



IX International Symposium on

Walnut & Pecan

Grenoble, France 12–16 June 2023

ABSTRACT BOOK



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KEYNOTE LECTURES

SESSION I: GERMPLASM, GENETICS, BIOTECHNOLOGY

Harnessing genetic diversity in *Juglans* for sustainable walnut production

Pat J. Brown, UC Davis

Walnut production is threatened by both biotic factors (*Xanthomonas* blight, insect pests, soil-borne pathogens, and cherry leaf roll virus or CLRV) and abiotic factors (declining winter chill, extreme heat, and water deficit/salinity). Both these threats are characterized by uncertainty: uncertainty about how quickly chemical inputs for biotic control will be phased out, and uncertainty about how climate change will impact production in different walnut growing regions. Adoption of new scion and rootstock cultivars is the most cost-effective long-term solution for the walnut industry but carries significant short-term risk for individual growers.

In this presentation I will highlight our efforts in the California Walnut Improvement Program (WIP) to generate new biological knowledge, breeding tools, and improved cultivars of walnut scions and rootstocks. Molecular data are having an increasing influence on scion breeding decisions, but the mutations underlying critical allelic differences remain unknown. An international collaboration to catalog global genetic diversity in *Juglans regia* resulted in the discovery of a selective sweep possibly associated with walnut domestication. Internal collaboration at UC Davis resulted in a new method for quantifying resistance to *Xanthomonas* blight and revealed a new source of potential resistance.

Diversity across the *Juglans* genus remains largely untapped for both rootstock and scion improvement. I will present our strategy for efficient generation and genotyping of *Juglans* hybrids. A large collaborative effort screening *J. microcarpa*/*J. regia* hybrid rootstocks for resistance to multiple soil-borne pathogens unexpectedly revealed a locus with large effects on crown gall resistance, Phytophthora resistance, and vigor. Backcrossing to *J. regia* is being used to develop CLRV-tolerant rootstocks that maintain vigor and stress resistance comparable to hybrids.

Adoption of any new tree cultivar carries a risk. Our goal in the WIP is to enable individual growers to make data-driven decisions by providing quantitative data, from relevant environments, with complete transparency.



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SESSION II: PLANT PHYSIOLOGY

Ecophysiological functioning of walnut and main risks in a changing climate

Thierry Ameglio and Guillaume Charrier, INRAE, France

Drought and frost stresses are the most important limiting factors that determine the ecological distribution and production of tree species. Walnut, in particular, has a low resistance to edaphic drought and frost, which explains its development in lowland areas with deep and fertile soils and its low development at high elevation in our climates.

The ecophysiological tools developed in the laboratory (determination of hydraulic conductance, carbon reserve, water potential, electrolyte leakage and continuous monitoring of trunk or branch diameter variations) allow us to evaluate the risks of drought or frost death throughout the tree's development cycle and to understand the periods of greatest sensitivity to these risks.

The construction of mechanistic models, based on physiological parameters that vary according to climatic data, makes it possible to predict these risks in future climates and to propose risk maps according to these constraints.

SESSION III: PRODUCTION, HARVEST, FRUIT QUALITY AND MARKETING

The consumption of fruit and vegetable in the food transition: what place in the kitchen?

Catherine Baros, CTIFL, France

The covid crisis has accelerated some tendencies in the food consumption, and habits are coming back. Furthermore, at short term the inflation in France has an influence on the purchase of most households. It is already necessary to adapt our consumption to the climatic change. The revegetation of food on the way may be a solution and an opportunity to develop the consumption of walnut and pecan, at home and out of home.

SESSION IV: PLANT PRODUCTION, ORCHARD MANAGEMENT, DEFENSE

Is crop happiness under our feet?

Daniel Wipf, INRAE, France

Soils are a resource threatened by increasing anthropogenic pressures (IPBES, 2018). Some agricultural practices are the cause of physical degradation of soils, but also of biological degradation, with a decrease in biodiversity and soil organic matter. Other so-called agroecological farming practices, on the contrary, have a positive impact on soil biodiversity. The talk will review agricultural practices in the broad sense of the term, with a focus on their impact on or integration of soil biodiversity, in the light of current societal and environmental challenges.



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SESSION V: PESTS AND DISEASES

Climate change and plant diseases: opinions, trends, and case studies

Florent P. Trouillas, University of California, United States

Climate change refers to long-term shifts in temperatures and weather patterns. Although these shifts may occur naturally, human activities since the 1800's have been broadly accepted as the main driver of climate change, primarily due to the burning of fossil fuels like coal, oil and gas. One major aspect of climate change is the increase of extreme weather events, including cold and heat waves, drought, heavy precipitations, floods and storms. In the current scenario of rapidly evolving climate change and high frequency of extreme weather events, plants are more frequently subjected to stresses of both abiotic and biotic origin. Because most plant pathogens respond to weather, changes in weather events due to climate change are likely to impact the frequency and severity of disease epidemics, thus threatening the global food supply. Numerous scientific reviews have attempted to foresee the impacts of climate change on plant health. Yet, scenario analyses of the potential impact of climate change on plant pathogens are limited. While the impacts of climate change are not easily determined, these could have positive, negative or no impact on individual plant diseases and will vary by region and by crop. Adverse impacts may include disease emergence and outbreaks, increased yield losses, the inefficacy of management strategies and changes in the geographical distribution of plant pathogens. The present review will summarize common opinions and trends related to climate change and plant diseases. Global case studies, adaptation strategies and field observations in California forests and fruit and nut crops will be discussed.



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ORAL COMMUNICATIONS

SESSION I: GERMPLASM, GENETICS, BIOTECHNOLOGY (Part 1)

(OC02): Growth of tall, unpruned bare rooted Chandler walnut trees on clonal Paradox VX211, RX1, and Vlach rootstock 2015-2022

Janine Hasey, Luke Milliron, Clifford Beumel, Clarissa Reyes, Charles Brannon, Dr. Bruce Lampinen (University of California, USA)

In California, finished bare root trees are typically delivered from the nursery around 1.8 m height. During the first leaf, trees are trained to a single trunk. The primary scaffolds develop during the second leaf and secondary scaffolds during the third leaf. One-year old nursery grown Chandler June buds on clonal rootstocks with large root systems were left unheaded at 2.7 m tall when they were dug and then planted in winter 2015. Subsequently, no heading cuts were made during the tree training years. These tall trees produced functional primary branches during the first growing season. By the end of the second growing season, trees had a well-developed structure much more advanced than a conventionally trained two-year old tree. Trees were mechanically harvested and produced high yields in the third leaf, with RX1 producing significantly higher yields. In 2019 through 2022, there were no significant yield differences between rootstocks. Chandler on all three clonal rootstocks produced approximately 6.7 metric tons per hectare in the sixth and eighth leaf. Only in 2022 were there significant nut quality differences between the rootstocks, with RX1 producing Chandler nuts with poorer quality. Rootstock circumference for VX211 was significantly greater than Vlach which was significantly greater than RX1. Chandler on VX211 had a significantly lower scion circumference compared to Vlach early on but there were no differences between scion circumferences from 2020 through 2022 among the three rootstocks. Surveys showed Vlach with the most crown gall, VX211 as intermediate, and very low infection in RX1 rootstock. July and October 2022 leaf samples showed trees on RX1 with significantly higher percent chloride compared to VX211 and Vlach. Using tall bare rooted trees with no heading cuts for eight years allowed earlier tree development and yields, reducing costs of orchard development while producing quicker and sustained returns.

Keywords:

June buds, unheaded, primary branches, crown gall, chloride

(OC03): Individual leaf area variation in a provenance collection of pecan (*Carya illinoensis*)

Cristina Pisani, Jennifer Randall, Quentin Read, Angelyn Hilton, Xinwang Wang, Warren Chatwin, Patrick Conner, Jordan Brungardt, Lenny Wells, Richard Hereema, **Clive Bock** (United States Department of Agriculture, USA)

Pecan (*Carya illinoensis*) is a nut tree native to North America. A collection of 889 trees representing 19 provenances covering the native range of the species is planted at the USDA-ARS Southeastern Fruit and Tree Nut Research Station, Byron, GA. The collection is being used to characterize traits of horticultural interest. Of these traits, individual leaf area (LA) is important as studies in other species have shown a direct relationship between LA and photosynthetic efficiency, which contributes to carbohydrate accumulation, yield, and nut quality. Thus, evaluating LA in pecan will provide insight into the breeding potential of the germplasm. In spring 2021 and 2022, ten fully expanded compound leaves were sampled from each of the 889 trees in the collection, the LA was measured, and leaflet counts per leaf were obtained. The LAs showed a similar range and distribution for each provenance for both years. The Kentucky (KY-W) (latitude 37.050) provenance trees had the largest maximum leaf area (799.9 cm²) and (808.5 cm²) in 2021 and 2022, respectively. Mexico (MX-O) (latitude 17.083) had the smallest maximum leaf area (415.7 cm²) in 2021, and Texas (TX-K) (latitude 29.233) (350.3 cm²) in 2022. Mean leaf areas varied significantly depending on the source of provenance in both years ($P < 0.0001$). Trends were similar in both years, with northern provenances having the largest leaves and southern provenances



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having smaller leaves. The maximum leaflet count in 2021 (17) was shared by several provenances, but in 2022 a provenance in Missouri had the highest leaflet count (19). The lowest leaflet counts were from a Mexican provenance (5) and a Texas provenance (4) in 2021 and 2022, respectively. This research provides insight into the diversity of leaf size and leaflet counts in pecan, providing knowledge for pecan improvement.

Keywords:

Leaf area index, *Carya illinoensis*, germplasm, native range, leaflet

SESSION I: GERMLASM, GENETICS, BIOTECHNOLOGY (Part 2)

(OC04): Growth of bare root June budded Chandler walnut trees compared to potted micrografted finished trees on clonal Paradox RX1 rootstock

Janine Hasey, Luke Milliron, Clifford Beumel, Dr. Bruce Lampinen (University of California Coop. Extension, USA)

California walnut growers have several nursery products for clonal rootstock including bare root or potted rootstock, or finished bare root one-year old June buds or two-year old nursery grafted trees. Another option, potted finished trees that are grafted in the container (micrografts), have limited availability. Planting bare root or potted rootstock and fall budding or grafting the following spring adds an additional year to the training, compared to finished trees where the trunk is trained during the first leaf. An orchard planted in 2016, alternated every two rows with either bare root Chandler June budded trees or potted Chandler micrografted trees both on clonal Paradox RX1 rootstock, to compare growth, yield, and crown gall incidence. Within the June bud rows was a replicated rootstock trial with RX1 as a treatment. Growth and yield (2018-2020) on these trees were compared to adjacent micrografted Chandler on RX1. Additionally, canopy light interception and yields were taken (2019-2022) comparing June buds to micrografts on a larger scale in the orchard. No heading cuts were made at planting or during training. At planting and after the first leaf, June budded rootstock was significantly larger than micrografted rootstock. By the third year and continuing through year six, both rootstock and scion on micrografts were significantly larger than on June buds. However, fourth leaf yields were significantly higher in June budded trees. Although there were no significant yield differences for fifth through seventh leaf, cumulative yield and yield efficiency were significantly greater in June budded trees. We observed delayed catkin production in micrografted trees. There were no significant differences in nut quality in 2022. RX1 had extremely low crown gall infection in micrografted trees. Our results show higher vigor on potted micrografted finished trees, but lower early yield compared to bare root June budded trees.

Keywords:

nursery products, yield, crown gall, canopy light interception, no heading cuts

(OC05): Field testing of resistance of novel hybrid clonal walnut rootstocks to soilborne biotic stresses

Dr. Katherine Jarvis-Shean, Janine Hasey, Charles Leslie, Dr. Greg Browne, Dr. Ali McClean, Natalia Ott, Dr. Andreas Westphal, Dr. Daniel Kluepfel (University of California, USA)

Continued breeding and selection strives for walnut rootstocks with improved, combined resistances to multiple soilborne pathogens, including *Agrobacterium tumefaciens* (At) (crown gall), *Phytophthora* species (Psp) (crown and root rot), and *Pratylenchus vulnus* (root lesion nematode). In a factorial field experiment, resistance to At and Psp in four new clonal rootstock candidates was compared to the three commercially available clonal rootstocks ('RX1', 'VX211' and 'Vlach') as standards. In May 2015, potted trees were planted in replicate genotype subplots nested within inoculation main plots (Psp, At, and non-inoculated). Trees were budded to 'Chandler' in September 2015, and failed grafts were re-budded in 2016 and 2017. Inoculation treatments were applied in May 2017. In 2020, crown gall incidence and gall size rating were measured at the trunk bases. In November 2020, all root systems were excavated and sampled to assess incidence of crown and root rot



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associated with Pssp. At the same time, root infections with *P. vulnus* of natural soil infestations were quantified in the non-inoculated replicates. VX211 and Vlach had the highest gall ratings in response to At, significantly higher than RX1. Novel K3 had significantly lower gall ratings than the three standard clones. Gall ratings on novel 11-991, STJM4 and 29JM8 were not significantly different from those of RX1 or K3. High incidence of crown and root rot (CR) from Pssp was found in K3, Vlach and VX211. The Phytophthora-resistant RX1 had no CR, as did 11-991; 29JM8 and STJM4 had low to zero incidence of CR. Novel K3 showed consistently low nematode infections, similar to the standard clones, whereas 29JM8 and STJM4 had higher numbers than the standards, with 11-991 intermediate. Budding success, tree mortality, and tree growth were also documented. The results showed high potential for using genetic resistance to combat these soilborne biotic stresses.

Keywords:

Hybrid Paradox rootstocks, Phytophthora, Agrobacterium, Nematodes, VX211, RX1, Vlach, 11-991, K3, 29JM8, STJM4

(OC06): Identifying walnut rootstocks with resistance to multiple soil-borne plant pathogens

Dr. **Andreas Westphal**, Dr. Zin Thu Zar Maung, Tom Buzo, Dr. Patrick Brown, Charles Leslie, Dr. Greg Browne, Natalia Ott, Dr. Ali McClean, Dr. Dan Kluepfel (University of California Riverside, USA)

Most US edible walnuts are produced on deep-rooting soils of California. Favorable growth conditions also provide conducive conditions for soil-borne pathogens to infect and thrive on susceptible walnut roots. Traditional rootstocks based on crosses of Northern California black walnut and English walnut ("Paradox") seedlings are susceptible to key soilborne pathogens, including: *Agrobacterium tumefaciens*, *Phytophthora* spp., and *Pratylenchus vulnus*. Soil fumigation has been relied upon to reduce preplant populations of phytopathogenic nematodes but has generally not provided effective management of *Agrobacterium* or *Phytophthora* on walnut. Recent releases of clonal rootstocks, RX1 (resistant to *Phytophthora* spp.), Vlach (high vigor), VX211 (tolerance to *Pratylenchus vulnus*) provide some protection from single soil-borne disease problems. Clearly, integrated management of soilborne walnut diseases would benefit greatly from rootstocks with improved genetic resistance to the nematode, *Agrobacterium*, and *Phytophthora* pathogens. In the current project, >300 accessions each from controlled crosses on two selected mother trees were tested for susceptibility to the three pathogens in respective greenhouse or field-testing programs. When compiling the data of all three testing systems, some accessions expressed only minimal susceptibility to all three pathogens and grew at mid to high vigor under nematode-infested field conditions. Multiple accessions showed greatly reduced susceptibility to two of the three pathogens under investigation. Orchard trialing is underway to validate putative pathogen resistance and horticultural performance of multiple elite walnut rootstock selections that have resulted from this interdisciplinary project.

Keywords:

plant-parasitic nematodes, crown gall, *Phytophthora* crown and root rot, resistant rootstocks

(OC08): Influences of rootstocks on pecan scion growth in a replicated test orchard

Dr. Xinwang Wang, Mr. Keith Kubenka, Dr. Angelyn Hilton, Mr. Braden Tondre, Dr. Warren Chatwin, Dr. Cristina Pisani, Dr. Clive Bock, Dr. **Richard Heerema**, Dr. Jennifer Randall (United States Department of Agriculture, USA)

The pecan tree (*Carya illinoensis*) is native to North America and grows in a wide range of latitudes and longitudes in the United States. In order to accelerate the pecan scion's flowering and fruiting time, grafting onto an established rootstock is necessary. However, it is challenging to breed or select an appropriate rootstock that adapts to all pecan growing areas due to multiple factors. Since micropropagation technology is not readily available for pecan, open-pollinated nuts are used as seedstock by growers. Breaking nut dormancy is required for most pecan seedstocks, but the effect of stratification or freeze on seedling growth is unclear. To investigate



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the effects of different rootstocks on the growth and development of the 'Pawnee' scion, the USDA ARS Pecan Breeding Program conducted an experiment using 12 rootstocks from different geographical regions. Open-pollinated seeds were collected and subjected to different treatments: frozen/not frozen and stratified/not stratified. The test was conducted in a randomized complete block design with 240 trees with 5 replicates in each block, and data was collected on several growth parameters over three years. The results showed that frozen nuts significantly increased certain growth habits of the germinated seedling but did not affect others. Stratified nuts did not significantly change any growth characteristics. The influence of rootstocks on scion growth depended on their genotype and origin. Southern rootstocks generally performed better than northern ones, with rootstocks from Mexico significantly increasing scion trunk diameter, plant height, and canopy width. Among the 12 rootstocks tested, three south provenances showed the best results for increasing scion growth parameters, while north provenances significantly delayed bud break time. 'Posey', a cultivar of northern provenance, was found to significantly increase scion leaflet size compared to other northern provenances. These findings provide valuable insights for rootstock selection and breeding goals in the pecan breeding program.

Keywords:

Carya illinoensis, provenance, stratification, germination, plant height, trunk diameter, canopy width

SESSION I: GERMPLASM, GENETICS, BIOTECHNOLOGY (Part 3)

(OC11): Behavior of five walnut rootstocks in 'Serr' and 'Chandler', under the edaphoclimatic conditions in O'Higgins and Coquimbo regions, Chile

Ms. Giovanni Lobos, Ms. Jaime Otarola, Ms. Gamalier Lemus (Instituto de Investigaciones Agropecuarias, Chile)

The main cause of death of walnut plants in Chile is by *Phytophthora* spp., due to the fact that the most widely used rootstock is *Juglans regia*, highly susceptible to the disease, with 93% of the orchards located between the regions of Coquimbo and Maule, presenting some degree of the disease. The controls applied are of high cost and low results. Starting in 2017, the first clonal rootstocks, RX1, VX211 and Vlach, were introduced in Chile, adding to those already used Paradox and J. regia. In order to evaluate its behavior and the described characteristics, two orchards were established, located in the regions of O'Higgins and Coquimbo, where five rootstocks were grafted with 'Serr' and 'Chandler'. The design contemplated eight repetitions and the experimental unit of three plants each. Four of the replicates were infested with *Phytophthora* spp, to see the response to this variable, compared to the uninfested replicates. The evaluations began in October 2017. Plant growth and development were evaluated through shoot length, leaf area, trunk cross-sectional area (ASTT), root growth, floral induction, productivity, and fruit quality. In addition to surviving the infestation. The results from the first five seasons indicate that the VX211, Paradox and Vlach rootstocks have induced greater vigor in the variety, which has translated into greater plant development and fruit production/cm² ASTT, and RX1 it presents a greater tolerance to water stress, while J. regia in both 'Serr' and 'Chandler', presented lower development and production and there is no clear effect regarding the infestation with *Phytophthora* spp on the development of rootstocks.

Keywords:

Disease management alternatives, *Phytophthora* spp., growth and development, production and quality of the nut.

(OC11b): Phenological and pomological comparison of "Diriliş" and "Chandler" cultivars

Assoc. Prof. Mehmet Sutyemez, Dr. Akide Ozcan, Dr. S.Burak Bukucu (Univ of Kahramanmaraş Sutcu Imam, Faculty of Agriculture, Dept. of Horticulture, Turkey)



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This study was carried out to compare the new hybrid walnut cultivar "Diriliş" and Chandler cultivars in terms of phenological and pomological characteristics. The research was carried out in "KSU SEKAMER" walnut breeding plots between 2018-2022.

In the study, it was determined that the "Diriliş" variety leafing 2-5 days later than the "Chandler" variety, and it comes to harvest 18-21 days earlier. Defoliation in "Diriliş" occurred about 25-35 day earlier than "Chandler". The kernel percentage was determined as 52-54% in Diriliş variety and 47-49% in Chandler variety. In terms of tree fruit yield, it was determined that the "Diriliş" variety was more productive than the "Chandler" variety. The conclusions proved that 'Diriliş' cultivar's has superior traits in being late leafing date, early harvest date, high yield, and good nut quality.

Keywords:

Walnut, breeding, Diriliş, Chandler

SESSION II: PLANT PHYSIOLOGY (Part 1)

(OC12): Presence of different phenolic groups in various tissues of *J. regia*

Mr. Aljaz Medic, Prof. Dr. Anita Solar, Prof. Dr. Hudina Metka, Prof. Dr. Robert Veberic (University of Ljubljana, Slovenia)

Walnuts (*Juglans regia* L.) are a very high-energy food that is not only rich in valuable nutrients, but also has the most diverse phenolic profile and the highest phenolic content among tree nuts, both in the kernel and in other plant tissues. In the kernel, phenols consist mainly of phenolic acids, flavanols, and tannins, and to a lesser extent tyrosols, dihydrochalcones, and flavonols. Other phenolic groups are also found in other walnut tissues, particularly naphthoquinones, many of which are considered toxic. Since there is little information on the content of phenolic compounds and the relative abundance of different phenolic groups in different walnut tissues, our goal was to compare which phenolic groups are present in different walnut tissues and which are the most important phenolic groups in different tissues. Using UHPLC-MS / MS, more than 150 different phenolic compounds were identified in different walnut tissues. While no naphthoquinones were found in the edible part of walnut (pelticle and peeled kernels), naphthoquinones were present in all other tissues studied (leaves, petioles, bark, buds, roots and husks). This was expected since juglone and other naphthoquinones are associated with toxic effects and thus walnut kernels would be potentially toxic if consumed. Overall, naphthoquinones were the major phenolic group found in the leaves, petioles, bark, and buds, hydroxybenzoic acids in the lateral roots, and hydrolyzable tannins were the major phenolic compounds identified in the walnut kernels. The highest content of total analyzed phenolic compounds was found in the main root of walnut, followed by lateral roots and buds, leaves, and one-year-old bark, while the lowest content was found in petioles and two-year-old bark.

Keywords:

Phenolic compounds, walnut, *Juglans regia*, naphthoquinones, leaves, petioles, bark, buds, roots, husk, kernel

(OC14): Annual evapotranspiration of pecan tree in an arid and hot zone in Mexico

Prof. Dr. Julio Cesar Rodriguez, Prof. Dr. Salah Er-raki, Prof. Dr. Fidencio Cruz-Bautista, Prof. Dr. Abdelilah Meddich, Prof. Dr. Carlos Lizarraga-Celaya, Prof. Dr. Alejandro E. Castellanos-Villegas, Prof. Dr. Andres Ochoa-Meza (Universidad de Sonora, Mexico)

Agriculture uses about 70% of the annual available fresh water. This resource is limiting agricultural activity in the arid and warm areas of Mexico. The presence of perennial crops with a long growing season significantly increases the amount of water used by them, compared to annual and short-cycle ones. Asparagus, table grapes and pecan walnut are crops that have increased their area in recent decades. Pecan walnut has had the largest



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increase in area and has migrated from surface to pressurized irrigation. However, the annual volumes used have decreased little. To determine the atmospheric demand (ET_o), the crop evapotranspiration (ET_c), and the crop coefficient (K_c) of the pecan tree and contribute to the efficiency of water use, a micrometeorological tower was installed in the center of an orchard of 108.0 ha in 2016. A covariance system was placed in it to determine ET_c and meteorological sensors to calculate ET_o. Additional sensors were installed in the soil to monitor moisture and water tension. The results show a mean annual ET_o of 1,865 mm (1,784 – 1,965), with a mean annual ET_c of 1,409 mm (1,287-1,651), with mean annual irrigation of 1,612 mm (1,415 – 1,898) and mean annual rainfall of 210 mm (153-272). The average annual water balance was +413mm (301-506), always indicating inputs are normally higher than outputs. The Water Use Efficiency (WUE=GPP/ET) annual average was 1.43 gC m⁻² mm⁻¹(1.28 – 1.58), gradually improving efficiency from 2017 to 2022. The results suggest that it is possible to reduce the volume of water per hectare and maintain gross primary productivity. Proposing in a first stage, the uniformity of soil moisture, increasing the number of dripline (four to five) and in a second stage the reduction of the volume of water, maintaining the monitoring of the moisture and tension of water in the soil, the water potential in stems and stomatal conductance of pecan trees.

Keywords:

Eddy covariance, crop evapotranspiration, reference evapotranspiration, irrigation, arid lands

(OC15): Seasonal Crop evapotranspiration and crop coefficient of pecan walnut in arid region of Mexico

Prof. Dr. Salah Er-Raki, **Prof. Dr. Julio Cesar Rodriguez**, Prof. Dr. Fidencio Cruz-Bautista, Prof. Dr. Carlos Lizarraga-Celaya, Prof. Dr. Alejandro E Castellanos-Villegas, Prof. Dr. Abdelilah Meddich, Prof. Dr. Andres Ochoa-Meza (University Cadi Ayyad, Morocco)

A multi-year (2017-2022) study was conducted to measure the seasonal variation of crop coefficient (K_c) and crop water consumption (ET) of pecan walnut in semi-arid environment of Sonora in Northwest of Mexico. K_c values were derived as the ratio between crop evapotranspiration measured with eddy covariance (ET_c) and the reference evapotranspiration (ET_o) estimated by the FAO-56 Penman-Monteith equation. The results showed that the seasonal ET_c and K_c was mainly controlled by leaf area index (LAI) and surface soil water content.

The magnitude of daily K_c was the lowest at the beginning of season (Jan-Mar). It increased continuously up to full development stage (Jul-Sep) and decreased during the maturity stage. The annual values of K_c were 0.91, 0.68, 0.77, 0.74, 0.69 and 0.76 from 2017 to 2022, with an average value being 0.76, which is very close to the value (0,75) recommended in the FAO-56 paper.

Keywords:

Crop coefficient, Eddy covariance, crop evapotranspiration, pecan walnut, arid land, Mexico

(OC16): Pecan nut weight is dependent on local shoot carbon supply-demand balance

Dr. Richard Heerema, Dr. Ciro Velasco Cruz, Isaiah Apodaca, Claudia Galvan, Dr. Omar Holguin (New Mexico State University, USA)

Wide variation in local carbon balance among individual shoot terminals in pecan (*Carya illinoensis*) tree canopies exists due to shoot-to-shoot differences in carbon supply (photosynthesis) or reproductive demand for carbon. Our objective was to test how nut quality parameters relate to the apparent balance of carbon supply and demand of pecan shoots. We used specific leaf weight (SLW) of two terminal leaflets as an indicator of apparent shoot carbon supply and the number of nuts per shoot (NPS) as an indicator of shoot reproductive carbon demand. In 2021, ripe 'Pawnee' and 'Western' pecan nut clusters and leaflets were hand sampled from individual shoot terminals representing the whole range of NPS and SLW in the canopies. Inshell nuts and shelled kernels were weighed and kernels were analyzed for total lipids. There was a significant increase in average individual nut weight with increasing shoot SLW. There was also a significant interaction of SLW and NPS on total



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cluster nut weight, with the greatest magnitude of the positive effect of SLW on total nut weight at high NPS. On the other hand, there was no significant relationship of SLW with total and average shoot kernel weight. Initial analyses did not indicate a relationship of total kernel lipids with either NPS or SLW. These data are consistent with the hypothesis that local shoot level carbon balance can limit pecan nut growth and that carbon imported from elsewhere in the canopy may not be sufficient to make up for a shoot's shortfalls in carbon needed for nut development. SLW is closely associated with cumulative leaf sunlight exposure, so these results highlight the need for maintaining good sunlight distribution in pecan canopies for development of high-quality nuts.

Keywords:

photosynthesis, carbohydrates, lipids, specific leaf weight, source-sink balance

SESSION II: PLANT PHYSIOLOGY (Part 2)

(OC18): Photosynthesis in pecan tree under different management conditions in the Northwest México

Prof. Dr. Julio Cesar Rodriguez, Prof. Dr. Salah Er-raki, E. Alejandro Castellanos-Villegas, Mr. J. Abraham Gamez-Lucero, Prof. Dr. Richard Heerema, Prof. Dr. Fidencio Cruz-Bautista, Prof. Dr. Abdelilah Meddich (Universidad de Sonora, Mexico)

In northwestern Mexico, trees with high water requirements are grown. One of them is the pecan walnut (*Carya illinoensis*), which presents, like other fruit trees, alternate bearing and vivipary. This region is characterized by high temperatures in spring, and relatively high humid and warm summers. In the search to understand if the high temperatures are impacting the accumulation of reserves and therefore the production of the next cycle. Monthly measurements of photosynthesis (P_n) and leaf water potential (ψ_{leaf}) were carried out in seven sites, under different management conditions in the Coast of Hermosillo, Mexico. These measurements were made from May to November 2022. For that, the LI6400 (LI-COR) and the Sscholander pump (MPS) were used, respectively. The first ones were carried out between 9:00 am and noon, while the second around 13:00. P_n measurements were made on the same branches, of the same trees, until the age of the leaves allowed it. In the case of ψ_{leaf} , measurements were maintained on the same trees throughout the measurement cycle. The results of ψ_{leaf} during fruit growth (May-July) varied from -0.57 to -1.02 MPa, with an average value of -0.71 MPa. During the fruit filling period (August) the ψ_{leaf} varied from -0.56 to -0.86 MPa, with an average value of -0.69 MPa. In the harvest period (September-October) the ψ_{leaf} increased due to the reduction of irrigation, varying from -0.50 to -1.94 MPa, with an average value of -1.09 MPa. During the post-harvest period to the beginning of senescence (November) some plantations managed to rehydrate the trees, varying the ψ_{leaf} from -0.79 to -1.22 MPa. On the other hand, the P_n data varied from 8.93 to 15.34 $\mu\text{mol CO}_2 \text{ m}^{-2} \text{ s}^{-1}$, with an average value of 12.41 $\mu\text{mol CO}_2 \text{ m}^{-2} \text{ s}^{-1}$, during the fruit growth stage (May-July). During the fruit filling period (August) the P_n varies from 7.73 to 16.18 $\mu\text{mol CO}_2 \text{ m}^{-2} \text{ s}^{-1}$, with an average value of 11.36 $\mu\text{mol CO}_2 \text{ m}^{-2} \text{ s}^{-1}$. In the harvest period (September-October) the P_n presented its greatest variation, due to the fact that some measurements were made prior to harvest during and post-harvest, ranging from 8.57 to 17.26 $\mu\text{mol CO}_2 \text{ m}^{-2} \text{ s}^{-1}$, with an average value of 12.32 $\mu\text{mol CO}_2 \text{ m}^{-2} \text{ s}^{-1}$. This greater variation is due to the fact that during the harvest period, hydration and drying periods alternate between each harvest. In post-harvest to the beginning of senescence (November), the P_n varies from 4.72 to 7.62 $\mu\text{mol CO}_2 \text{ m}^{-2} \text{ s}^{-1}$, mainly influenced by the early drop in air temperatures during this 2022 cycle. Finally, it can be preliminarily concluded that the average values between the period of fruit growth, fruit filling, and harvest did not vary significantly. However, monthly events of high P_n rates (greater than 20.0 $\mu\text{mol CO}_2 \text{ m}^{-2} \text{ s}^{-1}$) occurred, which corresponded to the minimum ψ_{leaf} (less than -0.7 MPa).

Keywords:

Alternate bearing, leaf water potential, leaf temperature, stomatal conductance, air temperature, arid lands, irrigation



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(OC19): Variation of bioactive compounds, fatty acids and volatile profile of walnuts (*Juglans regia* L.) during harvest and postharvest handling

Benedetta Ms. Fanesi, Marco Vanacore, Eugenio Cozzolino, Roberta Foligni, Ancuta Nartea, Samuele Crescenzi, Matteo Zucchini, Veronica Giorgi, Davide Neri, Deborah Pacetti, Paolo Lucci (Università Politecnica delle Marche, Italy)

Walnut (*Juglans regia* L.) is a highly appreciated nut because of its pleasant taste and utilization in many food preparations. Walnut kernel is rich in essential fatty acids (linoleic and linolenic acid), essential amino acids, minerals, and other bioactive compounds, e.g., tocopherols and carotenoids, which are strong antioxidants with vitamin activity. Nevertheless, walnuts are highly susceptible to oxidative rancidity during both processing and storage, that may significantly limit product shelf-life and consumer acceptance. Therefore, the proper harvesting and processing time are crucial for high-quality production. During ripening, walnut husk starts to crack until full opening, and then fruits fall. The harvesting should occur within 48 hours and walnuts should be air-dried within 12 hours after harvest at maximum 40°C to avoid undesired chemical changes. Despite the importance of harvest and postharvest handling on the nutritional and sensory characteristics of walnuts, only limited data is available.

In this study, changes in the composition of bioactive compounds (tocopherols and carotenoids), fatty acid and volatile profiles of walnuts harvested and processed at different times were evaluated. Samples (300 fruits each) were picked from trees with cracked and with fully opened husks, and then, after 24, 48, 72, and 96 hours from their fall. These samples were air-dried within 12 hours after harvest. Other walnuts were harvested and stored in bins for 24, 48, 72, and 96 hours before drying. The oil content of walnut kernel was assessed. Tocopherols and carotenoids were analysed by UPLC coupled with a PDA-MS detector, while the fatty acid profile was assessed by GC. Furthermore, SPME was employed to extract volatile compounds from ground walnut kernels, which were then analysed by means of GC-MS. Results from the investigation of chemical changes along walnut supply chain are helpful to optimize harvest and postharvest handling, and to obtain high-quality walnuts.

Keywords:

Vitamin, antioxidant, PUFA, aroma profile, handling time, walnut

(OC20): Yield of 'Chandler' walnut according to PAR interception and crop load: effect of pruning

Prof. Gabino Reginato, Miguel Valenzuela, Assoc. Prof. José Luis Henríquez (University of Chile, Chile)

The yielding condition of 'Chandler' walnut orchards was studied in central Chile, between latitudes 35° and 37°36', according to the PAR interception of the orchard and the crop load expressed in terms of fruits/m² PAR intercepted. In the southern part of studied area, it was observed that orchards over 10 – 12 years old reached high canopy volume and higher PAR interception (close to 100%), with trees which showing fruit set mainly at the top and on the outer side of the canopy. For that, several pruning experiments were performed during 4 years to improve canopy illumination using different strategies, either removing branches, heading back the trees or both, contrasting them with untouched trees (control).

Yield efficiency reached up to 1.5 kg per square meter of intercepted PAR which means around 11.25 metric t per ha that intercept 75% PAR, showing always a linear response to crop load expressed as fruits/m² PAR_i, which reached values up to 170. An inverse relationship was found between crop load and fruit size. Generally, in the areas with higher latitude it was found more fruit density (fruit per shoot) which lead to higher yields and less fruit size.

In general, pruning not necessarily reduces PAR interception but can increase the sunlight inside to the canopy. As the result of pruning, always crop load and yield were reduced and fruit size increased, but not necessarily with a noticeable increase in yield efficiency unless fruit size was positively affected by pruning besides to the effect of modifying crop load. Crop values was always less under pruning treatments, even though fruit size and



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fruit price were improved by pruning, because the reduction of yield was not compensated by the better fruit price.

Keywords:

Yield efficiency, fruit size, crop value

SESSION III: PRODUCTION, HARVEST, FRUIT QUALITY AND MARKETING

(OC21): Towards the geographical traceability of the Italian walnut: a case study based on stable isotopes signatures ($\delta(2H)$, $\delta(18O)$, $\delta(13C)$, $\delta(15N)$, $\delta(34S)$) and elemental fingerprinting

Erica A. Di Pierro, Pietro Franceschi, Daniela Bertoldi, Luca Ziller, Agostino Tonon, Roberto Larcher, Luana Bontempo, Federica Camin, Michela Troggio (Centro Ricerca e Innovazione, Fondazione Edmund Mach, Italy)

Juglans regia (L.) is cultivated worldwide for its nutrient-rich nuts. Due to the beneficial properties of its nutrients, walnut is a highly recommended food in the human daily diet and a crop of high economic interest. Italy is among the top five importing countries of walnuts in shell due to the strong decline in the Italian walnuts production in the early seventies combined to a constantly growing demand. Most of the imported walnuts in Italy come from the USA (50%), followed by France (20%) and Chile (14%), and a lower part also from Argentina and Australia. The current context is therefore favourable to promote the development of a local high-quality Italian walnut production. In this respect, tools capable of tracing the origin of walnut can support the process of valorisation and promotion of this product, especially by protecting the authenticity of its geographical provenance. The main aim of this study was to investigate to what extent stable isotope data (H, O, C determined in the lipid and defatted fractions, N and S of the defatted fraction) coupled with 56-element profiles could be used to differentiate Italian walnuts on a regional and global level. A total of 122 walnut samples from six countries and two years of harvest were analysed. Principal Components Analysis suggested a strong effect of the geographical origin, particularly at the regional level. However, values of $\delta(2H)$, $\delta(18O)$ and $\delta(13C)$ and some mineral profiles were significantly influenced also by the harvest year. A preliminary analysis focussing on a few samples of international origin confirmed that the proposed approach could be potentially applied for the discrimination of Italian walnuts. Overall, the results presented support the effectiveness of stable isotopes and multi-element analyses for walnut traceability and local origin protection.

Keywords:

Persian walnuts, food authenticity, isotope ratio mass spectrometry, inductively coupled plasma mass spectrometry, geographical origin discrimination

(OC22): The effect of cultivar, pressing temperature and kernel quality on the yield of walnut oil and its phenolic content

Dr. Anita Solar, Assoc. Prof. Ana Slatnar, Prof. Dr. Franci Stampar, Prof. Dr. Metka Hudina, Prof. Dr. Robert Veberic (University of Ljubljana, Slovenia)

Walnut oil is obtained by pressing walnut kernels. Since it contains some useful nutrients, it is one of the most appreciated vegetable oils with nutty and delicate taste. In the present study, we compared the effects of cultivar, pressing temperature, and kernel quality on the yield of the oil, the ratio of oil to bagasse pellets, and the content of phenolic compounds in the oils and the pellets. Five cultivars ('Franquette', 'Fernor', 'Fernette', 'Lara', 'Adams') and the mixture of cultivars were included in the experiment. Three pressing temperatures (30 °C, 40 °C, 50 °C) and three different walnut kernel qualities (healthy, lightly infested and heavily infested by walnut husk fly) were compared. The total phenolic content (TPC) of the samples was determined using the Folin-Ciocalteu phenol reagent method. It was expressed as gallic acid equivalents (GAE) in $\mu\text{g}/\text{kg}$ of oil. In the so-called cold-core pressing at 30 °C, the highest oil yield was obtained by the cultivar 'Adams' (37.3%) and the lowest by the cultivar 'Fernor' (28.6%). At a pressing temperature of 40 °C, significantly more oil was obtained than at 30



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and 50 °C. Less oil was obtained from kernels heavily infested with walnut husk fly than from non-infested kernels. The highest total phenolic content was found in the oil of the 'Franquette' cultivar, and the lowest in the oil of the 'Fernor' cultivar (84.6 vs. 15.2 µg GAE /l oil). At a pressing temperature of 50 °C, the total phenolic content was almost three times higher than at lower temperatures. The quality of the kernels also affected the TPC content, so that it was highest in the oil obtained from dark kernels heavily infested by the walnut husk fly.

Keywords:

Juglans regia L., cultivars, kernels, oil, phenolic compounds

(OC23): The influence of the harvest time and duration of drying on the quality of walnuts

Dr. Anita Solar, Prof. Dr. Rajko Vidrih, Aljaz Medic, Prof. Dr. Robert Veberic, Prof. Dr. Metka Hudina (University of Ljubljana, Slovenia)

Ripe walnuts contain 30 to 40% moisture, and even up to 50% in rainy years, which makes them extremely susceptible to various molds and spoilage processes if not processed properly. The most important thing is to harvest them as soon as possible and dry them quickly and efficiently. In a two-year study, we investigated the influence of harvest date and drying time (one to three days, S1 to S3) on the external and internal quality of fruits of cultivars 'Sava', 'Franquette' and 'Lara'. At the stage of technological maturity, we divided the fallen fruits into three groups. The fruits of the first group were harvested immediately (T0). After six days we collected the fruits from the second group (T6) and after 13 days from the third group (T13). The fruits of the 'Sava' and 'Lara' cultivars, which were dried for only one day, had the most moldy husks regardless to the harvest time, while drying for two days was enough for the husks to be completely free of mold in at least 96%. In the 'Franquette' cultivar, the shells were most moldy in walnuts that were harvested and dried 13 days after maturity. Between 4 and 16 % of their kernels were black. 4-8% had more than half of the mold surface and are less aromatic and more bitter than earlier harvested fruits. Bright kernels, which have the highest market value, were obtained from immediate harvesting and three-day drying. In the case of 'Franquette' T6S3, correlation between bitterness of the kernels and the total phenolic content was determined. Harvest and postharvest treatments also affected fatty acid content of the kernels, having more saturated fatty acids in combination T13S1 of 'Franquette' and 'Lara' cultivars. The contents of unsaturated fatty acids were the highest in the 'Franquette' and 'Sava' cultivars in kernels harvested after six days, and in the 'Lara' cultivar in kernels harvested after 13 days.

Keywords:

common walnut, *Juglans regia* L., harvest and postharvest treatments, fruit quality

SESSION IV: PLANT PRODUCTION, ORCHARD MANAGEMENT, DEFENSE (Part 1)

(OC24): Pecan cultivation in Puglia region, Southeastern Italy: fruits and truffle production

Assoc. Prof. Giuseppe Ferrara, Prof. Dr. Leonardo Lombardini, Dr. Andrea Mazzeo, Dr. Giovanni Luigi Bruno (Università di Bari, Italy)

In Italy, suitable pedo-climatic conditions for pecan cultivation can be found in the Po Valley, Puglia and Sicily regions. Considering the rising interests of growers and consumers for healthy food and diversified diets, pecan has the potential to be considered an alternative and valuable fruit crop. However, there is very limited scientific information regarding pecan cultivation in Puglia or other regions with similar pedo climatic conditions in Italy. The aim of this study was to 1) evaluate some quantitative and qualitative characteristics of fruits selected from established pecan cultivars over two seasons and 2) conduct preliminary studies to assess the potential of pecan seedlings of 'Shoshoni' and 'Shawnee' to establish ectomycorrhizal symbiosis with truffles (*Tuber borchii*, *T. aestivum* and *T. melanosporum*) collected in Puglia. 'Wichita', 'Shoshoni', and 'Pawnee' trees showed yields higher than 20 kg/tree, with small differences between on and off years, and a kernel dry weight of ≈3 g. The



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other cultivars investigated showed strong alternate bearing. Plant height, number of leaves, chlorophyll content (expressed as a SPAD unit) and stem diameter partially indicated the increase in ecological fitness in truffles-inoculated plants. The rate of mycorrhization, assessed 12 months after the inoculation, ranged between 41 and 64%, thus indicating that 'Shoshoni' and 'Shawnee' pecan seedlings are suitable hosts to establish ectomycorrhizal symbiosis with *T. aestivum*, *T. borchii*, and *T. melanosporum* cultivation under Puglia climatic conditions. The cultivation of pecan, especially of plants mycorrhized with commercial truffles, could be an important alternative to recover the rural marginal areas in Mediterranean regions and to improve organic farming production, sustainability, and biodiversity.

Keywords:

Nut, kernel, mycorrhization, *Tuber borchii*, *Tuber aestivum*, *Tuber melanosporum*

(OC25): French Pecan project - Adaptation of arboriculture to climate change through agroforestry pecan cultivation in France

Mr. Fabien Balaguer, **Mr. Mathieu Duflos** (France Agroforesterie, France)

The pecan tree *Carya illinoensis* is the tree that produces the pecan nut. Native to North America, there are dozens of cultivars adapted to different soil and climate conditions.

More resistant to heat waves and periods of drought than the common walnut, for example, it meets the need for diversification expressed by the nuts producers and offers production opportunities in France.

France is an important arboriculture territory, notably with the dynamism of its walnut and hazelnut supply chains. However, the arboricultural sector is already suffering the effects of climate change. Faced with this observation, the challenges of mitigating climate change and adapting agriculture to it arise.

To meet these challenges, the "French Pécan" project proposes innovative solutions through the adaptation of crops using pecan trees, and the adaptation of techniques through agroforestry. Moreover, the practice of agroforestry also plays a key real role in mitigating climate change, in particular by sequestering carbon in the soil and in the biomass.

In France, a few trials have been carried out by farmers wishing to explore new practices and new crops better adapted to climate change. However, pecan cultivation in France is still unknown and undocumented, the quantities produced remain anecdotal, and the upstream and downstream organisation of the sector does not exist. The "French Pécan" aim is to structure a French agroforestry and organic pecan supply chain that meets the needs of adapting arboriculture to climate change, mitigating the latter through agroforestry techniques, and diversifying farms.

Keywords:

Carya illinoensis, Pecan tree, agroforestry, supply chain, diversification, adaptation to climate change, arboriculture

(OC26): Performance of micropropagated walnut orchards trained to central leader in Emilia-Romagna, Italy

Dr. Daniela Giovannini, Dr. Federica Brandi, Mr. Sandro Sirri, Dr. Francesca Massetani, Dr. Eugenio Cozzolino, Prof. Dr. Davide Neri (CREA, Italy)

The central leader is the most popular training system in Emilia Romagna (Po valley, Italy), relatively simple to manage and suitable to mechanization of pruning. Although grafted trees are the most planted, there is an increasing interest towards the micropropagated plant materials, because they are homogeneous, pathogen-free, and relatively cheap. From 2016, various micropropagated Chandler trees of different size and nursery cycle length were field tested in commercial orchards. All these materials showed an intense vegetative growth since planting, and even the small (10-15 cm) 8-months-old plants were able to develop a 2-3 m long axis in the first year of planting, and numerous and well distributed branches along the axis in the second year of planting.



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Regardless of the type of nursery material planted, at the end of the third growing season the training of the trees is almost complete and in the following 2-3 years, a few cuts on the top and main scaffold branches are sufficient to finalise the central axis formation and get the tree ready for the full production phase. Micropropagated Chandler trees produce flowers since the second growing season; however, as the proterandry phenomenon is particularly marked in this material, especially in the first years of planting, adequate and timely pollination must be ensured (eg. with artificial pollination) in order to allow a satisfactory yield performance since the early years of the orchards. According to the interesting results with high planting density, we are currently investigating the most appropriate pruning interventions for maintaining the tree structure highly functional and ensure high productivity levels.

Keywords:

Pruning, nursery stock material, vegetative growth, training system, female flowers

SESSION IV: PLANT PRODUCTION, ORCHARD MANAGEMENT, DEFENSE (Part 2)

(OC28): Financial and design aspects of walnut agroforestry in the UK

Prof. Dr. Steve M. Newman, Mr. Tom Tame (BioDiversity International Ltd, United Kingdom)

The history of walnut production for nuts in the UK is presented based on trials planted as early as 1936 and research since 1986. Reflections are given on variety selection and management in the context of the desire for agroforestry approaches involving crops and or animals. Financial spreadsheet analysis shows possible break-even points and ongoing gross margins for a range of configurations. Suggestions for further actions including farmer led research are outlined.

Keywords:

Walnut, agroforestry, silvopastoral systems, silvoarable systems

(OC29): Effects of crop covers in walnut orchards

Delphine Sneedse, Eloïse Tranchand (SENURA, France)

The maintenance of the grass cover in walnut orchards facilitates the work of the harvester and allows convenient access to the plot. The main problem observed on the inter-row is the compaction of the soil caused by the regular passage of machinery. This inter-row can be conceptualized differently to limit the phenomenon or even improve the profitability of the plot. Results of trials conducted in GREENCOQ project showed that, in young orchards, a rotation of two successive crop covers, field beans sown in the fall followed by fodder sorghum sown in summer, making it possible to increase the organic matter rate. In the same project, in mature orchards, a cover crop, sown in the autumn, suggested improvement of the microbiological activity of soil, soil decompaction and nitrogen supply and the opportunity to reduce the use of chemical fertilizers.

Keywords:

Walnut, crop cover, soil decompaction

(OC30): Use of Mechanical Hedge Pruning to Transition Southeastern U.S. Pecan Production to a More Profitable and Sustainable System

Lenny Wells (University of Georgia, USA)

The southeastern United States is historically a major pecan producing region of North America. The state of Georgia alone accounts for one third of the U.S pecan crop. However, southeastern U.S. pecan production is



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currently at a market disadvantage due to low volume production per unit of land, increasing cost of production, and a declining price at the farm level. Additionally, pecan orchards in the region have been heavily damaged by major hurricanes and tropical storms intensified by climate change. With increasing agricultural water use and a growing population, agricultural irrigation efficiency is necessary for sustainability. Dormant hedge pruning has been evaluated in ongoing trials since 2013. Midday stem water potential (ψ) was higher ($P \leq 0.05$), indicating less water stress, on hedged trees than on non-hedged trees. Nut weight and percent kernel were increased ($P \leq 0.05$) by hedge pruning. From 2019-2022, comparisons were made in a separate trial of summer vs. dormant hedge pruning of pecan. There were no differences in pecan yield, nut weight, or percent kernel between summer and dormant-hedged trees. Shoot length was significantly ($P < 0.05$) reduced on summer hedged vs. dormant hedged trees. Results from these trials demonstrate that hedge pruning of pecan provides multiple advantages in the southeastern U.S., including enhanced spray coverage, minimizing alternate bearing, increased tree water efficiency, larger nut size, higher percent kernel, and a 60% reduction in wind damage from major hurricanes. Both summer and dormant hedge pruning led to similar advantages for pecan production. Highly productive, disease resistant cultivars combined with hedge pruning can help transition southeastern U.S. pecan production into a more profitable and ecologically resilient cultural management system for the 21st Century.

Keywords:

Carya illinoensis, stem water potential, wind resistance, water use

(OC31): High density walnut management

Mr. Gustavo Mendoza, Vittorio Bianchini, Miguel Carus (Nogaltec, Chile)

Planting walnut trees at high density is not something new, but the technologies developed to manage the productive structure efficiently are still being developed. In general, the tendency for fruit trees is to be planted in ever greater density. For the last 6 years we have been looking for high density alternatives for the Chandler variety. The plantation frames tested are 5 meters by 2 meters and 6 meters by 2.5 meters. The main objective is to improve the return on investment, and this is achieved by having greater production in the first years and, as a consequence, a greater accumulated production. On the other hand, the aim is to improve the quality of the fruit, mainly in terms of size and kernel yield. The most important challenge of a high-density orchard is light management, where different types of pruning have been tried, always looking for the right amount of light to have the highest percentage of productive wood and avoid weakening or death of internal structures. The results have been better in the majority of aspects against the traditional 7 x 5 frame in the Chandler variety. It is possible to obtain better results using the same resources than a normal density, this translates into a better use of the energy of the plant focused only on production and not on wasting energy forming large wood structures. There is also considerable savings in water and inputs during the first 5 years.

Keywords:

Walnuts, high density, light management

(OC32): Characteristics of spray coverage from large pecan orchard air-blast sprayers

Dr. Clive Bock, Dr. Ted Cottrell, Dr. Michael Hotchkiss (United States Department of Agriculture, USA)

Pecan (*Carya illinoensis*) is a particularly tall orchard crop. Achieving adequate coverage with sprays throughout the canopy profile is challenging. Air-blast sprayers are typically used to apply sprays from the ground. We performed a series of tests to ascertain the effects of spray volume, speed and use of a volute on characteristics of spray coverage in the absence of a tree canopy. Kromekote spray cards were positioned on a 20 m pole at heights of 5.0, 7.2, 9.4, 11.6, 13.8, 15.0, 17.2, and 19.0 m. Spray with Vision Pink dye for visualization was applied without a volute at 2.4 or 3.2 km/h and 470 L/ha, 940 L/ha, and 1870 L/ha, and with a volute at 2.4 km/h or 3.2 km/h and 470 L/ha and 940 L/ha. Spray coverage on cards was measured using image analysis. For those sprays



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applied without a volute there was greater coverage at 5 m compared to 19 m. But despite more coverage with higher volume, the effect was not constant with height as the difference in coverage between volumes applied declined with height, and at 19 m the differences among treatments were negligible and inconsistent. The effect of speed was relatively small compared to volume, and also not consistent at different heights. However, the volute-based treatments had greater coverage at 19 m compared to 5 m, although the effect was minimal with 470 L/ha. At 940 L/ha spray coverage was greater, particularly at heights greater than 13.8 m. Use of a volute at spray volumes less than 940 L/ha will be marginal. The results confirm previous studies on the effect of volume and speed on spray coverage in pecan tree canopies and further illustrate the limitations of orchard air-blast sprayers for spraying tall pecan trees.

Keywords:

Pecan insect pests and disease, disease control, fungicides, pecan scab, *Venturia effusa*

(OC33): Improving pecan nut yield by applying Kelpak® during flowering

Dr. Heino Papenfus, Pedro Larrain (KELPAK, Chile)

Kelpak® is a biostimulant product made from *Ecklonia maxima* seaweed. Numerous trials on a wide variety of crops have shown that Kelpak® can stimulate root growth, improve plant establishment, mitigate abiotic stress, improve fruit size, crop quality, and increase fruit and nut set which ultimately culminates to increase yields. Kelpak® has proven to be effective in improving yields in most nut crops including almonds, hazelnuts, macadamia nuts, pecan nuts, pistachios, and walnuts. During 2016-2019, three trials were conducted on pecan nut cultivars Choctaw and Ukulinga in White River, South Africa. Results showed that Kelpak® can significantly improve nut yield. To confirm these results in a high production area, a trial was conducted during the 2021-2022 season on pecan nut cultivar Wichita in Hartswater, South Africa. For each treatment, 20 trees were randomly selected to be included in the trial. There were two treatments which consisted of Kelpak® being applied as a foliar application at a dose of either 0.2% or 0.3%. The foliar applications were applied at 50% catkin elongation, pistillate flower and nut set developmental stages. Kelpak® applied at 0.2% and 0.3% improved nut set percentage and crack-out percentage significantly compared to the untreated control. Applying Kelpak® at 0.2% improved total nut yield numerically but only Kelpak® applied at a rate of 0.3% improved yield significantly compared to the control. By applying Kelpak® at 0.3% produced a total nut yield of 2970 kg/ha compared to 2560 kg/ha for the control, a yield increase of 16%. It is noteworthy that Kelpak® seems to follow a dose response curve in pecan nuts, with the higher dose of 0.3% producing better results than Kelpak® applied at 0.2%. This trial confirms that three Kelpak® applications at a rate of at least 0.3% during flowering can significantly improve yield in pecan nuts.

Keywords:

Seaweed extract, *Ecklonia maxima* extract, nut set, yield increase

(OC34): Surveying fungal diversity in pecan orchards of the Southwestern USA

McKenzie Stock, Dr. Richard J. Heerema, Dr. Jennifer J. Randall, Dr. Nicole Pietrasiak (New Mexico State University, USA)

Scarcity of water resources, high salinity, and alkaline soils limit the profitability and growth of the pecan industry in desert regions, such as the Southwestern USA. Symbiotic relationships between tree roots and mycorrhizal fungi may help mitigate these challenges by providing a wide range of ecological services to the host plant, such as increased drought and salt tolerance, improved nutrient uptake, and protection from pathogens. Furthermore, saprotrophic fungi play important roles in the soil ecosystem by decomposing dead plant material to aid in formation of soil organic matter, benefiting both water relations and mineral nutrition of plants. No fungal characterization study has been previously conducted for arid pecan-growing regions of the USA. This



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study provides a novel survey of the fungal communities found in pecan orchards in the Southwestern USA, including New Mexico, Texas, Arizona, and California. With engagement from regional pecan producers, we collected fungal fruiting body, mycelial, and tree root tissue from orchard soils. DNA was extracted from fungal tissues and Sanger sequenced. Our sequences were compared with reference sequences from DNA databases and phylogenetic trees were generated to aid taxon identification. We confirmed several species of ectomycorrhizal fungi in Southwestern pecan orchards, including *Pisolithus tinctorius*, *Scleroderma cepa*, and *S. laeve*. We also confirmed saprotrophic fungal species, including *Chlorophyllum molybdites* and *Agaricus deserticola*. Others, such as *S. bovista*, *S. citrinium* and *Russula* species, were also identified morphologically. *P. tinctorius* and *S. cepa* are both commonly found in commercial mycorrhizal inoculants due to their abilities to aid in water and nutrient uptake. *C. molybdites* and *A. deserticola* may decompose decaying wood, such as shredded mechanical prunings from pecan trees. By characterizing fungi present in the region, the important roles these fungi may play for pecan trees and for pecan agriculture at large can be better understood.

Keywords:

Microbiome, mycorrhizae, soil ecology, saprotroph, symbiosis, arid zone agriculture

SESSION IV: PLANT PRODUCTION, ORCHARD MANAGEMENT, DEFENSE (Part 3)

(OC35): SysNoix, a French innovative project to answer tomorrow's needs

Ms. Marine Barbedette, Marianne Naudin (SENURA, France)

For several years, agriculture has been evolving. These changes have been initiated by both environmental and societal awareness. The transition to a more respectful production mode has been accelerated by French government directives.

In this context, to help French walnut growers, the SENURA station has launched a project from 2018 to 2023, called « Sys'Noix », the objective of which is to reduce the Treatment Frequency Indicator (-100% herbicides, -50% fungicides, from -50% to 100% insecticides) and to maintain the economic sustainability of farms.

Two systems have been evaluated in comparison to the common growers' practices. The first has tested the combination of known levers (mechanical weeding, mating disruption...) on a traditional Franquette variety orchard. The second, and more innovative system, aims to redesign a Lara variety orchard with physical barriers. The proposed oral presentation will focus on this second orchard and on the results obtained since 2019 by installing rain covers and insectproof nets (TFI, production, pest pressure...).

This experimentation has enabled to test the system's limits and the evaluation of advantages and disadvantages of such an installation in a walnut tree orchard.

Keywords:

Walnut, physical barriers, reducing treatments, economic sustainability

(OC36): Ecklonia maxima seaweed extract improves nut set and nut size of 'Chandler' walnuts trees

Pedro Larrain, Alejandro Ochagavia, Hector Tabilo (KELPAK, Chile)

Improving yield is one of the most important challenges of the walnut industry. In the search for greater production, the use of seaweed extracts is well established, especially extracts of *Ecklonia maxima* which have shown to improve fruit set and size in various fruit and nut crops. Three trials were conducted on 'Chandler' walnut trees in Chile to determine whether *E. maxima* extract Kelpak® could improve walnut yield when applied on its own or in combination with Hydrogenated Cyanamide (Dormex®). The first trial during 2014-2015, showed that three applications of Kelpak® applied at a rate of 0.3% at catkin elongation, pistillate flower stage and 14 days later improved production by 26% compared to the control. During the 2017-18 season, Kelpak applied at 1% was compared to the application of 1.5% Dormex®. Both products were applied 30 days before sprouting



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(DBS). In this study, only Kelpak® improved yield significantly compared to the control. Kelpak® improved the overall yield by 21% and Dormex® improved yield by 6%. Finally, during the 2018-19 season two treatments consisted of Kelpak® being applied alone at different rates and application frequencies and a third treatment where Kelpak® was combined with 1.5% Dormex® at 30 DBS. Only the two Kelpak® treatments (without Dormex®) improved yield significantly above the control, increasing yield by 36% and 29% respectively, with no negative effect on nut quality or size. It is evident from three years of trials that Kelpak can improve nut set and yield in walnut without compromising nut quality or size.

Keywords:

Biostimulant, Seaweed extract, Ecklonia maxina extract, walnut set, walnut size

(OC37): Mitigation of salinity stress in 'Chandler' walnuts by the soil application of *Ascophyllum nodosum* extracts: effects on growth, yield and its impact on physical and biological soil properties

Prof. Victor Beyá-Marshall, Mr. Francisco Quintanilla, Prof. Dr. Thomas Fichet (University of Chile, Chile)

In Chile the majority of the walnut's orchards are in semi-arid areas under saline conditions. Therefore, mitigate the salinity stress is a relevant issue for the farmers, especially for 'Chandler', the most sensitive and planted variety. The aim of this study was to evaluate the effect of the application through drip irrigation of an extract of *Ascophyllum nodosum* (ANE; Acadian Organic®), on the vegetative and productive response in 'Chandler' walnut trees affected by salinity and its effect on the physical and biological soil properties. 4 L/ha of ANE was applied monthly, during two seasons (2021-2022, 2022-2023), from the beginning of shoot growth until one month before harvest (24 L/ha per season), compared with a control without amendment. The water irrigation quality was always close to severe conditions for walnut (EC 2.0 dS/m and chlorides between 250 and 300 mg/L). In 2021-2022, ANE applications mitigate salt damage in walnut trees, reaching a greater thickness of shoots, a higher SPAD index and better vegetative expression, represented by the NDVI spectral index. Consequently, a higher proportion of large sizes (>34 mm; 25 % ANE vs 13 % control) and better kernel filling, effect that was quickly evident in the first season of applications. The stress mitigation promoted a greater accumulation of reserves in shoots, with expected accumulated effect after 2 or 3 seasons of applications. On the other hand, the ANE applications generated significant improvements in the physical and biological soil properties, increasing the soil microbial activity and stimulating a greater organic matter accumulation, which allowed to reduce compaction, improve soil structure and increase soil macroporosity in depth. The latter is relevant for irrigation and salt management, since a greater stability of the soil porous system under irrigation cycles would allow an adequate water distribution in the soil profile, improving the displacement of salts and, consequently, reducing the saline stress.

Keywords:

Biostimulants, salt tolerance, saline soil, sustainable management, organic matter

(OC38): Evaluation of different training techniques in walnut orchard

Dr. Daniela Giovannini, Veronica Giorgi, Marco Vanacore, Samuele Crescenzi, Matteo Zucchini, Eugenio Cozzolino, Prof. Dr. Davide Neri (CREA, Italy)

In recent years, walnut cultivation has been steadily increasing worldwide (FAO statistics), and it is showing signs of recovery in Italy as well (AGRISTAT). In a modern walnut orchard, the challenge is to obtain early and high production, hence the tendency is toward denser planting patterns and training systems that promote early bearing and superior fruit quality. The aim of the work was to test different training techniques and evaluate the response of the trees. The trial was carried out in a commercial orchard in Emilia Romagna region (central Italy). The farm management consists in topping the central axis of nursery trees 150 cm above the soil during the first year in the field, leaving intact two buds below the cut, and then debudding on 30 cm of the axis. Other



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management methodologies were tested to avoid some critical aspects of the farm management: topping at 150 cm above the soil without debudding but with a summer pruning of lateral shoots (summer management), topping at 200 cm above the soil without debudding but with a summer pruning of lateral shoots (high topping management), and elimination of the apical bud with debudding until to 140 cm above the soil (no topping management). Results show a different distribution and length of the shoots along the axis, with high topping and no topping managements having a high density of short shoots in the apical section of the axis. While farm and summer management had a more uniform distribution of the shoots, that were more vigorous and so more suitable to form the future structure of the tree.

Keywords:

Debudding, summer pruning, high density, micropopagated nursery material

SESSION V: PESTS AND DISEASES (Part 1)

(OC40): The Characterization of *Xanthomonas arboricola* pv. *juglandis* Strains from Bacterial Blight and Brown Apical Necrosis of Walnuts in Türkiye

Dr. Damla Ertimurtas, Prof. Dr. Hatice Ozaktan (Ege University, Turkey)

Walnut bacterial blight (WBB) is one of the important bacterial disease in Türkiye. In recent years, Brown apical necrosis (BAN) which cause walnut immature fruit drops also threatened walnut production in Aegean and Marmara Region. *Xanthomonas arboricola* pv. *juglandis* (Xaj) is the causal agent of WBB. Xaj is also primary responsible pathogens of BAN which are the secondary agents of the disease are *Fusarium* and *Alternaria*. The purpose of the present study was to determine the prevalence and causal agents of WBB and BAN in Manisa, Balıkesir and Çanakkale in walnut plantations. In addition, 31 isolates were identified as *X. arboricola* based on classical diagnostic methods. For WBB, in pathogenicity tests on immature walnut fruits, 11 Xaj isolates did not cause any symptoms while the remaining isolates caused typical necrotic lesions on lateral surface. On the other hand, only 4 bacterial x fungal isolates caused typical BAN symptoms. All bacterial and fungal isolates produced typical symptoms for WBB and BAN on immature walnuts. Moreover, *A. alternata* was diagnosed in a high prevalence from typical BAN symptoms fruits. Furthermore, molecular identification of isolates selected to represent each region were performed using 27F/1492R universal primers for 16S rDNA sequence. All isolates had to highest homology to Xaj (%99.83-100). Repetitive PCR and MLSA using 7 housekeeping genes showed genetic diversity between Xaj isolates among different region. According to MLSA results, Xaj isolates grouped in 3 different clades. Differences between groups resulted different pathogenicity and virulence that are still being addressed. In this study demonstrated us that the phenotypic and genotypic variability among Xaj strains could be explained by different methods and provide a better understanding of WBB and BAN epidemiology.

Keywords:

Walnut bacterial blight, BAN, MLSA, repetitive PCR, *Xanthomonas arboricola* pv. *juglandis*

(OC41): Evaluation of the effectiveness of a bacteriophage-based formulation in laboratory and field trials on *Xanthomonas arboricola juglandis*

Lydie Leymarie Lachaud, Dr. Camila Morales, Sofía Bustos, Héctor Apablaza, Dr. Fernanda Flores, Marie-Neige Hébrard, Dr. Patricia Jargeat, Dr. Pablo Núñez (Agroadvance SpA and Comité des fruits à coques du Lot, France)

Xanthomonas arboricola pv. *juglandis* (Xaj) is a phytopathogen bacteria causing walnut (*Juglans regia* L.) blight disease, which means serious damage to plantations, generating economic losses for walnut producers. Disease control against Xaj, generally based on copper, are losing efficacy due to resistance acquisition by strains. An alternative solution to control Xaj and reduce symptomatology of walnut blight could be the application of bacteriophages, small viruses that infect and kill bacteria.



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The aim of this study was to evaluate the effectiveness of a bacteriophage-based formulation in laboratory and field trials. To this, Xaj strains were isolated from infected walnut from Chilean and French fields. The isolates were identified by PCR with specific primers for Xaj identification.

Subsequently, a laboratory preventive test was carried out in immature walnut fruits. For this assay, we used the most pathogenic Chilean and French Xaj strains, previously selected for their pathogenicity in walnut leaves. We inoculated a bacteriophage formulation, composed by three phages (F11, F13 and F31), inside immature walnut fruits and 24h later the pathogenic bacterium (Chilean strain T3H and French strain Xaj-12) and then we observed infection at 7- and 15-days post-inoculation of Xaj. The results showed that the preventive treatment with our bacteriophage formulation prevents Xaj infection in immature walnut fruits.

Finally, we carried out field trials in walnut plantations in Chile and France. The Chilean evaluation was performed in a Chandler variety plantation, while the France evaluation was carried out in a walnut ferner variety orchard. For comparative purposes, control plots without treatment were included in both trials. We observed that the application of our bacteriophage-base formulations reduces the pathogen pressure, compared to control groups both in Chile and France trials. These results suggests that the use of bacteriophages can be an alternative to fight Xaj in walnut plantations.

Keywords:

Xanthomonas arboricola pv. juglandis, phage therapy, walnut blight

(OC42): Phenotypic behaviour and assessing phage sensitivity in pathogenic Xanthomonas arboricola pv. juglandis

Dr. Maroua Oueslati, Dr. Tamas Kovacs, Ms. Dominika Bali (Biopesticide Ltd, Hungary)

Xanthomonas arboricola pv. juglandis (Xaj) associated with walnut blight, the most significant aboveground bacterial disease causing tremendous loss worldwide. Xaj uses numerous virulence factors for pathogenicity and fitness in plant hosts. We emphasize the diversity in the assimilation of substrates, in virulence such as motility, surfactant, biofilm formation and tolerance to copper and streptomycin of 18 strains, among them 7 newly isolated from Romania and Hungary. Current management practices are unsustainable and ineffective. Phage-based products aroused particular interest and demonstrated promising results under greenhouses and field conditions. In order to enhance their application, the characterization of XajHu 1,2,3 and 4 phages, isolated from wastewater was assessed including their morphological, physiological and genomic analyses and their combination with phylogenetically distinct phages was assessed.

Keywords:

Xanthomonas arboricola pv. juglandis, walnut blight, virulence, bacteriophages

(OC42b): Phenotypic behaviour and assessing phage sensitivity in pathogenic Xanthomonas arboricola pv. juglandis

Dr. Tamas Kovacs, Ms. Naila Bounedjoum, Ms. Dominika Bali, Prof. Gabor Rakhely (Biopesticide Ltd, Hungary)

Plants are constantly exposed to numerous abiotic pressure and biotic jeopardy. One major biothreat is bacterial pathogens causing various diseases, critically diminishing crop yields, and threatening worldwide food security. The necessity of developing sustainable biocontrol agents is markedly rising, such as the application of bacteriophages. Indeed, the reemerging phage therapy approach to control plant-pathogenic bacteria is highly promising: cost-effective, eco-friendly, extremely specific, and safe for the host plant. Phages were successfully tested as therapeutic agents against various phytopathogenic bacterium species. Xanthomonas spp., being the most economically influencing group. Accordingly, we recovered from Italian and Hungarian samples 12 bacteriophages infecting Xanthomonas arboricola pv. juglandis (Xaj), the causal agent of walnut blight disease. Before application in fields, following a unique pipeline, bacteriophages were characterized morphologically and



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genetically. Their efficacy was also analyzed, and basic life cycle parameters (burst time and burst size) were determined in vitro in the laboratory trials. Based on the characterization, we formulated a cocktail containing six bacteriophages and performed field trials for two consecutive years in Pitesti, Romania. The applications were implemented through foliar spraying the suspension on different *Juglans regia* varieties. The phage cocktail treatment protected walnut trees against Xaj effectively, albeit efficiency discrepancies were observed in accordance with the variety treated. Biosafety and ecotoxicity tests were performed to evaluate the bacteriophages' intervention effect on the ecosystem. The findings highlighted their safety and neutral effect on the environment. The presented results aim to assess the potential of bacteriophages as biocontrol agents and further emphasize their biopesticide activity against *Xanthomonas* spp.

SESSION V: PESTS AND DISEASES (Part 2)

(OC43): Epidemiology and Management of *Botryosphaeria* Canker and Blight of Walnut in California

Dr. Themis Michailides, Mr. Victor Gabri, Mr. David Morgan, Dr. ShuaiFei Chen, Mr. Giuseppe Fiore (University of California-Davis, USA)

The first report of a *Botryosphaeria* canker (caused by *B. ribis*) in California walnuts was a disease named "melaxuma" on "Franquette" walnuts in 1915. No additional studies were performed on this disease until in 2014 when growers reported significant yield reductions in their orchards. *Botryosphaeria* canker and blight is caused by 10 *Botryosphaeriaceae* species among which *Neoscytalidium dimittatum* that was reported in 1947 causing branch wilt of walnut (old name, *Hendersonula toruloidea*) in San Joaquin Valley of California. Infections occur in the spring when rainy weather spreads the pycnidiospores to susceptible tissues. Immature fruit, leaf and peduncle scars, and pruning wounds can be infected, while not any disease symptoms of the disease have been observed on leaves. Infections on fruit remain latent until late in August when hulls show a brown decay leading to fruit blight. Infections from one fruit can move to neighboring fruit and through the peduncle invade the spur (parental shoot). As the canker progresses into the spur, it kills both male and female buds representing the following year's crop. Both the blighted fruit and the killing of fruiting buds may partially explain deductions of yields in California walnut orchards. Fungicides that control *Botryosphaeria* panicle and shoot blight of pistachio were tested and shown to be very effective in controlling *Botryosphaeria* canker and blight of walnut. Various cultural and chemical disease management practices will be discussed.

Keywords:

Bot blight, *Juglans regia*, branch wilt, *Botryosphaeriaceae*, epidemiology, disease management

(OC44): Investigation on the involvement of *Botryosphaeriaceae* in recent walnut dieback in French orchards

Dr. Yohana Laloum, Ms. Cyrielle MASSON, Ms. Marie-Neige Hébrard, Ms. Aude MORONVALLE, Dr. Flora Pensec, Dr. Adeline Picot, Ms. Marie Belair, Prof. Gaëtan Le Floch, Ms. Agnès Verhaeghe (CTIFL, France)

A recent national survey revealed a recrudescence of wood dieback associated with blight, cankers and fruit necrosis in French walnut orchards. A preliminary study reported that these symptoms were associated with the presence of *Phomopsis*, *Colletotrichum*, *Botryosphaeria*, *Neofusicoccum* and *Fusarium*. Between 2020 and 2021, 180 symptomatic and asymptomatic samples (branches and fruits) were collected on 12 walnut orchards from the two main French production areas in order to focus on the nature and distribution of *Botryosphaeriaceae* species. Culturing fragments of wood canker and fruit necrosis revealed that two *Botryosphaeriaceae* species were mainly associated with these symptoms: *Botryosphaeria dothidea* and *Neofusicoccum parvum*, whatever the production area. Pathogenicity tests were performed in both greenhouse and experimental orchards on attached shoots. Results confirmed that *B. dothidea* and *N. parvum* induced necrosis on shoots. *N. parvum* isolates were the most aggressive fungi in all walnut tissues evaluated, compared to *B. dothidea*. This work is the first report to identify the *Botryosphaeriaceae* fungal species causing this complex disease in French walnut



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orchards. It would be helpful to better understand the influence of environmental conditions in the disease development for a better management control.

Keywords:

Botryosphaeriaceae, walnut dieback, diagnosis, French walnut orchards

(OC45): Colletotrichum acutatum in French walnut orchards – improving knowledge on the behavior of the fungus and its host

Ms. Cyrielle Masson, Ms. Marine Barbedette, Ms. Lucie Leurs (SENURA, France)

Since 2011, fruit losses due to the fungus *Colletotrichum* have been a great concern for French walnut growers. Two main species of the complex *Colletotrichum acutatum* are involved: *C. godetiae* and *C. fiorinae*. In order to find methods to manage the disease in the fields, studies were carried out to first improve knowledge on the pathogen and host's sensitivity: conditions of germination, varietal and ripening stage sensitivity... The effects of temperature and wetness duration on germination were investigated on cellulose acetate discs put on wet filter paper. The two species showed in the trials an optimal germination between 25 to 30°C, with 100% of germinated conidia after 15h of wetness duration. Concerning varietal and ripening stage susceptibility tests, there were performed on detached fruits under controlled conditions. Between the three most planted varieties in France, Franquette turned out to be the less sensitive than Fernor and Lara. In addition, fruits become more sensitive with ripening. These results aim to improve management and timing applications, combined to field trials.

Keywords:

Walnut, *Colletotrichum acutatum*, temperature, wetness, germination, varietal susceptibility

(OC46): Etiology of walnut fruit rot and twig dieback in South-Western France

Dr. Florent Trouillas, Ms. Marie-Neige Hébrard, Ms. Violette Aurelle, Dr. Patricia Jargeat, Ms. Eloise Tranchand (University of California, USA)

With an average of 40,000 tons produced annually on more than 20,000 hectares, walnut is France's second most cultivated orchard crop after apples. The South-Western region of Périgord is one the largest and most historical walnut producing region of France. During the summer of 2018, numerous brown to black necrotic walnut fruit were observed in orchards and harvest bins, with estimated yield losses ranging between 40 to 70%. During the following spring of 2019, field observations from these orchards also revealed abundant twig and shoot dieback in trees. Many fruit spurs had died following previous year fruit infections, thus leading to further reduction of the orchard production capacity. The objective of this study was to identify and characterize the main fungal pathogens associated with fruit rot and twig dieback of walnut trees in the South-Western France. In May 2019, twenty-one walnut orchards were surveyed. Declining shoots and twigs were sampled and symptomatic tissues were placed on Potato Dextrose Agar (PDA) for the isolation and morphological identification of fungal pathogens. In August 2019, walnut fruit expressing the black walnut syndrome were sampled and necrotic tissues were isolated in PDA. Fungal identifications were confirmed by morphology as well as by sequencing and phylogenetic analyses of the internal transcribed spacer rDNA. Isolation results from declining shoots and twigs showed a high proportion of *Diaporthe* (syn. *Phomopsis*) spp. associated with dieback, ranging from 45 to 100% of sampled shoots and twigs across the sampled orchards. Additional fungal species isolated from shoots and twigs included *Botryosphaeriaceae*, *Colletotrichum*, *Fusarium*, *Boeremia* and *Melanconium* spp. From the fruit sampling, *Diaporthe/Phomopsis* species also prevailed in orchards, followed by *Colletotrichum* and *Botryosphaeriaceae* spp. *Fusarium* species also were found occasionally from diseased fruits. Main species identified from this study included *Diaporthe eres*, *Neofusicoccum parvum*, *Botryosphaeria dothidea*, *Colletotrichum godetiae* and *Fusarium lateritium*.



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Keywords:

Walnut diseases, Diaporthe, Phomopsis, Colletotrichum, Botryosphaeria, Neofusicoccum, canker

(OC47): Transcriptome analysis under pecan scab infection reveals the molecular mechanisms of the defense response in pecan

Mr. Gaurab Bhattarai, Prof. Dr. Patrick J Conner, Prof. Dr. Jennifer J Randall, Dr. Horat Shadgou Rhein (University of Georgia, USA)

Pecan scab (caused by fungal organism *Venturia effusa*) is the most important disease of pecan (*Carya illinoensis*) in the southeastern United States. Pecan scab causes significant yield loss in susceptible cultivars, and its control requires numerous applications of fungicides throughout the growing season. Resistance breeding is complicated by the presence of multiple fungal genotypes which are only able to infect a narrow range of pecan cultivars. To understand the mechanisms of pecan response to pathogenic and apathogenic scab isolates, we performed a transcriptional analysis of the pecan cultivar, 'Desirable', in response to a pathogenic and an apathogenic scab isolate at three different time points (24, 48, and 96 hrs. post-inoculation). Differential gene expression and gene ontology enrichment showed contrasting gene expression patterns and pathway enrichment in response to the contrasting isolates. Weighted gene co-expression analysis of differentially expressed genes detected 12 different gene modules. Among them, module-3 and 4 had significant enrichment of genes involved in protein phosphorylation, defense response, response to fungus, response to chitin, receptor kinase activity, cytochrome p-450 activity, signal transduction, and transcription factors related to biotic stress, and module-5 showed enrichment of genes involved in cell wall modification, cell wall thickening, lignin biosynthetic process, and cell wall biogenesis. These genes were upregulated in the resistant reaction at the early stage of fungal infection (24h) compared to susceptible reaction. Transcriptional patterns observed in analysis of RNA sequencing data were verified with real-time quantitative PCR. These observations suggest that the early response of pathogen-related signal transduction and development of cellular barriers against the invading fungus are likely defense mechanisms employed by pecan cultivars against apathogenic isolates. The transcriptomic data generated in this study may provide the foundation for identifying candidate resistant genes in pecan against *Venturia effusa* and exploring the molecular mechanisms of disease resistance.

Keywords:

Pecan scab, disease resistance, RNA sequencing

SESSION V: PESTS AND DISEASES (Part 3)

(OC48): Discovering and Improving Microbial Biocontrol Methods for Managing Key Pecan Pests

Dr. David Shapiro-Ilan (United States Department of Agriculture, USA)

Pecan is plagued with a number of insect pests that can severely limit production. For the most part, these pests can be controlled using broad spectrum chemical insecticides. However, broad spectrum chemical insecticides are toxic to humans and other nontarget organisms. Therefore, due to environmental and regulatory concerns, alternative methods of control are needed. Microbial control may be a viable alternative for several important pecan pests. Microbial control is the use of insect pathogens (or their derivatives) to achieve pest management goals. The approach is safe to humans and the environment. A series of steps can facilitate effective microbial control programs. These steps include discovery and improvement. Improvement approaches encompass strain enhancement and stabilization as well as developing novel production, formulation, and application technology. Example case studies to be discussed include using pheromones to enhance control of pecan weevil (*Curculio caryae*) with entomopathogenic nematodes, inoculating pecan trees with endophytic fungi (e.g., *Beauveria bassiana*) to control aphids, and improved microbial formulations such as nanoparticle formulations for entomopathogenic nematodes and fungi. Methodology for these approaches will be presented. Results indicated



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that field experiments with pheromones and entomopathogenic nematodes caused high levels of pecan weevil control. Successful inoculation of *B. bassiana* into pecan seedlings resulted in suppression of pecan aphids. Novel nanoparticle formulations or sprayable gels protect entomopathogenic nematodes and fungi against ultraviolet radiation and desiccation. These approaches can be applied broadly in pecan for control of key pests. Moreover, the approaches may be applicable to other pests that occupy similar niches in other cropping systems (such as walnut).

Keywords:

Pecan, weevil, aphids, entomopathogenic, nematodes, fungi, Beauveria, Steinernema, Heterorhabditis

(OC49): Pilot-scale field trial of Sterile Insect Technique for codling moth management in walnut orchards

Dr. Ghais Zriki, Mr. Jean-Michel Leyre, Ms. Stéphanie Blachon, Jean-Baptiste Philibert, Jean-Michel Ricard, Ms. Agnès Verhaeghe (CTIFL, France)

The codling moth *Cydia pomonella* (Lepidoptera: Tortricidae) is a fruit-feeding insect and a major pest of pome fruit and walnut crops worldwide. The Sterile Insect Technique (SIT) have been successfully implemented as a key-component of area-wide approach to suppress codling moth populations in apple orchards since 1990. The CaropTIS project (2021-2024) aims at the implementation of SIT as a central strategy of management programs of *C. pomonella* in walnut crops in France. Since 2021, a SIT pilot-scale field trial has been conducted in walnut orchards in the Isère region. The objectives of this trial were 1) to setup an adapted SIT logistic package to walnut production context and 2) to evaluate field efficacy of sterile moths' releases in reduction wild population density and fruits damages. Sterile moths were released weekly (from April to September) in 4 experimental sites (orchards) with a total area of 17 ha. A trapping system was used to estimate sterile/fertile moths' ratios and to compare population densities between treated and control orchard. The percentage of damaged nuts were also evaluated at the end of 1st and 2nd generations of *C. pomonella*. Sterile moths survived up to 4 weeks under field conditions, mean recapture ratios of sterile moths were relatively low (4% along the release period). Relatively more wild moths were captures in control orchards compared to SIT ones. The population densities of wild moths were relatively low during the two seasons of release and less than 0,5% of damaged nuts were observed in both SIT and control orchards. The pilot field trial allowed the implementation and the evaluation of SIT logistic package in walnut orchards. The 3-year pilot trial will permit to identify the mains factors for a successful implementation and a long-term deployment strategy of SIT for codling moth management in walnut crops.

Keywords:

Cydia pomonella, moths, walnut production, Inherited Sterility, Biocontrol

(OC50): Efficacy and Persistence of the Entomopathogenic Fungi and Nematodes on Walnut Husk Fly

Dr. Jhalendra Rijal, Dr. Sudan Gyawaly, Chang Vue (University of California-Davis, USA)

Walnut husk fly (WHF), *Rhagoletis completa* Cresson, is a severe pest of commercial walnuts in California, United States. The WHF larvae feed on fruit husk and can cause shriveling and darkening of walnut kernels or shell staining. WHF control in walnut orchards involves multiple applications of synthetic insecticides throughout the entire summer when adults emerge and oviposit in the fruit husk. This approach can lead to an increased risk of secondary or other pests resurgence because of the detrimental impacts on the natural enemies. Therefore, we conducted studies to explore non-chemical pesticide alternatives, i.e., entomopathogens, that are more IPM-friendly in managing WHF populations in the orchard. In the study, we found that two entomopathogenic nematode species caused significantly higher mortality of walnut husk fly larvae compared to the control and entomopathogenic fungus treatment in the laboratory studies. Field-applied entomopathogens persisted in the field for at least four weeks. Further field studies are devised to target the ready-to-pupate larvae in the Fall and



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teneral adults during their emergence in the summer to confirm if entomopathogens can be effective against WHF. These bio-based tools can be an effective part of the WHF management program to establish Sustainable Pest Management (SPM) in California walnuts.

Keywords:

Rhagoletis completa, Walnut Husk Fly, Entomopathogen, walnut

(OC51): Preliminary findings on the improved monitoring of walnut husk fly through a new pheromone lure

Dr. Robert Van Steenwyk, Dr. Spencer Walse, Dr. Dan Kuzmich, Ms. Melissa Thayer (University of California, Berkeley, USA)

Walnut husk fly (WHF) is a significant pest of walnuts in the United States and Europe and has significantly increased in importance over the past 20 years. Control of WHF is based on repeated applications of insecticides to control the adults. Initiation of the spray program and monitoring the efficacy of the spray program is based on captures in yellow panel traps baited with an ammonium carbonate lure. An improvement in monitoring would aid in determining the proper time to initiate an insecticide control program and aid in determining the efficacy of the control program.

Sarles et al. (2018) discovered a male produced WHF pheromone (d-hexalactone and d-heptalactone). Field evaluations by Sarles et al. (2018) of a 50:50 (d-hexalactone:d-heptalactone) lure resulted in a significant increase in trap catch as compared to blank traps. Research conducted in California in 2022 and reported here shows: 1) d-hexalactone and d-heptalactone are aggregation pheromones that attract both male and female flies, 2) polymeric plug lures are superior to gray septa lures used by Sarles et al. (2018), 3) the combination of an ammonium bicarbonate lure and d-hexalactone lure increased trap catch over ammonium bicarbonate lure and d-hexalactone lure alone, 4) load rate of d-hexalactone in polymeric lures needs to be at least 200 mg to achieve the desired field longevity, and 5) d-heptalactone appears to be the more important component as compared to d-hexalactone.

Keywords:

Walnut husk fly, lactones, monitoring

(OC52): Analysis of the nutritional content of the Green Walnut Husk (GWH) and perspectives for the Walnut Husk Fly (*R. completa*) larval diet development

Ms. Solène Travaillard, Mr. François Verheggen (SENURA, France)

Rhagoletis completa is a major walnut (*Juglans* spp.) pest that feeds exclusively on the Green Walnut Husk (GWH), decreasing the final fruit quality. Research effort investigating this model is constrained by the current inability to fully rear this species under laboratory conditions. While Walnut Husk Fly (WHF) adult diet received some attention [1], larval dietary requirements remained unexplored. GWH mesocarp nutritional content is largely unknown, mostly because 1) only the seed of the walnut fruit is edible to humans and 2) it contains otherwise more interesting compounds with potential medicinal properties, such as juglone and other phenols [2]. Here, we propose characterization of the nutritional content of GWH (cultivar: Franquette) at the fruit stage corresponding to the peak infestation of WHF larvae. Our investigation covers macronutrients, minerals and vitamins, and includes amino acids, fatty acids and sterols profiles. Comparison of GWH nutritional content to a panel of 75 fruits pulps revealed that GWH is notably 1) rich in minerals such as selenium, iron and calcium, 2) rich in several vitamins including vitamins D, B12, K1, B2 and E, 3) rich in cholesterol and 4) rich in glucose. Using principal component analyse (PCA), we found that GWH nutritional content departs from other pulps, and that squashes (*Curcubita maxima*) are amongst the closest matches. These results provide novel characterization of an important walnut-production waste and will help to guide holidic (chemically defined) and oligidic (crude ingredients) larval diets compositions for WHF rearing.



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Keywords:

Juglans regia, Franquette, mesocarp, insect nutrition

SESSION V: PESTS AND DISEASES (Part 4)

(OC53): Understanding seasonal biology and exploring detection tools for Pacific flatheaded borer in walnut orchards in California

Dr. Sudan Gyawaly, Dr. Jhalendra Rijal (University of California, USA)

Flatheaded borer (Coleoptera: Buprestidae) infestation is a resurging problem of commercial walnuts, *Juglans regia*, in California's Central Valley. Reported as a problem of stressed weaker trees historically, the flatheaded borer problem has been increasingly reported in both young and mature and healthy walnut orchards for over five years. Since limited information is available regarding the biology and monitoring of this borer pest, we conducted studies to understand the extent of the problem, determine pest phenology, and explore potential monitoring tools. Our study showed that adult emergence occurs from May through July, with the peak activity in mid-June. None of the traps and lures evaluated over three years captured the Pacific flatheaded borer, *Chrysobothris mali*, populations at high numbers. However, this study helped us understand the occurrence of multiple wood-boring species, including the Pacific flatheaded borer in walnut orchards. In 2019, ten adult *Chrysobothris* sp. were captured in green prism traps ($n = 4$) and purple funnel traps ($n = 6$). All the specimens we caught on the green prism traps were *Chrysobothris mali*. In contrast, all six specimens (all females) collected in the purple funnel traps were *C. wintu*. The study results are discussed in relation to the abundance of these multiple species in walnut orchards and what it means for long-term orchard health. Ongoing studies focus on evaluating newer lure types and trap designs that may be useful for monitoring this pest in walnuts.

Keywords:

Flatheaded borer, walnut, pest monitoring

(OC54): Using a double-sided volute sprayer to control scab in tall pecan trees

Dr. Clive Bock, Dr. Michael Hotchkiss, Mr. Buck Paulk (United States Department of Agriculture, USA)

Pecan (*Carya illinoensis*) is an important specialty crop in the southern United States. Scab (caused by *Venturia effusa*) is the major biotic constraint to pecan production in the southeastern region. Control of scab relies on large orchard air-blast sprayers. Some have been adapted to include a volute on one side to project spray higher into the canopy due to a decline in coverage with height. A grower-designed and engineered double-voluted (DV) sprayer was assessed for spray coverage and scab control compared to a standard (S), non-voluted sprayer. Spray coverage studies showed that spray using the DV sprayer resulted in significantly greater spray coverage at heights measured >13.8 m (up to 19.0 m), with DV coverage ranging from 18.4 to 14.1%, compared to coverage using the S, which ranged from 7.9 to 2.9%. The slope in decline of spray coverage with height was significantly shallower with the DV sprayer, and spray profiles reflected these differences. Over three seasons, scab control



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was either equal too, or superior using the DV sprayer. In 2017, mean scab severity for the control was 9.07, for the DV was 0.19, and for the S was 0.24%, respectively; in 2018 severity was 84.3, 18.4 and 29.1%, respectively; and in 2019 was 32.7, 7.0 and 11.6%, respectively. There were no discernable gradients in scab severity with either sprayer in 2017, but in 2018 the difference was significant, with the DV having a shallower gradient, and in 2019 the slope was numerically less steep with the DV compared to the S. Thus, the DV provided greater spray coverage at heights >13.8 m when compared to the S sprayer, and disease control was numerically or statistically greater overall when using the DV. DV sprayers may offer economical, superior scab control in pecan orchards with trees >13.8 m.

Keywords:

Spray deposition, fungicides, pecan insect pests, disease management



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POSTERS

SESSION I: GERMPLASM, GENETICS, BIOTECHNOLOGY

(P01): Determining the chilling requirements to overcome dormancy of three pecan nut cultivars

Dr. Neus Aleta Soler (IRTA, Spain)

One of the most important factors limiting the geographical development of temperate deciduous fruit trees is the winter chilling requirements (CR), as it is necessary to break the endodormancy process. In Catamarca, a region located in the northern Argentina, pecan [*Carya illinoensis* (Wangench.) K. Koch] is becoming a nut tree alternative. The aim of this work was to determine the chilling requirements of the budbreak in three pecan cultivars growing in the new plantations: 'Stuart', 'Pawnee' and 'Western'. The study was made on one-year-old well lignified branches removed from trees in autumn 2018 and 2019. Short budsticks, with its terminal bud (TB) and only one lateral bud (LB), were used for biological control. Samples (220) were exposed to low temperatures ($5\pm 2^\circ\text{C}$) to promote artificial chilling accumulation under ten different treatments, from 0 to 1000 chilling hours (CH) at intervals of 100 CH. Then, shoots were forced to sprout in a greenhouse under controlled environmental conditions. Results were expressed as mean time (days) to reach budbreak (MTB). When the response to the increasing accumulation of CH did not show significant differences in MTB, it was considered that the cold requirements were satisfied. All cultivars sprouted even under no chilling accumulation (0 hours), although these treatments showed the highest MTB (80-120 days). TB and LB showed the same chilling requirements but differed in the MTB value. The 'Western' cultivar had the lowest CR, between 300 and 400 CH; 'Pawnee' showed intermediate CR (400-500 CH); while 'Stuart' showed the highest CR (600 and 800 CH). Results indicated that the increase of CH causes a decrease in the MTB value in all studied cultivars. This information, from the biological test, will allow to better select cultivars and will contribute to determine the most suitable expansion of pecan tree in Catamarca considering future climate scenarios.

Keywords:

Biological test, budbreak, endodormancy, *Carya illinoensis*, mean time to budbreak, chill hours.

(P02): Seasonal dynamic of sap macro and micro elements composition in walnut (*Juglans regia*) cv. Lara

Dr. Julien Toillon (ANPN (Association nationale des producteurs de noisettes), France)

For walnut growers, optimizing fertilization is an essential tool to combine productivity and environment sustainability. The key for a good management is a balanced supply of necessary nutrients, at the right time. Knowledge of the seasonal pattern of mineral uptake by trees and considering the various factors that can influence it, is therefore essential. The objective of this trial was to test sap analysis as a diagnostic tool. This study aims at better understand the seasonal changes in sap composition in walnut leaves, and to constitute references in walnut cv. Lara (*Juglans regia* L.). Leaf samples were taken from four walnut orchards every two weeks, from flowering to mature fruit. The concentration of different nutrients in the sap of these leaves was measured. At the same time, various contextual data were collected: phenological stages of the trees, soil characteristics, meteorological data, fertilization, yield and quality of the fruits produced. Each nutrient presented a specific seasonal evolution of its concentration in sap, which allowed the identification of critical phenological stages. Moreover, contextual data influence on sap analyzes results was highlighted by the observation of inter-plot orchards and/or inter-annual variability. The proposals made are intended to improve the reliability of the results and to confirm the hypotheses put forward, to eventually design a decision support system to optimize fertilization management.

Keywords:

Physiology, walnut, sap composition, mineral dynamic



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(P03): Climate change and walnut budbreak phenology: Assessing cultivar-specific chill and heat requirements in NE Spain

Dr. Neus Aleta Soler (IRTA, Spain)

In recent years, temperatures are gradually increasing worldwide. A negative effect on reproductive phenology is being detected in many temperate woody species, including walnut (*J. regia* L.). In the Iberian Peninsula, budbreak of some walnut cultivars is often erratic which consequently affects their productive response. Thus, the knowledge of cultivar-specific chill and heat requirements becomes crucial for selection of the best adapted genotype at each site. The main goal of this study, carried out on 11 commercial cultivars, is to assess the effect of chill and heat accumulation on budbreak date. A statistical approach (Partial Least Square) based on phenological (sprouting date) and meteorological data, which considers temperature changes occurring in the chilling and forcing phases of budbreak, was assessed using the R package 'chillR'. Data was obtained from the walnut tree collection of IRTA (Constantí-Tarragona) and recorded from 1986 to 2022. The budbreak trend was significant for six of the cultivars studied showing mostly a delay tendency. Results shown that both chilling and heating requirements affect sprouting with marked differences between cultivars. Chilling requirements, measured in chill portions, were notably higher in *J. regia* than for other temperate fruit trees. They ranged from $42,36 \pm 5,64$ to $87,96 \pm 9,47$ in 'Serr' and 'Ronde de Montiganc', respectively. On the other hand, heat requirements, measured as growing degrees hours, were estimated between 6.502 ± 1127 and 13.259 ± 1.907 , in 'Serr' and 'Franquette', respectively. In early cultivars, the budbreak date was closely linked to the cumulated chilling, while in mid-late cultivars both the chilling and heat accumulation affected sprouting. In a current Mediterranean climatic scenario, with sudden temperature changes becoming widespread in winter-spring, an early cultivar is subjected to a high risk of suffering frost damage because of its low heat requirements for sprouting. This information will support varietal selection, which is crucial for planning new orchard, especially in species with a long productive life, such as walnut.

Keywords:

Juglans regia, commercial cultivars, chill portions

(P04): Application of BBCH codification to walnut (*Juglans regia* L.) phenophase

Dr. Julien Toillon (ANPN (Association nationale des producteurs de noisettes), France)

A wide range of walnut (*Juglans regia* L.) cultivars are produced around the world. They are generally classed among them according to their phenology (bud break, flowering and nut fall). To date, phenological stages have been recorded using alphanumeric codes, although data analysis is difficult with such codes. This study converted the alphanumeric codes to fully numerical codes using the Biologische Bundesanstalt Bundessortenamt und Chemische Industrie (BBCH) scale. A walnut BBCH scale was created for the main English walnut cultivars, which are the main cultivated cultivars around the world production area. The scale was constructed with at least two digits (0 to 9) for the main growth stages and three digits for flowering, fruit development, and fruit ripening. The first number indicates the general growth stage, the second number indicates the growth stage at a specific time, and the third digit, when present, indicates a proportion at that stage. The walnut BBCH scale describes in 48 stages within 7 of the 10 available stages. The BBCH stages used are 0 (bud development), 1 (leaf development), 5 (inflorescence emergence), 6 (flowering), 7 (fruit development), 8 (fruit ripening), and 9 (senescence). To avoid stage recording errors between male and female flowering during data collection, it was chosen to use a 2-digit code for male flowering and a 3-digit code for female flowering. The high value of this new scale is the addition of new information on kernel development and ripening stages which were not evaluated in the former phenological scale. It will help producers and technical staff to have a better idea of the fruit ripening and potential harvest date.

Keywords:

Phenology, botanical coding system, nut tree species



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(P05): Environmental suitability of walnut (*Juglans regia*) in south-central Chile under projected climate changes
Prof. Gabino Reginato (University of Chile, Chile)

Walnut (*Juglans regia*) cultivation is an important economic activity in Chile due to the growing demand for the nuts. Crop zoning allows the identification of areas suitable for the establishment of fruit orchards using various techniques such as statistical analysis, climate modeling, remote sensing and participatory evaluation. To anticipate and adapt to changing conditions and ensure crop sustainability and efficiency, it is essential to make projections under climate change scenarios. The impact of climate change on walnut orchards was evaluated based on the climate change scenarios proposed by the IPCC Sixth Assessment Report. Using literature review and expert knowledge, the FAO ECOCROP model was calibrated and evaluated to quantify the impact of baseline (2010), 2030, 2050 and 2070 climate on the territorial suitability of walnut in south-central Chile. The study used the Shared Socioeconomic Pathways scenarios, which provide monthly average data on precipitation and temperature (mean, maximum and minimum) in each decade. The scenarios obtained indicate that walnut is not significantly affected in the future in the current growing areas; however, this behavior is due to the use of irrigation. Towards the south of Chile, this future change has a positive effect, with an increase in the area suitable for cultivation, also under irrigation conditions. The change of global suitability in the areas of preferential suitability for walnut showed that the crop is potentially adaptable to future climatic changes, with an increase towards the south of the current crop frontier.

Keywords:

Crop zoning, climate modeling, crop suitability, FAO ECOCROP

(P06): GERMAINE, CHARLETTE and DORIANNE: three new French varieties being registered for a release in 2025, a tribute to three figures of walnut sector development in France
Ms. Eloïse Tranchand (Station expérimentale de la noix de Creysse, France)

GERMAINE, CHARLETTE and DORIANNE result respectively from the crosses “Franquette” x “Chandler” and “Fernor” x “Chandler” made in 1991 and the cross “Fernor” x “Shinrei” made in 1998, by INRA (National Institute for Agricultural Research) Bordeaux center. After 15 years of observation by the experimental stations of Creysse and of Rhône-Alpes (SENuRA) coordinated by Technical interprofessional Center of Fruits and Vegetables (Ctifl), they have been in process for registration since 2020 with a theoretical release in 2025, jointly obtained by INRA-Creysse Experimental Station-SENuRA. All three have very promising characteristics for the walnut sector: high lateral production, very good kernel quality, large caliber and good tolerance to pests. Their names were chosen in tribute to three important figures in the development of the French walnut sector: Eric GERMAIN, creator of varieties at INRA Bordeaux since 1977, accompanied by Francis DELORT from 1990, as well as Gérard CHARLOT, Ctifl technical manager of SENURA from 1982 to 1996 whose main work focused on walnut nutrition.

Keywords:

Variety, production, phenology, pests, history of French walnut sector

(P07): Horticultural field performance of new putative resistant walnut rootstock genotypes compared to seedling Paradox and standard clonal Paradox rootstocks
Janine Hasey (University of California Coop. Extension, USA)

In California, most walnuts are produced on either seedling or clonal hybrid Paradox rootstocks. Seedling Paradox is susceptible to the soilborne pathogens *Agrobacterium tumefaciens* (cause of crown gall disease), and *Phytophthora* species (crown and root rots) while commercially available clonal Paradox rootstocks have varying degrees of resistance. A breeding program began in 2012 to identify rootstocks with high resistance to these pathogens and pathogenic nematodes. We report here on four orchard trials representing differing soil and



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disease challenges in California's walnut growing regions. Chandler was budded onto four experimental hybrid rootstock genotypes with putative resistance to *Agrobacterium* or *Phytophthora*. These bare root June budded trees were planted in 2016 at the four locations and are currently maintained. For comparison, the trials included commercially available clonal VX211, RX1, and Vlach, and seedling Paradox rootstocks. Rootstock performance, as assessed by tree mortality incidence, scion and rootstock growth, canopy light interception, yield, and nut quality, varied by location. Tree mortality, mainly resulting from failure to establish rather than from disease, was lowest on commercially available clonal rootstocks and experimental rootstock 11-991. Scion growth rates among trees on Vlach, VX211, RX1, and experimental rootstocks 11-991, K3 and 29JM8, exceeded the growth on seedling Paradox in 2021. Chandler on Vlach, RX1, K3, and VX211 had higher mean yields compared to other rootstocks across all sites in 2021. Chandler on Vlach yielded well at each site while RX1, K3, and STJM4 each yielded well at three of the locations. In 2021, 11-991 had the highest scion uniformity and Paradox seedling was the least uniform. Seedling Paradox exhibited significantly more crown gall than any of the commercial or experimental clonal rootstocks. Our results indicate that some experimental rootstocks examined here are horticulturally similar to the commercially available clonal rootstocks that continue to perform well overall.

Keywords:

Chandler, horticultural rootstock performance, hybrid Paradox rootstocks, VX211, RX1, Vlach, 11-991, K3, 29JM8, STJM4

(P08): Microsatellite fingerprinting and evaluation of genetic relationship for new perspective walnut genotypes from Nikita Botanical Gardens

Dr. Sergey Khokhlov (Nikita Botanical Gardens, Russia Federation)

Study was conducted with the aim to investigate genetic polymorphism and relationship for set of new promising walnut genotypes from the collection of genetic resources of Nikita Botanical Gardens (Yalta). Twelve «WGA» SSR-markers were used for the genotyping of 21 walnut accessions among which set of modern perspective genotypes are presented along with older Crimean cultivars. Specific SSR-profile was established for all studied genotypes. Totally, 71 alleles were identified for whole SSR-markers set. The number of alleles for each locus ranged from two (WGA 79, WGA 4) to eight (WGA069, WGA276) with average about six alleles per locus. Cluster analysis revealed two main clusters, one of which contains varieties, consisted of two groups. Noteworthy that one cultivars, which characterized by very late flowering period was allocated into the out-groups. Relationship among more modern and older walnut cultivars and breeding selections is discussing.

Keywords:

Walnut, polymorphism, micro-satellite, molecular identification, genetic similarity

(P09): Agronomic and quality evaluation of pecan tree varieties collection in the Southwest of Spain

Dr. Margarita López Corrales (CICYTEX (Centro de Investigaciones científicas y tecnológicas de Extremadura), Spain)

In 2010, a collection of 19 pecan tree (*Carya illinoensis* Koch) varieties was established at Cicytex (Scientific and Technological Research Center of Extremadura) (Guadajira, Spain). The aim was the evaluation of the agronomic behaviour and the fruit quality in the edafoclimatic conditions of the Southwest of Spain. A pollination chart from 2013-2021 has been worked out taking into account the lack of synchrony between the receptivity of female flowers and the maturation of the pollen grain of these varieties. Once the trees started producing, a pecan nut maturity calendar was determined, as well as the average production (kg/tree) of fresh and dried nuts after five days, the average weight of the nuts and kernels (fresh and dried) and the nut yield (relation between the kernel and the shell). All the cultivars got into production in 2013, although 'Osage', 'Wichita', 'Mohawk' and 'Cape Fear' have shown a jump-start with values above 4kg/tree in 2014. 'Osage' and 'Pawnee' stood out for being the more



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precocious cultivars with harvest in the last week of October, while 'Houma' was the latest cultivar with mature nuts in the third week of November. The largest accumulated productions were obtained by 'Mohawk', 'Kiowa' and 'Bitem T-4' with 63.1, 61.4 and 59 kg/tree respectively. Furthermore, 'Mohawk' and 'Kiowa' showed the nuts and kernels with largest average weight, while "Wichita" was the cultivar with largest fruit yield with 58.54% for 2013-2021 period. These results have revealed the adaptation and productivity potential of certain cultivars of pecan tree in areas of Southwest of Spain.

Keywords:

Carya illinoensis, production, phenology, yield, weight, kernel

(P09b): Phenology of ten pecan cultivars in two well differentiated regions of Spain

Dr. Margarita López Corrales, María Guadalupe Ms. Domínguez Yagüe, Dr. Abelló Laia, Dr. Ana María Fernández, Mr. Cordero Manuel, Mr. Fernando Pérez Gragera, Dr. Neus Aletà (CICYTEX (Centro de Investigaciones científicas y tecnológicas de Extremadura), Spain)

The pecan tree [*Carya illinoensis* (Wangenh.) K. Koch] has its natural area of expansion in the southwest of USA and Mexico. Currently, its fruit is looking for its place among the nuts and it is spreading in large parts of the world. In the south of Europe, the species exists as garden tree, introduced by 'Indians' from the 19th century. However, as a crop, the interest is very recent and the adaptation of the main cultivars in Europe is still little known. In Spain, some of the main varieties cultivated in the USA have been studied in collection in two well differentiated stations: Finca de la Orden (Lobón-Badajoz) in southwestern Spain (Extremadura) and Mas Bové (Constantí-Tarragona) in northeastern Spain (Catalonia). The phenology of ten varieties, sprouting, flowering (female and male), and fruit ripening are presented. Nine of the varieties are from the USA and one is a local selection from Catalonia. The data series analysed range from the second year of planting to 10-12 years of trees. Differences were observed in some traits that can affect directly the choice of both main varieties and pollinators to be used for planting in the different areas.

Keywords:

Carya illinoensis, adaptive traits, dicogamy.

SESSION III: PRODUCTION, HARVEST, FRUIT QUALITY AND MARKETING

(P14): Effects of Modified Atmosphere Packaging on in-shell stored fresh walnuts

Dr. Miltiadis Christopoulos (Hellenic Agricultural Organization – Demeter, Greece)

The need for consumption of foods with high nutritional value is well established and, according to the recent studies, the fresh (raw, non-dried) walnuts met this criterion. However, there is a narrow time availability of fresh walnut due to their rapid deterioration during storage. The objective of the present work was to study the effects of Modified Atmosphere Packaging (MAP) on in-shell (nut) stored fresh walnuts. Fresh walnuts (*Juglans regia* L. cv. Chandler) fruits were harvested at 26% (w/w) kernel moisture, de-hulled, in-shell walnuts (nut) were packaged (500 g / package) in four different commercially available packaging types and stored at 4°C and 90% RH. The packages were (i) open top polypropylene (PP) punnet sealed with polyethylene (PE) film of 9 µm thickness (code: PE); (ii) open top polypropylene (PP) punnet sealed with bi-layer polyamide (20 µm thickness) / PE (60 µm thickness) film (code: PA/PE); (iii) macro-perforated polyethylene terephthalate (PET) clamshell (code: PET); open top polypropylene (PP) punnet without sealing (Code: Control). Weight loss, composition of package atmosphere, kernel moisture, color (CIELAB L*, a* and b*), texture (peak force at 3 mm puncture and force area to peak) and respiratory (O₂ consumption, CO₂ production, RQ at 20°C) characteristics were evaluated after 0, 1 and 2 months (mo) of storage. The WL was intense in control (27% in average) and PET (13% in average), whereas almost negligible WL was observed on PE (0.3% in average) and PA/PE (0.04% in average).



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The 'fresh' character (threshold of 20% kernel moisture) was maintained in all packages after 1 mo storage, but at 2 mo only PE (26%), PA/PE (26%) and PET (21%) samples exhibited kernel moisture higher than 20%. The control kernels had 11.7% moisture after 2 mo and this intense dehydration resulted in harder kernels than the other packages, as revealed by texture analysis. The package atmosphere was 13.1% O₂ / 3.7% CO₂ in PE (in average) and 4.4% O₂ / 18.8% CO₂ in PA/PE (in average), whereas no atmosphere modification was recorded in control and PET. During storage, a sharp decrease in all kernel and nut respiratory attributes were found in controls and PET, but the use of PE and PA/PE packaging limited these decreases. However, in PA/PE samples there was an indication of anaerobic respiration evolution (nut respiratory quotient of 1.47 in average). The PA/PE stored nuts showed the mildest kernel browning (the highest L* and the lowest a* and b*), whereas the kernel color was very similar among control, PE and PET. Conclusively, the storage period of fresh walnuts could be extended beyond the 1 mo only using MAP, but further research is needed for the selection of the optimum package properties.

Keywords:

Fresh nut, MAP, color, texture, respiration, *Juglans regia*

SESSION IV: PLANT PRODUCTION, ORCHARD MANAGEMENT, DEFENSE

(P10): Pruning strategy depending on the variety (Franquette, Fernor, Lara)

Ms. Delphine Sneedse (SENURA, France)

Pruning remains one of the most important levers for the management of the orchard, allowing the entry of light, the aeration of the foliage, the renewal of fruiting branches and the balance of leaf and root volume. This having consequences on the tree resistance to climatic risks, pests, diseases, and fruit productivity, quality and size.

New varieties are planted in high density, supposed to increase yield in the first years compare to a low density, but, at some point, the trees cannot develop anymore: crowding occurs.

Both French experimental stations of Creysse and of Rhône-Alpes (SENURA) carried out trials, for more than 10 years, to acquire the necessary knowledge to formalize rules to help growers to adapt their pruning practices depending on the varieties. Interviews with walnut growers were also conducted to define the types of farming systems. A tool was created, from a given orchard situation (variety, age, density, tree structure, pedoclimatic conditions) and technicality of the producer (material, management of irrigation, fertilization), making it possible to adapt their planting of trees' strategies.

Keywords:

Pruning, walnut, density

(P11): Microbiome observations of fully-watered and deficit-watered micropropagated rootstock pecan trees

Dr. Richard Heerema (New Mexico State University, USA)

Carya illinoensis (pecan) trees are among the top valued crops in the United States, and in recent years pecan production has expanded worldwide. However, due to changing climates, water quality and availability has become an issue that needs to be addressed. Improved water practices that do not negatively impact tree health and pecan nut production will need to be implemented to ensure that the available water is used judiciously. Therefore, we studied the impact that deficit watering would have on the health and overall microbiome of greenhouse-grown potted pecan trees. In this study we sought to identify the effects of 70% and 60% deficit watering on potted micropropagated pecan trees and their associated fungal communities in a two-year study and further compare to our previous microbial study that defined the pecan core microbiome. Our findings indicated no significant differences in leaf fungal communities between control and water deficit plants in 2018,



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however, significant differences were observed between the two groups in the 2019 study. In both years, Cladosporiaceae was found to be relatively the most abundant in the control groups. Pleosporaceae was identified to be most relatively abundant in the deficit treatment group and previously reported as potentially conferring drought tolerance in other plant species. In 2018 the study proceeded with a slow adoption of water deficit conditions and this appeared to assist the plant in adapting to the water deficit conditions as opposed to the study performed in 2019 where water deficit conditions began abruptly. We found that growth parameters of the trees in 2018 and 2019 were similar. To the best of our knowledge, this is the first time a drought study with genetically identical pecan rootstock was conducted.

Keywords:

Water deficit, phytobiome, *carya illinoensis*, fungi, plant health

(P12): Exploring the molecular basis of the scarlet pellicle trait in English walnut (*Juglans regia* ‘Robert Livermore’)

Noah G. Feinberg (University of California, USA)

Quality in walnuts (*Juglans regia*) is largely determined by the color of the seed coat or pellicle, generally ranging from a pale cream color to a dark brown. Consumers primarily demand light-colored kernels, though the market for more unique pellicle colors is growing. This is exemplified by the Californian variety ‘Robert Livermore’, released by UC Davis in 1999, which yields kernels with scarlet pellicles that have grown in popularity among consumers. However, planting and production are limited as the tree itself possesses average horticultural characteristics and a challenging shell to crack. The latter is of particular note, as it leads to scuffing or breakage of the kernels, creating a lower-value product more susceptible to oxidation during storage. For this reason, it would be advantageous to understand and translate the underlying genetics of the “scarlet pellicle” trait to other genotypes with better horticultural attributes. Here we present the first step towards that goal with a comparative molecular analysis of ‘Robert Livermore’ and its non-red parent variety, ‘Chandler’. Transcriptomic and metabolomic datasets of kernel development for these two varieties were generated to uncover the biological nature of this phenotype. Analysis of these datasets has provided key insights into the general development and maturation of the walnut pellicle, as well as the unique features of the two genotypes. Importantly, the accumulation of an anthocyanin was observed uniquely in ‘Robert Livermore’ and corresponds with the intensity of pellicle color during development. Targeted metabolomics identified this anthocyanin as cyanidin 3-O-galactoside, otherwise known as ‘ideain’, a red-colored anthocyanin observed in many plant species. Subsequent querying of the transcriptome with the sequences anthocyanin-associated proteins revealed candidate genes involved in the biosynthesis and regulation of anthocyanins in ‘Robert Livermore’, notably a predicted UDP-Galactose flavonoid glycosyltransferase (JrUC3GalT) and MYB transcription factor (JrMYB113-like) that warrant further investigation.

Keywords:

English Walnut, Seed Development, Pellicle, Plant Pigmentation, Anthocyanins, Cyanidin 3-O-Galactoside, Transcriptomics, Metabolomics

(P13): Fruit effect of exogenous application of calcium chloride in Chandler commercial walnut orchards

Assist. Prof. Helen Kalorizou (University of Patras, Greece)

Chandler walnut orchards were tested for their fruit characteristics after foliar calcium chloride applications as calcium nutritional delivery scheme. Treated fresh fruits were found to be heavier and dimensionally bigger in comparison to control. Furthermore analyzing green husk weight and thickness, calcium chloride supplemented trees provide heavier green husks at the same tissue thickness. No dimensional changes in walnut endocarp and kernel fresh weight were observed among treatments. Quantitative free calcium analysis in husks, endocarp and



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kernel reveals lower husk free calcium levels in treated trees. Data suggest that no major effect on commercial value of walnut kernel was added due to calcium chloride treatments; however, husk byproducts and waste production for circular economy purposes is enhanced. Recorded lower free cellular Ca²⁺ concentrations on treated husks in conjunction to increased weights without thickness advancement potentially suggest an extensive signaling role for exogenous CaCl₂ and/or calcium overfunctional management mediated by outer tissues of walnut fruits.

Keywords:

Calcium chloride, Chandler Walnuts, fruit size

(P13b): Carbon partitioning in a walnut-maize agroforestry system through arbuscular mycorrhizal fungi

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Agroforestry can be defined as a dynamic, ecologically based natural resource management system that integrates trees into farms. Agroforestry systems allow to improve diversity and sustain plant production, to improve the social, economic and environmental conditions of land users. In France, agroforestry systems could associate open-air fruit trees or forest trees with grasslands, field crops or market gardening, and promote and optimize ecosystem services provided by soil micro-organisms.

Arbuscular mycorrhizal fungi (AMF) are forming a mutualistic symbiosis with roots of the majority of land plants, as open-air fruit trees and field crops. They improve water and nutrient supply and protect plants from pathogens. AMF form a net of hyphae in the soil, namely the Common Mycelial Network (CMN), which both stabilizes the soil and can link plants of the same or different species.

Diversity of AMF associated with walnut and maize roots is different. One Cluster AMF could form a CMN between roots of both plants. When analysing AMF diversity, the genus *Funneliformis* was mainly associated with maize roots, while no specific Cluster was found on walnut roots.

The natural discrimination of maize (a C₄ plant) and walnut (a C₃ plant) for ¹³C during the photosynthesis allowed to trace carbon fluxes between plants through the CMN. $\Delta^{13}C$ analysis of mycelium living in the root vicinity suggested that some carbon from walnut trees can be transferred to maize plants. Our results suggest that in temperate agroforestry system, AMF may be involved in the redistribution of nutrients between connected plants.

Keywords:

Agroforestry, walnut, maize, mycorrhiza

(P13c): Walnut rootstocks mycorrhization: when microorganisms help micro-propagated plants to adapt

Dr. Laurent Jouve, Marianne Siquin, Dr. Céline Allewaert, Dr. Sharhiar Hessami, Thomas Brun (TARNAGAS, France) – anciennement OC27

Micropropagation of walnut trees, and more specifically of selected rootstocks, is a widely used method. In the past, this method was not considered a viable solution due to the high mortality rates after acclimatization. We therefore tried to validate the relevance of this method, from an economic point of view, for Vlach (*J. hindsii* x *J. regia*) and RX1 (*Juglans microcarpa* x *J. regia*) rootstocks or varieties propagated on their own roots, such as Chandler or Fernor, through the measurement of survival after acclimatization. We have shown that acclimatization of micropropagated walnut trees can be carried out on a large scale, with a survival rate of over 85% in trials with economically interesting numbers.

We have evaluated the value of mycorrhization in the acclimatization process. Two types of endo-mycorrhizae were tested during acclimatization: *Glomus iranicum* var. *tenuihypharum* and *Rhizophagus irregularis*. While survival did not seem to be statistically improved with the use of this symbiosis, growth was significantly



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increased in the two rootstocks tested. They also enhance the response of the plants to sanitary obstacles outside in vitro conditions.

Keywords:

Micropropagation, rooting, acclimatization, *Juglans regia*, Fernor, Chandler, Vlach, RX1, Arbuscular mycorrhiza, *Rhizophagus irregularis*, *Glomus iranicum*

SESSION V: PESTS AND DISEASES

(P15): Fungal community associated with shoot blight and branch dieback of 'Pieral' Lara walnut variety
Loredana Cretu G. Stelian (Romania)

Field surveys conducted in a commercial walnut orchard from Sahateni (Buzău county) in Romania highlighted the occurrence of shoot blight and branch dieback caused by Botryosphaeriaceae and Diaporthe (Phomopsis) complex. Branch dieback with internal wood discolorations and shoot blight were observed in the 'Pieral' Lara walnut variety. Fifty samples from branches showing typical symptoms of dieback and internal wood discolorations were collected in early spring of 2020 and fungal community associated with branch dieback was analyzed through agar plate method. Results are presented as incidence (%) of detected isolates. Isolates belonging to the genera *Alternaria*, *Botryosphaeria*, *Chaetomium*, *Epicoccum*, *Phomopsis* and *Sordaria* were identified in the fungal community associated with branch dieback. Most of the detected isolates were of the genus *Alternaria* (*A. alternata*), with an incidence of 28%. Isolates of *Botryosphaeria dothidea* have been detected with an incidence of 12% and those of the genus *Phomopsis* with an incidence of 8%. *Dothiorella sarmentorum*, a species that is reported in *Botryosphaeria* complex was also detected (8%). Among the species known for their antagonistic potential, *Sordaria fimicola* (20%) and *Epicoccum nigrum* were also identified (10%). Fungal microbiota isolated from buds located on the branches with dieback symptoms was represented by isolates belonging to the genera *Alternaria* (32%), *Cladosporium* (16%), *Epicoccum* (6%), *Aureobasidium* (4%), *Fusarium* (4%) and *Aspergillus* (2%). As in the fungal community detected in branches with dieback symptoms, potential antagonists as *Epicoccum nigrum* and *Aureobasidium pullulans* isolates have been detected. Our results highlight the fungal community associated with shootblight and branch dieback of English walnut, as a first report in Romania. Further studies are needed to know the dynamics of this microbiota and its role in the development of the disease.

Keywords:

Juglans regia, branch dieback, fungal community, walnut microbiota

(P16): Anthracnose Disease of Walnut in California and its Management
Dr. Themis Michailides (University of California- Davis, USA)

Walnut (*Juglans regia*) is a major nut crop in California, USA, with approximately 160,000 ha planted. Among the few fungal diseases affecting walnut in California, anthracnose, caused by *Marssonina juglandis* shows only in certain regions and only when there is rain in the spring. The same pathogen also infects black walnut (*Juglans nigra*) in Sacramento Valley. Although the disease was found sporadically in various counties, San Benito Co. has shown endemic levels of the disease with lesion symptoms on both leaves and fruit in rainy springs. The pathogen overwinters as perithecia formed in drooped leaves on the ground which release ascospore in early spring (April) as the young leaves and fruit start developing. Although sanitation by removing leaves from the ground is not economically feasible, the disease can be successfully controlled with timely fungicides, starting early in the spring. The first application is applied when the emerging leaves are half the size of the final size of the leaves. The second and third applications are applied at about 2-3 weeks after the previous application. Initially fungicides tried were Bordeaux mixture, Liquid lime sulfur, Quilt Xcel, Inspire Super, tebuconazole, Topsin-M,



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Pristine and Syllit. The most effective fungicides were Luna Experience, Pristine, and Syllit. The fungicides Bordeaux mixture and liquid lime sulfur showed no efficacy. More efficacy fungicide trials were tested in subsequent years and will be discussed in detail.

Keywords:

Juglans regia, Marssonina juglandis, acervulli, fungicidal control

(P17): Efficacy of two nematode species against the Walnut husk fly

Dr. Erick D.M. Campan (Université Paul Sabatier Toulouse, France)

The walnut husk fly, *Rhagoletis completa* (Tephritidae), arrived in France in 2007 and has since spread very widely in the various production areas starting from Rhône-Alpes district (Centre-East of France). Populations can be controlled by chemical treatments, but biological control (Kaolin, pheromones) is not fully effective.

Our research aims to find and develop an effective means of controlling the walnut husk fly, that does not use products derived from synthetic chemistry and accordingly to the respect of environment.

A first strategy was to test two species of entomopathogenic nematodes: *Steinernema feltiae* and *Heterorhabditis bacteriophora*. These nematodes, via the symbiotic bacteria they harbour, kill the infected insects very quickly (48h according to the bibliography).

In the laboratory, these two species attack the larvae of the walnut husk fly when they are on the ground. Fifteen days after treatments, the mortality rate varies between 14 and 48% according to the trials; which is greatly above the natural mortality encountered in the control batches. While *S. feltiae* was more effective than *H. bacteriophora* in orchard soil, both species have a similar effectiveness in potting soil.

Pupae and adults are not susceptible to nematodes, except at emergence of imagos, especially with *S. feltiae*.

Keywords:

Biological control, *Rhagoletis completa*, entomopathogenic nematodes

(P18): Walnut Husk Fly - Monitoring population dynamics and density with yellow sticky traps

Dr. Anita Solar, Rok Veber (University of Ljubljana, Slovenia)

The walnut husk fly (WHF) is a major pest of walnuts in Slovenia, causing significant damage to the fruit, leading to crop losses and reducing the market value by up to 80%. The control strategy of WHF is based on monitoring the population with special traps consisting of a yellow sticky plate and an attractant of ammonium carbonate. By monitoring population density, farmers can determine the need for control measures that reduce pest population and damage. In this paper, we present the seasonal dynamics and density of the WHF population at four sites. In Pesniški dvor, Petrovče, Buče and Maribor orchards, two traps were placed at two different heights in the period 2020-2022. In the Pesniški dvor orchard, which is located on a prominent slope, four traps were set to determine whether altitude plays a role in the density of WHF. The results show that populations of WHF were greatest in 2021 at all sites surveyed. Traps hung at 5 or 6 m height had up to 12 times the number of flies trapped compared to traps hung at 2 m height. 163 trapped flies were the absolute maximum. It was counted at the Buče site at a height of 6 m in the third decade of July 2021. At all sites and in all three years except Petrovče in 2020, the first flies appeared in the second decade of July. At three sites we recorded a population peak: in the third decade of July (Buče) or in the first decade of August (Pesniški dvor) or in the second decade of August (Petrovče). At the Maribor site, there were two population peaks: in the second decade of August and in the first decade of September. The number of WHF was lower at the foot of the hill than at the top of the hill (Pesniški dvor).

Keywords:

common walnut, *Juglans regia* L., *Rhagoletis completa* Cresson, counting, population peak



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(P19): First report of *Ilyonectria liriodendri* causing black foot of walnut in Chile

Prof. Gabino Reginato (University of Chile, Chile)

Walnut (*Juglans regia*) is an important fruit crop in Chile with about 42,000 ha planted in the central part of the country. During autumn of 2021 declination of walnut orchards was observed in Metropolitan and O'Higgins regions, the affected trees showed lack of shoot growth, death of terminal shoots and regressive death, leaf chlorosis and defoliation, the roots presented black lesions or were completely black and dead, trees death was also observed. The disease was present in new orchards (2 years old) as well as in old orchards (18 years old). From the affected roots *Cylindrocarpon* like fungi were consistently isolated, that were identified according with the morphology of their conidia and conidiophores. The pathogen has been reported previously from walnut nurseries in Italy and Chile. Monosporic cultures of two isolates obtained from affected roots were used for the molecular identification. ADN was extracted and amplification of the *tub2*, *his3* and *tef1* gene regions was performed. The amplicons were sequenced by Psomagen (USA) and a multilocus phylogenetic analysis was performed. Both isolates were identified as *Ilyonectria liriodendri*. To test the pathogenicity on walnut, the isolates were inoculated on wounded walnut shoots and stored at room temperature. After 4 weeks, symptoms of necrosis and black coloration of the shoots was observed, the pathogen was re-isolated completing Koch's postulates. This report is the first detection of *I. liriodendri* causing black foot of walnut in Chile. A survey of the disease is being conducted in order to unravel its etiology and to understand the conditions that have favored the pathogen to attack walnuts.

Keywords:

Cylindrocarpon, multilocus phylogenetic analysis, orchard declination

(P20): Project ParasiT: using egg parasitoids as biocontrol agents against walnut and chestnut lepidopteran pests

Jean-Baptiste Philibert (SENURA, France)

Walnut and chestnut orchards represent the second-vastest plantation of France and they share several specific challenges of cultivation such as high canopy, difficulty of access, and a relative lack of efficient treatments against insect pests. The fruit-boring caterpillars represent a particular danger in the context of climate change which increases the number of generations per year and facilitates the arrival of new species.

The goal of the project ParasiT is to contribute to the development of alternative means of defense against five species of lepidopteran pests (*Pammene fasciana*, *Cydia splendana*, *C. fagiglandana*, *C. pomonella* and *Ectomyelois ceratoniae*), two on walnut and three on chestnut, via the identification, rearing and introduction of native oophagous parasitoids. Micro-wasps of the genus *Trichogramma* are already a widespread and effective method of biological control in other agricultural systems (e.g. maize, apple...). In the previous project LICHEN, several species of *Trichogramma* were identified in nut orchards' canopies on supplied *Ephestia kuehniella* eggs. To confirm these findings, the project ParasiT aims to sample the diversity of indigenous parasitoids specific to the studied pests by using the sterile eggs of their own hosts. Potential candidates' efficiency will then be evaluated both in laboratory and in the field, with particular care for eventual non-target impacts they could cause, in order to select the best suited ones for the subsequent mass releases.

Keywords:

Biological control, *Trichogramma*, *Cydia pomonella*, *Ectomyelois ceratoniae*