



## Proceedings of the VI EpidemioBrasil Brazilian Workshop of Plant Disease Epidemiology



**Chapecó, Santa Catarina, Brazil**

**14 and 15 September 2022**

**Realização**



**Promoção**



**SOCIEDADE BRASILEIRA DE  
FITOPATOLOGIA**





**Proceedings of the VI EpidemioBrasil**  
**Brazilian Workshop of Plant Disease Epidemiology**

**Chapecó, Santa Catarina, Brazil**

**14 and 15 September 2022**

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## **Preface**

The VI Brazilian Workshop of Plant Disease Epidemics – EpidemioBrasil, takes place in Chapecó, Santa Catarina, Brazil, in 14 and 15 September, 2022. The meeting is held at the “Centro de Treinamento da Epagri de Chapecó”. The scientific program was developed looking to contemplate the main phytopathological problems in Brazil and the efforts of the scientific community to generate knowledge about them. There are registered attendees from the Brazilian states Santa Catarina, Paraná, Rio Grande do Sul, São Paulo, Espírito Santo, Minas Gerais, Bahia, Goiás, Distrito Federal and Pernambuco and from other countries such as Argentina and United Kingdom. The first edition was held in Viçosa (2004), and the subsequent editions occurred in Campos do Jordão (2007), Bento Gonçalves (2010), Curitiba (2013), Cruz das Almas (2016) and has served as the forum for presentation and discussion of the most current research in plant disease epidemiology. The historical record of these conferences is available at the Brazilian Society of Phytopathology (SBF - *Sociedade Brasileira de Fitopatologia*) website. The formal presentations at the conference are divided between oral and poster format. Manuscripts prepared by the authors of the above presentations comprise this book. We would like to acknowledge the conference secretaries Agnes Etges and Sônia Maria Bortolanza, the event manager Régis Rogério da Rocha. The staff at Epagri Training Center has been exceptionally helpful in setting up the rooms for the meeting and dealing with the many last-minute adjustments that are a part of any such meeting. This Workshop edition is supported by FAPESC. Lastly, we would like to acknowledge the conference participants themselves for volunteering to chair, moderate and facilitate the sessions and subsequent discussions.

**Maria Cristina Canale**

**Cristiano Nunes Nesi**

**Armando Bergamin Filho**

**Emerson Del Ponte**

**Lilian Amorim**

**Louise Larissa May De Mio**

**Francisco Laranjeira**

## Program – Wednesday, 14 September 2022

### Programação VI EpidemioBrasil

Quarta-feira, dia 14 de setembro de 2022				
08:00 - 08:40	Recepção dos conferencistas - boas vindas - abertura	Dra. Maria Cristina Canale	Epagri	Anfitrião
		Dr. Cristiano Nunes Nesi	Epagri	Anfitrião
08:40 - 10:00	Geographic patterns in genetic diversity of the meadow spittlebug <i>Philaenus spumarius</i> , an insect vector of <i>Xylella fastidiosa</i>	Dr. Saskia Hogenhout	John Innes Centre, Reino Unido	Palestrante
		Prof. Dra. Lilian Amorim	Esalq/USP	Coordenação
10:00 - 10:30	Coffee break			
10:30 - 12:30	Sessão 1: Epidemiologia e manejo de doenças transmitidas por vetores	Dra. Maria Cristina Canale	Epagri	Coordenação
10:30 - 11:00	Ecology, epidemiology, and management of vector transmitted diseases	Prof. Dr. Armando Bergamin Filho	USP - ESALQ	Palestrante
11:00 - 11:30	Dalbulus maidis and corn stunt pathogens monitoring program in Santa Catarina, Brazil: Scientific advancements and challenges	Prof. Dr. Fábio Nascimento da Silva	UDESC	Palestrante
11:30 - 12:00	Epidemiology and control of citrus Huanglongbing	Dr. Renato Beozzo Bassanezi	Fundecitrus	Palestrante
12:00 - 13:30	Lunch time			
13:30 - 15:00	Sessão 2: Perspectivas para o manejo de doenças de plantas no Brasil	Prof. Dr. Eduardo Gomide Mizubuti	UFV	Coordenação
13:30 - 14:00	Epidemiological aspects of Asian grapevine leaf rust	Prof. Dra. Lilian Amorim	USP - ESALQ	Palestrante
14:00 - 14:30	Epidemiology and the bright future of tailored solutions	Dr. Braz Hora	Bayer Cropscience	Palestrante
14:30 - 15:00	Research by the private initiative and its contributions to the management of plant diseases	Dra. Caroline Rabelo Costa	PlantCare Pesquisa Agrícola	Palestrante
15:00 - 15:30	Coffee break			
15:30 - 18:00	Sessão 3: Avanços no monitoramento e avaliação de risco	Dr. Emerson Medeiros Del Ponte	UFV	Coordenação
15:30 - 16:00	Warning models for the occurrence of Phoma leaf spot on coffee plants	Prof. Dr. Humberson Rocha Silva	UFRPE	Palestrante
16:00 - 16:30	Weather favorability and inoculum monitoring for coffee leaf and soybean rusts	Dr. Fernando Dill Hinnah	SmartAgri	Palestrante
16:30 - 17:00	Early warning system: Wheat blast in Bangladesh	Dr. José Maurício Cunha Fernandes	Embrapa	Palestrante
17:00 - 17:30	Alert system and predictions for the control of apple tree diseases in the state of Santa Catarina	Dr. Leonardo Araújo	Epagri	Palestrante
17:30 - 18:00	Debate sessões 2 e 3	Prof. Dr. Waldir Cintra de Jesus Junior	UFSCar	Debate
		Dr. João Américo Wordell Filho	Epagri	Debate
Dinner at the city center (adesion)				

## Program – Thursday, 15 September 2022

### Programação VI EpidemioBrasil

Quinta-feira, dia 15 de setembro de 2022				
<b>8:30 - 10:00</b>	<b>Sessão 4: Ferramentas estatísticas e computacionais de código aberto</b>	<b>Dr. Cristiano Nunes Nesi</b>	<b>Epagri</b>	<b>Coordenação</b>
08:30 - 09:00	Towards an open research and education in plant disease epidemiology	Prof. Dr. Emerson Medeiros Del Ponte	UFV	Palestrante
09:00 - 09:30	EpidemioR: a diverse collection of epidemiological studies tailor-made with R	Prof. Dr. Walmes Marques Zeviani	UFPR	Palestrante
09:30 - 10:00	<i>Coffee break</i>			
10:00 - 11:00	<b>Speed talk session</b>	<b>Dra. Isabela Primiano</b>	<b>Fundecitrus</b>	<b>Coordenação</b>
11:00 - 12:30	<b>Poster session</b>	<b>Dra. Heloísa Thomasi</b>	<b>UFPR</b>	<b>Avaliadora</b>
12:30 - 13:30	Lunch time			
<b>13:30 - 15:00</b>	<b>Sessão 5: Dispersão de propágulos e resistência de fungos a fungicidas</b>	<b>Prof. Dra. Lilian Amorim</b>	<b>Esalq/USP</b>	<b>Coordenação</b>
13:30 - 14:00	Can fungal aerobiology tools help tracking fungicide resistance alleles and block the spread of fungicide resistance in the agroecosystem?	Prof. Dr. Paulo Ceresini	UNESP, Ilha Solteira	Palestrante
14:00 - 14:30	Fungicide resistance evolution in global populations of the wheat pathogen <i>Zymoseptoria tritici</i>	Dr. Guilherme Rossato Augusti	Sumitomo Chemical	Palestrante
14:30 - 15:00	Aerial spread of smut spores during peanut harvest	Dr. Juan Pablo Edwards Molina	INTA, Argentina	Palestrante
15:00 - 15:30	<i>Coffee break</i>			
<b>15:30 - 18:00</b>	<b>Sessão 6: Perspectivas de trabalhos em rede e colaboração</b>	<b>Dra. Maria Cristina Canale</b>	<b>Epagri</b>	<b>Coordenação</b>
15:30 - 16:00	The plant diseases epidemiology in the tropical plant health network scope	Dr. Adriano Augusto de Paiva Custódio	IDR-PR	Palestrante
16:00 - 16:30	From the individual to the collective: Challenges of plant health, a view of the governmental plant protection agency	Ms. Fabiane dos Santos	Cidasc	Palestrante
16:30 - 17:00	Debate sessões 5 e 6	Prof. Dra. Louise Larissa May De Mio	UFPR	Debate
		Dr. Francisco Ferraz Laranjeira	Embrapa	Debate
17:00 - 17:10	Entrega dos prêmios Speed talk e pôster	Dra. Isabela Primiano	Fundecitrus	Coordenação
17:10 - 17:30	Escolha do próximo local de realização do EpidemioBrasil e Encerramento	Dra. Maria Cristina Canale	Epagri	Coordenação



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# ABSTRACT LECTURES

## **Geographic patterns in genetic diversity of the meadow spittlebug *Philaenus spumarius*, an insect vector of *Xylella fastidiosa***

Roberto Biello<sup>1</sup>, Qun Liu<sup>1</sup>, Sam T. Mugford<sup>1</sup>, Alan Stewart<sup>2</sup>, Claire Harkin<sup>2</sup>, Katherine Lester<sup>3</sup>, Rebecca Cairns<sup>3</sup>, Michael Wilson<sup>4</sup>, Simon Conyers<sup>5</sup>, Duncan Allen<sup>5</sup>, Damian De Marzo<sup>5</sup>, Gerard Clover<sup>1</sup>, and Saskia A. Hogenhout<sup>1</sup>

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*Xylella fastidiosa* is a plant pathogen which is transmitted exclusively by xylem sap-feeding insect species to over 500 plant species, including crops, ornamentals and trees. The bacterium was limited to the Americas and in the past ± 40 years was introduced multiple times into Europe causing a range of plant diseases, including the economically damaging Olive Quick Decline Syndrome (OQDS) in Italy that has led to the eradication of entire, often ancient, olive groves. OQDS was detected in 2013 and spread throughout southern Puglia within just five years. The meadow spittlebug/froghopper *Philaenus spumarius* (Linnaeus, 1758) (Hemiptera: Aphrophoridae) was identified as the predominant insect vector of *X. fastidiosa* in Italy and is one of the most common insect species in the United Kingdom (UK). *P. spumarius* is native to the Palearctic region but has also been unintentionally introduced to other areas, such as North America and New Zealand. The insect is considered a eurytopic and polyphagous species, tolerating a wide range of environmental factors and feeding on a range of plant species. However, our understanding of the levels of *P. spumarius* genetic variation, including if there are cryptic (sub-)species and their migration rates, is incomplete. To investigate these, we characterized the global genetic diversity and population structure of this insect species. Firstly, we generated a high-quality chromosome-level assembly of the 2.7 Gb *P. spumarius* genome. We then re-sequenced whole genomes at 10x coverage of ~440 individuals from the UK and the rest of Europe. The data provided insights into how *P. spumarius* has dispersed globally. We found that *P. spumarius* is split into three divergent mitochondrial lineages across Europe with two located in the UK (one in the north and one in the south) and the third in southern Europe. The whole-genome sequencing data showed a weak genetic structure among the UK populations that supports isolation by geographical distance with evidence of admixture between the two lineages probably resulting from secondary contact. The *P. spumarius* populations that occur in the *X. fastidiosa* outbreak regions of southern Italy do not only belong to a different mitochondrial haplogroup than those in the UK, but also have higher migration rates, distinct phenology and a different plant host preference compared to most UK populations. This research has provided key information that will help to mitigate the impacts of potential *X. fastidiosa* outbreaks in the UK and elsewhere.

## Ecology, epidemiology, and management of vector transmitted diseases

Armando Bergamin Filho

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Traditional botanical epidemiology considers two types of diseases: (i) **polycyclic diseases** – when inoculum that causes infection is produced during the epidemic in or on individual plants that had been previously infected in the course of current epidemic, and (ii) **monocyclic diseases** – when inoculum that causes infection is not produced in or on individual plants that had been infected in the course of current epidemic, but in the soil, on secondary hosts, or in infected crop plants of the same host in other fields. In general, diseases of the first type present a logistic disease progress curve and its management is based on reducing the rate of infection, whereas diseases of the second type present a monomolecular disease progress curve and its management is based on reducing the initial inoculum. This article deals with plant diseases that depart in their structure and behavior from these two prototypes, because they borrow elements from both of them. We address epidemics in which the primary inoculum has a continuous and dynamic role, and in which the secondary inoculum also contributes to epidemic build-up. We call this group as **polycyclic diseases with continuous primary spread**. They are usually caused by bacteria or virus transmitted by vectors. This epidemiological structure generates less clear-cut disease progress curves. We focus here on this type of disease because this more complex, combined, pattern is actually quite common, often leading to severe plant disease epidemics, with impacts at the farm, community, and country scales, and because such epidemics are among the most difficult to manage. The management of such epidemics needs addressing both, primary and secondary inoculum; more importantly, these actions to be effective have to be performed in an area-wide, regional basis. Our analysis leads us to assess past errors and current courses of action for diseases of this type. It brings us to recognize, in addition to the conventional tools for management with local effects, the critical importance of collective action, of more difficult implementation.

Support: FAPESP and CNPq.

## ***Dalbulus maidis* and corn stunt pathogens monitoring program in Santa Catarina, Brazil: Scientific advancements and challenges**

Fábio Nascimento da Silva<sup>1</sup>, Jacson Ferreira<sup>2</sup>, Eduardo Silva Gorayeb<sup>1</sup>, Vivian Borges de Souza<sup>1</sup>, Daian Marcos Savaris<sup>2</sup>, Maria Cristina Canale<sup>2</sup>, Leandro Prado Ribeiro<sup>2</sup>, Matheus Rodrigues Magalhães Albuquerque<sup>1</sup>, Meyrielle Pires de Camargo<sup>3</sup>, Vinicius Stock<sup>3</sup>

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*Dalbulus maidis* (DeLong & Wolcott, 1923) (Hemiptera, Cicadellidae) is an insect vector of the pathogens that cause corn stunt diseases (maize bushy stunt phytoplasma, corn stunt Spiroplasma, and maize rayado fino virus). In recent years, a considerable increase in the incidence of maize leafhopper and corn stunt complex was observed in Santa Catarina (SC). Therefore, several actions have been undertaken by the action committee against maize leafhopper and associated pathogens, including the program for monitoring the progression of maize leafhopper and its infectivity ("Programa Monitora Milho SC"). This program aimed to monitor *D. maidis* populations in different regions of SC and its infectivity with the corn stunt-associated pathogens, providing subsidies for the postemergence integrated management of maize in a regionalized context. In 2021/2022 (first and second crops), 22 and 14 representative production areas from different regions of SC were monitored, respectively. In each selected area, four yellow sticky traps were placed and inspected weekly to evaluate the insect-vector population and subsequent extraction of nucleic acids from insects collected to detect pathogens associated with the corn stunt complex. To support these activities and the integrated management of this pathosystem, the following experiments were performed: (1) determination of the time for collecting the traps that would allow the adequate diagnosis of pathogens from the insect tissues, (2) determination of the prevalence of the three pathogens in symptomatic corn plants collected in production areas, (3) development of a tool to detect three pathogens simultaneously, and (4) evaluation of the genetic variability of *D. maidis* using the mtCOI gene. In experiment (1), 15 insects were collected at different exposure times (1, 3, 7, and 14 days) from the traps in the field; the quality of the genetic material was monitored via spectrophotometry and agarose gel electrophoresis. In experiment (2), 28 samples of symptomatic corn plants were collected in four production areas in SC. The presence of mollicutes and viruses was determined by PCR and RT-PCR, respectively. In experiment (3), the nucleic acid extraction procedure was optimized using tissues isolated from insects and plants. The multiplex reaction was developed using specific primers to detect the three pathogens in the same reaction. In experiment (4), the insects were collected from different production areas in Brazil, including SC, which were characterized for variability and phylogenetic relationship using the nucleotide sequence of the mtCOI gene. The time established for the traps to remain in the fields was 7 days, providing high-quality genetic material and adequate time for the logistics of the established monitoring program. The three pathogens were detected in the plants in the production areas in SC, with the virus and Spiroplasma being the prevalent pathogens. However, phytoplasma was also detected in some samples. This result indicates that the virus should also be evaluated in the second cycle of the monitoring program. For this, the multiplex technique proved to be sensitive, cheaper, and faster in detecting the three pathogens in insect and plant tissues. The low genetic variability of *D. maidis* was observed using the mtCOI gene. During the 40 weeks of monitoring, which included first and second crops, 2,848 traps were monitored, with the exponential growth of *D. maidis* populations over the weeks and a population peak at the end of the monitored period (second half of April). In addition, total infectivity was variable, reaching 80% within a few weeks. These data were periodically made available to the productive sector. Support: FAPESC 2021TR1246, FAPESC 2021TR1284, BAYER, CAPES and CNPq.

## Epidemiology and control of citrus Huanglongbing

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Huanglongbing (HLB) is an important citrus disease worldwide due to its rapid spread, severity of damage to fruit production and quality, and difficulty in controlling it. In Brazil, it is mainly caused by the bacterium '*Candidatus Liberibacter asiaticus*', transmitted by the Asian citrus psyllid *Diaphorina citri*. The absence of resistant varieties and curative measures means that disease control is mainly based on exclusion, eradication and protection measures. Aspects of the epidemiology of this disease make its control extremely difficult and complex. The perenniality of the citrus makes it exposed for many years to infections, needing to be protected for the same period. The incubation period ( $\geq 4$  months) much longer than the latent period ( $\sim 14$  days) allows infected plants to serve as an inoculum source even before they can be detected by visual inspections or even by PCR (irregular distribution of the bacteria in the plant) and makes it almost impossible to eradicate the disease after its introduction. The presence of inoculum sources external to commercial orchards and the long-distance spread of the vector (a few kilometers) makes the primary spread of the disease as important or more important than the secondary spread for the progress of the epidemic and that eradication of diseased plants only in commercial orchards have almost no effect on reducing the progress of the disease. In addition, the slower progression of the severity of symptoms in adult trees than in young plants, with the maintenance of a residual production for a few years, leads growers not to immediately eliminate diseased adult trees and keep potential sources of inoculum inside the orchard. The transmission of the bacteria by the psyllid in the initial stages of sprouting (V1 to V4), preferred by the psyllid for feeding and oviposition, generates the need for a higher frequency of insecticide sprays to maintain an effective barrier of protection during the production and development of new tissues. Secondary infections are almost completely controlled with fortnightly insecticide applications to break the psyllid development cycle from egg to adult ( $\geq 14$  days) in infected plants and to control adult psyllids that acquire the bacteria on diseased plants before the period of latency of the bacteria in the vector ( $\geq 12$  days) is completed. On the other hand, primary infections by infective psyllids that developed in diseased plants (completed latency period of the bacteria in the vector) and migrated to commercial orchards are not fully controlled even with weekly applications of insecticides during the vegetative flushing period (poor coverage, growth of new tissues without insecticide, rain-wash). However, some characteristics have helped to suppress the disease. The decreasing gradient of the psyllid population and diseased trees from outside to inside the orchards has been used to direct the monitoring of the vector and the inspection for diseased plants, as well as the application of control measures that reduce the primary dispersion to the interior of the property, such as: density and planting direction, planting of varieties with more or less vigor, tree pruning, trap-crop, more intensive applications of insecticides and application of repellent kaolin. The seasonality of the psyllid population (citrus shooting period) made it possible to direct the highest frequencies of insecticide application, as well as the period of greater expression of disease symptoms has directed actions to detect and eliminate symptomatic plants. Thus, for the effective control of HLB, all producers in a given region must act in a coordinated and joint way to reduce inoculum sources and control the vector both inside and outside the orchards, which is quite difficult to accomplish in practice due to the diversity of the citrus growers' profile. In cases where regional management was carried out, satisfactory results were obtained, and this is the way until more effective and economically and environmentally sustainable measures, such as the development of resistant plants, are available. Support: FAPESP (project #17/21460-0) and CNPq (project 304253/2020-0).



## Epidemiological aspects of Asian grapevine leaf rust

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Asian grapevine leaf rust (*Neophysopella tropicalis*, syn *Phakopsora euvitis*) was first observed in Brazil in 2001, in Paraná (Tessmann et al., 2004), and, in the following decade, the disease has spread to the main grape-growing regions in the country (Primiano et al., 2017). Soon after its detection, a great advance in the behavior of the pathogen as well as in the rust management was achieved by the research group led by Prof. D. Tessmann. More recently, the epidemiology group at ESALQ has been dedicated to understand the epidemiological development of the disease at two hierarchical levels: the plant and the population. The damage caused by the pathogen and the disease monocyclus was evaluated at the plant level and the temporal progress of the disease was characterized in grapevine fields with and without plastic cover. The colonization of leaf tissue by the pathogen is similar to that of *Phakopsora pachyrhizi* in soybean, with the formation of satellite uredinia (Primiano et al., 2019). Consequently, the infectious period is long: at least 30 days. Unlike other rusts, net CO<sub>2</sub> assimilation in symptomatic leaves is greatly reduced in uninjured areas, with a large imbalance in carbohydrate partitioning (Nogueira Jr. et al., 2017). Grapevine cells infected by *N. tropicalis* show modifications similar to those presented by senescent leaves, with the formation of gerontoplasts (Rasera et al., 2019) and this modification seems to be related to early defoliation induced by the disease. Although the symptoms of rust usually appear after the grapes are harvested, from December onwards in the Southeast region, early defoliation delays the sprouting of the plants in the following season. The accumulation of carbohydrates in the roots is reduced in symptomatic plants, which can potentially generate cumulative damage by shortening the lifetime and decreasing the vigor of the grapevines. This behavior suggests that rust, despite being polycyclic, causes polyetic damage to plants. Disease progress curves evaluated in six consecutive seasons of Niagara Rosada, cultivated in different management systems, showed that plastic covering is not efficient for reducing the incidence or severity of the disease when the inoculum concentration is high in the field. The amount of inoculum close to the plant canopy is greater in plants with plastic cover than in plants without plastic cover (Simões et al., 2022), as the plastic acts as a barrier to the dispersal of the inoculum. In turn, the short leaf wetness periods on plants covered by plastic significantly reduce the germination of urediniospores. Although plastic cover is an excellent option for controlling downy mildew (*Plasmopara viticola*), its efficiency has not been demonstrated for Asian grapevine leaf rust. Chemical control remains an efficient method, but the possibility of overexpression of the *sterol 14 $\alpha$ -demethylase* gene and the absence of an intron at position 143 of the *cytochrome b* gene at *N. tropicalis* are a threat to the emergence of populations of the pathogen resistant to the DMI and QoI fungicide groups (Santos et al., 2021).

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## **Epidemiology and the bright future of tailored solutions**

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Bayer Crop Science is working to offer not just outstanding products, but also the best tailored solutions options for farmers face the current and future challenges that can impact agriculture's yield. The main goals are reduce impact on the environment, increase food security and help farmers to combat the continued pressure of biotic and abiotic diseases on our global ecosystems. The next frontier of value creation is enhancing those digital solutions to optimize yields, create efficiencies and improve returns. We are putting insights by our plant breeders, data scientists, agronomists and plant epidemiologists to advance on scientific solutions designed for farmers of all scales in all geographies through our continued progress in developing digital tools based on customer feasibility, desirability and viability assessment. Tailored solutions require an integrated approach, exploring diverse dataset. We are going to show cases exploring data and expertise from Plant Epidemiologists and Data scientists to address key diseases for soybean farmers (Asian Soybean Rust - ASR) and for corn farmers (Northern Corn Leaf Blight – NCLB, and Maize White Spot – MWS). All models showed high accuracy and a powerful tool to disease management based on genetics and fungicide control. Through these tailored solutions, empowered by our digital platforms, we provide growers with the key to be as sustainable and successful in every part of their operation as they can based on yield optimization and cost reduction.

## **Research by the private initiative and its contributions to the management of plant diseases**

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Brazil has become a major player in the global agricultural business. With investments in research and development (R&D), rural extension, and subsidized rural credit, agribusiness has been consolidating itself as the driving force of the Brazilian economy. In the face of incentives and efforts out of science and technology (S&T) institutions and, especially from rural producers, sharp productivity gains have been observed over the last few decades. The technologies currently used in the field include nanotechnology, biotechnology and information technology and these have increased production processes generating products with excellence and high impact for the sector, as well as the expansion of bioinformatics in the analysis and sharing of scientific data. These trends are based on the intense digital transformation that has been taking place in agriculture. However, intensive agricultural crops have several challenges related to external and internal factors over the production process, among some, phytosanitary issues are crucial for high yields and knowledge in plant protection, aiming to a correct disease management, has become essential to keep Brazilian agribusiness competitive in the world stage. Losses associated with the incidence of pests, diseases and weeds limit production, and in a tropical country such as Brazil, two scenarios aggravate this prospect: the so-called "green bridge", which is the uninterrupted sequence of commercial crops, a practice made possible by the irrigated areas and plant material adapted to different regions and environmental conditions favorable to the development of these diseases. Solutions to mitigate sanitary risks must involve a combination of measures and tools with action by the private and public sectors, in which the availability of resources often determines actions. Technology, so exalted and widespread today, is a great ally in the convergence between research and the market, contributing to the development of new products, whether machines, seeds, agrochemicals, production structures and, more recently, apps and startups, which can be managed for the monitoring, prevention or management of diseases. The public and private sectors, in proportions, have been alternating over time, but in recent years, there has been a strong participation of private investments proportionately as large local corporations and, mainly, multinationals, have managed to change the scenario of return of investments. Agro startups (Agtechs) bring disruptive and incremental innovations quickly and efficiently, helping to improve the processes which involve disease prevention and/or control. Mapping produced by Embrapa in 2020/2021, described more than 1500 Agtechs operating currently in the country that inside and outside the farm in the most diverse areas such as smart agriculture, automation, biotechnology, drones, satellites, among others, bringing tools ranging from the use of pesticides, phytochemicals, application technology, to the perspective of using RNAi, biological agents as growth promoters, and immunity generators etc. Within the innovation center, private agricultural research stations enable this diversity of studies in the field and in laboratories, as well as their applicability, to be conducted in an effective and accelerated way and with high technical-scientific rigor that the processes require for the elucidation of problems, in order that its use on a large scale can be carried out by researchers, consultants and rural producers.

## Warning models for the occurrence of Phoma leaf spot on coffee plants

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Brazil is the world's largest producer and exporter of coffee. However, crop productivity is affected by several diseases, including Phoma leaf spot, caused by *Phoma* spp., which causes damage between 15 and 43%. Thus, it is necessary to adopt efficient strategies to manage this disease. Most cultivars planted in Brazil are susceptible to Phoma leaf spot, and chemical control is the most adopted strategy in the field. However, it is necessary to rationalize the use of fungicides, as they are expensive and can cause negative impacts on man and the environment. Thus, this work aimed to develop warning models for the occurrence of Phoma leaf spot on coffee plants, based on the monitoring of meteorological variables. The experiment was carried out under field conditions, in the county of Santo Antônio do Amparo-MG, in a field of *Coffea arabica* L. cultivar Catucaí Amarelo 2 SL, in an area of 7.65 ha, implanted with a spacing of 3.7 x 0.7 m and age of four years at the beginning of the research. A regular grid of 85 points was demarcated throughout the area, with the aid of a Topcon® Hiper Lite L1 L2 GNSS receiver, spacing 30 x 30 m between points. At each georeferenced point, symptoms were evaluated in five coffee plants. Meteorological information was recorded using a Campbell Scientific Inc.® automatic weather station installed at the experimental area. Disease incidence assessments took place between September 2013 and August 2015, with monthly assessments. 52 meteorological variables were generated from the primary variables related to temperature, humidity and wind direction and speed. These variables were used in two lag approaches in relation to disease assessment dates. The meteorological variables and lag periods with the highest correlation with the disease were pre-selected. Subsequently, the multiple linear regression models (MLRM) were adjusted, using as selection criteria: significant regression coefficients by the t test ( $p \leq 0.05$ ), lower AIC and higher values of  $R^2$  and  $R^2^*$ . Of the 52 meteorological variables, 34 showed a significant correlation with the incidence of the disease. The variables related to the number of days with leaf wetness (positive correlation), number of hours with relative humidity less than or equal to 70% (negative correlation) and greater than or equal to 80% (positive correlation), and number of hours with temperatures between 15 and 20°C (positive correlation) and 25 and 30°C (negative correlation) were the ones with the highest association with the disease over time, considering the two forms of lags adopted in the analysis. A total of 126 MLRMs were fitted to the incidence progress curve, considering the overall mean of 85 points. Four of these models showed high precision and accuracy, and two of them allowed estimating the disease two weeks in advance.

Support: CAPES and CNPq

## Weather favorability and inoculum monitoring for coffee leaf and soybean rusts

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Rusts are biotrophic pathogens, requiring living tissues to infect, colonize and multiply. They move field to field usually carried on low level winds. Currently, *Phakopsora pachyrhizi* and *Hemileia vastatrix* are the two major rusts happening in Brazil, causing losses every growing season. The losses are more frequently accounted as lower profits due to higher cost on fields protection. To help growers on their decisions, forecast models have been developed since 1960's. In this case, we developed four different models in order to understand coffee leaf rust, using 18 years of monthly data from three different sites on Minas Gerais, after Gompertz model choice for monthly evolution calculation. The models were developed using weather data of common weather measurements as temperatures, relative humidity and derived complex estimation to obtain dew formation data. The models tested different periods of time before first visible symptoms, considering latency period and the temporal resolution of incidence assessments, to understand which weather measurements and when they drive coffee leaf rusts. After comparing the models with independent data, the best one presenting 9.5% of false negatives was chosen, allowing the move towards seven field trials. Sprays scheduled by schemes based on accumulated favorability performed better than scheduled standards, decreasing AUDPC. Field trials on two site presented exceptions: Campinas, a site surrounded by diseased plants, had incidence out of control at all fungicides schemes (including the scheduled with a greater number of sprays), even with medium weather favorability; Rio Claro presented no disease, in an isolated coffee farm, even with high weather favorability. The hypotheses for these exceptions seemed to be due to the lack of inoculum measurements. At this moment, in 2019 we started the development of spores collector devices, in order to allow inoculum measurement for biotrophic pathogens, with a better measurement of the epidemiological vertices. The devices are now spread in the country mainly for soybean rust management, with an objective to become the standard device as it has known air volume sampled every day, higher than most of other devices, and allowing fast analysis (20 minutes) on microscopic due to air concentration. Two examples of soybean rust spores quantification were obtained by researchers at Passo Fundo and Santa Maria. In Passo Fundo, the disease has reportedly never been found before January 10<sup>th</sup>. Empirical criteria are frequently used, therefore on this date two sprays usually have been made, without leaf incidence or spores presence assessments. Using a spore collector at 2020/21 season, spores were detected on January 28<sup>th</sup>, and leaf incidence 12 days after on February 11<sup>th</sup>. At the 2021/22 season, in Santa Maria spores were first detected on February 7<sup>th</sup>, while disease incidence only on March 1<sup>st</sup>. The practical usage of the spores collector is developed on Jaguari-RS region since 2020/21 season, with 20 devices deployed on a 8000 ha area of commercial growers, to support consultant decision. Using the spore's information, with weather favorability helped to decrease in 22.6% the amount of fungicides, resulting on R\$ 912.000,00 of savings. At 2021/22 a less weather favorable season, the reduction on the same area was about 41.66%. The system from Smart Agri has been used for spraying decision joining spore's collector devices and weather favorability maps information with consultants' experiences and knowledge.

## Early warning system: Wheat blast in Bangladesh

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Wheat blast, a disease with destructive potential, is caused by a lineage of the ascomycete fungus *Magnaporthe oryzae*, capable of infecting several species of the Poaceae family, including the genus *Triticum*. Significantly damaging wheat blast epidemics are sporadic and limited to tropical wheat-growing areas in South America. Unexpectedly, wheat blast was reported in Bangladesh and Zambia in 2016 and 2020, respectively. The urgent need to address a poorly studied disease has mobilized the scientific community. An example of a collaborative effort between CIMMYT, EMBRAPA, and the University of Passo Fundo resulted in developing an alert system for the risk of blast epidemics in Bangladesh. The system was co-developed with the Bangladesh Maize and Wheat Research Institute (BWMRI), the Bangladesh Meteorological Department (BMD), and the Bangladesh Agricultural Extension Department (DAE) between 2017 and 2019. This co-development led to the endorsement of the system for institutional use to inform extension agents in Bangladesh about the risks of an outbreak in late 2019. National partners in Bangladesh now endorse the alert system. It is being used to advise farmers nationally about when and where epidemic risks are anticipated. Currently, 6,500 extensionists in the DAE are receiving real-time alerts on the risk of wheat blast occurrence. The wheat blast alert system consists of a computerized structure that integrates several layers of geographic, climatic, and biological information, which is processed and the results communicated through textual and graphical outputs to target users. However, since the 2016 epidemic, the wheat blast has been reported in some wheat fields but at relatively low incidence levels that do not characterize an epidemic state. Therefore, the alert system developed has never been fully validated. The objectives of this work were: 1) To use a backcasting approach to evaluate the effectiveness of the wheat blast simulation model as the core of an alert system in Bangladesh and Brazil, 2) To generate a narrative of disease alerts over time, and 3) To compare the cumulative sum of alerts with disease incidence from experimental data. Wheat blast incidence was determined in wheat field samplings in the grain-forming period at different locations from 2018 to 2021 in Bangladesh. The number of conidia similar to *Magnaporthe oryzae* was also determined using a rotorod air sampler at various locations (2019-2022). In Brazil, wheat blast incidence was obtained from 45 uniform fungicide trials conducted over seven years at multiple locations. The meteorological organizations in Bangladesh (BMD) and Brazil (INMET) provided the weather data as input to the model. A wheat blast incidence from field sampling and uniform fungicide trials greater than 20 % denoted an epidemic event. Similarly, five or more notifications during the post-heading represented a high risk. Using a confusion matrix, we could assess model simulation performance compared to field observation records in Brazil and Bangladesh. The validation results were promising as the accumulation of notification alerts backcasting the wheat blast risk of infection in Bangladesh and Brazil approximated the disease intensity observed in the experimental data in epidemic and non-epidemic situations. There is a need, however, to improve the understanding of local disease hotspots observed in Bangladesh and drive broader scale model improvement. Nevertheless, notification alerts can be applied to future seasons of wheat blast management, making them an entirely early warning system predictive application using a combination of observed and short-term weather forecast.



## **Alert system and predictions for the control of apple tree diseases in the state of Santa Catarina**

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Apple tree disease control has been based on the use of pesticides, a practice that may bring about some consequences such as environmental contamination, increased production costs, and risk of developing fungicide resistance. Thus, a strategy to replace the schedule-based applications of agrochemicals for preventing diseases is the adoption of warning and forecast systems, which help fruit growers making decisions on fungicide spraying time. The rational use of agrochemicals can reduce production costs and environmental contamination and enable the maintenance of single-site fungicides for a longer period. Therefore, in this work, we will describe the positive and negative aspects, as well as the perspectives for the use of warning systems in the State of Santa Catarina, addressing its current application on integrated management of apple tree diseases such as apple scab, Glomerella leaf spot, European canker, bitter rot and white rot. Support: Cnpq, FAPESC.

## **Towards an open research and education in plant disease epidemiology**

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Plant disease epidemiologists usually make heavy use of mathematical, statistical and computational approaches when analyzing epidemic data. Early on, computers have been one of the main tools in the plant epidemiologist's tool-box. However, most of these tools were specialized and relied on proprietary software. These may quickly become deprecated and problems related to technology life-time, cost, portability, compatibility and reproducibility may affect the rate of adoption and long-term sustainability. Recently, open source free software environments (e.g R or Python) have greatly evolved and gained popularity among researchers and teachers willing to freely share reproducible code, teaching notes or even ebooks. The growing community of R users has contributed new (or updated) libraries that have greatly facilitated the use of this environment by non-programmers. In this talk, I will showcase software, documented scripts for the analysis of research data, and publications produced in my group and by other colleagues using R statistical software with the goal of facilitating and promoting transparent research as well as training in the field of plant pathology and epidemiology.

## **EpidemioR: a diverse collection of epidemiological studies tailor-made with R**

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EpidemioR is an open and online book that demonstrates the use of R software for the development, application and evaluation of methods for data analysis in plant disease epidemiology. In general, analysis approaches are seen that range from the application of R to the analysis of inoculum dispersion, modeling and forecasting of epidemics, multiple regression analysis and non-linear regression, among others. Without departing from the objectives of epidemiological research, we seek to innovate in the use of statistical methods so that there is better use of the data, which is achieved mainly with: appropriate graphic visualizations, contemporary methods of analysis and appropriate to the characteristics of the investigation and open source and reproducible production. The emphasis of the material is on problems, designs and analysis approaches common to the epidemiological area such as those that assess time to an outcome (inoculation, sporulation), proportion data (germination, etc.), growth curve data (non-linear models) and analysis of disease assessment data (incidence and severity). This material is produced due to the collaboration of professors and students of the Postgraduate Program in Plant Production, with the collaboration of professors and external researchers.



## **Can fungal aerobiology tools help tracking fungicide resistance alleles and block the spread of fungicide resistance in the agroecosystem?**

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Wheat blast (WB) and the Asian soybean rust (ASR) are considered extremely important and devastating fungal diseases affecting wheat or soybean crops. WB is caused by the ascomycetous fungus *Pyricularia oryzae* *Triticum* lineage (*PoTI*), while ASR is caused by the basidiomycetous fungus *Phakopsora pachyrhizi* (*Pp*). Populations of these two pathogens have shown the prevalence of high levels of resistance to three of the major groups of fungicides used intensively for managing wheat and soybean diseases in Brazil, which included Qols, DMIs and SDHIs. The onset of double or triple fungicide resistance in populations of *PoTI* or *Pp* in Brazil indicated that the anti-resistance strategies adopted (if any at all) in the country in the last two decades have failed, requiring fast actions and additional approaches to rationalize fungicide sprays, blended within an integrated management (IDM) program. The joint adoption of anti-resistance and IDM strategies should be coordinated locally, taking into account the particular circumstances of fungicide resistance in each region. However, despite the ever increasing knowledge of these two pathogens' population biology and epidemiology, there has been no concerted effort in the country towards the development of a nationwide plant disease forecasting system. Since a national disease forecasting system that should be guiding the spraying timing decisions is completely lacking, preventive calendar-based environmentally-impactful fungicide spraying schemes persist, resulting in excess fungicide applications, disease control failure due to fungicide resistance emergence and spread, high yield losses, and extreme environmental impact. Therefore, under such circumstances, IDM strategies are simply impossible to adopt. In order to rationalize fungicide inputs (e.g. choice, timing, dose-rate, spray number and mixing/alternation) for managing WB or ASR, we need high performance monitoring tools, enabling quantitative measurement of pathogen's inoculum levels and detection of fungicide resistant alleles, in combination with disease forecasting. This talk aims to answer the following question: "Can fungal aerobiology tools help tracking fungicide resistance alleles and block the spread of fungicide resistance in the agroecosystem? Our expectation is that, after this talk, your answer will be a definite "yes, it can". These aerobiology tools can be, fundamentally, novel real-time disease surveillance and fungicide resistance monitoring tools to foster a smart and sustainable crop protection platform in Brazil aimed at delaying evolution and spread of resistance of existing fungicides (azoles and Qols) and new actives (SDHIs) and to future new actives entering the market. As practical examples of application of aerobiology tools, we used an automated, compact, high-volume, spore sampling device able to process 10 L air.min<sup>-1</sup>, from Burkard Inc., UK, (<https://www.youtube.com/watch?v=1qehH5zlq6w>) positioned in a major wheat and soybean cropping region, in Londrina, Paraná State, coupled with a real time quantitative PCR (RT qPCR) assay, with the following objectives: 1) Monitor specific fungal DNA from *PoTI* and *Pp* airborne conidia or urediniospores continuously released from 2019 to 2021, both within and off-season, and 2) Determine the relationship among weather variables and the amount of *PoTI* or *Pp* DNA in airborne spore samples. *PoTI* and *Pp* inoculum was consistently detected during the 2019 and 2020 wheat or soybean cropping seasons, but amounts varied significantly between seasons, with higher amounts detected in 2019. However, high peaks of *PoTI* DNA were continuously detected in both 2020 and 2021 off-seasons. Our sampling strategy was very useful for the purpose of describing the annual aerobiology of multiple pathogens of agricultural importance, in the same airborne spore samples. Statistically significant but low correlations were found between the levels of pathogens molecular detection and the weather variables, indicating

that a system based on weather variables has very low predictive value for determining levels of airborne *PoTl* or *Pp* inoculum. These observations do not invalidate the potential of the continuous direct monitoring of *PoTl* or *Pp* airborne inoculum as prior detection of airborne spore levels of the pathogens can prevent major crop yield losses by ensuring that timely and appropriate disease management practices are adopted. We also revealed the prevalence of Qol resistant (Qol-R) and Qol sensitive (Qol-S) *cytB* alleles in *PoTl* airborne spores samples from PR by a combination of PCR-amplification and pyrosequencing. For the ASR, in a countrywide parallel study from 2020/21 growing season, we assessed the prevalence in *Pp* airborne inoculum of the *sdhC*-I86F mutation for resistance to SDHI fungicides, which represented ~64% of the samples (N = 25/39), especially in MG, MS, MT and PR states. This discovery is particularly worrisome since these fungicides have been just recently labeled for ASR and resistance seemed widespread already. Next steps should be to open the discussion about the importance, rational, feasibility, partnerships, and source funding of a concerted effort for a national real-time disease surveillance and fungicide resistance monitoring systems built upon epidemiologists expertise and fundamentally based on a network of spores samplers installed across the country coupled with molecular detection of relevant pathogens via RT qPCR (or another molecular detection specific system, such as loop-mediated isothermal amplification (LAMP)), with fully public open access to any sector of the Brazilian society interested in reducing unnecessary fungicide sprays, blocking the spread of resistance in the agroecosystem, and its environmental impact.

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## **Fungicide resistance evolution in global populations of the wheat pathogen *Zymoseptoria tritici***

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The fungal pathogen *Zymoseptoria tritici* (teleomorph: *Mycosphaerella graminicola*) causes worldwide Septoria tritici blotch (STB) in wheat, the most important foliar wheat disease in NW-Europe. In absence of complete host resistance, fungicides are the main method to control STB. However, the continuous use of fungicides on wheat has selected resistant strains of *Z. tritici*, reducing their efficiency for disease control. In NW-Europe, methyl benzimidazole carbamates (MBC) and quinone outside inhibitors (QoI) are no longer used for STB control because of complete resistance, leaving azoles (demethylation inhibitors-DMI) and succinate dehydrogenase inhibitors (SDHI) as options for disease management. Decline of fungicide effectiveness has been observed in other countries where STB also poses as a threat for wheat production. This research has assessed the fungicide sensitivity of 10 globally distributed populations of *Z. tritici*. European isolates appear to be replacing the local genetic background in South America and New Zealand and for this reason populations from these continents are less sensitive for azole fungicides and are evolving convergently towards the more resistant NW-European genotypes. Nonetheless, the North American populations are slowly evolving azole resistance independently to European populations. QoI and MBC resistance are widespread all over the globe, although at lower frequency for non-European populations. Mutants with altered sensitivity to SDHI fungicides are already present in European field populations. No shifts in bixafen sensitivity were detected outside Europe, but 60% of Oregon isolates are insensitive to up to 1ppm of fluopyram. Support: CAPES.

## Aerial spread of smut spores during peanut harvest

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Peanut smut (*Thecaphora frezii*) is one of the most important diseases of peanut in Argentina. The pathogen transforms peanut kernels into spore masses, leading to yield reductions or losing commercial value of the grains. The release of spores from infected pods during the harvest process is supposed to be the main mechanism of pathogen dispersion to neighboring fields, which makes *T. frezii* a particular pathogen: a soil-borne organism infecting underground organs with aerial dispersion. We conducted an observational study in six peanut fields at the harvesting moment to estimate the extent of spore dispersal. We distributed passive spore traps placed at 100, 200, 300, and 400 m away from each harvesting field's centroid in the four cardinal directions. Three time slices were sampled: from the beginning of harvest to 90-, 180-, and 270-minutes during harvest. Wind speed and direction were recorded at each trap. Additionally, spore size and proportion of different smut spore types were assessed (from a single unit spore to a 5-multinuclear propagule). A generalized additive model was fitted to the spore count data (considered to belong to a tweedie distribution). Based on the use of AIC, the model that best fit the data, included: the smoothed terms for the cartesian coordinates of the spore traps placed around the harvest fields (x and y); the smoothed time slice variable (time\_slice in minutes); the wind direction in relation to the traps' placement (degree\_dif); the traps' distance from the harvest field, (distance\_m); and the field and traps themselves as a random effects, field and xy. The response versus fitted values clusters randomly around the 1:1 line and presence of autocorrelation in the residuals was checked. The steepest gradient (more negative) of spore deposition was observed towards upwind traps in contrast to those located downwind. Along the studied range of distances we observed an increase in the amount of spores the three time slices included. No statistical differences were observed in the proportion of the spore types trapped, upon Pearson's Chi-squared test ( $P = 0.1959$ ). Despite some experimental limitations (such as low trap height), our work provides the first study inspecting the role of wind in the short distance dispersal of *T. frezii*, and needs further efforts for model training and validation, which can support the design and implementation of integrated pest management strategies.

## **The plant diseases epidemiology in the tropical plant health network scope**

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The Brazilian agribusiness supply chains now have an even stronger support for scientific research on diseases, pests and weeds in agricultural systems of grain and fiber crops such as cotton, corn, soybeans, and wheat. A nationwide initiative called Tropical Plant Health Network (RFT) arises to organize, strengthen and optimize existing collaborative networks between public and private research centers. This national cooperative research network has developed a website (<https://www.fitossanidadetropical.org.br/>) to centralize important information that was previously spread among different research groups and institutions in the national territory. This work proposal aims to optimize existing collaborate networks for multiple biological targets between public and private research centers to develop applied studies in plant health. The idea was to consolidate almost 20 years of experiences in collaborative research in this sub-area of agricultural sciences and agronomy, and at the same time form a basis for the development of future research networks. The RFT was created to strengthen the integration between projects, researchers, and specialties, to enable the opening and acceleration of innovative scientific research fronts, to improve communication with the various sectors of agriculture and, above all, to reduce the dispersion of efforts. Our purpose has been to contribute to the success of farmers and agribusiness companies in overcoming plant health problems and to provide information to promote the maintenance of yields and the sustainability of crops. For farmers, the information obtained by the RFT could be a valuable tool to support assertive decisions, which enable them to increase efficiency in the use of inputs, promote yield stability, obtain fibers and safe food, and increase their income, while respecting the natural resources. With activities in plant pathology, agricultural entomology and weed science areas, RFT already brings together more than 34 public and private research centers and several supporters from funding organizations and industry sectors. This document aims to present to the researchers from VI Brazilian Workshop on Plant Diseases Epidemiology, in Chapecó/SC, the advantages and benefits, the potential reach and structuring process of the RFT. Support: FAPEAGRO. Acknowledgements: Soybean Trials Cooperative Network, Wheat Trials Cooperative Network and Cotton Ramularia Network.



## **From the individual to the collective: Challenges of plant health, a view of the governmental plant protection agency**

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In agriculture, categorically, the movement of people and commodities worldwide has favored epidemics in crops, caused by emerging plant diseases resulting from the introduction of pathogens and strains in new geographic areas, which may have the dispersion and establishment favored by climatic conditions and ecological interactions in this new location. Brazil has more than 300 plant pathogens listed as quarantine pests and fungal pathogens lead the global biological invasions, generating not only impacts on agricultural production and access to trade, but also the loss of direct and indirect jobs, mainly in economically vulnerable populations. In addition, they are often associated with environmental and public health problems, whether correlated with the use of pesticides or the loss of biodiversity. Faced with scarce resources, the task of defining and maintaining preventive containment, eradication or suppression actions for each of these organisms is hard. The strategy adopted by several countries, including Brazil, is the prioritization of pests, and the elaboration of contingency plans or management strategies for them. However, the feasibility of the planned phytosanitary measures, even if they cover both public and private institutions, depends on the understanding of the risks associated with the activities of everyone involved. The biggest challenge is when the measures to be applied depend on changes in behavior and habits, especially those related to greater organization and greater work force in a synchronized way across the entire area of occurrence of the disease. In this context, the risk management of emerging plant diseases needs to consider the characteristics of the landscapes, the cultural aspects of the population, the dynamics and response capacity inherent to the production chain involved. To exemplify the different perceptions and challenges, we took as a basis the occurrence of plant diseases that have official control, that is, they have measures regulated in specific legislation of the state due to the potential to cause losses in crops of economic importance in the territory of Santa Catarina, for instance: European canker (*Neonectria ditissima*), Passionfruit woodiness disease caused by *Cowpea aphid-borne mosaic virus* (CABMV), *Huanglongbing* (HLB), caused by "*Candidatus Liberibacter asiaticus*". In addition to the imminent risk of the entry of *Fusarium oxysporum* f. sp. *cubense* race 4 Tropical (Foc R4T). We understand that the success of the measures adopted elapse from the early detection and diagnosis of the pathogen and the characteristics of the pathosystem. Thus, surveillance programs cannot ignore the processes that determine the dynamics and spread of the disease. Strategies that involve regional management were adopted more easily when the interest came fundamentally from the productive sector, when there is a demand for laws and regulations and predicted sanctions and the obligation to adopt phytosanitary measures by all those who may be responsible for the plant disease dissemination, which justifies the coordination of plant health programs by government agencies. We will discuss how the challenges listed can be mitigated, punctuating experiences, achievements and perspectives of plant health, in the view of the plant protection agency of Santa Catarina (Cidasc) and how we can advance in a greater integration between research, education, technical assistance, rural extension and plant health sector, consolidating the one health concept.

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# **SPEED TALKS AND POSTER SESSION**

## Evaluation of a generic model to predict garlic rust on San Valentim cultivar in Caçador, SC

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Garlic rust caused by the fungus *Puccinia porri*, is a disease of widespread occurrence in all regions of the world and, during the cultivation of susceptible plants, there are environmental conditions favorable to their progress. The objective of this work was to validate a generic model for the prediction of garlic rust. An agrometeorological station was used to collect the meteorological data that were inserted in the model used to predict the occurrence of rust. For the development of the model, the life of a pathogen was taken as a basis, creating an easily parameterizable structure. All processing takes place through the exchange of data on the number of organs created and the available area of each one (healthy area) at that moment and through information on the area that the disease takes place (infected area). From the combination of temperature, relative humidity ( $\geq 90\%$ ) and precipitation (input data) variables, the model performs the simulation and provides as output data the amount of spores present in the environment. Field experiments were carried out with cultivar San Valentim, in the 2019, 2020 and 2021 harvests, at the Experimental Station of Caçador (Epagri). The inoculation of plants with the fungus *P. porri* occurred naturally in the field. From the cloves differentiation phase, the weekly evaluations of incidence and severity of rust in the field began. To assess severity, a diagrammatic scale was used. Data collected in the field were compared with simulated data to validate the model. It was verified by the simulated and observed data that the years 2019 and 2020 were quite epidemic for garlic rust. In the 2021 harvest, there was less severity compared to previous years. The simulated and observed data showed a similar trend in the rust epidemic, indicating that the generic model is correctly predicting rust. The garlic rust prediction model could be a useful tool for technicians and producers, in the decision making of the ideal moment to carry out the disease control. Support: FAPESC.



## **Conidia release and ascospores ejection of *Neonectria ditissima* in apple branches in São Joaquim, Brazil**

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Brazilian conditions during spring/summer, with prolonged periods of leaf wetting, high temperatures, and relative humidity, are ideal conditions for apple diseases. Only in 2012 European canker caused by *Neonectria ditissima* was officially confirmed in Brazilian apple orchards. The disease has high potential to damage fruit production, and reduces the productivity. Due to the need to eliminate symptomatic branches, and/or all apple tree to contain epidemics within the orchards, knowledge of *N. ditissima* spore release is required to optimize management practices, because it facilitates prediction of European canker infection risks and improves disease management decisions. Thus, the objective of the study was to investigate the *N. ditissima* spore release in apple plants and detached branches over time. In São Joaquim, Santa Catarina, in Brazilian South region, both conidia and ascospores of *N. ditissima* can be released from cankers in apple tissues that are alive and/or dead during all year. Peaks of spores release of *N. ditissima* were observed in traps when there is rainfall, irrespective of the season in Brazil. Ascospores peaks were more observed and higher in comparison to conidia collected in both traps and apple tissues. Symptomatic apple detached branches actively release spores of *N. ditissima* in the three years of evaluation, however in smaller quantity in comparison to traps with alive plants. Thus, management measures should be adopted for the control of European canker dissemination in apple orchards in Brazil.

## Mechanical transmission of *Xylella fastidiosa* to *Prunus salicina* using pruning shears

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Plum leaf scald (PLS) is a systemic disease that causes great economic losses to the fruit growers. *Xylella fastidiosa*, the causal agent, inhabits the xylem vessels and causes the dieback of plum trees. This bacterium is known to be transmitted by sharpshooter leafhoppers and by propagative techniques. However, spatial-temporal analysis showed that leaf scald symptoms appeared in trees along the same planting row in São Paulo and Paraná orchards, which raised the hypothesis that the disease could also be transmitted by pruning shears. Therefore, this study aimed to evaluate the dissemination of *X. fastidiosa* subsp. *multiplex* to *Prunus salicina* by pruning shears. An adult plum tree ('Reubennel') located at a commercial orchard in the municipality of Araucaria (Paraná, Brazil) naturally infected by the bacterium *X. fastidiosa* represented the source-plant. Ten non-infected six-month old plants of each genotype ('Reubennel', 'SC7' and 'SC15') were taken to the field in cages with anti-aphid screen protection in order to avoid the contact with leaf scald vectors, representing the test-plants. All plants (including the source-plant) were tested by polymerase chain reaction (PCR) before the onset of the assay to confirm the presence or absence of *X. fastidiosa*. A 103-bypass pruning shear (Okatsune, Hiroshima, JP) was disinfected before the beginning of the experiment and between genotypes. One cut was made along the infected symptomatic branch (source-plant), immediately followed by one cut on green shoots of the test-plant. This protocol was repeated for six times per each test-plant, totaling six cuts. Plants were placed in an isolated greenhouse with anti-aphid screen protection. The *X. fastidiosa* detection was performed at 90, 270 and 450 days after pruning using polymerase chain reaction and quantitative polymerase chain reaction (qPCR). The results showed that no leaf scald symptoms were visualized in the test-plants until 510 days after pruning. The leaf samples analyzed by conventional and quantitative polymerase chain reaction were negative to *X. fastidiosa* in the first (90 days after pruning) and the second (270 days after pruning) evaluations. However, one sample of 'SC7' genotype was detected as positive by qPCR in the third evaluation (450 days after pruning; cycle threshold  $\leq 28$ ) but was not detected by conventional PCR test. Even with a low rate of transmissibility (4%), contaminated pruning instruments were able to disseminate the disease in healthy seedlings, but without the appearance of leaf scald symptoms. 'SC7' was more sensitive to drought when compared to the other genotypes. In general, water stress increased the bacterial concentration in the xylem vessels. For this reason, we believe that this feature may have facilitated the detection of *X. fastidiosa* in these plants. Likewise, we intend to repeat this experiment using at least two detection methodologies. Nevertheless, in consequence of these first results, the sanitation of pruning instruments using 70% alcohol, sodium hypochlorite and quaternary ammonia is recommended, as a way of preventing the disease dissemination within the orchard. Support: CAPES.

### **First report of ‘*Candidatus Liberibacter asiaticus*’ associated with three symptomatic ‘Ponkan’ citrus tree in Xanxerê, Santa Catarina**

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*Huanglongbing* (HLB) is considered the most threatening disease of citrus. Etiological HLB agents are the Gram-negative bacteria in the Rhizobiaceae family, ‘*Candidatus Liberibacter asiaticus*’, ‘*Ca. L. americanus*’ and ‘*Ca. L. africanus*’. ‘*Ca. L. asiaticus*’ is the main HLB bacteria with occurrence in Brazil, which transmission is aided by the Asian-citrus-psyllid *Diaphorina citri* Kuwayama (Hemiptera: Liviidae). The Brazilian South state Santa Catarina does not figure among the main citrus producers States in Brazil, but the orchards are mainly cultivated in small farms, which brings up *Citrus* spp. as an important agronomic alternative for family farmers. During an inspection service demanded by the Department of Plant Health and Agricultural Inputs of the Secretariat of Animal and Plant Health and Inspection (SDA) of the Ministry of Agriculture, Livestock and Food Supply (MAPA), regulated by a recent released ministerial order (*Portaria* 317, 21 may 2021), a suspect plant was spotted in a productive orchard in Xanxerê, Santa Catarina (geographical coordinates: -26.8774415, -52.406414). The branches of the mandarin plant (*Citrus reticulata* Blanco cv. Ponkan) had been already severely pruned by the grower few weeks before, as he noticed an unusual appearance and a decrease of production. When visited by the plant health officer, it was already emitting a new flush, with the typical appearing of blotchy mottle leaves, with downwards curvature and thickening of veins. A leaf was detached from this plant as a sample and addressed to a laboratory for molecular diagnosis. The laboratory carried out the protocol described in the *Bulletin* EPPO (44:376, 2014) for HLB pathogens detection, which involved the use of primer HLBr combined with primers HLBas, HLBam or HLBaf in a qPCR diagnostic protocol for ‘*Ca. L. asiaticus*’, ‘*Ca. L. americanus*’ and ‘*Ca. L. africanus*’, respectively, using HLBp as a FAM probe. To confirm the positive qPCR result for ‘*Ca. L. asiaticus*’, a conventional multiplex PCR was performed with the forward primers OI1 and OA1 combined with reverse primer OI2c to detect ‘*Ca. L. asiaticus*’ and ‘*Ca. L. americanus*’. A 1160 bp band was produced and the amplicon was sequenced for further *in silico* distinction using XbaI digestion of the amplicon. After ‘*Ca. L. asiaticus*’ detection in that plant, other plants in the very same orchard were inspected and surveyed for molecular diagnosis. HLB bacteria was also detected in other two ‘Ponkan’ tree that was displaying HLB symptoms as described above and producing lopsided fruit, with seed abortion and stain of the vascular columella. A contingency plan was adopted by the Plant Health Agency of Santa Catarina State (CIDASC), in which the infected plant with HLB bacteria has been eliminated by the grower. In addition, yellow sticky traps were installed within the orchard in Xanxerê in order to verify the presence of *D. citri*. Also, the orchards within a radio of 4 km are under inspection. The Plant Protection Agency is currently tracking back the origin of the plants in order to find out whether the diseased plants were introduced via nursery plant or the pathogen was transmitted via resident insect vector. Acknowledgement: Agrônômica Laboratório de Diagnóstico Fitossanitário e Consultoria.

## **The population of the Asian-citrus-psyllid, *Diaphorina citri*, may be low in Santa Catarina**

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The Asian-citrus-psyllid, *Diaphorina citri* Kuwayama (Hemiptera: Liviidae), is the insect vector of ‘*Candidatus Liberibacter asiaticus*’, the main bacterial pathogen associated with *Huanglongbing* (HLB), currently the main citrus disease. HLB have been spotted in cities in Paraná state which are close to the northern border of Santa Catarina state, in South of Brazil, and infected citrus plants have been already reported in Misiones (Argentina) (western neighboring country). In addition, the Asian-citrus-psyllid was already trapped in Santa Catarina in 2006, however with a very expressive capture fluctuation, sometimes without success (Chiaradia et al., Revista de Ciências Agroveterinárias, 2008). A monitoring program for *D. citri* population was established in November 2016 in localities of Santa Catarina and it is currently ongoing in order to assess the presence of psyllids in commercial orchards along Santa Catarina state and their infectivity. Public agencies involved in research and extension (Epagri) and inspection (Cidasc) services are periodically checking 10 citrus orchards situated in Apiúna, Araranguá, Balneário Rincão, Celso Ramos, Chapecó, Itajaí, Mafra, Pouso Redondo, São Lourenço do Oeste and São Ludgero. Yellow sticky traps are installed in the upper part of plants located in the border and interior of the orchard, and they are replaced every 20 days, in two flushing periods of the year: February - March and September - October, so performing three replacements in each period. Orchards are monitored with 4 to 12 traps, depending on the size of the orchard. The yellow traps are addressed to laboratory for examination using a stereomicroscope. Hitherto, 3,580 traps were examined and only five psyllids were found (Araranguá, 1 psyllid, November/2017; Itajaí, 2 psyllids, March/2021; Balneário Rincão, 2 psyllids, February/2021). Insects were removed from the trap and sent to laboratory for individual qPCR analysis, and ‘*Ca. L. asiaticus*’ has not been detected in any of these specimens. We speculate the climate conditions in Santa Catarina are not appropriate for full development of the Asian-citrus-psyllid populations, and perhaps atypical environmental circumstances that hamper the insect capture may have occurred during this study. We hypothesize a low population of *D. citri* in Santa Catarina, which might result in an inefficient ‘*Ca. L. asiaticus*’ dissemination. This could favor a successful establishment of a contingency plan if the pathogen was eventually detected in the State. Acknowledgements: Helvécio Della Coletta Filho (Instituto Agronômico de Campinas) for psyllid qPCR diagnosis; lab technicians Carmen Cella, Neusa Maciel and Zelinda Meneguzzi (Epagri, Chapecó), for traps examination; officers of Epagri and Cidasc, for field monitoring.

## **Nucleic acid extraction and multiplex polymerase chain reaction analysis for the detection of maize stunt causal agents in plants and insect tissues**

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Presently, the corn stunt complex (CSC) is one of the most important diseases affecting corn crops. This complex is associated with infections of at least one of the following pathogens: maize bushy stunt phytoplasma (MBSP), corn stunt spiroplasma (CSS), and maize rayado fino virus (MRFV). Currently, the control of CSC is focused on their vector *Dalbulus maidis* (DeLong and Wolcott), which combines monitoring programs with chemical spraying and biological control in the field. Owing to its RNA genome composition, MRFV detection is performed separately from mollicutes, which contributes to the omission of virus diagnosis in CSC monitoring activities and epidemiological studies. In this study, we developed a complete CSC diagnostic method comprising an RNA/DNA extraction protocol and a triplex polymerase chain reaction (PCR) for detecting all CSC pathogens in a single reaction step. Therefore, an MRFV-specific primer pair was designed for use along with the widely used F2n/R2 and CSSF2/CSSR6 primers for mollicute detection. Total nucleic acid extraction was performed using the CTAB-based protocol. MRFV-specific primer was designed using the Primer3 software (v. 0.4.0) and the two complete MRFV sequences (from the USA and Costa Rica) available in the GenBank. *In silico* tests were conducted to determine the primer specificity and secondary structure formation using BLASTn and IDT oligo Analyzer tools, respectively. The newly MRFV-specific designed primer pair was tested *in vitro* for its specificity and sensibility and subjected to a temperature-gradient PCR to adjust the best virus detection conditions. The MRFV primer was then combined in a triplex PCR with the mollicute-specific primers using samples from different Brazilian states, retrieved from infected insects and plants. The designed MRFV primer pair was species-specific, sensible, and did not form secondary structures or dimerize either with each other or with the other primers used to detect mollicutes. The primer was used to amplify a 270-bp fragment comprising the 5' end of the polyprotein gene of MRFV-infected samples from different geographical locations and tissues (both plants and insects) in a multiplex reaction. Three positive bands comprising the three pathogens (with sizes of ~270, 500, and 1250 bp, for MRFV, CSS, and MBSP, respectively) were visibly separated in the agarose gel, thereby yielding a clear interpretation of the outcomes. The results revealed that the proposed method was effective, cheaper, faster, and easier to perform relative to the other methods reported in the literature. Therefore, we recommend this tool, which is helpful for basic, applied, and epidemiological analyses and for monitoring programs conducted to evaluate the viral incidence in the corn stunting complex and corn crops. Support: FAPESC 2021TR1246, CAPES, and CNPq.



## **Aphid fauna (Insecta, Hemiptera) associated with transmission of plant viruses to vegetable brassicas in the green belt of São Paulo**

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Recent surveys, carried out in vegetable brassicas producing regions of Brazil, revealed the predominance of viruses whose spread in the field is associated exclusively to aphids, including turnip mosaic virus (TuMV, *Potyvirus*), cauliflower mosaic virus (CaMV, *Caulimovirus*), cole latent virus (CoLV, *Carlavirus*) and cole mild mosaic virus (CoMMV, *Carlavirus*), transmitted in a non-circulative manner, and turnip yellows virus (TuYV, *Polerovirus*), transmitted in a circulative manner. In this context, the State of São Paulo stands out as the largest national producer with about 50% of the areas destined to vegetables occupied with a wide range of species and varieties of brassicas, which the green belt of the city of São Paulo (GBSP) is the largest center of production and distribution, and where interactions of aphid fauna in the transmission and spread of these viruses is still poorly studied. Aiming to evaluate the population dynamics of aphid species with potential for transmission of TuMV, CaMV, CoLV, CoMMV and TuYV, two commercial fields located in the GBSP, in the municipalities of Biriba Mirim (23° 34' 22"S; 46° 02' 20"W) and Ibiúna (23° 39' 23"S; 49° 13' 21"W) were chosen for carrying out these surveys. The captures and identifications of the winged (migrant) aphid fauna were carried out fortnightly in the 2019, 2020 and 2021 agricultural years using Moericke yellow traps. The data obtained were analyzed considering the ecological parameters of specific diversity (Shannon Index) and occurrence and frequency. The aphid fauna of the two monitored areas was characterized by low diversity, being composed of about fourteen polyphagous and oligophagous species. The polyphagous species *Aphis fabae/solanella*, *A. gossypii* and *Myzus persicae* behaved as common species, while *Aphis craccivora*, *A. nasturtii*, *Aulacorthum solani* and *Macrosiphum euphorbiae* were classified as intermediate species. The oligophagous species *Brevicoryne brassicae* and *Lipaphis erysimi*, considered pests of brassicas, behaved as common species and, in turn, *Aphis nerii*, *Hyperomyzus lactucae*, *Nasonovia ribisnigri* and *Uroleucon ambrosiae* were classified as intermediate species, and *Pentalonia nigronervosa* and *Greenidea* sp. as rare species. Considering the population dynamics of the aphid fauna in the monitored areas, it was possible to estimate that the common polyphagous species are closely related to the spread of TuMV, CaMV, CoLV and CoMMV, while the common oligophagous species are involved with the transmission of TuYV. The understanding of the ecological and epidemiological aspects of the aphid fauna associated to plant viruses, recurrent in the GBSP, will help, above all, in the adoption of management strategies aimed at reducing production losses, since there are no effective resistance genes in commercial varieties of brassicas. Support: FAPESP (PROC. Nº 2018/17287-4; 2014/22594-2), CNPq/IC (IB/2021-2022), CNPq/PQ (PROC. 305336/2021-5); *This study was financed in part by the Coordenação de Aperfeiçoamento de Pessoal de Nível Superior - Brasil (CAPES) - Finance Code 001.*

## **Assessment of the resistance of PIWI varieties and selections to grapevine vine downy mildew and the genotype interaction vs environment**

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The production of grapes for wines in regions between 900 and 1,400 meters of altitude in Santa Catarina has been increase. However, the control of fungal diseases, such as downy mildew (*Plasmopara viticola*), raises the cost of phytosanitary treatments, as well as offering risks to the environment and human health. Resistant *V. vinifera* varieties called PIWI, the acronym from the German word “Pilzwiderstandfähige”, which means fungus resistant, have been released in last two decades, mainly in Europe. Thus, the aim of this work was to evaluate the temporal dynamics of grape downy mildew in PIWI varieties and selections with resistant genes to powdery and downy mildew in different edaphoclimatic conditions. The experiment was conducted in two vineyards, the first one located in the Agricultural Experimental Area of the Federal University of Santa Catarina, Campus of Curitibanos, Curitibanos, SC, and at the Epagri Experimental Station, Videira, SC, in the 2018/19 growing seasons. The experimental design used was bifactorial, with four replications, arranged in complete randomized blocks, with nine Piwi genotypes and a susceptible control (factor 1) evaluated in two locations (factor 2). Graphs of the area below the disease severity progress curve (AUDPC) of each variety and selection were made, comparing the susceptible genotype in relation to the onset of symptoms (OS), maximum ( $S_{max}$ ) and minimum ( $S_{min}$ ) value of severity, time to reach maximum disease severity (TRMDS). Samples were comprised 30 leaves, including young and old leaves, with different responses. Using the Buffara scale, the lowest value of AUDPC was presented by the selection Gf.2004-043-0004 (3398.65) in Curitibanos; while in Videira the lowest AUDPC was obtained by Cabernet Cortis (22403.55). The AUDPC values of the witness were 1677.87 and 2689.89, in Curitibanos and Videira, respectively. The beginning of the appearance of symptoms (BAS) value in Curitibanos ranged from 20 to 24 for all varieties and selections PIWI, except for the control that reached 32. In Videira, the BAS values for the varieties were higher (ex: 42 in Gf.2004-043-0013, but lower in the control (15). The  $S_{max}$  in Curitibanos was revealed by Cabernet Cantor (85.8), in Videira by Cabernet Cortis (56.3). In both locations, the control obtained the highest OS value (75). TRMDS was 116 and 100 in the selection Gf.2004-043-0004, in Curitibanos and Videira, respectively, very similar to the value presented by the control (100). Due to the statistical significance of the interaction between genotypes versus environment, breeders should select varieties better adapted to each location. Financial Support: FAPESC/TO2017TR1844, FAPESC/TO2021TR002096 and CNPq. Acknowledgments: RGV and UFSC.

**Keywords:** *Plasmopara viticola*; Severity; Resistance to fungal diseases.

## **Spatial and temporal incidence of maize rayado fino disease in a second crop season experimental field in Chapecó, Santa Catarina, Brazil**

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The *Maize rayado fino virus* (MRFV) is the causal agent of the maize rayado fino (MRF) disease and is transmitted to plants by the insect vector *Dalbulus maidis* (Hemiptera: Cicadellidae). The main symptom is characterized by numerous small chlorotic spots along leaf veins, and the disease has the potential to significantly reduce grain yield. To better understand this disease dynamics, we evaluated the spatial distribution pattern and the temporal evolution of MRF incidence on second crop season maize in an experimental field in Chapecó (Western Santa Catarina). The genotype used was P30F53 VYHR, sowed in a field of 24 rows spaced in 0.9 m and 30 m long, in February 2019. All the 2880 plants that composed the experimental field were visually evaluated at 5, 8 and 11 weeks after sowing, corresponding approximately to V8, V11 and R2 phenological stages, respectively. Plants were assigned as diseased or healthy and mapped. The statistical analysis of spatial and temporal incidence of MRF was carried out with software R, using Rcitrus package. The disease incidence was 6.8%, 33.4% and 53.6% at 5, 8 and 11 weeks after sowing, respectively. The dispersion index was calculated to three different quadrat sizes (2x6, 3x9 and 4x12) and indicated aggregated distribution of the disease for all quadrat sizes in all evaluation dates ( $p < 0.05$ ). The Monte Carlo test also indicated aggregated pattern of MRF distribution in a 3 m radius, with respective 2.1 and 1.6 observed and estimated diseased neighbors near to other infected plants at 5 weeks ( $p = 0.005$ ); 8.4 and 8.1 observed and estimated diseased neighbors, respectively, at 8 weeks ( $p = 0.003$ ) and 13.5 and 13.0 respective observed and estimated diseased neighbors at 11 weeks ( $p = 0.0017$ ). The dispersal kernel showed higher aggregation of disease in the border of the experimental field. The disease gradient increased from the borders to the center of the experimental field along the three evaluations, suggesting primary dissemination of MRFV in the bordering plants by infective leafhoppers that probably emigrated from older adjacent maize crop. It was verified a 5 fold increase in the disease incidence on the second evaluation in relation to the first assessment, evidencing that a secondary dissemination of MRFV inside the experimental field also happened, which resulted in the inoculation of plants in the center of the experimental field, in a later developmental stage of the crop. These results help to understand MRF incidence and evolution within a crop situation, and can be useful to plan accurate management strategies as seed treatment, early chemical control of the vector, especially on the crop borders and sowing synchronization in order to avoid the establishment of new fields close to older contaminated crops.

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## **Dispersão de *Pseudocercospora vitis*, agente causal da mancha das folhas da videira, nas condições climáticas da região metropolitana de Curitiba**

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A mancha das folhas da videira (MFV) é causada por *Pseudocercospora vitis*. No Brasil, a MFV ocorre em cultivares de *Vitis labrusca*, na maturação dos frutos ou após a colheita. O controle da MFV é realizado com a aplicação de produtos em parte aérea, porém a eficiência de controle não tem sido satisfatória. Neste sentido, estudos epidemiológicos, como a dispersão de inóculo no ciclo de cultivo, são importantes para posicionar as ferramentas de controle - que estão disponíveis e que têm sido desenvolvidas. Desta forma, o objetivo deste trabalho foi monitorar a presença de conídios de *P. vitis* ao longo das safras 2020/21 e 2021/22, no município de Campo Largo, Paraná. Para isso, armadilhas caça-esporos foram instaladas na área experimental, constituídas de lâmina de vidro de microscopia impregnada com vaselina. As lâminas foram colocadas no interior de um cilindro aberto, na altura de um metro, que se movia conforme a direção do vento. O número de conídios de *P. vitis* foi avaliado semanalmente, entre setembro (início da brotação) e março (final do ciclo vegetativo), com auxílio de microscópio. As variáveis climáticas de temperatura e molhamento foliar também foram monitoradas nas duas safras, por meio de sensores instalados dentro da área experimental. Conídios de *P. vitis* foram observados a partir da segunda semana após a brotação (SAB) em 2020/21 e 2021/22. Em ambas as safras, o número de esporos cresceu significativamente após a 17ª SAB (janeiro), período de pré-colheita dos frutos. Entretanto, o somatório de conídios após a 17ª SAB foi maior na safra 2020/21 em comparação à 2021/22. Em 2020/21, a temperatura média das primeiras semanas após a brotação (até 17 SAB) oscilou de 16,2 a 22,6°C e sete períodos de molhamento foliar com duração maior do que 24 horas foram observados. Em 2021/22 as temperaturas foram mais amenas (abaixo de 20°C) e apenas um período de molhamento foliar com duração superior a 24 horas foi verificado até a 17ª SAB. Conclui-se que o inóculo de *P. vitis* está disponível para infecções muito tempo antes do período em que normalmente ocorre a expressão de sintomas da MFV no Brasil. Além disso, os resultados sugerem que a quantidade de esporos de *P. vitis* presente na fase de pré-colheita dos frutos pode estar associada com as condições climáticas no início do ciclo da videira e ao longo período de incubação de MFV. Suporte: CAPES.

## Infectivity of corn leafhopper, *Dalbulus maidis* (Hemiptera: Cicadellidae), with Maize bushy stunt phytoplasma and *Spiroplasma kunkelii* in Santa Catarina State, Brazil

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Great amount of white-pale insect of no more than 0.5 cm flying off corn plants has been observed by maize producers in Santa Catarina State (SC), Brazil. In fact, it was registered a great loss in 2020/21 year-crop due to corn stunt disease complex incidence, which is caused by two mollicutes, the Maize bushy stunt phytoplasma (MBSP, 'Candidatus Phytoplasma asteris', 16Srl-B), the corn stunt spiroplasma (*Spiroplasma kunkelii* Whitcomb), and the virus (*Maize rayado fino virus*), all pathogens transmitted by the corn leafhopper, *Dalbulus maidis* (DeLong & Wolcott) (Hemiptera: Cicadellidae). We intended to verify the natural infectivity of *D. maidis* with both mollicutes during the maize first crop in 2021/22 year-crop. In this sense, we installed yellow sticky traps in 22 corn crops of different municipalities in SC (14 crops in the West region, 3 crops in the North region, 2 crops in Serrana regions, 2 crops in Itajaí Valley and 1 crop in South region). The traps were weekly replaced during 27 weeks (from July, 2021 to January 2022) and immediately addressed to the laboratory for molecular diagnostics. If there were up to 3 insects captured by the traps, we performed the diagnosis of all of them individually; if more than 3 insects, we performed diagnosis in triplicates until 7 samples. After a CTAB DNA extraction of those insect samples, a multiplex PCR was performed using primers R16F2/R16R2 and CSSF2/CSSR6 for MBSP and *S. kunkelii* detection, respectively. We analyzed 1571 samples. We obtained 161 positives samples for MBSP (10.24%) and 254 positives samples for *S. kunkelii* (16.16%). In spite of finding some corn leafhoppers in the early weeks of the assay, we started to get positive-PCR for both Mollicutes from the 4<sup>th</sup> week on, when corn crops have all already been sown. Corn crops in Itapiranga and Palmitos, both in the West region, presented the highest number of week events of MBSP-positive samples, respectively, 44.4% and 50%, whereas corn crops in Caxambú do Sul, União do Oeste, Chapecó, São Miguel do Oeste (all located in the West region) and Ituporanga (Itajaí Valley) presented, 47.8 to 55.6% of *S. kunkelii*-positive events. The 4<sup>th</sup> and the 22<sup>nd</sup> were critical for MBSP detection, in which we got 83.3% and 100% of positive samples for MBSP. Several weeks were important for *S. kunkelii*, as we found more positive-events at 4, 6 and 7 weeks after the beginning of monitoring (more than 80% of positive events) and from 22<sup>nd</sup> week on after traps installation (more than 65%). In this sense, there was more *D. maidis* infectivity with *S. kunkelii* along the first planting and both mollicutes were detected in the leafhoppers when maize was already sown. The crops located in the West region showed more insect-infectivity with both pathogens. We noticed a fluctuation of insect-infectivity through the weeks. A crop season surveillance such as we performed shall help to determine the best time-points and locations for *D. maidis* infectivity assessment with MBSP and *S. kunkelii* to establish an efficient and achievable monitoring program and risk analysis for the corn stunt disease complex.

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***Dalbulus maidis*, the insect vector of *Maize rayado fino virus* (MRFV), does not distinguish between two maize genotypes in field conditions but seems to prefer asymptomatic plants**

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The corn leafhopper *Dalbulus maidis* (DeLong & Wolcott) (Hemiptera: Cicadellidae) is the vector for the *Maize rayado fino virus* (MRFV), and recent studies have demonstrated its ability to discriminate different maize genotypes (Virla et al. 2010; Faria et al. 2021). When a persistently transmitted virus is involved in a plant-insect interaction, a series of modifications can also occur that aids transmission and dissemination process, involving changes in the insect vector's behavior (Mauck et al. 2012). This study aimed to verify the presence of *D. maidis* on MRFV-symptomatic and non-symptomatic plants of P4285 hybrid (resistant to order Lepidoptera) and SCS156 Colorado (an open pollination variety) in a field experiment. The incidence of MRFV diseased plants and plant production was also recorded in both genotypes. A field study was carried out in the second season after maize genotypes were sown at Epagri experimental field in Chapecó (Santa Catarina, Brazil) in January 2021. The experiment was set with 6 parcels for each genotype, with 6 rows of 5 m and spaced 0.8 m. Plants in the rows were spaced with 0.2 m. Corn leafhoppers were visually counted in the maize whorl at 9, 12, 14 and 16 days after sown (d.a.s.). When it was no longer possible to count the insects directly from the plant, yellow sticky traps were installed in the center of the plot at 1 m high on the 23<sup>rd</sup> d.a.s. The traps were replaced weekly, for three consecutive weeks. The number of *D. maidis* in the plant whorl or captured in the traps was not statistically different for P4285 hybrid or SCS156 Colorado. Maize plants were displaying symptoms of MRFV at 14 d.a.s. We evaluated the incidence of MRFV-symptomatic plants by observing 10 plants in the center of the plot. There were more MRFV diseased plants in P4285 than in Colorado ( $\bar{x}$  = 2.50 and 0.16, respectively,  $P$ -value = 0.001). We counted the number of *D. maidis* in 10 MRFV-diseased plants in each plot, and there was more leafhoppers in asymptomatic plants than in symptomatic, disregarding the genotype ( $\bar{x}$  = 7.3 and 5.4, respectively,  $P$ -value = 0.008). Plants (~12 each plot) with and without MRFV symptoms were tagged during experiment conduction, and their cobs were harvested during physiological maturation in order to check plant production. The production of each maize genotype was affected by MRFV disease, as SCS156 Colorado produced 42% less if MRFV symptomatic plants, while P4285 produced 45% less in comparison with asymptomatic plants. In conclusion, *D. maidis* seems not to distinguish between P4285 and SCS156 Colorado in field conditions. However, a higher incidence of MRFV in the hybrid was observed in the early days of the crop, which may be due to a shorter incubation period of the virus *in planta*. Insects were more numerous in asymptomatic plants of either genotype, suggesting either a preference for symptomless plants or a manipulation of the virus for spreading. In the end, MRFV affected the productivity of both maize genotypes.

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## **Temporal transition of microbial communities and chemical properties of soil related to *Fusarium* wilt disease in one banana field**

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*Fusarium* wilt of banana (FWB), caused by *Fusarium oxysporum* f. sp. *cubense*, is of widespread occurrence in Brazil. The average incidence of FWB was estimated to be 11% and biotic and abiotic soil properties are hypothesized to influence the temporal and spatial dynamics of the disease. The main purpose of this work was to assess the changes in biological and nutritional factors associated with plants affected by FWB over time, under field conditions. Soil samples from six asymptomatic banana mats (cv. 'Maçã', Silk subgroup), grown in the field, were collected in 2018 and another sample set from the same six mats, later symptomatic, were collected in 2020. Metabarcoding analysis using environmental DNA was conducted with the Illumina Miseq platform (2x250 bp). For bacterial communities 515f-806r primers targeting the V4 region of the 16S SSU rRNA and for fungal communities ITS1-ITS2 primers were used for the internal transcribed spacer (ITS) region. Amplicon sequence variants (ASVs) were assigned to taxa and the relation between phytosanitary states was determined. Changes in the microbial communities over time were assessed by permutational multivariate analysis of variance (permanova) and association network. For soil analysis, organic matter content, micronutrients and routine chemical attributes were assessed. Principal component analysis (PCA) biplot was used to assess the relationships between main chemical variables and FWB. A total of 3120 fungal and 6830 bacteria ASVs comprised the microbial communities associated with banana mats. The highest bacterial and fungal richness were observed in symptomatic mats. The fungal communities varied according to the phytosanitary state, bacterial communities not and no temporal difference was detected when the same banana mats were sampled in 2018 and 2020. Network analysis was conducted with 180 fungal and 249 bacterial family taxa. In symptomatic plants 14 hubs were present for both communities while for asymptomatic plants 12 hubs were detected for fungal and 16 for bacterial communities. PCA biplot showed that the capacity of effective cationic exchange, cation exchange capacity, sum of bases and concentration of Calcium and Zinc apparently were positively/negatively related with asymptomatic/symptomatic plants. Phytosanitary states, symptomatic and asymptomatic, in one banana field are related with alterations in microbial communities and nutritional composition of soil over time.

### **Pathogenic specificity of *Austropuccinia psidii*, the causal agent of myrtle rust**

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The myrtle rust (*Austropuccinia psidii*) has been associated with more than 480 plant species belonging to the family Myrtaceae. Intraspecific diversity, based on pathogenic and morphological variability, has already been reported among isolates of *A. psidii* from different hosts. However, there are few studies that have comparatively quantified the pathogen and disease in guava (*Psidium guajava*) and rose apple (*Syzygium jambos*). The objective of this work was to verify the specificity of monosporic isolate of *A. psidii* from guava 'Paluma' and its compatibility with rose apple. Young leaves of rose apple potted plants were inoculated with suspensions of monosporic isolates of *A. psidii* from guava and rose apple at the concentration of  $2 \times 10^4$  urediniospores mL<sup>-1</sup>. The monocyclic components evaluated over time were the incubation and latent periods, density of lesions and disease severity. Lesion density and disease severity were evaluated on the upper leaf face in two areas of 2 cm<sup>2</sup> per plant. The severity of the disease was quantified with the Quant software based on scanned images of 2 cm<sup>2</sup> of leaf per repetition. Additionally, histological analysis with confocal laser microscopy was performed to characterize the infection of the same isolates of *A. psidii* in rose apple. For this, leaf samples were collected at 1, 2, 9 and 13 days post inoculation, and fixed in trichloroacetic acid in 4:1 ethanol/chloroform. After leaf diaphanization, leaf tissue samples of approximately 0.5 × 0.5 cm<sup>2</sup> were fixed in a staining solution containing 20 µg mL<sup>-1</sup> of WGA-Alexa Fluor 488, 50 µg mL<sup>-1</sup> propidium iodide, 20 µg mL<sup>-1</sup> BSA and 0.1% Triton X in PBS pH 7.4, vacuum infiltrated and incubated over night at 10 °C. Confocal laser microscopy was conducted in sequential scan mode at Leica SP8 microscope. The incubation and latent period was similar for both isolates. High density of sporulating lesions was observed in rose apple inoculated with rose apple *A. psidii* isolate. Necrotic lesions were observed in rose apple inoculated with guava isolate and sporulating lesions were only occasionally observed in this interaction. The mean severities of the disease in rose apple inoculated with rose apple and guava isolate were, respectively, 19.9% and 6.4%. The monomolecular and logistic models of population growth (Bergamin, 2018) were fitted to the temporal evolution of lesion density and disease severity, by means of nonlinear regressions, with Statistica software (Statsoft). The initial process of infection of the two isolates of *A. psidii* in rose apple was similar. For the two isolates, haustoria with a globular structure with a highly rugose surface was observed within the mesophyll cells. Although sporulating lesions were not observed in rose apple leaves inoculated with guava isolate, there was extensive colonization by the pathogen and haustorium formation at 2 days post inoculation.

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## Optimizing the frequency of insecticide application to control primary infections of Huanglongbing in citrus edge blocks

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Huanglongbing (HLB) is caused by '*Candidatus Liberibacter asiaticus*' (CLAs) and transmitted by the Asian citrus psyllid (ACP) *Diaphorina citri*. HLB is a polycyclic disease with continuous primary spread by infective ACP coming from outside the citrus orchard. This migrant ACP population presents a decreasing gradient in relation to the orchard edge. Thus, the prevention of new primary infections in orchard edges, originating from ACPs that acquired the bacteria in inoculum sources outside the commercial orchard, is essential for the HLB control. While fortnightly insecticide sprays are sufficient to prevent secondary infections, weekly insecticide sprays are required to control primary infections, because of the continuous arrival of infective ACP and low residual control of insecticides applied especially during citrus flushing. Therefore, the objective of this work was to optimize the frequency of insecticide application in different widths of the citrus block edge strip located on the orchard border to control primary infections of HLB depending on ACP density. For this, a theoretical approach to estimate the number of HLB-symptomatic trees in a hypothetical plot was developed. A hypothetical 3-years old citrus block was considered, with 497 m of width by 500 m of length, spacing of 7 m between rows and 2.5 m between trees (72 rows of 201 trees, total of 14472 trees), parallel to the orchard border. The several scenarios refer to the different widths of the edge strip that received the weekly insecticide spray, ranging from the first row to the entire block. In the first scenario, the first row of the plot was sprayed every 7 days, while the remaining 71 rows were sprayed every 14 days; and the last scenario corresponded to the entire plot (72 rows) sprayed every 7 days. The number of HLB-infected tree in each row was estimated by the number of migrating ACP (data from Fundecitrus Alert System - FAS), the gradient of ACP from the edge (experimental data), the mean percentage of ACP with CLAs obtained (data from literature), the infection efficiency (data from literature), and insecticide spray efficiency (experimental data). Different densities of migrating ACP were compared: the average ACP population from FAS, 0.5x FAS, 2x FAS, 3x FAS, and 5x FAS. All the other variables were kept constant throughout the simulation period. Accumulated benefit and costs after 5 productive years were considered in the analysis and were calculated according to tree's age (data from CEPEA and Fundecitrus). Benefit consisted in the income received from remaining bearing trees and cost in the expenses with insecticide spray, removal of infected trees, and reset. The economic approach consisted in calculate the additional profit for each scenario using the Net Present Value method. Additional benefit and costs related with each scenario were compared with fortnightly frequency of insecticide spray in total area. The accumulated number of trees with HLB symptoms estimated for 1 year from FAS density ranged from 289 symptomatic trees (2% of the trees present in the plot) in the scenario with the highest edge strip width (497 m) sprayed every 7 days to 455 symptomatic trees (3.1% of the trees present in the plot) in the scenario with the first row sprayed every 7 days. In areas with 0.5X, 1X, or 2X, the edge strip width that offers the highest additional profit was 14 m, 77 m, and 329 m, respectively. In scenarios with high ACP populations (3x and 5x FAS), it was economically viable to spray the entire plot every 7 days. Primary ACP spread is not completely bypassed by insecticide sprays. Growers should be motivated to continuous participate in area-wide control campaigns to reduce ACP density within the region and, consequently, reduce the edge strip that will receive weekly insecticide spray frequency. Support: FAPESP (projects #17/21460-0 and #19/19481-5) and CNPq (project 304253/2020-0).

## **Assessment of *Alternaria* Brown Spot outbreaks on tangerine orchard by using an ordinal rating scale**

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The *Alternaria* Brown Spot (ABS), caused by *Alternaria alternata*, is a major threat to orchards of tangerines and their hybrids in Brazil. The pathogen affects young leaves, shoots and fruit resulting in leaves abscission, twig dieback and fruit drop in orchards severely affected by ABS. The progress of ABS in the field is still not well comprehended and complex to assess. Outbreaks of ABS have been reported in the Paraná state, mainly in the municipality of Cerro Azul, the largest producer of tangerines in Brazil. However, there are no epidemiological studies for ABS in this citrus growing region. Thus, this study aimed to propose a new ordinal rating scale and to assess the progress of ABS in the field. A field trial was conducted during the season 2021-22 in a commercial ‘Ponkan’ tangerine (*Citrus reticulata*) orchard planted in 2019 in Cerro Azul (24°57'27.36"S, 49°22'1.58"W, altitude of 333 m). Tangerine trees were not sprayed with fungicide along the experiment assessments from October/2021 to June/2022. An ordinal rating scale was proposed to quantify ABS symptoms based on the disease levels, where: Level 0 – no ABS symptoms; Level 1 – young shoots only with ABS on leaves (< 50% lesioned area); Level 2 – young shoots only with ABS on leaves (> 50% lesioned area); Level 3 – ABS on leaves and twigs of young shoots (< 50% lesioned area); Level 4 – ABS on leaves and twigs of young shoots (> 50% lesioned area); Level 5 – ABS on young shoots and twigs, leaf abscission and diseased fruit. Two ABS outbreaks were observed, the first at the end of December/2021 and another one in March/2022. ABS outbreaks occurred in periods with temperatures above 25 °C and relative humidity above 80%. Levels 1 and 2 were the most observed during the first and second sprouting period. In addition, young shoots from the first sprout were affected by ABS rapidly and probably served as inoculum source for the second sprout, where the levels 3 and 4 were significantly higher. This experiment provided new information on ABS progress in the field and a potential scale for the disease quantification to be used in other studies with this pathosystem. Support: CAPES.



### Effect of early harvest on wheat *Fusarium* Head Blight severity

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Mycotoxins are toxic metabolites produced by fungi while crops are still on the fields or even after harvest. Their occurrence is related to the biosynthesis capacity of fungi that infect the kernels, the environmental conditions during cultivation and storage. The time elapsed between harvesting and storage in rural properties, commercialization, processing and transport often allows adverse conditions that favor contamination, development of fungi and the production of mycotoxins. In this context, an adequate management of the crop combined with the anticipation of the harvest time may contribute to the better use of the productive potential of the cultivars used, ensuring that drying should be performed as soon as possible, aiming at maintaining the nutritional and sanitary quality of the kernels. Thus, the objective of this work was to evaluate strategies to reduce mycotoxin content and preserve the nutritional quality of wheat kernels. Three wheat cultivars were used: (TBIO Duque (susceptible to *Fusarium* Head Blight (FHB)); TBIO Audaz (moderately susceptible to FHB) and ORS Feroz (moderately resistant to FHB)) and one of triticale (BRS Zenite (moderately resistant to FHB)) with fertilizations based on soil analysis and the recommendations for the crops. Three spraying strategies and five harvest times were evaluated: Strategy 01 – Control (without application); Strategy 02 – Application of Carbendazim Nortox<sup>®</sup> (carbendazim (500g/L) 0.8 L/ha at the beginning of the flowering stage and Tebufort<sup>®</sup> (tebuconazole (200 g/L) 0.75 L/ha) at 7 and 14 days after flowering; Strategy 03 – Fox Xpro<sup>®</sup> (trifloxistrobin (150 g/L) + prothioconazole (175 g/L) + bixafen (125 g/L) 0.5 L/ha) + Aureo<sup>®</sup> (soybean oil methyl ester (720 g/L) 0.5 L/ha) at the beginning of the flowering stage and Miravis Pro<sup>®</sup> (pydiflumetofen (62.4 g/L) + prothioconazole (75 g/L) 0.9 L/ha) at 7 days after flowering. Harvest times started at the physiological maturation (PM) and were carried out with intervals of 7 days each. A randomized block design was used with treatments arranged in the scheme of subdivided plots (genotypes, applications, harvest time). Crop yield decreased for the control but did not differ from the treated plots and between cultivars, with a significant reduction for the harvests carried out after the PM. Hectoliter weight (HW) was also significantly reduced after PM, there was a difference between genotypes and between spraying strategies. For DON (deoxynivalenol), an interaction was observed between genotypes and spraying strategies, with higher values of DON in triticale (control), but with no effect among wheat cultivars. The incidence of *Fusarium graminearum* in harvested kernels was higher in triticale than wheat genotypes, higher when fungicide applications were not done and increased in harvests performed after crops had reached their PM. From the residues of ANOVA, a significant correlation was observed between the levels of DON and the percentage of kernels infected by FHB, ranging from a confidence interval of 0.27 to 0.52. Support: Epagri; Cooperalfa; Embrapa.

## Diversity of oomycetes associated with soybean seedlings disease in Brazil

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Soybean diseases caused by species of oomycetes have gained considerable attention worldwide due to high yield losses impinged by a diversified group of pre-emergence and seedling pathologies. Our objectives were to assess the abundance, diversity and pathogenicity of oomycetes associated with soybeans in Brazil. During the 2019/20 and 2020/21 seasons, soybean seedlings affected by damping-off and root rot were observed in different states of Brazil. Soil samples were collected in 59 municipalities in five states: Rio Grande do Sul, Santa Catarina, Paraná, Mato Grosso do Sul and São Paulo. The samples underwent leaf baiting and bioassay methods to recover oomycetes. The isolates recovered were identified using both morphological and molecular characters. A total of 272 isolates were recovered, of which 130 isolates were obtained from the 2019/20 season, while 142 isolates were obtained from the 2020/21 season. *Pythium* was the predominant genus, with 205 isolates, followed by *Phytophthora* with 63 isolates and *Phytophythium* with 3 isolates. *Phytophthora* isolates were classified as *Phytophthora sojae* based on species-specific PCR assays. The pathogenicity of *P. sojae* isolates was evaluated by hypocotyl inoculation method. Ten seedlings of susceptible cv. Williams were inoculated by injecting approximately 0.1 mL of the mycelial slurry of each isolate in the hypocotyl. The number of dead seedlings was recorded to score the reaction as pathogenic ( $\geq 60\%$  of seedlings killed) or non-pathogenic ( $< 60\%$  of seedlings killed). All *P. sojae* isolates were pathogenic. Regarding the other isolates previously classified as *Pythium*-like, the pathogenicity was evaluated by the sand-cornmeal method. The inoculum (475 ml of sand, 25 ml of cornmeal, and 125 ml of water distilled) was prepared with mycelium discs from colonies grown on V8 medium and kept in a growth chamber at 25°C for 10 to 14 days. The sand-corn inoculum was mixed with vermiculite in a 1:4 ratio (v:v) and distributed in 500 ml plastic cups. Ten soybean seeds of cv. NS 5959 IPRO were placed on the surface of the infested substrate. Each treatment, i.e. isolate, was applied to 8 cups containing 10 seeds each. The assay was carried out in a completely randomized design. The number of germinated seeds was counted 14 days after sowing. The pathogenic *Pythium*-like isolates were submitted to a direct colony PCR and sequencing of the internal transcribed spacer (ITS) 1 of the ribosomal RNA gene and cytochrome c oxidase subunit 1 (COI) of the mitochondrial gene. Of the 13 pathogenic isolates characterized to date, 10 were confirmed to belong to the *Pythium* genus, being 5 isolates of *Pythium irregulare*, 4 isolates of the *Pythium myriotylum*, and 1 isolate of the *Pythium pleroticum*. Three other isolates were classified as *Phytophythium cucurbitacearum*. Based on the results it is advisable to expand and conduct a deeper evaluation of the ecology of oomycete communities and the epidemiology of damping-off and root rot in soybean fields. Understanding the diversity of oomycetes in soybean cultivated areas and the implications of such diversity to the intensity of the epidemics of damping-off and seedling root rot is highly relevant given the increase in both the number of outbreaks and the number of reports of new oomycetes pathogenic to soybean.

### **Comparative epidemiology of coffee leaf rust (*Hemileia vastatrix*) at different altitudes**

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Brazil is one of the main coffee producers and exporters in the world. The occurrence of diseases in coffee plants is one of the limiting barriers to increasing coffee production and productivity, especially coffee leaf rust, caused by the fungus *Hemileia vastatrix* Berk. & Broome (Pucciniales: Pucciniaceae). In view of the above, the objective of this work was to characterize the temporal progress of coffee leaf rust at different altitudes. The experiment was carried out on three crops of conilon coffee, Robusta Tropical variety propagated by seeds at different altitudes (<100m; >100m and <500m; >500m) in the southern region of the state of Espírito Santo during the period from November 2017 to December 2019. In each area, a sampling grid was demarcated, consisting of 80 points spaced every 15 m, each point was composed of a single plant. Monthly assessments of disease incidence were performed, and in each evaluation, the number of diseased leaves in the upper third of the four quadrants was quantified. To describe the temporal progress of the disease, the rust progress curves were plotted and the area under the disease progress curve (AACPD) was calculated. The empirical models (Monomolecular, Logistic, Gompertz and Exponential) were adjusted to the disease incidence data and the choice of the best model was performed based on the coefficient of determination ( $R^2$ ), Lin's coefficient of agreement (CCC) and residual standard error (RSE). The model adjustments were performed using the “epiffiter” package and the analyses were performed using the R® software (version 4.1.3). Based on the results obtained, it was found that two epidemics of the disease occurred during the period evaluated, with the highest AACPD value observed at altitudes >100m and <500m. The model that best described the epidemics in the evaluated areas was the logistic model, with the highest infection rates at altitudes <100m and >100m and <500m for the first and second epidemics, respectively. It was concluded that the intensity of coffee rust was higher at altitudes >100m and <500m.

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## Convolutional Neural Network to estimate the severity of Late Blight on potato leaf discs

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Automating the quantification of severity can make this process faster and less reliant on human training. Automated disease severity assessment is key to high-throughput phenotyping assays such as those for fungicide sensitivity or plant resistance evaluation, for example. In this study, we modified a machine learning algorithm of the Convolutional Neural Network (CNN) U-Net to (1) segment the leaf disc and sporulation area; and (2) estimate the severity of late blight (*Phytophthora infestans*) on potato leaf discs, by estimating the pathogen sporulation. The photos were obtained using a Motic digital camera (Moticam 10 - 10.0MP) adapted to the stereomicroscope (Olympus SZ61 model SZ2-ILST), at 10X. No previous treatment or background removal was done for this study. We annotated two regions on 435 photos using polygons to select the areas of the leaf disc and the sporulation. The annotation was done with the VGG software, available at: <https://bit.ly/3OqO32F>. The following steps were done on the Google Colaboratory environment and the pipeline is available at: <https://bit.ly/3zoKp57>. JSON files were imported with annotation coordinates and each VGG annotation was converted into class masks. The masks were mapped according to numeric index: 0 to the background; 1 to leaf disc; and 2 to sporulation. The images received the index as a shade of gray. Class indices were then converted to a one-hot vector with  $K \times H \times W$ .  $K$ ,  $H$ , and  $W$  are, respectively, the number of classes of the problem, image height, and image width. We applied three operations to augment the data: (1) horizontal mirroring with 50% chance; (2) vertical mirroring with 50% chance; and (3) modification of up to 10% in brightness, contrast, and saturation and up to 5% in hue. The U-Net was modified to be used with our dataset, and the modifications were: (1) replacement of a 2x2 max pooling layer and a 2x2 up-Conv layer by 3x4 layers to accommodate the height:width ratio of images; (2) increase the number of network outputs from 1 channel to 3; (3) apply standard ImageNet normalization to approximate the mean of the values of the network inputs to 0 and the variance to 1; and (4) use of a CrossEntropyLoss function which is more suitable for multi-class classification problem. The network was trained with 80% of the dataset and tested with the other 20% of the dataset. Each image was segmented into three regions by the network: background, leaf disc, and sporulation. The sporulation rate was then automatically estimated by  $(Y_{\text{leaf}} / (Y_{\text{leaf}} + Y_{\text{spore}}))$ , where  $Y_{\text{leaf}}$  = number of pixels of disc leaf class and  $Y_{\text{spore}}$  = number of pixels of sporulation class. The model was evaluated according to the percentage of hits performed in estimating the sporulation rate. We define a hit as a prediction that differs by up to 5% from the actual value. Our U-Net was able to learn with 201 epochs and presented 0.19% error, and 0.67% of accuracy in 95% of the dataset. This study is a starting point for using a CNN to automatically estimate the sporulation of *P. infestans* in potato leaf discs.

## Progress of stalk rots in maize

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Among the pathogens that damage maize, fungi that cause stalk rots (SRs) can be highlighted, because these fungi alter and reduce the resistance of plants at the base of their stalk. This alteration compromises the translocation of water and nutrients from the soil to the aerial part of the plants, reducing crop yield and grain quality. Thus, the objective of this work was to evaluate the progress of stalk rots from roots to the internodes of maize, its effects on yield and its relationship and progress with the phenological stage of the crop. The experiments were conducted in the harvest seasons of 2019/20 and 2020/21 in no-tillage fields using the corn hybrids P3456R (moderately resistant to SRs) and P32R22 (moderately susceptible to SRs) with a plant density of 70.000 plants/ha. Root and stalk (first, second and third internodes, from bottom to top) were evaluated for the incidence of SRs in four phenological stages: V8 (eight fully expanded leaves), beginning of grain filling (R1 - silking), physiological maturation (PM) (R6 - 55 to 65 days after R1) and pre-harvest (10 - 15 days after PM). The incidence of SRs was quantified according to the methodology proposed by Reis et al. (1998), considering symptomatic plants those that presented discoloration of the first or second internode and/or those with lower resistance of the stalk to the pressure of fingers (thumb and index fingers). The symptomatic and asymptomatic stalks and roots were cut and taken to the Phytosanitary Laboratory of Epagri/Cepaf, where ten random fragments of the internal tissue of the roots and internodes were removed. The fragments were disinfested with 1% sodium hypochlorite for three minutes and transferred to gerbox containing a culture medium of potato-dextrose-agar (PDA = Merck: 40g L<sup>-1</sup>) + A (antibiotic = 200mg L<sup>-1</sup> streptomycin sulphate). SRs were classified as caused by *Fusarium verticillioides* and *F. graminearum*. Initially, the stalks were visually evaluated regarding the SRs (external and internal visual diagnosis), with significant correlations of 0.84 and 0.91 for the harvest seasons of 19/20 and 20/21, respectively. The incidence of *F. verticillioides* predominated on the root in both harvest seasons. In the evaluations of the internodes, a predominance of *F. verticillioides* was observed for the second harvest season. For stalks, overall, the incidence was low in the initial stages (V8 and silking) with incidences below 5%. In grain filling and physiological maturation, the incidences were up to 50% with no significant differences observed between grain filling and physiological maturation, independent of the internode. For the harvested kernels, a predominance of *F. verticillioides* was observed (21.7% and 45.6% in 19/20 and 20/21, respectively) in relation to *F. graminearum* (0.1% and 1.4% in 19/20 and 20/21, respectively). Spearman's linear correlations were significant and ranged from 0.48 to 0.67 between the incidences observed in the internodes 1, 2 and 3. No significant correlations were observed for the incidences of rots between roots and stalks and between stalks and kernels. Support: Epagri; Cooperalfa; Embrapa.



## **Mycelial growth and sporulation of small-spored *Alternaria* isolates associated with potato brown spot in Brazil**

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In recent years potato brown spot has gained the attention of growers and consultants due to the increase in the occurrence of epidemics countrywide. Allegedly, climate change is implicated with higher temperatures in potato growing regions in Brazil contributing to increase the frequency and severity of brown spot epidemics in potato growing regions. To date, there is no information regarding the effects of temperature on basic epidemiological/ecological variables associated with small-spored *Alternaria* spp. from Brazil. Therefore, this study aimed to evaluate the influence of different temperatures on mycelial growth and sporulation of small-spored *Alternaria* isolates sampled from Minas Gerais, Paraná, São Paulo, Rio Grande do Sul, Santa Catarina, Goiás, and Bahia, the main potato-producing states in Brazil. Fifteen isolates were used in all experiments. Mycelium discs (0.5 cm in diameter) from each isolate were transferred to the center of Petri plates containing PDA medium. The plates were kept at 15, 20, 25, 30, and 35 °C and 12 h photoperiod. Colony diameter measurements were performed daily until the 10th day of incubation. The experiment was conducted twice. The number of conidia produced was estimated with a spectrophotometer based on the absorbance reading at 600 nm (OD600). The experiment was set in a completely randomized design with 15 isolates, 5 temperatures, and 4 replicates. Temperature affected both mycelial growth and conidia production. The highest mycelial growth was observed at temperatures between 25°C and 30°C, on the other hand, greatest sporulation was observed at 15°C. There was no effect of the origin of the isolate regarding the response to temperature, for both variables assessed. Isolates from different states had similar responses to temperature.

## Combined effect of myrtle rust and water deficit on photosynthesis of guava plants

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Plants grow and reproduce in harsh environments containing a wide variety of biotic and abiotic factors (Taiz et al., 2015). The effect of abiotic stresses on plant development has been extensively studied; however, few is known about the interaction between abiotic and biotic stresses (Ramegowda and Senthil-Kumar, 2015). The objective of this study was to evaluate the guava rust progress under water deficit, and the impacts of the combination of these two stresses on plant photosynthesis. Potted 'Paluma' guava plants were exposed to moderate water deficit and *Austropuccinia psidii* infection, applied individually and in combination. Disease progress and leaf gas exchange were evaluated over time. The relative net photosynthesis of a diseased leaf ( $P_x/P_o$ , where  $P_x$  is the net photosynthesis of each diseased leaf and  $P_o$  is the average of the net photosynthesis on healthy leaves) was related to disease severity ( $x$ ) in diseased plants with and without water deficit by  $P_x/P_o = (1 - x)^\beta$  (Bastiaans, 1991).  $\beta$ -value represents the relationship between the virtual lesion (green leaf area adjacent to the lesion where photosynthesis is null) and the visual lesion. Photosynthetic variables, such as maximum carboxylation and electron transport rates, were estimated at 15 days postinoculation from curves of photosynthesis response to increasing chloroplast  $CO_2$  concentration, according to Sharkey et al. (2007). The rust lesion density was higher in guava plants submitted to water deficit than in those without water deficit. The water deficit did not affect the rust latent period. The area under the  $CO_2$  assimilation curve was significantly smaller in diseased plants submitted to water deficit than in all other treatments. Severe reduction of relative net photosynthesis ( $P_x/P_o$ ) in the green leaf area adjacent to the lesions occurred when rust was associated with water deficit.  $\beta$ -values were 4.6 and 2.5 in diseased plants with and without water deficit, respectively. Rust significantly reduced the maximum carboxylation rate allowed by ribulose-1,5-bisphosphate carboxylase/oxygenase (Rubisco) and the rate of photosynthetic electron transport, causing biochemical limitations to photosynthesis of guava leaves. The increase in water use efficiency, observed in guava plants submitted to water deficit, was impaired by rust. This suggests that *A. psidii* interferes in the stomatal control of guava leaves. Taken together, our results show that water deficit increased rust intensity and that the photosynthesis reduction imposed by the combination of stresses was more severe than those caused by the stresses applied individually to the plants.

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## **A resistência da goiabeira ‘Suprema’ à ferrugem tem custo fotossintético e depende da concentração de inóculo**

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O Brasil é o maior produtor de goiabas de polpa vermelha do mundo, as quais são produzidas principalmente no Estado de São Paulo, responsável por 32% da produção nacional. Embora seja a cultivar mais comercializada no Estado, a goiabeira ‘Paluma’ apresenta alta suscetibilidade à ferrugem (*Austropuccinia psidii*; Gonçalves et al., 2022), que, na ausência de controle químico, pode reduzir até 80% da produção de frutos (Martins et al., 2014). Em contrapartida, a cultivar ‘Suprema’, também conhecida como ‘Tailandesa’, apresenta baixa suscetibilidade à ferrugem (Sales et al., 2021). O alto nível de resistência à ferrugem, aliado à sua alta produtividade, têm impulsionado o plantio da cultivar ‘Suprema’ no Estado de São Paulo. O objetivo deste estudo foi avaliar os efeitos da concentração de inóculo de *A. psidii* na suscetibilidade da cultivar ‘Suprema’ e na fotossíntese de folhas de goiabeira. Folhas jovens de goiabeiras ‘Suprema’ envasadas foram inoculadas com suspensões de *A. psidii* ajustadas para  $0$ ,  $10^4$ ,  $10^5$  e  $10^6$  urediniósporos  $\text{mL}^{-1}$ , em três repetições. Após a inoculação, as plantas foram mantidas em câmara úmida, no escuro, a  $23\text{ }^{\circ}\text{C}$  por 24 h. Em seguida, as plantas foram transferidas para casa-de-vegetação. A fotossíntese líquida foi avaliada em áreas de  $2\text{ cm}^2$  do limbo foliar, após a estabilização da severidade da doença, com analisador de gás por infravermelho. A severidade da doença foi quantificada com o programa Quant, a partir de imagens digitais obtidas nas mesmas áreas onde a fotossíntese líquida foi avaliada. As severidades médias da ferrugem foram 5,3%, 77,3% e 79,3% em goiabeiras inoculadas com  $10^4$ ,  $10^5$  e  $10^6$  urediniósporos  $\text{mL}^{-1}$ , respectivamente. A fotossíntese líquida relativa de uma folha doente ( $P_x/P_o$ , onde  $P_x$  é a fotossíntese líquida de uma folha doente e  $P_o$  é a fotossíntese líquida média de folhas sadias) foi relacionada à severidade da doença ( $x$ ) através do modelo  $P_x/P_o = (1 - x)^{\beta}$  (Bastiaans, 1991).  $\beta$  representa a relação entre a lesão virtual (áreas verdes ao redor das lesões onde a fotossíntese é nula) e a lesão visual. Os valores de  $\beta$  relatados para as ferrugens são baixos, como para a ferrugem na goiabeira ‘Paluma’, que apresenta  $\beta = 2,1$  (Gonçalves et al., 2022). Surpreendentemente, o valor de  $\beta$  para a ferrugem na goiabeira ‘Suprema’ foi de 6,2, indicando uma redução severa na eficiência fotossintética do tecido verde adjacente às lesões. As reduções fotossintéticas causadas por *A. psidii* na ‘Suprema’ tiveram magnitude de danos similar à dos causados por patógenos necrotróficos ou hemibiotróficos. Nossos resultados relevam que a resistência da cultivar ‘Suprema’ à ferrugem tem custo fotossintético. Além disso, a resistência deixa de se manifestar sob altas concentrações de inóculo de *A. psidii*. Agradecimentos: FAPESP 2020/07746-1 pela bolsa de iniciação científica do primeiro autor e FAPESP 2019/13191-5 pelo auxílio financeiro.

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## **Integration of the tutoring system, leave removal with inoculum and the interval of application of fungicides in the management of early blight of tomato**

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Early blight (*Alternaria solani*) is one of the main diseases of tomato and can cause direct and indirect damage if adequate management strategies are not implemented. Disease control is mainly based on systematic applications of fungicides, often without epidemiological criteria. The objective of this work was to evaluate the effect of integrating cultural practices associated with the use of fungicide in the management of the disease. The experiments were carried out at the Universidade Federal do Espírito Santo, Alegre, ES, Brazil, for 2 years, in a randomized block design in subdivided plots with three replications. The main plots consisted of 3 staking systems (radical, triangular and vertical), the subplots consisted of 3 intervals between fungicide applications (no application and applications at intervals of 7 and 15 days) and the subsubplots comprised three systems of leaf removal (no leaf removal and leaf removal with severities  $\geq 16\%$  and  $\geq 32\%$ ). For each treatment, the area under the disease progress curve (AUDPC), healthy leaf area duration (HAD), healthy leaf area absorption (HAA) and tomato yield were calculated. The application of the treatments was efficient in the management of the disease, and the vertical staking provided higher productivity regardless of inoculum removal and the interval of fungicide application. The effect of leaf removal reduced disease progress and increased productivity, with leaf removal with severity values  $\geq 16\%$  being the most effective system, allowing to reduce the number of fungicide applications without compromising management effectiveness.

Support: CNPq.

## Temporal analysis and quantification of damage caused by eucalyptus rust

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The eucalyptus rust (caused by *Austropuccinia psidii*) is one of the most important diseases of this crop, making it necessary to know about the progress of the disease over time and the damage it caused. Given the importance of this issue and that the pathogen *P. psidii* is presenting the eucalypts, the present study aimed to perform a temporal analysis of rust in the field and quantify damage. This study was conducted in the field in the experimental area of Suzano Pulp and Paper Company, located in the municipality of Mucuri - BA. Planted 200 seedlings of clone 2156, hybrid of *Eucalyptus grandis* x *Eucalyptus dunnii*, and 200 seedlings of clone 2392, hybrid of *Eucalyptus grandis* x *Eucalyptus urophylla* in 3x3 m spacing. The experimental design was a randomized block design with 10 replications in plots of 10 plants for each treatment, and the treatments with and without chemical control. The experiment was divided into two phases, Phase 1: started in April 2005 with the planting of seedlings and adaptation of the seedling, we assessed the incidence of eucalyptus rust, height and biomass of the plant, and the disease incidence and plant height were evaluated in all blocks of the experiment and the biomass in the blocks (1, 2, 3 and 4). For the assessment of biomass was necessary to cut the plants in these four blocks, which occurred when the plants reached six months of age; Phase 2: This phase lasted until 2009, during the four years of the experiment evaluated the height and the diameter of the plants in the remaining six blocks (5, 6, 7, 8, 9 and 10). With incidence data was performed temporal analysis of rust by evaluating the area under the disease progress curve (AUDPC). Quantification of the damage caused by rust was performed using the time variable (m), biomass (kg) and timber volume (m<sup>3</sup>) by statistical analysis. When it was not performed chemical control, it was found that the clone 2156 was more resistant toward clone 2392, which can be considered more susceptible, as it showed greater AUDPC. The application of the fungicide triadimenol (Bayfidan) was effective for clone 2392, leading to decreased incidence of rust and providing greater height, biomass and wood volume of plants. This clone was more susceptible to rust, however more productive relative to clone 2156.

## **Feasibility of integrating genetic resistance, fungicide and silicon in the management of Fusarium Head Blight of wheat**

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Wheat crop is of great importance to Brazil, and the Southwest region is the main producer in the State of São Paulo. Productivity can be compromised by several factors, with diseases playing a leading role. Among the diseases, Fusarium Head Blight (FHB) (*Fusarium graminearum*) stands out, which, in addition to causing a reduction in production, can greatly affect the quality of the grains due to the production of mycotoxins (such as deoxynivalenol - DON), which represent a huge risk to human and animal health. Disease management is a huge challenge for world wheat. The management of the disease is done using fungicides, cultivars with a certain level of resistance and cultural practices. However, a strategy used alone is not effective in management. There is a need to analyze the effect of the combination of other strategies. The objective of this work was to evaluate the feasibility of integrating genetic resistance, fungicide and silicon in the management of FHB, under rainfed and irrigated conditions. Eight management combinations (MC) involving genetic resistance (2 cultivars, moderately resistant and susceptible), application or not of fungicide (triazole + strobilurin) and use or not of potassium silicate were tested. The experiment was carried out in randomized blocks, split plot, with 3 replications. The evaluations consisted of quantification of disease intensity (incidence, severity and FHB index), grain yield and quality (DON concentration). Based on the results, it can be concluded that there was a significant difference between the cultivars, use or not of fungicide and application or not of silicon in terms of FHB management. The use of cultivars with a level of resistance is important in the management of the disease. The use of fungicide is essential to reduce the disease intensity (incidence, severity and FHB index) and DON concentration, as well as to maintain wheat production. The application of silicon contributed to potentiate the effect of fungicide and genetic resistance. The management of FHB can be carried out using the integration of variety with level of resistance, use of fungicide and application of silicon.

Support: CNPq.

### Spatiotemporal dynamics of bacterial wilt in eucalyptus

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Bacterial wilt (*Ralstonia* spp.) is one of the main diseases of eucalyptus, and is responsible for substantial losses to producers. For an efficient and scientifically-based management of this disease, it is necessary to understand the various factors involved in its development. Therefore, there is a need for a better understanding of the epidemiology of *Ralstonia* spp. on *Eucalyptus* spp. This study aimed to determine the spatial-temporal dynamics of bacterial wilt in eucalyptus under natural infection conditions. An experiment was conducted on a commercial plantation in the municipality of Itinga, Maranhão state, Brazil, using the clone FGCA0385 (*Eucalyptus urophylla* var. *platyphylla*). The study was conducted in four plots/repeats composed of 450 plants each, subdivided into nine rows with 50 plants per row and a spacing of 3 × 3 meters. Disease incidence quantification was performed for one year. The spatial dynamics of the disease was determined using the dispersion index, the modified Taylor law, and the analysis of the dynamics and structure of foci. For temporal dynamics, the curve of the disease incidence progress was plotted, and the data were analyzed by simple linear regression analysis fitted to three empirical models: logistic, monomolecular, and Gompertz. The distribution pattern of eucalyptus bacterial wilt occurred randomly distributed, which was confirmed by the Index of dispersion. As for the analysis of the dynamics and structure of the foci, 69 foci of the disease occurred, 44 of which were unitary, with an average number of plants per focus of 1.63, and the foci had greater length in the direction of the planting line. The epidemics were best described by the monomolecular model, with an estimated incidence of 27.77% in the fourth year of the study for eucalyptus bacterial wilt.

Support: CNPq.











The irregular landscape of Chapecó region ensues the existence of small properties where diversified economic activities which characterize family farming are conducted. This justifies the existence of the Family Farming Research Center, of the Agricultural Research and Rural Extension Company of Santa Catarina State (Epagri). The Center has been developing agricultural research for 70 years, and contributes to the permanence of families in rural areas with income and life quality.



Source: Google Maps