whiteflies from outside the plantations. Spatial disease progress showed an aggregate pattern, varying with the disease incidence, implying the presence of a source of inoculum nearby.

# MONOCYCLE AND FUNGICIDE SENSITIVITY OF *Phakopsora pachyrhizi* POPULATIONS FROM ORGANIC AND CONVENTIONAL PRODUCTION SYSTEMS

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Currently, the soybean Asian rust (*Phakopsora pachyrhizi*) is one of the most important economical threats for soybean growers in South America. The disease is controlled mainly by the use of triazole and strobirulin fungicides. Because of the risk of economic damage, growers often apply more than two fungicides sprays to control the disease. A failure of control was already observed in some years in different regions of Brazil. The intensive spray program could be contributing to the selection of isolates with low sensitivity to fungicides and, consequently, low efficiency in control of Asian rust. The objectives of this study were to compare the monocycle and tebuconazole sensitivity of *P. pachyrhizi* populations from organic and conventional production systems. The inoculum was removed from soybean leaves infected by *P. pachyrhizi* from commercial fields located at Planalto-PR (organic production system - OPS) and Ponta Grossa-PR (conventional production system - CPS). To assess the monocycle, detached leaves were inoculated by urediniospore suspensions (3 x $10^4$ urediniósporos.mL$^{-1}$) from each system. After inoculation, all leaves were assessed daily by counting the total number of pustules per leave until the end of the disease monocycle. To assess the tebuconazole sensitivity, the FRAC methodology of detached leaves was followed. The fungicide concentrations were: 0; 0.05; 0.25; 0.50; 1.0; 2.0; 4.0 and 8.0 µg.mL$^{-1}$. After 15 days, the disease severity was assessed using a diagrammatic scale and the fungicide concentration at which 50% reduction of population activity ($EC_{50}$) was calculated to compare the populations from OPS and CPS. These experiments were repeated twice. The values of $EC_{50}$ were lower in population from OPS (0.41) than in population from CPS (1.60) in both assays. There was high variability in the monocycle results of two populations. The parameters related to initial inoculum and rate were higher in population from OPS than population from CPS. Due to the high variability, more studies should be done, mainly with monouredinial isolates from each population.

# DISPERSAL of *Colletotrichum acutatum* AMONG CITRUS PLANTS BY HONEYBEES IN SCREENHOUSE

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Post bloom fruit drop (PFD) caused by *Colletotrichum acutatum* is an important disease in citrus orchards when the flowering period coincides with frequent rain and mild temperatures. This situation results in a high disease progress rate and in a random spatial pattern of diseased trees (Silva Jr. et al., 2013). This unexpected behavior of the disease suggests that the pathogen has additional mechanisms of dispersal, such as pollinator insects, since only rain splash could not explain so fast and severe epidemics. The present work was undertaken to characterize the role of honeybees in the dispersal of *C. acutatum* among citrus plants in an insect-proof screenhouse. Fourteen healthy Valencia sweet orange potted plants were distributed in three lines: four of them were protected from flower-visiting insects within individual insect-proof cages and the other ten plants remained without cages during the experimental period. The temperature and humidity in one plant inside the cage and in other outside were recorded every hour. A bee colony kept inside a wooden box was located at one end of the screen house. The honeybees were set free, and allowed to the free movement inside the screen house. At the other end, one Valencia sweet orange potted plant, inoculated with PDA disks containing spores of the fungus, served as source of inoculum. Every day, all the plants were gently sprayed with water, simulating evening dew. Daily, all the plants were visually evaluated and if symptomatic flowers appeared, they were collected, isolated in PDA medium and submitted to PCR assay to determine the fungal species responsible for the flower infection. The number of symptomatic flowers per plant was counted. Samples of honeybees were collected and examined by scanning electron microscope. The experiment was performed twice. Honeybees can disperse *C. acutatum* from inoculated citrus plant to healthy citrus plants. On average, 2.8 flowers per plantshoweddiseasesymptomsat9.9 days after the development of susceptible tissue. No plants inside the cages showed disease symptoms.

Silva Junior, G. J.; Spósito, M.B.; Marin, D.R.; Ribeiro Junior, P.J.; Amorim, L. Spatio-temporal characterization of citrus postbloom fruit drop in Brazil and its relationship to pathogen dispersal. *Plant Pathology.*Accepted for publication. 2013.

# Abstracts

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## Short Oral
## and
## Poster Presentations

(Listed in numerical order)

# EFFECT OF TEMPERATURE AND WETNESS PERIOD ON GUAVA STYLAR END-ROT CAUSED BY *Neofusicoccum parvum*

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Brazilian guava (*Psidium guajava* L.) yield was around 342.000 t in 2011. Due to the highly perishability of guava, most of the fruit goes to the domestic market and only a small portion is exported. One of the problems that devaluate the fruit are the diseases and among them stylar end rot. *Neofusicoccum parvum* is one of the causal agents of guava stylar end rot, which can reach 40% of incidence in post-harvest in Brazil. The objective of this study was to determine the favorable environmental conditions for the stylar end-rot monocycle development. Isolates of *N. parvum* were collected on symptomatic fruits and the species was confirmed by morphological characterization and phylogenetic analysis of the ITS and *β*-tubulin regions. Germination of conidia was evaluated at temperatures of 10, 15, 20, 25, 30, 35 and 40°C in combination with 4, 6, 12, 24 and 48 h of wetness period, and the beta generalized-monomolecular model was fitted to data. Guava cv. Kumagai were inoculated with conidial suspensions of *N. parvum* and maintained at temperatures of 15, 20, 25, 30 and 35°C and wetness periods of 6, 12, 24 and 48 h. The diameter of the lesion seven days after inoculation was evaluated, and the beta generalized model was fitted to data. The optimum temperature for the conidia germination was 30ºC and the maximum germination occurred after 12 h wetness period. The optimum temperature for lesion growth was around 30ºC independently of wetness period. The lesion diameter was 5.76, 5.90, 6.96 and 7.90 cm at wetness period of 6, 12, 24 and 48 hours, respectively. The favorable environmental conditions for disease development are temperatures around of 30ºC combined with wetness periods higher than 6 hours.

# LIGHT INTENSITY AFFECTS MONOCYCLIC PARAMETERS OF *Mycosphaerella musicola – Musa* spp. PATHOSYSTEM

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Sigatoka is one of the most important diseases occurring in banana plantations. Although being less damaging than black sigatoka, yellow sigatoka (*Mycosphaerella musicola*) still prevails in some regions. The objective of this study was testing the hypothesis of light interference in monocyclic parameters of yellow sigatoka epidemics. Treatments were Grande Naine seedlings kept under no shading conditions (post-inoculation) but originally grown under shade or not (acclimatizing conditions), and seedlings kept under shade but grown in one of those conditions. Only the leaves 1 and 2 were inoculated. Daily evaluations were performed for 60 days, when the following variables were registered for each inoculated leaf: time until symptoms onset, presence of infectious lesions, disease severity according to Stover's scale. Raw data were used to determine incubation, latency and infectious periods as well as time to reach the "disease development time" (DDT) and severities Stover 4 and Stover '>6'. Logistic regression was used to assess the relative risk of independent variables to infection success, formation of reproductive structures and development of high severities (Stover 4) or widespread leaf necrosis (Stover '>6'). The effects on the epidemiological periods and time until a given severity were assessed by survival analysis. None of the explanatory variables influenced the probability of a given leaf being infected. The risks of sporulation, of reaching high severities or necrosis were lower for plants under post-inoculation shading regardless acclimatizing conditions. Post-inoculation light intensity affected the incubation, latency and infectious periods, DDT and severity. On average, the disease development was five days faster in plants grown with no shading.

# EFFECT OF THE LEAF WETNESS ON *Diplodia macrospora* INFECTION IN CORN

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The fungus *Diplodia macrospora* (Earle) can cause ear and stalk rot in corn (*Zea mays* L.). In this study it was hypothesized that the severity of this disease can be influenced by leaf wetness. The objective of this study was quantifying the period effects of leaf wetness on disease severity in corn seedlings. The seedling inoculation of two simple hybrids (P32R48 and DKB 240) and one open-pollinated variety (SCS 155) were conduced in growth rooms (Eletrolab) planting four seeds in plastic vase containing about 500 g substratum. The monosporic isolate Dm 54 was used in inoculations. The seedlings were inoculated at stage V1 (10 to 12 days of age) by spraying a conidial suspension ($5 \times 10^4$/mL, 2.3 mL/seedling) with a De Vilbiss atomizer at the air pressure of 55 kPa and then transferred to a moist chamber for 0; 6; 12; 18; 24; 30; 36; 42; 48 and 54 h leaf wetness at $24 \pm 0.5$ºC and 95% air relative humidity and 12 hours photoperiod. The experiment was repeated twice with seven replications. The seedling leaf surface was allowed to dry before returning them to the growth room at constant temperature of $24 \pm 0.5$ºC until evaluation. The disease evaluations were done seven days after inoculation estimating the percentage of leaf tissue damaged by *D. macrospora* (severity). Values were assigned individually to each leave of the plant and then averaged. The Gompertz model reparametrized was ajusted (Zeviani, 2013) to estimate the period of leaf wetness to observe 99% of maximum severity $Y = b_1 * \exp(\log(0.99) * \exp(b_2 * (1 - T/b_3)))$ where Y = severity observed and T = time with leaf wetness and $b_1$ = upper asymptote (maximum severity); $b_2$ = parameter without direct interpretation and $b_3$ = period with leaf wetness to reach 99% of maximum severity. Data analyses were performed using the R statistical system. The corn cultivars differed as maximum severities in different periods of leaf wetness ($b_3$). The hybrid P32R48 and DKB240 showed confidence interval 24.8 to 35.8 and 23.2 to 27.2 hours of leaf wetness, respectively. The variety SCS 155 showed confidence interval between 39.6 to 46.7 hours of leaf wetness and this variation may be associated with high genetic variability of an open-pollinated variety. The determination of the period of leaf wetness is important for maize breeding to select corn resistant for *D. macrospora*.

Zeviani, W.M. Parametrizações interpretáveis em modelos não lineares. Tese (Doutorado em Estatística e Experimentação Agropecuária). 146 p. Lavras: UFLA, 2013.

# *Bacillus* spp. AND *Pseudomonas* sp. AS INHIBITORS OF *Colletotrichum* GROUP *acutatum IN VITRO* AND INTERFERENCE IN MONOCYCLE COMPONENTS OF GLOMERELLA LEAF SPOT

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The control of Glomerella leaf spot (GLS) in Brazil is based on fungicide sprays and new alternatives must be tested. In apple, few biological control studies are found, and most of them are focused on post-harvest pathogens. Therefore, the objectives of this work were: (i) verify mode of action of bacterial isolates and the commercial product Serenade® - based on *Bacillus subtilis* - against the *Colletotrichum acutatum* group, and (ii) evaluate the influence of bacterial isolates and the commercial product Serenade® in monocycle components of GLS. To understand the mode of action of bacterial isolates in *Colletotrichum acutatum* group, 'in vitro' tests were performed evaluating conidial germination, pairing colonies, action of thermostable metabolites and antifungal volatile compounds. The influence of bacterial isolates in the GLS monocycle was verified by treating seedlings in a greenhouse and after inoculating with *Colletotrichum acutatum* group ($1 \times 10^4$ conidia ml$^{-1}$). Seedlings were transferred to chambers maintained at 25 ºC and 12 hours light regime. Disease severity (GLS) was evaluated at daily basis by counting typical lesions caused by *Colletotrichum* spp. on all leaves during 12 consecutive days. Nonlinear models for incidence and severity were also adjusted, and made a comparison of epidemiological parameters between treatments. *Bacillus* sp. isolated from apple phylloplane inhibited more than 60% of *Colletotrichum acutatum* group conidial germination. Mode of action of *Bacillus* sp. and *Bacillus alcalophilus* on *Colletotrichum acutatum* group is through the production of fixed and volatile compounds which inhibit mycelial growth. The primary mode of action of the commercial product Serenade® on *Colletotrichum acutatum* group is the production of thermostable metabolites capable of inhibiting mycelial growth by 100%. In the GLS monocycle, it was possible to adjust the monomolecular model for incidence and logistic model for number of lesions. There were significant differences between epidemiological parameters of GLS in seedlings treated with bacteria isolated from the phylloplane of apple and Serenade® compared to control indicating potential of using biological control to management of GLS in the field.

# EFFECT OF TREE EXPOSITION ON COFFEE RUST PROGRESS CURVE

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Leaf wetness duration (LWD), temperature and sun radiation influence different components of the coffee rust (*Hemileia vastatrix*). The exposure of the plant to the sun radiation can modify the microclimate of the coffee tree canopy and consequently the temporal progress of coffee rust disease. The objective of this work was to compare the progress of coffee rust in the north and south exposition side of the coffee tree. In this experiment, we used four years old coffee tree of *C. arabica* cv. Catuaí Vermelho (IAC 144), planted in a spacing of 3.6x0.70 m (3,968 plants ha$^{-1}$) and located at 21º21'16''S and 45º06'48''W and 900 m of elevation. Disease incidence was assessed eighteen times at 30-days intervals, for two seasons, 2009/2010 and 2010/2011. In a nondestructive assessment method was used, eight leaves per tree were sampled in each assessment, four from each exposition side of the tree or from the side facing the sun (north and south). The leaves were from the third or fourth node of plagiotropic branches, randomly selected from middle third of the canopy. Disease was assessed in eight coffee trees in each experimental plot (n = 64). Disease incidence was determined by the percentage of leaves with rust. The experimental design was a complete randomized block, with eight replications and two treatments: north and south exposition side. The area under the disease progress curve (AUDPC) was calculated. Statistical analysis (*p<0.05*) of the AUDPC was performed by using the Student`s least significant difference (LSD) test. Two peaks of the disease were observed, one in May 2010 (72% south side and 33% north side) and the other in June 2011(25% south side and 9% north side). In the first season, the incidence of rust was higher than in the second season due to the biennial production of coffee. In both seasons, the highest AUDPC occurred in the south exposition side and the lowest AUDPC was in the north exposed side of the trees. The less exposed coffee foliage to direct sun radiation in the south side may have increased the LWD (Santos et al., 2008) and reduced the leaf temperature, consequently favoring the coffee rust progress.

Santos, E.A.; Sentelhas, P.C., Pezzopane, J.E.M.; Angelocci, L.R.; Monteiro, J.E.B.A. Spatial variability of leaf wetness duration in cotton, coffee and banana crop canopies. *Scientia Agricola*, Piracicaba, BR, 65, 2008.

# DEVELOPMENT OF RISK ANALYSIS SYSTEM FOR DETECTION OF PATHOGENIC ACTIVITY OF *Rhizoctonia solani* IN SOIL

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Rhizoctonia canker caused by *Rhizoctonia solani* is an important disease of cowpea and common bean in the Northeast of Brazil. The objective of this study was to develop a risk analysis system for detection of pathogenic activity of *R. solani* in soils for planting with cowpea and beans. The study was conducted in three stages: a) evaluation of the efficacy of baits to quantify the saprophytic activity of *R. solani* in soil; b) determination of the relationship between saprophytic and pathogenic activities of *R. solani* in soils; c) determination of the sample sizes to quantify the saprophytic activity of *R. solani* in soil. For evaluation of the baits, soils from three locations were placed in trays and infested with an isolate of *R. solani* (50 mg of colonized substrate kg$^{-1}$ of soil). After seven days, the soil samples were transferred to plastic pots (5.0-5.0-6.5 cm), and six different baits were sown: beet, cowpea, corn and sorghum seeds, cowpea stems and wooden toothpick segments. After 48 h at 25 °C, the baits were transferred to Ko & Hora modified medium. The toothpick bait allowed the detection of higher levels of saprophytic activity in all soils and was evaluated with regard to eight isolates and six inoculum densities of *R. solani*, demonstrating a high efficacy in all situations. For analysis of the relationship between the saprophytic and pathogenic activities, 12 soils were collected from areas destined for cowpea and common bean cultivation. The saprophytic activity was evaluated using toothpick baits, and pathogenic activity was evaluated by the distribution of soils in trays, followed by sowing cowpea and the assessment of the Rhizoctonia canker severity. A significant positive correlation (r = 0.7698; P≤0.01) was found between the saprophytic activity (SAP) and pathogenic activity (PAT). The regression equation PAT = 1/(0.5822-0.0056 SAP) was estimated with high precision (R$^2$ = 0.9930; P≤0.01). To determine the ideal sample sizes for quantification of saprophytic activity, soil samples were taken in 16 areas destined for cowpea and common bean cultivation. Forty-eight soil samples of 300 g were collected in each area (1 ha), and samples were analyzed for saprophytic activity of *R. solani* by the toothpick method. The spatial pattern of the soil samples was estimated using Lloyd's Index of Patchiness and ideal sample sizes were calculated based on degrees of acceptable error. The spatial pattern of the samples was predominantly aggregate and considering a 10 and 20% rate of acceptable error, the mean ideal sample sizes were 56 and 14 soil samples of 300 g, respectively, for each 1.0 ha of field.

# CLIMATIC CONDITIONS FOR DEVELOPMENT OF EUROPEAN CANKER OF APPLE IN SOUTHERN BRAZIL

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The presence of European canker of apple, a disease caused by the fungus *Neonectria ditissima,* has recently been detected in orchards from apple growing region in Brazil. Researches from various regions in the world have pointed out air temperature and rainfall regime thresholds to the establishment of the risk levels. The fungus develops in temperatures ranging from 5 to 25°C, with optimal of 11 to 16°C. Additional risk for the disease is the occurrence of precipitation in more than nine days per month. Besides to the environmental conditions, it is necessary to have wounds in the plant for the disease to develop. The aim of this study was to verify conditions for disease occurrence by analysis of recent climatic series data in two Brazilian apple production areas – Vacaria (28°30'49''S; 50°52'58''O) and São Joaquim (28°16'33''S; 49°56'05''O), according to Latorre et al. (2002) and Beresford & Kim (2011) approaches. The frequency of daily hours were analyzed in temperature ranges relative to different risk levels and the occurrence of rainfall – the amount and frequency of days with rain – in the period from 2008 to 2012. In Vacaria, the months of May and June showed, respectively, an average of 57% and 63% of days with precipitation and 10.2 and 8.4 hours per day with optimal temperature. In São Joaquim, where leaf drop comes earlier, the same indicators obtained in the period resulted in more than 50% of days with rain and an average of 10.1 hours per day with optimal temperature. The climatic conditions of late autumn and early winter, which corresponds  to the period of leaf fall in apple trees, was characterized as favorable to the development of European canker of apple. Once the pathogen inoculum gets sufficient concentrations, the risk of epidemics in the orchards of Vacaria and São Joaquim is high. There is a small proportion of periods unfavorable for disease development. In these regions, wounds and inoculum availability play a more important role and need to be better known. Disease control may be achieved through strategies like the reduction of ascospores and conidia sources before autumn.

Beresford, R. M.; Kim, K. S. Identification of regional climatic conditions favorable for development of European canker of apple.Phytopathology, v. 101, n. 1, p. 135–146, 2011.

Latorre, B.; Rioja, M. E.; Lillo, C.; Muñoz, M.The effect of temperature and wetness duration on infection and a warning system for European canker (*Nectriagalligena*) of apple in Chile.Crop Protection, v. 21, n. 4, p. 285–291, 2002.

# THE ROLE OF SEED-BORNE INOCULUM IN BROWN SPOT EPIDEMICS AND EFFECTS ON CROP STAND AND YIELD OF IRRIGATED RICE

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The objective of this study was to evaluate the effect of *Bipolaris oryzae* seed-borne inoculum levels on the development of brown spot (BS) epidemics and rice yield. Eleven field trials were conducted at two locations (Bagé and Cachoeirinha) in RS state during three growing seasons (2008/09, 2009/10 and 2010/11). Healthy seeds of IRGA 424 cultivar were artificially infected with a highly pathogenic isolate using a water-restriction technique. Incremental levels of seed-borne inoculum (0, 3, 6, 12, 24 or 48%) were obtained through mixture of varying proportions of infected and healthy seeds. The trials were conducted in a completely randomized block design with 4 replicates, each consisting of 10 planting rows (spaced 0.17 m) of 5 m. Plots were spaced 0.5 m. The disease-variables evaluated were: BS incidence (INC) assessed at a 6 to 8-day interval in 50 plants/plot collected at random; BS severity (SEV) assessed at a 6 to 8-day interval during the reproductive stages on 12 marked flag-leaves; and incidence of *B. oryzae* infection (INF) in harvested kernels. Additionally, crop stand (CS, seedlings/m$^2$) was assessed at the stage of four expanded leaves and crop yield (YLD, kg/ha) was assessed at harvest. A generalized linear mixed model was fitted to count, proportion and continuous data using the appropriate link functions. BS epidemics initiated at the vegetative stages in only three trials during the 2009/10 season, which received above-normal rainfall early in the growing season. For those, disease progressed faster in treatments with higher levels of seed-born inoculum and INC reached 22 to 44% at the end of the season across the plots. Treatments significantly affected CS in 8 of 9 trials. Maximum CS reduction was 50% and was related with seed-born inoculum levels. Seed-borne inoculum levels did not affect final SEV (mean = 1.7% [0.1-5.6%]) and YLD (mean = 8.7 tons.ha$^{-1}$ [6.4-11.1]). Also, INF was not affected (mean = 13.8% [5.2-29.5%]) by the treatments. The lack of correlation between initial development of BS epidemics, negatively affecting crop stand, and the later disease progress on flag-leaves and yield suggests that further crop development compensates for the lower crop stand, and that inoculum from sources other than the seeds influenced the BS epidemics.

# MONITORING OF INOCULUM QUANTITY AND DISPERSAL OF *Colletotrichum* spp. ASSOCIATED WITH GLOMERELLA LEAF SPOT ON APPLE

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Glomerella Leaf Spot (GLS) caused by *Colletotrichum* spp. is one of the most serious apple diseases in Brazil. The aim of this study was to describe the conidia density of *Colletotrichum* spp dispersed by wind and by runoff within the plant. Traps to collect conidia were used. Also conidia dispersion by insects was assessed. The experiment was carried out over three consecutive years in areas with (A1 and A2) and without chemical treatment (A3). Spore traps were placed at different heights from the ground (0.3 and 1.0 m) and replaced weekly for the first and second seasons and, every 15 days for the third season. The presence of conidia was observed in all traps and also on the wind trap located above the plant canopy(2.5 m of the ground). The number of conidia trapped was quantified and the area under the inoculum density curve (AUIDC) was calculated based on the variation of inoculum amount over time. With the use of pheromone, individuals of *Grapholita molesta* and *Bonagota cranaodes* were collected every 15 days and incubated on semi-selective media to observe the grown of *Colletotrichum* spp. during 7 days. Leaf wetness and favorable days for the occurrence of the disease were calculated using the data of temperature, relative humidity and rainfall. The release of conidia was dependent on environmental conditions and occurred between November (2009/10 and 2011/12 seasons) and January (2010/11 season) at A1 and A2, and between October (2009/10 season) and November (2010/11 season) at A3, extending to the first half of April in all areas. The highest capture of conidia occurred in January and February, in all seasons. The application of dithiocarbamate and strobilurins reduced the number of conidia during the seasons (A1 and A2), but did not interfere in the beginning of conidia release and was not effective in reducing the AUIDC at 2009/10 season. The majority of captured conidia occurred near the ground and these were observed both in air trap and in water runoff trap within the plant. It was not detected conidia of *Colletotrichum* spp. on *G. molesta* and *B. cranaodes* indicating that these insects should not be dispersal agents of the pathogen.

# COMPOSITION OF THE *Fusarium graminearum* SPECIES COMPLEX POPULATIONS IN WHEAT CROPPING ENVIRONMENTS IN SOUTHERN BRAZIL

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The *Fusarium graminearum* species complex (FGSC) comprises several toxigenic species that cause Fusarium head blight (FHB) in wheat. In this study, a great number (n=671 isolates) of pathogenic isolates (isolated from FHB-infected spikes) was obtained from a 3-year survey (2009-2011) conducted on commercial wheat fields in PR (n=216) and RS (n=455) states. In RS, FHB incidence, latitude and longitude, elevation (m), grain developmental grain stage at sampling and previous summer crop (corn or soybean) based on visible stubble, were recorded for each field. Also in RS, 2011 year, three wheat fields, 174 to 275 km apart, were selected and FGSC isolates were obtained from: a) within-field stubble collected during elongation crop stage; b) air above canopy during flowering; and c) *Fusarium*-damaged mature kernels. All isolates were identified to species/trichothecene genotype through a multilocus genotyping (MLGT) assay or sequences of the TEF1-alpha gene combined with multiplex PCR. Five species were found in the regional survey: *F. graminearum* (Fgra, 557/671), *F. meridionale* (Fmer, 86/671), *F. cortaderiae* (Fcor, 17/671), *F. austroamericanum* (Faus, 6/671), and *F. asiaticum* (Fasi, 3/671). All Fgra were of 15-acetyl(A)-Deoxynivalenol(DON) and all Fmer (excepting one 15-ADON) and Fasi were of the nivalenol (NIV) genotype. Both Fcor and Faus were of NIV or the 3-ADON genotype. Frequencies of the 15-ADON, compared to grouped 3-ADON and NIV genotypes, differed significantly (based on a chi-square test) between the two states. In RS, trichothecene genotype frequencies were affected by FHB incidence level (30% incidence cut-off) and grain stage during sampling, and not affected by previous crop and elevation. Among the 189 isolates, Fmer was dominant (37/54) in the stubble population, followed by Fcor (16/54) and Fgra (1/54). Conversely, Fgra was dominant (23/34) in the airborne population, followed by Fmer (6/34) and Fcor (5/34); and Fgra was dominant (85/101) in the spike population, followed by Fcor (10/101), Fmer (5/101) and Faus (1/101). Fasi was not found in these three fields and the trichothecene genotype-species correspondence was the same as that of the regional survey. Our data update on the FGCS members affecting wheat in Brazil and raise potential food and feed safety concerns regarding the increased prevalence of NIV at certain regions. The uncoupling of the dominant FGSC populations from stubbles, air above canopy and spikes suggests host/niche adaptation.

# IN VITRO EVALUATION OF THE SENSITIVITY OF *Phomopsis amygdali* TO TEMPERATURES AND PHOTOPERIODS

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*Fusicoccum* canker or twig blight caused by *Phomopsis amygdali* is a harmful and economically important fungal disease. This disease, which primarily affects the lower branches of trees, occurs severely in humid environments during the spring and fall, causing damage to crop productivity. The aim of this study was to evaluate the sensitivity of the pathogen to different temperatures and photoperiods. Two *in vitro* experiments were carried out: the first to determine the best temperature and the second to assess the influence of photoperiods on the mycelial growth of the fungus. For both the experiments, the fungus was grown in Petri dishes containing potato dextrose agar (PDA) medium. In experiment 1, the dishes with the pathogen colonies were subjected to 7 temperatures (5, 10, 15, 20, 25, 30, and 35°C) in a biochemical oxygen demand (BOD) incubator. In experiment 2, the pathogen was subjected to 4 different photoperiods: continuous light, 10 h of light/10 h of darkness, 12 h of light/12 h of darkness, and continuous darkness, under the previously determined temperature that provided the best mycelial growth. Mycelial growth was assessed by calculating the average of 2 diametrically opposed measurements of the colonies at 3, 5, 7, and 9 days after the transfer of the pathogen, by using a ruler. The experimental design was completely randomized. The means were compared using Tukey's test at 5% significance. The highest mycelial growth of *P. amygdalei* occurred at a temperature of 20°C, with the mycelia reaching the borders of the dish after 9 days. The tested photoperiods were not found to affect the mycelial growth of the fungus.

# IN VITRO SENSITIVITY OF *Phomopsis amygdalli* TO ESSENTIAL OILS

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The irrational use of pesticides has caused the accumulation of harmful compounds in the environment, leading to the emergence of plant pathogen populations resistant to chemical substances in addition to causing environmental imbalance. In conjunction with these facts and with the current growing demand for pesticide-free foods by consumers, several research projects are being conducted aimed at identifying natural products for disease control. The aim of this study was to evaluate the in vitro fungitoxic activity of essential oils as an alternative method in the control of *Phomopsis amygdali*, the causal agent of twig blight. Four essential oils were tested: eucalyptus (*Eucalyptus* ssp.*)*, clove *(Syzygium aromaticum)*,oregano *(Origanum vulgare*), and peppermint *(Mentha*spp.) in 5 concentrations (0, 1, 5, 10, and 20%). The pathogen was transferred to Petri dishes containing potato dextrose agar (PDA) medium. The oils were distributed at four equidistant points inside the dish. Evaluations were made by calculating the average of 2 diametrically opposed measurements of the colonies at 3, 5, 7, and 9 days after the transfer of the pathogen, by using a ruler. The treatments were arranged in a completely randomized factorial design, and mycelial growth data were subjected to analysis of variance and analyzed using polynomial regressions. The oregano and clove oils were found to linearly reduce mycelial growth with increased dose, whereas the eucalyptus and peppermint oils did not affect the growth of *P. amygdali*.

# INCREASE OF CITRUS FRECKLE SPOT AND HARD SPOT ON FRUIT WITH CITRUS BLACK SPOT (*Guignardia citricarpa*) UNDER DIFFERENT POST-HARVEST TREATMENTS

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The citrus black spot (CBS) is a disease caused by the fungus *Guignardia citricarpa* that causes superficial lesions and premature fruit drop. The CBS causes major economic impact to the citrus industry due to yield reduction in orchards and fruit depreciation. The pathogen can cause lesions on fruits, branches, and stems, but rarely on leaves. The symptoms occur both in field and post-harvest. Apparently healthy fruit may show symptoms of CBS up to thirty days after harvest. Late symptoms appearance is the main reason of rejection of sweet orange lots exported from Brazil to EU. The objective of this study is to evaluate the effect of carnauba wax and its combination with fungicide imazalil on increase of lesions caused by CBS on oranges. Three treatments were tested: carnauba wax, the combination of carnauba wax and fungicide imazalil, and control (no carnauba wax and no fungicide). The treatments were carried out by processing machines in a packinghouse. Fifty fruits were submitted to each treatment. The number of freckle spots and hard spots was evaluated every four days, while the number of pycnidia was evaluated every seven days, during the fruit shelf life. No hard spot lesion was observed in any fruit from all treatments, but freckle spot occurred in all treatments. At the end of the evaluation period, an average of 10.18 freckle spots per fruit occurred on the carnauba wax treatment; 8.28 on the control and 5.38 on the treatment with fungicide imazalil + carnauba wax. The number of pycnidia did not differ among treatments by t test ($p < 0.05$).

# TEMPORAL PROGRESS OF LEAF BLIGHT AND DOWNY MILDEW ON *Vitis labrusca* GRAPES

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Viticulture has been explored in both tropical and subtropical regions in Brazil. However, viticulture may be unfeasible in regions with high disease pressure. The Metropolitan Region of Curitiba (MRC) - Paraná State is expanding the *Vitis labrusca* grape cultivation, which is affected by two major diseases: downy mildew caused by *Plasmopara viticola* and leaf spot caused by *Pseudocercospora vitis*. To understand the temporal progress of epidemics of the two diseases in MRC and to recommend diseases management strategies, a field trial was carried out in the Pinhais municipality in the MRC with cultivars Concord and Bordo, used for juice produced regionally. In the 2008-2009 and 2009-2010 growing seasons, incidence and severity of both diseases was evaluated at intervals of 7 to 21 days. The disease progress curve data was fitted to nonlinear models and the regional weather data was recorded. The downy mildew incidence was above 80%, and the disease severity was adjusted to the Richards and Logistic models for the seasons 2008/09 and 2009/10 respectively. Early defoliation caused by the disease was observed, and leaf blight severity was fitted to Logistic and Exponential models for the seasons 2008/09 and 2009/10 respectively. A significant increase in disease severity was observed at the end of each season. The weather was humid and rainy, with temperatures around 20°C from November to April. The region where this study was carried out is very favorable to downy mildew and the disease increased significantly after the first symptoms were observed. Our results indicate that control measures must be implemented preventively starting from early November. Leaf blight disease progresses slowly and can be controlled until up to 5% disease incidence, which occurs late in the crop season in mid-March in the MRC.

# ANTHRACNOSE INCIDENCE IN DIFFERENT RUSTIC GRAPE CULTIVARS IN CAMPO LARGO, PR, BRAZIL

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Grapevine anthracnose is a severe disease that affects several grape production areas. This disease is considered a tropical disease, therefore the evaluation of grape cultivars before of the vineyard implantation is important for choosing the most suitable cultivar to each region. Rustic Grapes are an important option for vintners in the Curitiba Metropolitan Area, however, there is little diversification of cultivars in this region. The objective of this study was to evaluate the anthracnose incidence progress in grape leaves in three scion cultivars (Concord, Bordô and BRS Carmem) in combination with three rootstocks (1103 Paulsen, VR 043-43 and IAC 766) in Campo Largo (PR). The experiments were carried out on commercial orchard and the plants were planted in ridges with conduction system in semi-trellis. The disease incidence was assessed between September and March 2010/2011, 2011/2012, and 2012/2013 each 14 days, in the last 10 leaves in the apex of the plant. Six plats of each different combination cultivar rootstock were considered, totaling 54 plants. In the area under the disease progress curve (AUDPC), in the first harvest, BRS Carmen was the most susceptible and Concord the least. In the second and third cycle, the cv. Bordô had the highest disease incidence, differing from the other cultivars in the second year and only of Carmen in the third. The unfolding of the interaction showed difference between rootstock in BRS Carmen, IAC 766 was the most susceptible in 2010/2011 and VR 043-43 the least in 2011/2012, however in 2013, within the Concord cultivar, VR 043-43 was the most tolerant rootstock. The results showed for this area and seasons, the cultivar Concord in VR 043-43 was the least susceptible to anthracnose incidence.

# ATTRIBUTES OF LESIONS AND SPATIAL PATTERNS OF RICE BROWN SPOT FIELD EPIDEMICS AT THE LEAF SCALE

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In this study, we determined attributes of rice brown spot (BS, caused by *Bipolaris oryzae*) lesions and analyzed their spatial patterns at the leaf scale through image analysis. Three hundred and fifty symptomatic leaves were collected during the course of BS development in irrigated rice plots during an epidemic year in southern Brazil. Each leaf was digitally scanned and the images enhanced with an image editing software. ASSESS 2.0 was used to measure disease severity (SEV) and selected 100 leaves representing the SEV range. For this set, the following lesion attributes were determined using ASSESS: lesion count (LC) per leaf, relative grid position of the lesion centroid and lesion area (mm²). Algorithms were applied to generate a variable number of quadrats (0.7x1 cm each), according to the leaf size, and determine LC per quadrat using R software. For instance, a 20-cm long leaf had 40 (2 x 20) quadrats. For the quadrat count data we applied a Kolmogorov-Smirnov (KS) test of complete spatial randomness (CSR), a spatial analysis by distance indices (SADIE, aggregation index, $I_a$) and calculated the Morisita's index ($I_M$). Taylor's power law (TPL) was used to summarize LC across all leaves by fitting a linear regression model for the relationship between log-transformed variance and log-transformed mean LC data. Additionally, for leaves where LC was >50, nearest neighbor analysis (NNA) was conducted. Linear regression models were fitted to relate SEV and LC data. Mean BS SEV was 3.3% and ranged from 0.04 to 28.8%; 50% of the leaves showed SEV<2%. Mean LC/leaf was 24.5 and ranged from 1 to 293 lesions per leaf. In a linear regression model, LC explained 85% of the variability in BS SEV. For the quadrat-based analysis, lesions of 76, 66 and 66 leaves were distributed at random according to $I_M$, $I_a$ and KS statistics. However, a tendency towards clustering was found with the increase of BS SEV. For instance, the 24 leaves with SEV>8%, 15, 19 and 18 leaves showed clustering of lesions according to $I_M$, $I_a$ and KS statistics, respectively. In the TPL analysis, positive slope coefficient differed significantly from 1, suggesting clustering of lesions for higher mean BS SEV. NNA detected clustering of lesion for 30 out of 34 leaves with LC>50. This study provides for the first time empirical data useful for modeling brown spot epidemics at the leaf scale and generating hypothesis related to the dynamics of the disease spread.

# INFECTIOUS PERIOD OF CITRUS BLACK SPOT ON SWEET ORANGE

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Citrus black spot (CBS) is an important fungal disease, caused by *Guignardia citricarpa*. The risk of introduction and spread of CBS to new unaffected citrus-producing areas is a major concern for those orchards attempting to remain free of the disease. It has never been demonstrated that *G. citricarpa*-infected fruit have established infections in commercial diseased-free areas. The period of production of spores in fruit lesions is an important variable in a model for risk assessment. The objective of this study was to evaluate the infectious period of CBS on sweet oranges. Twenty infected oranges treated with carnauba wax with 'hard spot' symptoms of CBS were kept in a humid chamber until 'freckle spot' symptoms developed. When the first pycnidium formed, 50 μl drop of sterile distilled water was placed on one freckle spot lesion per fruit. After four hours, the water drop was transferred to a tube which contained 250 μl of water with Tween-20 (1%). Every 48 hours over 40 days, the number of pycnidia and the number of conidia formed in each lesion were counted. The experiment was repeated twice. The first pycnidia were observed 2-7 days after the appearance of typical freckle spots. The number of pycnidia increased until 30-40 days, and ranged from 1 to 31 per lesion. Conidia liberation started at 2-12 days and stopped 16-38 days after pycnidia formation. The number of conidia ranged from 0 to 109,875 per lesion. The average infectious period was 15 days and ranged from 0 to 26 days.

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