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Microbial biostimulants tend to reduce chestnut trees mortality caused by chestnut rot and canker in nursery

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Abstract:

*Gnomoniopsis castanea (*Syn. *G. smithogilvyi*, an endophytic fungus, recently identified in Europe and Switzerland, is the the main cause of the chestnut brown rot but also a chestnut canker agent, in nurseries and orchards. In previous works, it was detected as an endophyte in chestnut material (roots, fruits, leaves) of rootstocks and grafts, whereas *Cryphonectria parasitica*, the mainly known agent of chestnut blight (or canker), was never detected as an endophyte. Rootstock material was slightly contaminated, while grating material of all varieties tested was highly contaminated by the endophytic fungus *G. castanea*. Two

microorganisms, *Trichoderma hamatum* UASWS1405 and *Pseudomonas putida* UASWS0946 had shown a high level of protection against *G. castanea* in climatic chambers experiments and could provide with a practical solution to reduce chestnut mortality in nurseries and orchards. For this reason, commercial formulations of these microorganisms were produced and two in planta experiments were designed at the State Nursery of Canton Ticino. These experiments were carried out from September 2017 to August 2018 to evaluate the biostimulant and biocontrol efficacy of these organisms. A metagenomic experiment was also performed to understand how these two organisms may influence the microbiota of chestnut trees. The first field experiment carried out in Ticino aimed to evaluate the biostimulating and curing effects of these microorganisms on trees infected by *G. castanea,* in which the pathogen's presence had been previously confirmed by a specific molecular diagnostic test. The commercial formulations of the two antagonists were inoculated in the substrate by wetting, under several modalities. As we observed that the contamination mostly occurs at grafting, a second experiment aimed to evaluate the potential use of these antagonists as protective treatments applied at grafting by soaking grafts in suspension of microorganisms and to measure the survival rate of grafted trees in the nursery. Molecular diagnostics showed that *G. castanea* was present in almost all the mother trees (89 samples analyzed) used for grafting in the nursery. Old varieties and local varieties were the most infected of *G. castanea* presence, while infection by *C. parasitica* remains globally very low in all the mother trees samples. In the 200 young of the biostimulant experiment, the presence of *G. castanea* was about 10% of the trees. Compared to controls, soaking grafts in aqueous suspensions 18h hours before grafting had no significant effects on trees survival, while the first experiment by wetting of potting soil showed a promising biostimulant activity on tree growth and a reduction of mortality in all modalities using the 2 microorganisms alone or combined.

Keywords: *Gbomoniopsis castanea Gnomoniopsis smithogilvyi*, chestnut canker, biostimulant, biocontrol, *Trichoderma hamatum, Pseudomonas putida*

Castanea sativa burr growth in its final stages

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Abstract:

The daily growth pattern and vascular flows of *Castanea sativa* Mill. (cv. “Marrone di Castel del Rio”) burrs, were monitored during a sensitive burr growing phenological stage (nut filling), through plant-based sensors (fruit gauges) connected to a wireless data-logger system. Environmental parameters were also recorded by a weather station placed in the middle of the orchard. The daily burr growth pattern was generally characterized by a rapid shrinkage in the early afternoon, followed by nocturnal swelling until the early morning. Daily burr growth was highly associated to chestnut orchard environmental parameters: positively influenced by air relative humidity and precipitation while negatively affected by vapour pressure deficit. Vascular flows confirm that the daily shrinkage was due to high burr water losses by transpiration. The xylem was fully active and able to replenish the amount of water lost during the day by the transpiration process. Phloem inflow was the major component for the net daily burr growth. These observations, coupled to the increase in nut dry mass content typical of this stage, suggest a passive model of burr phloem unloading. This study aimed both to establish the influence of environmental conditions on daily burr growth and to identify the vascular flow model supporting burr growth during a period of strong demand by the nut. Knowing and monitoring, in real-time, the burr daily growth could be promising for the adoption of smart and sustainable practices for chestnut orchard management.

Keywords: sweet chestnut, chestnut physiology, environmental parameters, vascular flows, plant-based sensors

Evaluation of commercial products for the control of *Gnomoniopsis smithogilvyi* and *Cydia splendana* in chestnut trees

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Abstract:

The chestnut market faces a complex challenge, controlling the fungus *Gnomoniopsis smithogilvyi*, which causes the brown rot and the pest *Cydia splendana* which causes nut tortrix. For G. *smithogilvyi* there were tested *Gliocladium catenulatum* (Prestop®) and potassium phosphonate (Alexin®) while for *C. splendana* there was tested emamectin (Affirm opt®). Two essays were prompted to evaluate their efficiency in separate or together and in two period treatments. The orchards are 20 years old, of the "Judia" variety. In the essay a) trees were sprayed three times, weekly spaced, during flowering phenophase only for G. *smithogilvyi*, with potassium phosphonate (T1) and tebuconazole (T2) and water (T3). In the essay b) treatments were done in the beginning of bur growth (first week of September) aiming the control of both, being emamectin (T4), emamectin + *Gliocladium catenulatum* (T5), emamectin + potassium phosphonate (T6) and water (T7).

Concerning essay a), one week after harvest, *in vitro* tests detected the presence of *G. smithogilvyi* in 8% of the samples from the T2 and 17% in the T3 while after one month of storage (at 4ºC), fungus was detected in 10% and 20% of samples from T1 and T3, respectively. In relation to essay b) any positive detection was found one week after harvest, although after one month storage, fungus was detected in samples from all treatments: T4, T5 - 60%, T6 – 40% and T7- 50%. This study reinforces the need to control the fungal load of chestnuts in the field, to reduce the post-harvest nut rot.

Concerning nut tortrix, in essay b) T6 was the most efficient, with 19% of infested nuts while in the other, there were 32% (T4), 39% (T5) and 40% (T7).

Acknowledgments:

"This work is supported by Commercial Chemical Massó, S.A."

Keywords: *Castanea sativa*, brown rot, nut tortrix, *Gnomoniopsis smithogilvyi, Cydia splendana*

Why are male-sterile chestnut trees more productive than male-fertile trees?

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Abstract:

In European chestnut (*Castanea sativa*) and in its hybrids, a non-negligible fraction of the trees are male-sterile: they have aborted stamens. These male-sterile trees are widely cultivated. Male-sterile trees are also found in natural chestnut forests, making European chestnut one of the few known gynodioecious* tree species (*plant species composed of both female and cosexual individuals). We first show that these male-sterile trees have much higher fruit set than male-fertile trees. To explore the mechanisms responsible for this differential reproductive success, we then used an innovative spatially explicit Bayesian model, combining large-scale paternity analyses and fruit set studies. In male-fertile trees, massive and synchronous flowering results in high rates of self-pollination, on average 74% in our study site in southwestern France. Since cross-pollen is more competitive than self-pollen, we estimate that 48% of female flowers are eventually fertilized by self-pollen. Due to strong late acting barriers against self-pollen, most (95%) flowers fertilized by self-pollen abort. Hence, only 4% of the fruits originate from self-fertilization. These results show that nearly half of the female flowers of male-fertile chestnut trees are wasted due to self-pollination. To maximize fruit production, we therefore advise to use varieties with reduced male fertility. However, the orchards should also include a

fraction of fully male-fertile varieties necessary for orchard pollination. We illustrate this point with simulations of fruit set in virtual orchards.

Keywords: Spatially explicit mating model, paternity analyses, emasculation experiment, female advantage, gynodioecy, fruit set, ovule discounting, sexual interference

The phenolic profile of different chestnut (*Castanea sativa* Mill.) accessions at different locations in Slovenia

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Abstract:

The European chestnut (*Castanea sativa Mill.*) is a member of the genus Castanea. Chestnuts are consumed throughout Europe, America, and Asia. Fresh chestnut fruits are rarely consumed raw, but are processed in various ways to improve their organoleptic properties (flavour, aroma, texture), fruit digestibility (making nutrients more bioavailable), and shelf life. The chestnut is geographically distributed in three major areas. In Europe with *Castanea sativa* Mill., in Asia with *Castanea crenata* Siebold & Zucc. and *Castanea mollissima* Bl., and in North America with *Castanea dentata* Borkh. The European chestnut probably originated in the eastern Mediterranean, later spreading throughout Europe, including present-day Slovenia. Although Slovenia is a small country in size, it has great natural differences in climate, having a sub-Mediterranean climate, a temperate continental climate, and an alpine climate, with chestnuts growing naturally in all these climates. Different climatic conditions (solar radiation, precipitation, temperatures, etc.) also affect the phenolic composition and profile of the chestnuts themselves, thus influencing their taste and health benefits. Our objective was therefore to sample different chestnut accessions throughout Slovenia to determine whether or not chestnuts grown at different sites have different phenolic profiles and whether or not there is a relationship between the different accessions at a site. The aim was also to investigate whether and how the different locations, and thus the different climate, influence the phenolic composition of wild chestnut accessions and thus their taste.

Keywords: phenolic compounds, chestnut, *Castanea sativa* Mill., climate effect

ımpact of SiK in promotion of chestnut tolerance against *Cryphonetria parasitica*

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Abstract:

The European chestnut (*Castanea sativa* Mill.), is an environmentally and economically important species in Europe, mainly for fruit production. However, it has been strongly affected by the chestnut blight (*Cryphonectria parasitica)*, a severe disease with negative consequences for trees. Exploring efficient alternative strategies that can help chestnut to increase their resistance against this phytopathogen in the short term is essential. Silicon (Si) fertilization is classified as an elicitor with a prophylactic effect allowing the rapid activation of biochemical defence in plants against invader pathogens, being an efficient inducer of resistance against chestnut blight. The present study evaluated the influence of Si fertilization on the protection of chestnut plants against *Cryphonectria parasítica.* Four treatments of seedling plants with 0, 5, 7.5, and 10 mM Si were done. One month after, half of each group was inoculated with the fungus. Results showed that higher concentrations of Si (7.5 and 10 mM) increased significantly the antioxidant enzyme activity, catalase (CAT) and superoxide dismutase (SOD) measured 60 days after the inoculation with this ascomycete. Data also showed that Si fertilization could reduce chestnut plants' disease severity and mortality rate. It was observed that 10% of Si-treated plants died at the end of the study, whilst all the non-treated died. Complementarily, using *in vitro* test, impregnated discs with phenolic extracts prepared from 7.5 and 10 mM showed a higher inhibition area (IA) against *C. parasitica* than the control treatment. Moreover, in *vitro* tests, done with Si impregnation in a PDA medium to evaluate its fungi toxic capacity, results showed that 7.5 mM and 10 mM Si solutions promoted a total capacity of suppression (100%) of *C. parasítica* mycelium growth as

opposed to the control Petri plates where the percentage of inhibition was 0%. These results indicate that fertilization with Si can be effective in the reduction of the rate of *C. parasitica* infection, so it should be successfully implemented in orchards, improving the resistance of chestnut trees against this biotic stress.

Acknowledgments:

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Keywords: blight disease, chestnut, *Cryphonetria parasitica*, phenols and silicon

European chestnut in Slovakia: overview of health condition and its cultivation for the future in the term of climate change

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Abstract:

European chestnut (*Castanea sativa* Mill.) is one of the oldest non-native tree species in Slovakia. Chestnut here is a minor fructiferous tree species; however, it significantly contributes to the preservation of the traditional agricultural landscape. Moreover, it is also precious melliferous tree species and suitable tree for the establishment of agroforestry systems. Nowadays, it is widespread at more than 220 localities with limited occurrence in hills from south-eastern Slovakia. Chestnut grows mostly in extensive old orchards or in mixed forest stands with other tree species. Currently, the health condition, as well as chestnut production, has rapidly declined as a result of the enormous dying out of chestnut individuals infected by the fungus *Cryphonectria parasitica* (Murr.) Barr. During the last decades, the fungus gradually spread to almost all chestnut localities. The presence of hypovirus in *Cryphonectria parasitica* populations in Slovakia was confirmed, but at a very low frequency (in 6 isolates from 161). The typical naturally hypovirulent cankers were scattered and rare, which indicates that hypovirus has not been established in *Cryphonectria parasitica* populations

in Slovakia yet and the health state of *Castanea sativa* is still intensely affected by chestnut blight. Based on expected climate change, but also within the ongoing massive dieback of chestnut individuals, intensive cultivation of chestnut is not expected in the future. For phytosanitary reasons and for preserving the landscape character, it would be advisable to grow chestnuts in small groups together with other tree species with similar ecological requirements. The mentioned issues, including necessary legislative regulations, could in the future be included in the proposal of potential management measures for the needs of adaptive chestnut cultivation in Slovakia.

Keywords: *Castanea sativa*, health status, *Cryphonectria parasitica*, silviculture, occurrence, perspectives

Artificial Neural Networks unravel the key factors influencing in vitro solid culture of Boletus reticulatus mycelium for chestnut mycorrhization

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Abstract:

Galician native forests are known for their rich biodiversity, featuring prominent tree species such as oaks (*Quercus robur*) and chestnut trees (*Castanea sativa*). A diverse range of wild mushrooms develops in this forest, including ectomycorrhizal fungi, which have been valued for their food and medicinal properties since ancient times. Among them, the *Boletus reticulatus (*bellowing to *Boletus edulis* complex) stands out due to commercial interest, based on its exceptional culinary and nutritional value. Artificial mycorrhization has been used to improve not only tree growth and development but to enhance mushroom production for commercialization. Traditionally, mycelium for mycorrhization of chestnut trees has been obtained from carpophores and/or spores, as it is easily

prepared, relatively affordable, and does not require specialized equipment. However, this approach is seasonally dependent, and may carry mycelia/spores from other fungi and even pathogens, hindering the selection of specific genotypes. To overcome those limitations, the production of mycelium through i*n vitro* culture using pure single and élite strains is highly recommended. Today, there is limited knowledge regarding the growth conditions and nutritional and climatic requirements of *B. reticulatus*.

This study aimed to identify the key factors influencing the mycelium growth of *B. reticulatus* under *in vitro* conditions in solid culture. The mycelium was isolated from carpophores collected in the chestnut forests. The studied factors included: culture time, genotype, sampling site, temperature, and culture media composition. Specifically, his impact on presence/absence and the area of growth were analyzed over 59 days.

The results were analyzed by neurofuzzy logic, a combination of artificial neural networks and fuzzy logic, revealing that only culture time and genotype were the key factors for the presence/absence of growth, while for the growth area were culture time, genotype, and culture medium, although the interaction culture time and temperature also played a significant role.

Overall, these findings provide valuable insights into the crucial parameters involved in the development of a novel protocol for *in vitro* culture of *B. reticulatus*, which can be used in mycorrhization procedures.

Keywords:

Chestnut mycorrhization, ectomycorrhizas, in vitro culture, Boletus reticulatus

The effects of chestnut apical buds position on vegetative and reproductive annual growth

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Abstract:

The growth and fruiting pattern of chestnut annual branches was investigated in a traditional sweet chestnut (Castanea sativa Mill.; cv. “Marrone di Castel del Rio”) orchard. The development of the annual laterals, from the top four apical flower buds, was described in one year old branches, randomly selected in the orchard. A total of 60 laterals was weekly measured in length and diameter from bud break (07 BBCH stage) to a week before burr fall (83 BBCH stage). On the same laterals, once female inflorescenses were well differentiated and fully receptive (65 BBCH stage), the burr diametral growth was also weekly measured and the number of burrs, in each lateral, counted. At harvest, nut quality (fresh weight and diameter) was also assessed. For each lateral, shoot length and burr diameter absolute growth rate (AGR) were calculated. The vegetative growth of laterals showed a rapid increase in the first part of the season, reaching a peak, in absolute growth rate, in the middle of May. Later, their growth reduced and being constant throughout the rest of the season. Laterals showed a reduced vegetative vigour, form the apical to the bottom part of each branch, although a limited presence of burrs in the lower ones. Once laterals stop completely their growth (middle of June), burr started to increase their growth rate, likely acting as the major sink. The apical bud, although carrying the highest number of burrs per lateral, showed the highest growth rate along the season with also improved nut quality traits recorded at harvest. These preliminary results highlight the importance of the apical bud in sustaining chestnut yield and quality.

Keywords: *Castanea sativa* Mill., chestnut physiology, shoot growth, burr growth

Chestnut leaf and burr composting techniques as alternatives to burning

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Abstract:

Chestnut husks and leaves present several problems in their management. They must be removed from the field before the following year's fruit fall to facilitate their harvest. In addition, they represent a source of inoculum of Gnomognopsis castaneae. For these reasons, in most cases chestnut crop residues are burnt, causing emissions into the atmosphere and risking the ignition of fires. Biomass management is a central issue on the road to greater sustainability in agriculture and biomass composting has often proven to be a viable solution, but chestnut crop residues have a high resistance to degradation due to their high C/N ratio. In order to find a solution to the problem, an experiment was set up to evaluate the feasibility of composting leaf and burr with the addition of different nitrogen-rich materials using a methodology that can be replicated even by small farms. In December 2021, 21 composters were built in two different locations in Piedmont. The composters were made of wire mesh, a cheap and easily workable material so that they can be replicated by farmers. The composters were filled by pressing the litter by hand and six different treatments were carried out by adding cattle manure, poultry manure, urea, ammonium sulphate, Prodigy® Plus and an ad hoc product from the company Gobbi®. In addition, two leaf piles were created at both locations. This research was realised within the project “Progetti pilota per la Cooperazione ed il miglioramento della Competitività della Castanicoltura regionale - 3C” (joint contribution of the European Union, the Stato Italiano and the Regione Piemonte under the "Programma di Sviluppo Rurale PSR 2014 – 2020, Mis. 16 Cooperazione, Op. 16.2.1 Attuazione Progetti Pilota nel Settore Forestale").
The expected results of the trial are the formation of compost useful for replenishing organic matter in the soil and the reduction of G. castaneae. The analysis on the presence of G. castaneae and the chemical composition of the litter after one year are still under processing, but the measurements taken on the horizons present in the composters offer already interesting information. The composters treated with Gobbi® LG135 present less undegraded litter, while those treated with cattle manure present the larger amount of humus.

Keywords: leaf, compost, sustainable agriculture, *Gnomognopsis castaneae*, crop residues

Effects of thermohidroterapy against Dryocosmus kuriphilus in dormant rooted *Castanea sativa* plants

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Abstract:

Dryocosmus kuriphilus is responsible for the chestnut gall disease (ACGW). Hot water treatment (HWT) has been proved to be effective for disinfecting *Castanea* spp. dormant scions against this pest. However, the effect of HWT on infested rooted chestnut plants has not been studied yet. To establish an effective disinfection protocol for this kind of plants, a randomized factorial trial was carried out evaluating four temperatures (46, 49, 52 and 55 ºC) and two soaking times (5 and 10 minutes). Chestnut plants without HWT were included as controls. The presence of galls on new shoots, plant survival and growth (length increase), bud sprouting as well as stem damage caused by burns were evaluated. The phenological and phytopathological monitoring was also carried out.

Five minutes soaking at 46 or 49 ºC was not completely effective in killing ACGW larvae. The 55 ºC treatment for 5 minutes eliminated the galls but burn damage was observed on 83% of the stems, decreasing by half the percentage of sprouted buds and a 6% the growth when compared to plants treated to lower temperatures. HWT at 55 ºC for 10 min was lethal for a 33,33% of the dormant plants; in the remaining ones, the stems suffered severe burn damage which had a negative impact on the evaluated parameters. Ten minutes soaking at 52 ºC produced stem burns on 33% of chestnut plants. All the surviving plants subjected to HWT had a similar or higher increase in stem lenght than the control ones and also experienced an advance on the phenological stages.

In conclusion, HWT protocols by applying 10 min soaking at 49 ºC or 5 min soaking at 52ºC were effective in killing ACGW larvae while retaining good percentages of stem growth and bud break.

Keywords: Asian gall wasp; chestnut; hot water bath; integrated control

Chestnuts are (entirely) insect-pollinated?

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Abstract:

Are chestnuts pollinated by wind? by insects? Or by both? For almost 150 years, this question has been in the air. The huge production of tiny pollen grains transported by wind over long distances seemed to point towards wind pollination. However, the brightly colored flowers, the strong spermatic smell, and the sticky pollen grains of chestnuts are characteristic of insect pollination. To clarify this question, we performed insect-exclusion experiments. The results clearly show that in the absence of insects, fruit production collapses almost entirely: chestnuts are therefore insect-pollinated. Which insects pollinate chestnuts? To answer this question, we monitored 16 chestnut trees during the entire flowering period in an experimental orchard. We identified 4203 arthropods from 129 taxa corresponding to at least 101 species. The most abundant orders were Coleoptera, Hymenoptera and Diptera. However, only beetles and calyptrate flies regularly visit both male and female flowers. Wild bees and honeybees visit male flowers of chestnut to collect pollen but fail to visit rewardless female flowers. The male parts of bisexual catkins play a key role in pollination: they attract walking insects close to female flowers, increasing the probability of a contact with the stigmas. Installation of beehives will not improve pollination service in chestnut orchards. Instead, the presence of wild insects is critical for fruit production. The preservation of these insects is therefore critical to the sustainable management of chestnut orchards.

Keywords: Insect-pollination, wild pollinators, insect-exclusion experiments, insect monitoring, beetles, flies, deceptive female flowers, mating facilitation

Developmental anatomy and histochemistry reveal the neo-ontogenesis of the leaf tissues of *Castanea mollissima* (Fagaceae) towards the galls of Chestnut

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Abstract:

Castanea species (Fagaceae) show a worldwide distribution in Asia, Europe and North America. *Dryocosmus kuriphilus* Yasumatsu (Hymenoptera, Cynipidae), the Asian chestnut gall wasp (ACGW), an invasive gall maker native to China, has become one of the major chestnut tree pests around the world. ACGW induced galls in leaves, stems, petioles, and midveins. Gall formation interfered with the normal development of vegetative and reproductive structures, reduced leaf area and the formation of female flowers, which jointly lead to a reduction in wood and nut yield. Understanding the morphological changes of host organs is critical for assessing how galls grew on leaves. Herein, qualitative and quantitative approaches were employed to elucidate how cell divisions, elongation patterns, and tissue organization for the development of intralaminar gall morphology were induced by ACGW.

Oviposition and larval feeding affected the formation and maintenance of galls. The gall was covered by the epidermis out of the upper epidermis, which was divided into: vegetative tissue layer, thin/thick-walled tissue layer, vascular bundle layer, cortex, epidermis and other structures from inside to outside. Before bud splitting, tiny ACGW larvae induced the formation of two layers of hypertrophied cells, and cell division occurred around their larval chambers. After sprouting, galls formed and served as a nutrition pool. The vegetative tissue in the gall was formed by special parenchyma tissue, which was close to the feeding site of the larva and began to form shortly after gall induction, with dense cytoplasm, protruding nucleus and accumulation of primary metabolites. As the larvae continued to feed, a sclerenchyma sheath developed around the vegetative layer. In young galls, larval activity resulted in damaged cells with disorganized protoplasm near the larval chamber. A vegetative layer surrounded the larval chamber and these cells contained numerous mitochondria and liposomes. The vacuolated parenchyma cells comprised the gall cortex as well as vascular tissues, which transport water and nutrients to other plant tissues.

The gall insects controlled host plant tissues and stimulated the primary and secondary metabolites. The histochemical analyses have shown that, proteins and reducing sugars were detected in the inner tissue compartment. The parenchyma, with hypertrophied cells, accumulated auxins, besides starch and phenolics. Starch was broken by phosphorylase activity, and then the invertases broke the sucrose into glucose and fructose, which were densely detected in the development of these galls. The ROS (Reactive Oxygen Species) was detected in nutritive cells, vascular bundles, and photosynthetic parenchyma, and the scavenging of ROS molecules performed by the phenolics modulates cell reprogramming.

Keywords: Chinese Chestnut, *Dryocosmus kuriphilus*, gall differentiation, histochemical analysis, Reactive Oxygen Species

Impact of the cooking process and chemical and enzymatic methods on the sensory characteristics of chestnut and, in consumer approval

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Abstract:

The COVID-19 pandemic has brought attention to the importance of sensory interactions in the human mouth, as some infected individuals experienced a loss of sensation on the palate. Meanwhile, consumer demand for high-quality sensory attributes and high nutritional value in food has increased, prioritizing healthy foods. Chestnuts (*Castanea sativa*), with their unique properties, are versatile nuts in gastronomy, increasing exceptional qualities in food dishes. This study aimed to investigate the influence of cooking chestnut processes, chemical composition, α-amylase activity, and sensory profile on two cultivars of chestnuts: Longal and Judia. The study was carried out in three phases: the chemical phase, in which the content of starch, soluble sugars, crude protein, and crude fat was analyzed; the enzymatic phase, in which the α-amylase activity of the taster was quantified during the nut tests; the sensory phase, through the execution of the QDA test (quantitative descriptive analysis), applied to a trained panel. The results show that the chestnuts, of both varieties, are low in soluble sugar and fat, but high in starch, which has been shown to influence the enzymatic activity in the mouth cavity of the consumers. At the sensory level, it was denoted that the roasted Judia and Longal chestnuts were the most appreciated since they are the most consumed by the consumers. Overall, nuts' biochemical and sensory properties have a potential impact on consumer acceptability. Understanding these properties can help in the development of new food products with these nuts and improve the quality of existing ones.

Keywords: *Castanea sativa;* chemical analysis; QDA test; consumer acceptability; α-amylase activity

Silicon fertilization in chestnut plants: an efficient solution to ink disease

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Abstract:

Castanea sativa has been severely affected by ink disease (caused by *Phytophthora cinnamomi)*, a problematic pathology that in Portugal was responsible for the disappearance of more than 50% of the chestnut-producing area since the beginning of 20th Century. Unfortunately, it continues to be one of the most devastating root rot pathogens of chestnut, being essential to finding alternative strategies that can help the trees to increase their resistance against it. In this context, silicon (Si) fertilization appears as a possible inducer of tolerance against *P. cinnamomi* infection, acting as a plant protector against this and other biotic stresses in different crops of the world. This study's main objective was to evaluate Si effect on plant protection against *P. cinnamomi*. Four groups of chestnut seedlings were fertilized with 0, 5, 7.5, and 10 mM Si, and one month after half of them were inoculated with the oomycete. The mortality rate of chestnut trees treated with Si was proportional to the concentration of Si applied, 40%, and 10% in the 5 mM, 7.5 mM, and 10 mM Si groups, respectively. In the *in vitro* study, 24 hours after incubation, the Petri plates containing Si+PDA didn't present any growth of *P. cinnamomi*, demonstrating 100% of percentage inhibition (PI) while in the control (0 mM Si) there was seen a total growth of the pathogen. Moreover, in another *in vitro* study, 12 days after incubation of SiK with the PDA medium to evaluate its fungi toxic capacity, results showed that 10 mM Si presented a stronger inhibition zone (90%) against *P. cinnamomi* comparatively to the control treatment (0%). Results indicate that Si can act as an elicitor that triggers rapid action by the biochemical defence system. The present research suggests that Si fertilization could be successfully used in the control of ink disease increasing plant protection against the oomycete through the action of both physical and chemical defence mechanisms and by stimulating the antioxidant system. In this way, it might constitute a

potential tool that can be used by producers to improve the protection of plants and consequently improve the production and quality of the chestnut fruit.

Keywords: chestnut, ink disease, phenols, *Phytophthora cinnamomi* and silicon

Comparative growth and yield performance of sweet chestnut in high-forest and coppice systems in young plantations

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Abstract:

We compare growth and yield of sweet chestnut in coppice and high-forest systems up to 24 years of age in Northern Portugal. In the coppice, data from 4 plots submitted to different management models for small (P1), medium (P2) and large (P4) diameters, including a plot without intervention (P3), were used. Management model in P4 (1 shoot at the end of rotation) is converging to a similar product as high-forest over time. Site index (SI) for the coppice is 15 m of dominant height (hdom) at 20 years. The same SI was used when selecting data from permanent plots in high-forest for the comparison. Individual-tree equations previously developed were used to obtain stem volumes. Biomass of stem was obtained from volume using wood density. Results show that coppice for similar ages and SI, produces more stem volume per hectare than high-forest in any situation up to 24 years. At this age, height of the mean tree (hg) and hdom are lesser in the high-forest than in the coppice's management model with similar target (P4). In contrast, diameter of the mean tree (dg) and dominant diameter (ddom) are similar in both systems. Results also show that P4 in coppice system produced more 100 m^3/ha of stem volume than the high-forest at age 24. However, mean annual increment (MAI) in stem volume in high-forest is still increasing as opposed to the coppice. Thus, coppice system produces more biomass in

young ages than high-forest (MAI in biomass: 9 t/ha/year against almost 4 t/ha/year, respectively). Managing coppice for a final target similar to high-forest introduces flexibility in sylviculture, allowing both high amount of biomass production in young stages, and long-life products at the end of rotation.

Keywords: *Castanea sativa* Mill., volume, biomass, sylviculture, forest management

Metagenomic characterization of soil bacterial communities in young chestnut orchards in northern Portugal

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Abstract:

Soil microorganisms are key factors in biogeochemical cycles and drivers of soil productivity. However, the soil bacterial community associated with the European chestnut (Castanea sativa Mill.) is poorly described. In this sense, this study aimed to characterize the soil bacterial community in young chestnut trees in Northern Portugal and its dynamics over the year. Soil samples were taken in three periods of the year (spring, summer and autumn) from two young chestnuts orchards located in Parada (41º38'12.53" N; 6º42'42.94" W) and Salgueiros (41º54'12.73" N; 7º01'40.95" W) at elevations 740 and 1008 m, respectively. Soil DNA was extracted and its 16S rRNA amplicons were sequenced using the Illumina MiSeq platform. Overall, the bacterial core of chestnut orchards is mainly composed by the four main phyla: Proteobacteria, Acidobacteriota, Actinobacteriota and Chloroflexi in both sites. These phyla were identified in all seasons of the year. However, the phylum Chloroflexi, for its metabolic and phenotypic diversity, was the one that stood out the most in altitude in autumn. Acidobacteriales and Acidobacteriaceae (Subgroup 1) of Acidobacteriota and α-Proteobacteria (Rhizobiales, Sphingomonadaceae, Rickettsiales and Micropespsaceae) and γ–Proteobacteria (Burkholderiales and Xanthomonadales) of Proteobacteria, and Thermoleophila and Acidimicrobia of Actinobacteriota were dominant in all seasons and in both sites. The study provided insight

into the bacterial community associated with chestnut trees and will allow for monitoring of potential changes in the ecosystem due to climate change.

Keywords: Chestnut, bacteria, 16S rRNA, Illumina MiSeq

Late season irrigation improves chestnut physiology and nut quality

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Abstract:

Italy is among the major producers of chestnuts around the world. Most of the traditional Italian chestnut orchards are still non-irrigated, as typically located in mountain-hill areas where climate conditions in the past were not limiting vegetative and reproductive growth. Nowadays, the rise of summer temperatures and the decrease of rainfall are negatively impacting chestnut physiological performance and productivity. Adoption of scheduled irrigation practices, given the limited water availability/possibility of storage typical of these areas, should become part of chestnut orchard management. The purpose of this study was to test the adoption of late season irrigation on sweet chestnut physiology, yield, and nut size. This research was carried out in a traditional chestnut orchard of the "Marron Buono di Marradi" ecotype, in the Tuscan-Emilian Apennines (Marradi, Italy). Between August and September, two treatments were carried out: non-irrigated and irrigated trees. Plant water status and leaf gas exchanges were monitored. Tree yield and nut size were recorded at harvest. Preliminary results showed that, in September, irrigated trees had higher physiological performances and a less negative water status, than non-irrigated trees. Nut size was significantly smaller in non-irrigated trees than in irrigated ones, while yield was not statistically affected by the irrigation treatment. Despite the mild and rainy season, late summer irrigation was beneficial for improving sweet chestnut physiological performances and nut quality.

Keywords: *Castanea sativa* Mill., leaf gas exchanges, water relations, nut yield

Application of commercial disinfectants to improve chestnut fruit sanitation

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Abstract:

Fresh European chestnut (*Castanea sativa* Mill.) has intermediate perishability characteristics between fresh and dried fruits. Weight loss, infestation by insects or live larvae, decay and fungal development are some of the problems observed after harvesting and storage that lead to significant economic losses along the chain. The conservation methods currently used by the chestnut industry (conventional hydrothermal treatment, 50 °C/45 min) are not enough to minimize the problems observed during storage. Thus, this work aimed to test an alternative way of post-harvest treatment of fresh chestnuts, using an appropriate disinfectant based on sodium hypochlorite, available on the market and used in the food industry. The treatments applied were as follows: (TA) immersion in a hot water bath at 50 °C/45 min (actual industry treatment) and (TB) immersion in a cold water bath with sodium hypochlorite (100 mg/L of active chlorine) for 10 min. After 30, 60, 90 and 120 days of chestnut storage in industry refrigerated chambers (0-2 °C, without relative humidity control), the population of aerobic mesophilic and fungi was evaluated. The results showed that the disinfectant sodium hypochlorite could be an alternative to the conventional method, since it significantly reduced the microbial load of total aerobic mesophilic and fungi up to 60 days of chestnut storage.

Keywords: Chestnuts, Aerobic Mesophilic, Fungi, Sodium hypochlorite

Stem volume ratio equations to variable merchantable limits for sweet chestnut in Portugal

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Abstract:

The volume is a fundamental dendrometric characteristic that helps estimate the amount and value of wood that can be harvested, and provides insight into land productivity. However, accurately measuring trees for volume calculation is both costly and time-consuming. Therefore, equations that estimate volume based on dendrometric variables, such as DBH (diameter breast height) and total or merchantable height, are highly important for forecasting forest production. However, the total stem volume alone does not indicate the amount of wood available for specific uses, such as sawtimber or biomass. Stem volume ratio equations are essential as they provide flexibility in determining the volume of wood based on the tree's various usage categories and potential changes in market demands over time. These equations allow for alternative options in obtaining volume, considering variable top diameter or height. Forest managers can use these equations to estimate the volumes of multiple logs from a single stem during integrated logging and for forest inventory purposes that require yield estimates by product categories. Therefore, this study's primary objective was to develop volume ratio prediction equations based on tree dendrometric variables, including DBH and total height, for high-forest sweet chestnut (***Castanea sativa*** Mill.) stands located in northern Portugal. Both linear and nonlinear regression estimation methods were employed, with data analysis based on information collected from 466 standing trees measured using a telerescope for precise volume determination. Model validation involved destructive analysis of 39 felled trees distributed across the existing diameter classes (10-65 cm) in three adult chestnut stands. The study employed the least squares method to fit several linear and nonlinear equations to select models, with the best-fitting equations presented.

Keywords: volume ratio-equations, *Castanea sativa* Mill., merchantability prediction equations, yield

Enhancing in vitro solid culture of B. edulis and B. reticulatus through elicitation with indoleacetic acid and oxalic acid

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Abstract:

The rapid urbanization trend has resulted in the neglect of rural areas worldwide, leading to economic and social challenges. However, rural regions possess abundant resources that, if properly harnessed, can significantly improve the well-being of their communities. One such resource is the chestnut tree, which has been utilized for centuries due to its timber and edible fruits. Spain's substantial chestnut production provides a valuable opportunity to explore complementary economic activities, such as mushroom harvesting. Despite this potential, the slow growth of *Boletus edulis* complex *in vitro* culture poses a significant obstacle. In this study, an innovative approach using organic acids as potential growth elicitors was developed, aiming to enhance the chestnut mycorrhization process with *Boletus edulis* complex and contribute to the economic development of rural areas.

To address the slow mycelial *in vitro* growth challenge, a novel cultivation protocol utilizing organic acids as growth elicitors were designed. Specifically, the effects of indoleacetic acid and oxalic acid at three different concentrations on the mycelium growth of *Boletus edulis* and *Boletus reticulatus* were investigated. The mycelium growth was evaluated using image analysis techniques, providing quantitative data for analysis and comparison. This methodology focused on identifying effective concentrations of organic acids that promote optimal mycelium growth and

could be implemented in a sustainable cultivation process at the industrial level.

Preliminary results revealed that the supplementation of oxalic acid at higher concentrations stimulated greater mycelium growth in both *Boletus* species, indicating its potential as a growth elicitor. These findings suggest that organic acids play a crucial role in promoting the development of mycelium, thus offering a promising avenue for improving the cultivation of commercially valuable boletus mushrooms. Further optimization of the cultivation protocol based on these results holds the key to unlocking the economic potential of mushroom production in rural areas.

Keywords:

Rural areas development, Mushroom cultivation, Boletus edulis complex, Sustainable agriculture, Organic acids, Growth elicitors