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Title:

The fungal community associated with individuals of the ambrosia beetle Xylosandrus germanus captured in chestnut groves

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Abstract body text:

Xylosandrus germanus (Blandford) (Coleoptera: Curculionidae, Scolytinae), is a highly polyphagous ragweed beetle, introduced in Europe for several decades now. It is currently an invasive species in forest ecosystems and plantations throughout Europe. In Italy it is widely present in the northern regions, preferably associated with coppice and fruit chestnut groves (Rassati et al., 2016; Dutto et al., 2018); recently it has also been reported in chestnut groves in central Italy (Rassatti et al., 2020). This species, like all ambrosia beetles, establish very stable to occasional associations with a large fungal community that includes both mutualistic symbionts that support the diet of the larvae in the tunnels, and phytopathogenic species that contribute to the damage on the infested host. The present study analyzed the fungal communities associated with individuals of *X. germanus* captured in chestnut groves in Friuli-Venezia Giulia, Lazio and from chestnut groves in the United Kingdom where the insect was recently reported. For the study of metacommunities and the identification of fungal taxa, it was decided to use High Throughput Sequencing techniques followed by bioinformatics processing of the data. The four populations studied showed significant differences in terms of species composition and abundance which probably reflect differences in the structure of the reference chestnut. However, the four populations have a 'core biome' of 43 taxa, where we find the typical symbionts such as *Ambrosiella grosmanniae*, *A. caenulata*, and *Fusarium solani* species complex but also phytopathogenic species such as *Gnomoniopsis castanea*. *Diaporthe foeniculina*, *Biscogniauxia mediterranea*, all taxa with marked endophytic habitus in the wood. Numerous other phytopathogenic species have been identified in the various sites associated with *X. germanus*, among which *Cryphonectria parasitica*, *Sclerotinia pseudotuberosa*, *Tubakia suttoniana* species complex. The possible ecological consequences of these new insect-fungus associations, both as a direct effect of the invasion and as an indirect effect on the biology and epidemiology of associated fungi, represent a new frontier of study in the context of global changes.

Keywords:

Ambrosia beetles, symbiosis, phytopathogenic fungi, biological invasions

Establishment of a biological control program of the blight fungus (*Cryphonectria parasitica*) adapted to a chestnut production site in western Hungary

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Abstract:

Chestnut blight disease caused by the fungus *Cryphonectria parasitica* (Murr.)Barr is a devastating tree disease in forests and areas prominent with chestnut cultivation in Central-Europe. The virulent strain of the pathogen is responsible for the deadly necrotic lesions or cankers on infected chestnut trees. The discovery of hypovirus (CHV1) in Europe has opened wide possibilities for biological control of *C.parasitica* in sweet chestnut populations once considered lost. Inoculation of virulent chestnut cankers with Cryphonectria hypovirus leads to non-lethal and inactive cankers. It is imperative to analyze the diversity of vegetative compatibility types of *C. parasitica* to develop improved, site-specific biological control applications and disease management.

Infected bark samples were collected (in 2022) from the site Nagykanizsa (Zala county, western Hungary). Isolates were cultured in the laboratory and vegetative compatibility between isolates was evaluated on PDA media.

An important task of the laboratory work was also to identify local hypovirulent (mycovirus- carrying) fungal strains and to determine their usability (conversion capacity) in biological control technologies.

The main aim of our research was to highlight the perspectives for chestnut blight disease control with special emphasis on hypovirulence strains of *C.parasitica*. During our surveys, we visited a production site in west-Hungary (Nagykanizsa-Bagóhegy/Zala county) where infected bark samples had not yet been collected or hypovirulent fungal strains had not been used earlier. With our tests, we wanted to establish the successful field treatments.

Keywords: Biological control, *Cryphonectria parasitica*, hypovirulence, virulence, vegetative compatibility, in vitro conversion.

Past and present comparison of the incidence of chestnut blight in South Tyrol (northern Italy)

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Abstract:

Cryphonectria parasitica is one of the most important pathogens of the chestnut tree (*Castanea sativa*). The fungus causing chestnut blight was introduced to Europe in 1938 and subsequently spread throughout the continent. In the Autonomous Province of Bozen-Bolzano (South Tyrol) in northern Italy, chestnut trees are an important landscape element of the slopy zones of the Etsch-Adige and Eisack-Isarco river valleys. Furthermore, chestnut represents the third common permanent crop by area, which contributes to an additional income of many farmers. The pathogen has been present in this area since 1958 and in the1990s, hypovirulent strains of *C. parasitica*, carrying a mycovirus, were widely inoculated in order to biologically control the disease. However, there was a lack of investigation and monitoring in the following years to assess the long-term success of these measures. Therefore, the present research aimed to survey the phytosanitary situation in at least twelve chestnut groves, where infection rates of chestnut blight were assessed in 1991, just before the release of hypovirulent strains. The current incidence of the disease is compared to past data and related to recently performed molecular genetic investigations, with the final goal to improve the knowledge and control of the disease.

Keywords: Sweet chestnut; plant disease; bark cankers; disease control; *Cryphonectria* hypovirus I

Gall wasp susceptibility of chestnut cultivars in an organic South Carolina orchard

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Abstract:

Between years 1995 and 2000, 28 grafted *Castanea spp*. cultivars and 8 OP seedling populations were planted on a Cecil loamy sand soil near Seneca, South Carolina. The design was completely randomized with 2 replications per genotype and 4-20 trees for each seedling population. The orchard site was a former native forest and was not irrigated nor received fertilizer after establishment. Weeds were controlled the first few years, but no cover sprays were applied. Once established, the trees received no cultural practices other than mowing. *C. sativa* type cultivars eventually died from chestnut blight. In Summer 2004, twig galls of the oriental chestnut gall wasp (*Dryocosmus kuriphilus*) were found in the planting. Years 2006 and 2007 had the most infestation with galling noticeably decreasing in 2008. Subjective ratings of the level of canopy twig galling from 0 (none) up to 5 (severe) were taken for each tree in March 2009. Galling was not noticeable after 2009 until 4-5 years later. The same ratings were used in February 2023 to score galling the previous 3 years. All trees were galled except 'Lockwood', which was the only *C. crenata* cultivar planted. The next least galled cultivars for both 2009 and 2023 were all *C. mollissima* ('AU-Leader', 'AU-Cropper', ‘Carr’, ‘Crane’, 'Meiling', and seedlings of 'AU-Cropper' and

‘Augenstein’). Hybrids 'Colossal' (*C. sativa* x *C. crenata*) and 'Layeroka' (*C. mollissima* x *C. sativa*) were severely galled and most died from blight. The gall wasp infestation began, peaked and collapsed within 6 years in the first occurrence and appeared to be ending within a similar time span in the second infestation. Though not verified, native or introduced parasitic wasps were suspected to have been biological control agents in the collapse of the infestations.

Keywords: *Castanea mollissima, Dryocosmus kuriphilus, Cryphonectria parasitica*

Chestnut mosaic virus: grafting-transmissibility and the interference with plant canopy

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Abstract:

Chestnut mosaic disease (ChMD) was described several decades ago, but its etiology has remained for long time unknown. Recently, combining grafting indexing and high throughput sequencing (HTS) techniques, it was possible to detect the genome of a novel DNA virus named chestnut mosaic virus, a badnavirus in two independent ChMD accessions (French and

Italian). The virus was found to be highly associated to symptomatic plants (> 91%). Typical symptoms are characterized by light and dark green patches on the leaves, accompanied by shoots with asymmetric blade deformation. From May to September, the severity of symptoms increases during the season, starting with leaf curling, light mosaic, and finally with desiccation of the leaf lamina. The severity of symptoms is inversely related to the vigor of plants and the quantity of production. The virus is transmitted by grafting and some chestnut hybrids (i.e. Maraval) are particularly sensitive so much that they are suggested for wood indexing during the volunteer certification program. After the recent reconstruction of ChMV genome and the setting up of a molecular diagnostic protocol, the detection of the virus was also recorded in asymptomatic plants. Most plants infected with badnaviruses are generally symptomless, but abiotic stress (such as temperature shifts and depletion of nutrients) can break the latency of the virus, with reemergence and increase of severity of symptoms. This situation, which can depend on the viral variants and chestnut genotypes, opens questions on the role of asymptomatic plants in the epidemiology of the virus, and the importance to select “ChMV-free” plants from which collect propagating materials (scions, buds) for grafting in nurseries and orchards. In the context of climate change, both viral spread and virulence could increase, with the risk of severe outbreaks.

Keywords: asymptomatic plants, Badnavirus, *Castanea* spp., molecular tools, virus transmission

Determination of the susceptibility levels to root rot disease of some rootstock candidates in chestnut

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Abstract:

Although many diseases threaten chestnut cultivation, especially two of them stand out. These are chestnut blight (*Cryphonectria parasitica*) and root rot (*Phytophthora* spp.) diseases. Root rot disease is challenging to control because it completely dries out the infected plants. Establishing orchards with resistant rootstocks/varieties is the most effective control against root rot disease. The study was conducted at Ondokuz Mayıs University Faculty of Agriculture between 2019-2020. It was aimed to determine the resistance level of the seedlings of some chestnut cultivars/genotypes grown in the Black Sea region to *Phytophthora cambivora* and *P. cinnamomi*. In the study, seedlings of seven complex hybrid chestnut cultivars/genotypes (A9, A11, A30, A41, 'Ali Nihat (A25), 'Akyüz' (A14), and 'Macit 55 (A100)), 2 European chestnut cultivars ('Erfelek' and 'Salıpazarı') ') and 1 *Castanea crenata* x *C. sativa* hybrid ('Marigoule') were tested. Six years old seedlings which were raised in the pots were inoculated with *Phytophthora cambivora* and *P. cinnamomic.* The root rot symptoms were followed up once a month for four months. A total of 600 seedlings were inoculated. The experiment was carried out according to the randomized plot's design in the factorial arrangement with ten rootstocks, two Phytophthora species, three repetitions and ten seedlings in each repetition. As a result of the study, the A9 genotype had the smallest lesion size among all genotypes. Also, the lowest survival rate was obtained from the same genotype. At the end of the study, there was no statistically significant difference in the survival rate between genotypes. However, the survival rate was numerically higher in the seedlings belonging to the A41 genotype. In this respect, it can be said that the seedlings belonging to the A41 genotype can be more tolerant than the other genotypes tested in the study.

Keywords: *P. cambivora, P. cinnamomi, Castanea spp.*

Anthropogenic and landscape features are associated with ink disease impact in Central Italy

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Abstract:

Ink disease of sweet chestnut (*Castanea sativa*) caused by the oomycetes *Phytophthora cinnamomi* and *P. x cambivora* is the limiting factor for chestnut cultivation in several European regions. The objective of this study was to explore how the spatial landscape heterogeneity affects the distribution pattern of ink disease over a wide chestnut area in Central Italy using an approach that combined remote sensing, ground truthing activities and GIS. A multivariate model was developed that explained a large proportion of the variance of the impact of the disease using the density of roads and drainage networks as predictor variables. The association of these landscape elements, specifically with ink disease foci, provides a practical tool to improve the accuracy of monitoring this disease and the preparation of risk maps. Within the project MOCA funded by the Government body of the Latium Region, a risk map for Ink disease was developed on a GIS and made available through a web site to offer a tool for decision makers for mitigation measures planning.

Keywords: Multivariate model; ink disease; chestnut; risk map

Hybrid de novo genome assembly and comparative genomics of three different isolates of *Gnomoniopsis castaneae*

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Abstract:

The first genome assemblies of Gnomoniopsis castaneae (syn. G. smithogilvyi), the causal agent of chestnut brown rot of kernels, shoot blight and cankers, are provided here. Specifically, the complete genome of the Italian ex-type MUT401 isolate was compared to the draft genome of a second Italian isolate (GN01) and to the ICMP 14040 isolate from New Zealand. The three genome sequences were obtained through a hybrid assembly using both short Illumina reads and long Nanopore reads, their coding sequences were annotated and compared with each other and with other Diaporthales. The information offered by the genome assembly of the three isolates represents the base of data for -omics strategies applied to the fungus and to develop markers for population studies at a local and global scale.

Keywords: Castanea sativa; brown rot; *Gnomoniopsis smithogilvyi*; omics strategies

Preventive biological protection method on young chestnut seedlings for controlling the chestnut blight disease

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Abstract:

Chestnut blight fungus (*Cryphonectria parasitica*) is considered to be the most dangerous pathogen of sweet chestnuts worldwide. Pest control is a major challenge as traditional crop protection methods have not been effective in stopping it. The only effective control against bark cancer is the treatment of infected trees with strains of reduced virulence (hypovirulence). The aim of our research was primarily the preventive use of hypovirulent strains, thus producing already "protected" chestnut propagation material for planting. For our field artificial inoculation experiments, we used 4 fungal isolates collected in Hungary, of which two were virulent and two were hypovirulent strains. Necrosis caused by pathogens was measured at three different times and at different vegetation periods, following the outdoor infection. We have shown that treatments with hypovirulent strains are not at all risk-free from a plant health point of view. Within less than a year, virulent strains of the blight fungus completely destroyed the treated seedlings. Of the two hypovirulent fungal strains, Nagymaros NM showed only partial shoot death on the seedlings, while those treated with Rezi R8 hypovirulent fungal strains were completely asymptomatic and callusform. Based on the results of the studies it can be stated that hypovirulent strains prove to be suitable for preventive (pre-immunization) control on sweet chestnut seedlings, but due to the special characteristics of different áreas, it has been extremely time and energy consuming to find the most suitable hypovirulent fungal isolate

Keywords: chestnut blight disease, prevention, hypovirulence

Presence of Gnomoniopsis castaneae in chestnut areas in Spain and possible association with *Dryocusmus kuriphilus* infestation

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Abstract:

An intense survey was carried out in 2022 in different chestnut areas in Spain including the Cominudad Valenciana, Andalucia, Estremadura, Comunidad de Madrid and Catalunya. Over sixty trees were tagged, georeferenced, and characterized for their social status, and presence of *Dryocosmus kuriphilus* galls. From each tree samples were taken in September from leaves, shoots and, where present, burrs and *D. kuriphilus* green and old galls. Isolation trials were carried out to assess and quantify the presence and isolation rate of *Gnomoniopsis castaneae* using a systematic isolation protocol. Preliminary results confirm the presence of *G. castaneae* both in areas with presence and absence of *D. kuriphilus* infestation although the incidence was higher in *D. kuriphilus* infested areas. Interestingly, incidence of *G. castaneae* along altitudinal gradients followed a Gaussian distribution with a maximum in a range of between 800-1000 msl. *Gnomoniopsis castaneae* was not isolated from chestnut trees at altitudes ove 1100 msl.

Keywords: Chestnut 'brown rot'; ecology; epidemiology; Iberian peninsula; distribution maps

Presence of *Gnomoniopsis castanea* (syn. *smithogilvyi*) in chestnuts, rootstocks and grafts of chestnut varieties in Ticino

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Abstract:

Gnomoniopsis castanea (Syn. *G. smithogilvyi)* is an endophytic fungus, recently identified in Europe and Switzerland, as the main agent of chestnut brown rot and as a chestnut canker agent. It seems to cause a high mortality in chestnut nurseries and orchards. The presence of this fungus and of the chestnut canker agent *Cryphonectria parasitica* was searched for in the propagation material of 6 chestnut tree varieties*,* used by the Ticino Cantonal Nursery to restore fruit orchards.Sixty root samples, 41 shoot samples from germinated chestnuts and 17 chestnut rootstock samples were analysed, along with 112 samples from 56 rootstock/graft pairs, in order to determine whether the pathogen was transmitted by the rootstock or the graft. DNA extraction was followed by specific amplification primers for *Gnomoniopsis castanea* and *Cryphonectria parasitica.* It was possible to detect the presence *of G. castanea* as an endophyte, but *C. parasitica* was never detected. Six of the 60 roots analysed from seed chestnuts were contaminated with *G. castanea* (in varieties *Lüina, Torcione Nero*, *Marrone Michelangelo*, *Marrone Lattecaldo* and *Bouche de Bétizac),* as well as 2 out of the 41 shoots from seed chestnuts (*Lüina* and *Bouche de Bétizac*), and 2 out of 17 rootstocks (*Lüina* and *Torcione Nero* varieties)*.* Regarding the 112 samples from 56 rootstock/graft pairs, *G. castanea* was found in 12% of the analysed rootstocks and 60% of the grafts. These results showed a weak presence of *Gnomoniopsis castanea* in rootstock propagation material and an important contamination of grafting material in all varieties, confirming that *G. castanea* is an endophyte.

Keywords: *Gnomoniopsis castanea, Gnomoniopsis smithogilvyi*, endophytic fungus

Protection from ink disease of Castanea sativa orchards: first steps of INKAS Project the rural development program for Marche region (Central – Eastern Italy)

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Abstract:

Chestnut (*Castanea sativa* L.) is a multifunctional species which plays a key role in the economy of mountainous areas of Marche region (central-eastern Italy), it. More than 4,000 ha are covered with chestnut and about 1,000 ha are cultivated for fruit production, with more than 500 farms involved. Since some decades, there has been a gradual increase of chestnut orchards left without any cultivation for economic and social reasons as well as phytosanitary problems. At the beginning of 21st century, introduction of Asian chestnut gall wasp had an important and negative impact on the vigor and on the production of plants, with the recrudescence of ink disease, caused by *Phytophthora* spp., which from restricted areas step by step is undermining the chestnut regional heritage. At the end of 2022, rural development program founded INKAS Project, whose main objective is the protection of existing regional chestnut heritage, from the relentless advance of ink disease, through a multidisciplinary approach. The phytosanitary status of the chestnut orchards has been determined by a collaborative monitoring survey starting from pivotal areas, in agreement with the principal owners. The survey was carried out by a randomised mapping of trees considered potentially infected through the acquisition of main data (diameter, GPS coordinates, presence of symptoms and disease severity), in order to provide a general picture of phytosanitary status and identify active outbreaks of the disease. Starting from these data we planned to monitoring the ink disease spreading through high resolution remote sensing methods at landscape scale and by a specialized dog trained to recognize "volatile markers", released by plants infected by *Phytophthora* spp. in nurseries and farms. The management of ink disease will be carried out by endotherapic applications and by organic compounds amended to the soil, which allow to have a beneficial impact on soil microbiota able to improve the healthy of plants and to suppress the pathogen community.

Keywords: Chestnut, multidisciplinary approach, *Phytophthora*, outbreak area, analysis of satellite images

Wasps and weevils in Michigan chestnut orchards: biology, distribution, and management

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Abstract:

Chestnut growers in Michigan, the leading producer of commercial chestnuts in North America, face increasing pressure from native and invasive insect pests, reflecting the expansion of this relatively young industry. Lesser chestnut weevil (*Curculio sayi* Gyllenhal), a native species which originally infested American chestnut (*Castanea dentata*), is a particularly serious problem. Adult female weevils lay eggs through burs into developing nuts in late summer. Larval feeding in fall on mature kernels ruins nuts and when densities are high, growers may lose entire harvests. Our research has addressed pre- and post-harvest tactics to reduce weevil damage. We monitored adult weevil emergence using traps and canopy sampling to identify optimal timing for insecticide sprays. Post-harvest treatment to control larvae in infested nuts included submerging nuts for intervals of 5 to 30 min in water heated to 22.5 to 60 °C. Effects of hot water treatments on larval emergence, feeding damage and nut quality were evaluated.

Asian chestnut gall wasp (ACGW) (*Dryocosmus kuriphilus* Yasumatsu), an invasive species native to China, was first detected in the US in Georgia in 1975. High densities of galls caused by larval feeding inhibit tree growth, reduce tree vigor, and decrease nut production. A parasitoid of ACGW, *Torymus sinensis* Kamijo, was released in the US in the 1970's. This specialized parasitoid has become established in several eastern states, via natural dispersal and additional introductions. In 2015, ACGW was detected in an orchard in Berrien County, in the southwest region of Michigan. We monitored ACGW distribution and spread along with the *T.*

sinensis parasitoid in Michigan from 2017 to 2022. As of 2022, ACGW is established in at least 25 orchards across 14 counties, spreading 29 km per year on average. The *T. sinensis* parasitoid, first detected in Michigan in 2017, appears to follow ACGW, generally lagging 1-3 years behind ACGW establishment. In addition to monitoring regional distribution, we analyzed annual spread of ACGW within individual orchards to assess spatial-temporal dynamics of this invader.

Keywords: chestnut weevil, Asian chestnut gall wasp, insect pests

Identification of two pathogenic fungi, *Neofusicoccum parvum* and *Neopestalotiopsis sp.*, on European chestnut (*Castanea sativa* Mill.)

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Abstract:

Two different lesions were observed in young chestnut plants. In April 2021, the symptom was a depressed bark with dark reddish coloration on the stem and in April 2022, a brownish-brown vascular lesion in the stem cross-section, without external symptoms.

Samples were analyzed to determine the etiology of the damage, using the same methodology: surface-sterilization, plated on potato dextrose agar (PDA) and incubated at 25ºC. Fungal colonies were consistently isolated: strains LPPA-971 and LPPA-975 for the first and second case, respectively.

For molecular identification, internal transcribed spacer (ITS) and beta-tubulin (Bt-II) were amplified and phylogenetic trees were carried out using Mega6.

By BLAST analysis ITS sequence of LPPA-971 showed 99.63% identity and Bt-II 100% with those corresponding to *Neofusicoccum parvum*.

ITS sequence of LPPA-975 had 99.80% of identity with *Neopestalotiopis* sp. and *N. australis*, Bt-II 99.76 % with *Neopestalotiopsis* sp. and *N. clavispora*. Phylogenetic tree with concatenated sequences didn't allow to identify the species.

For each fungus, one to three branches of ten young chestnut plants were inoculated by inserting colonized half PDA plugs into artificially wounded tissue and then wrapped. Five plants were used as controls introducing half PDA plug without the fungus. Plants were placed in a plastic tunnel, with drip irrigation and grown under natural conditions. The assays were conducted twice.

Regarding LPPA-971, ten days after inoculation, all the chestnut plants presented bark cankers similar to the original sample, being very virulent.

LPPA-975 caused a weak canker and a brown rot in the vascular system of the plants, showing a more rapid advance down than up the stems.

In both inoculations, no symptoms were observed in the controls. Colonies of both fungi were reisolated from the cankers, fulfilling Koch's postulates.

Keywords: ITS, Bt-II, phylogenetic trees, etiology, pathogen detection

Pathogenicity of *Gnomoniopsis smithogilvyi* on four varieties of sweet chestnut from Galicia (Spain)

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Abstract:

Gnomoniopsis smithogilvyi is an emergent pathogen that causes brown rot on nuts and burrs of sweet chestnut (*Castanea sativa* Mill.) in the main European producing countries, significantly reducing the production of this

crop. In Galicia (Northern Spain), this fungus was detected for first time in 2021. In this region *C. sativa* is a specie with high economic value, because has 66% of Spain's plantations and produces 90% of the country's total nut production. The aim of this work was to evaluate the pathogenicity of several *G. smithogilvyi* strains on four varieties of nut of sweet chestnut from Galicia (Negral, Longal, Parede, and Xudía). Nuts were surface disinfected and a superficial wound was made in the pericarp of each nut. A 2 mm-mycelial agar plugs of a 10-day old culture of each *G. smithogilvyi* strain was inoculated. Ten nuts were established for each strain and ten nuts inoculated with sterile PDA agar discs were used as a control. Two replicated tests were carried out. All of nuts were kept in a moist chamber at 25ºC. Two inoculated and two control nuts were inspected for the presence of rot symptoms every seven days. Three weeks after inoculation the remaining nuts were examined and a Disease Severity Index (DSI) using a scale of 0 to 4 to measure the damage was used. All nuts varieties were affected by the pathogen with a range of disease of 0.8–2.75, while the controls remained healthy. Significant differences in severity between fungal strains of *G. smithogilvy*i for Parede variety were observed but no for the others. In all inoculated nuts, the pathogen was re-isolated from symptomatic tissues and the identity of the reisolated was confirmed as *G. smithogilvyi* by morphological and molecular analysis.

Keywords: Castanea sativa, chestnut disease, *Gnomoniopsis castaneae*, nut rot, emerging pathogen

Evaluation of the biological control of *Cryphonectria parasitica* in four orchards of sweet chestnut in Galicia (Spain)

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Abstract:

In Galicia (NW Spain), the presence of *Cryphonectria parasitica* (Murrill) Barr, the causal agent of chestnut blight disease, is well known. Surveys conducted since 2003 in sweet chestnut areas of this region have allowed to establish a situation map of the affected trees by this disease and to know that Galician populations of *C. parasitica* are characterized by a low diversity of vegetative compatibility (vc) groups, with three predominant vc types, reproduction mainly asexual, and a reduced presence of hipovirulent strains (CHV-1 subtype E), although compatible with the virulent dominant strains. These population characteristics are widely considered favourable for the dissemination of hypovirulence and for increasing the chances of success of the disease biocontrol. The aim of this work was to evaluate the effectiveness and the dissemination capacity of a field application of local hypovirulent strains of *C. parasitica* for the canker control in symptomatic chestnut in Galicia, in experimental permanent orchards. In 2019, four orchards of sweet chestnut affected by *C. parasitica* were selected in the province of Lugo with 380 total trees (29% infected by the pathogen). The inocula was applied into 61 sweet chestnut trees. Holes of 1 cm diameter, 5 cm apart from each other, were made around each lesion and filled with the corresponding inoculum. At the time of inoculum application, the type (active, active-healing, and healed), length and width of the canker were recorded. Every six months a review to check the status of the inoculated cankers was carried out. After two years 90% of the inoculated cankers showed a significant improvement, as there was no advance of the lesion and evolved to healed cankers (28%).

Keywords: *Castanea sativa*, chestnut blight disease, CHV-1, hipovirulence, field inoculation.

First experiences with chestnut brown rot (*Gnomoniopsis castaneae*) in Austria

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Abstract;

Brown rot (*Gnomoniopsis castaneae*) is considered the most important disease of sweet chestnut fruits in most production areas. In Austria, major damage due to this fungus was observed for the first time in 2018. Since then, the associated yield losses have fluctuated between 10% and 80%, depending on the weather conditions. However, the fungus does not always occur as a pathogen but also lives as a saprophyte or as an endophyte in asymptomatic plant parts. The frequency of isolations of *C. castaneae* from male and female flowers, annual shoots, two year old shoots sampled in spring was compared to the infection rate at harvest. Male flowers showed a higher infection rate than female flowers, 2 year old shoots higher than young shoots, but there was no correlation to the number of infected fruits. In the following year samples of flowers respectively fruits were taken monthly from June to October. Although latent infection was detected in 90 % of the fruits in September, only 3% of them were symptomatic at harvest time. The infection rate in autumn is reflected in the infection rates in spring: highly infected orchards lead to higher infection rates in annual and perennial shoots in the following year. The isolation of *G. castaneae* from fruits at harvest time showed a significantly higher degree of infection than expected from the visible symptoms. Subsequent heat treatment drastically reduced the isolation rate, whereas it increased sharply after 8 days of cold storage.

Keywords: *Gnomoniopsis castaneae*, brown rot, disease cycle, latent infection