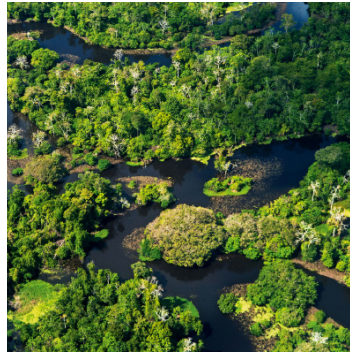


Pests and diseases



■ Monitoring of thrips in avocado 'Hass' in the municipality of Ziracuaretiro Michoacan, Mexico

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The growing of avocados in Michoacan, Mexico poses significant damage caused by insect pests, particularly the class Thysanoptera affecting soft tissues of leaf shoots, flowers, young leaves and developing fruit, causing pericarp damage and reducing its commercial value, especially for the exporting market. Monitoring this pest allows to develop more precise control plans in micro-regional environmental conditions. The study aimed to: evaluate the presence and incidence of thrips (*Franklinella* and *Scirtotrips*) in the town of Ziracuaretiro Michoacán during a cycle previous to harvest; compare catches of thrips in monochromatic traps (yellow and blue) and evaluate thrips and "scab" *Sphaceloma persea* damage in preharvest and postharvest fruit and their correlations. Direct sampling from terminal shoots, inflorescences and weeds was relized every fifteen days, so was the counting of adults in traps and the healthy fruit and thrips and "scab" damage percentage was quantified.

The highest thrips presence recorded was: in shoots, inflorescences and weeds in March and June (lower presence: April, October and September); in blue traps the largest catches were found in May (lowest presence: January, June, August and September). Traps registered higher catching averages of thrips / tree. At harvest, thrips damage averages were: 39% low and 11% high. Similar to "scab" damage with direct correlation value of 0.917.

■ The use of Fertimar®, a seaweed based biostimulant, as an effective fungicidal control of *Lasiodiplodia theobromae* in avocado plants (*Persea americana*)

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Lasiodiplodia theobromae is a phytopathogen that severely affects Avocado plantations in any developmental stage. It causes infections in the insertion area between the scion and the rootstock, plant "dieback", canker formations, and fruit pedicle necrosis. The objective of this study was to evaluate Fertimar's ability to activate defense mechanisms in plants infected with *L. theobromae* A3 under controlled conditions. Fertimar was applied using three different doses: 200, 300, and 400 g/200L. The plant material used was Hass variety grafted on 1.5 year old Zutano. Both scion and rootstock were inoculated by creating a wound and placing an 8mm PDA disc colonized with *L. theobromae* directly in contact with the cambium. Fertimar was applied 3 times, with a 7-day interval. As a preventive treatment, plant inoculation was done 5 days after Fertimar applications were completed; whereas for the corrective treatment, Fertimar applications began 5 days after plant inoculation. Image analysis program ASSESS 2.0 was used to determine the area of infection in cm² once the trial had ended. Preventive applications of Fertimar controlled the infection of the scion by 80-84%, while corrective applications controlled the infection by 35-42%. However, no significant differences were observed on the rootstock infection for both treatments. Applications of Fertimar at a dose of 300 g/200L and 400 g/200L, as preventive and corrective methods respectively, limited *L. theobromae* infections in avocado plants, emphasizing its role as an elicitor and proving to be an alternative to the synthetic fungicides currently being used to control this phytopathogen.

■ Defense mechanisms in avocado rootstocks to *Phytophthora cinnamomi*

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In the study of plant resistance to root diseases, often is done the evaluation at macroscopic level, by selecting healthy-looking plants or surviving plants, however, should be considered the histological mechanisms of plant resistance against pathogens, such as the root pathogen *Phytophthora cinnamomi*. The aims of this study were to identify the presence of mycelium of *P. cinnamomi* in the root xylem, and their implication in tyloses and deposition of phenols formation as histological defense mechanisms in the avocado rootstocks genotypes, Duke-7, Thomas, Tepetl, Atlixco and Tepeyanco. Seedlings were inoculated with *P. cinnamomi* in controlled soil temperature at 17 and 28 °C. Histopathological observations showed the presence of hyphae in root xylem, formation of tyloses, and accumulation of phenols in dead plants, plants with wilting symptoms and asymptomatic plants of Tepetl, Atlixco and Tepeyanco. The contents of mycelium in the root xylem of dead plants of Duke-7, Thomas and Tepetl at 17 °C showed no significant difference, however at 28 °C the contents of mycelium in Duke-7 was highly significant ($p < 0.05$). In plants with wilting symptoms, the presence of mycelium at 17 °C was bigger in Tepetl, and therefore is considered susceptible. Atlixco showed similar mycelial contents at 28 °C and is considered tolerant to *P. cinnamomi*. In plants with wilting symptoms and asymptomatic plants, both defense mechanisms, the tyloses formation and the phenolic deposition compounds contributed to the defense against the oomycete. The genotypes Tepeyanco, Atlixco and Tepetl showed tolerance-resistance probably because they activate their chemical and structural defense mechanisms previously to the *P. cinnamomi* infection.

■ Totipotency of avocado seedlings in the resistance to *Phytophthora cinnamomi*

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The identification of resistance in seedlings of native Mexican race avocado, naturally respond to infection by the oomycete *Phytophthora cinnamomi*. This response is due to genetic variation based on the survival and totipotential capability after inoculation with the oomycete. The aim of this work was to induce resistance in seedlings after inoculation and totipotential "formation of new seedlings." In selecting for resistance avocado seedlings were of six genotypes of avocado Atlixco, Tepeyanco, Tepetl, Toliman, Colín V-33 and Thomas (of 5 cm height and of 15 to 20 cm height) two trials, were seedlings inoculated with a virulent isolate of *P. cinnamomi*, in the second trial only seedlings of genotypes Tepeyanco and Atlixco (5 cm height). Were inoculated seedlings in advanced stages of growth (of 15 to 20 cm height) and juvenile stage (5 cm height) to detect resistance to *P. cinnamomi*. Atlixco seedling population is an important source of resistance as it had the largest number of new shoots of seedlings and totipotent asymptomatic seedlings. The seedlings genotypes Toliman, Tepetl and Thomas are considered susceptible. Colín V-33 can be classified as tolerant-resistant rootstocks to *P. cinnamomi*. Atlixco and Tepeyanco are considered tolerant- resistant avocado wilt and canker caused by *P. cinnamomi*.

Control the avocado root rot caused by por *Phytophthora cinnamomi* with different isolates of *Trichoderma* in the Chavimochic irrigation project in Peru

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The Avocado root rot caused by *Phytophthora cinnamomi* is one of the major diseases of Chavimochic irrigation in northern of Peru. The use of antagonists such as *Trichoderma* is one of the most used control alternative to prevent and reduce the damage of this pathogen to root level. The purpose of this study was to evaluate different strains of *Trichoderma* to control *Phytophthora cinnamomi* under greenhouse conditions in Chavimochic Irrigation. Avocado seedlings “Zutano” were inoculated three times with five *Trichoderma* strains with the dose of 1x10⁸ ufc per ml. The treatments were: *Trichoderma* sp (Chav01), *Trichoderma harzianum* (Chav02), *Trichoderma harzianum* (UNALM01), *Trichoderma viride* (UNALM02), *Trichoderma* sp (SOLA01). Additionally, an inoculated control with *P. cinnamomi* and one without inoculation were used. Inoculation of *P. cinnamomi* was done by incorporating sterilized wheat with mycelial growth of *P. cinnamomi*. Different parameters at 30, 45 and 60 days after inoculation of the pathogen were evaluated. It was found for the parameter of healthy root percentage that all isolates showed statistical differences with the inoculated control, but did not with the no inoculated. Among the treatments, *Trichoderma* sp (Chav01), followed by *Trichoderma harzianum* (Chav02) have proven to have the highest percentage of healthy root, these two isolations were taken from healthy avocado trees of Chavimochic Irrigation. In general, local strains have shown the best control while strains introduced from other areas have responded poorly.

Etiology of the wet rot root and steam in *Persea americana* Mill. Var., *drymifolia*

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The phytopathogens that cause damages and destroy the radical system are of great importance in the avocado producer areas around the world because of the economic losses for the producer, which goes from the 10% until the 100%, not being the exception the avocado region of Michoacan, Mexico. The goal of the present investigation was to identify the causal agent of the wet rot root and steam in *Persea americana* var., *drymifolia*. The symptoms observed in the infected trees were; yellowing, leaf curl, crust cracking, in mycelium roots from white color to yellowing and abundant rhizomorphs, in summer in the damaged parts of the trees it is produced abundant fruiting bodies of the fungus, the infected trees die from 4 – 6 months since the beginning of the firsts symptoms. The collect of the roots and crust with symptoms of radicular rot was made in three avocado orchards in the community of San Francisco Corupo (19°36'832" north latitude, 102°15'962" west longitude, 2221 msnm), and an orchard of the community of Santa Ana Zirosto (19°31'559" north latitude, 102°16'866" west latitude, 2567 msnm) of Michoacan. Isolations were made by phytopathological techniques, the obtained isolated were identified, their pathogenicity was obtained by the inoculation of avocado six month seedlings and in steams: manifesting the symptoms in both cases, of the isolations was identified the *Armillaria* spp. fungus

In vitro control of bacteria asociated to the banding of the avocado steam

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The banding of the avocado fruit steam is one of the most important diseases, taking losses of up to the 45% of the production, it is widely distributed in the avocado producer area of Michoacan, Mexico, associating diverse causes; nutritional and water failures, fungus and bacteria, the objective was to isolate and identify associated bacteria to the symptom of banding of the avocado steam and to determinate its antibiotic sensibility. Avocado fruits were collected with symptoms of the illness, and isolations were made by phytopathological techniques, the isolated colonies were identified, their pathogenicity was determined by the tests of: potato rotting, in situ impregnation of the avocado steam with the bacterial strain and the infiltration of the bacterial solution (1.5x10⁸) to the fruits. Afterwards susceptibility to different groups of antibiotics of the bacteria colonies was made: oxytetracycline, streptomycin + oxytetracycline, streptomycin + oxytetracycline + copper, gentamicin + oxytetracycline, kasugamycin and an absolute control. Two bacteria colonies were isolated, the first one was a mixed culture, identifying; *Ralstonia pickettii* and in the second one *Klebsiella oxytoca*. With the exception of *R. pickettii* this species are reported as phytopathogenic. The two colonies oxidized the potato tissue. In situ it is produced the banding of the fruit steam just with the bacteria infiltration. When it was directly inoculated it was only produced a blackness of it without the detachment of the fruit. *E. ludwigii* and *K. oxytoca* presented sensibility to the tested antibiotics; *R. pickettii* was only sensible to the mixture that contained copper. But none of this was sensible to the kasugamycin.

Insights into the phylogeny and expression of the *Persea americana* (Mill.) NPR1-LIKE gene family

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The Non-expressor of pathogenesis-related genes1 (NPR1) is an important transcription cofactor in many if not most plant species. NPR1 controls the expression of many pathogenesis related (PR) genes which are essential to the establishment of systemic acquired resistance (SAR). Furthermore, cross-talk between the salicylic acid (SA) and jasmonic acid/ethylene (JA/ET) pathways is largely regulated by NPR1. Clearly, NPR1 plays a significant role in pathogen defense responses. Avocado (*Persea americana*) is an economically important fruit crop in many countries worldwide. The most significant threat in terms of production is Phytophthora root rot (PRR). The causal agent, *Phytophthora cinnamomi*, is a hemibiotrophic oomycete which infects the feeder roots of avocado trees which in turn become necrotic, reducing the overall uptake of water and nutrients. Despite this there is limited molecular data which characterizes the interaction between avocado and *P. cinnamomi*. Thus, understanding the role of NPR1-like transcription factors in avocado could provide novel insights into the avocado – *P. cinnamomi* interaction. Five NPR1-like sequences were identified and subsequently annotated using FGENESH. Predicted protein sequences were subjected to maximum-likelihood phylogenetic analyses along with 34 other known NPR1-like protein sequences. Furthermore, conserved NPR1-like domains and motifs were annotated. Expression of these five sequences was described in the roots after salicylic acid, methyl jasmonate and *P. cinnamomi* treatment at several time-points. Additionally, their expression was defined in various tissue types as well as compared in tolerant and susceptible rootstocks during *P. cinnamomi* infection. The results of this study suggest a defensive role for three of the NPR1-like sequences and a developmental role for the remaining two. Thus we provide an important resource for further study and classification of this gene family in avocado, a vital step in understanding the role of NPR1 during *P. cinnamomi* infection.

Identification of species of mistletoe associated with avocado in Michoacan, Mexico

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Mistletoes are parasitic plants with cosmopolitan distribution, affecting diversity of wild or cultivated perennials (forest, fruit or ornamental type). As a consequence of the damage the infested plants may die. The avocado is among the susceptible hosts, without any reports that describe the susceptibility of the attack by the different varieties in existence or the mistletoe species associated with these plants. The aim was to identify species of mistletoe associated with wild and cultivated plants of *Persea* cohabiting in the state of Michoacan, Mexico. The study was conducted in 2014, in the municipalities of Uruapan, San Juan Nuevo, Tingambato and Ziracuaretiro in Michoacan, Mexico. These municipalities have an altitudinal gradient ranging from 1260-2360 m and are representative of the avocado producing region in Michoacan. Twenty commercial orchards of avocado 'Hass' were chosen and 10% of the trees were sampled to detect parasitism by mistletoe in the trees. In the gardens of the surrounding areas, either backyard or local roads, trees were sampled of an avocado Mexican race known as "Criollo" plus 'Hass' trees with no management. Were characterized sample trees, growth stage of the parasitic plant and geographic location, botanical material was collected for identification were observed mature reproductive shoots of mistletoe. No avocado trees 'Hass' were found parasitized by mistletoe. Plants of the "criollo" type with damage by mistletoe were observed. Associated with these plants and correlated with the altitude of their location, it was identified *Phoradendron velutinum* in altitudes around 2000 m in areas of San Juan Nuevo; *Psittacanthus calyculatus* was at the same altitude range in Tingambato, *Struthanthus condensatus* was collected at 1600 m in Uruapan; *S. venetus* was collected at 1330 m in Ziracuaretiro.

Validation of an integrated program borer branches *Copturus aguacatae* Kissinger (Coleoptera: Curculionidae: Zygopinae) in orchards in Michoacan, Mexico

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The borer branches *Copturus aguacatae* Kissinger is one of the most economically important pests in avocado plantations in Mexico, the damage is caused by develop galleries of larvae along the bone in secondary and terminal branches of the trees. Indirect damage is also generated by quarantine regulations that prevent the mobilization of fruit from infested orchards. The objective was to validate a strategy based on pest monitoring for the occurrence of different biological states of the insect and based on that make pruning branches with rates of damage, supplemented with foliar sprays of biopesticides in a grove of avocado 'Hass' 20 years in Ziracuaretiro, Michoacan, Mexico. Validation was performed during the years 2012 to 2014. The orchard previously had an 85% of infested trees. During the dry season (January to May), with intervals of 15 days revisions were made to the total leaf area of trees, proceeding to prune branches and destroy damage indices (grainy white powder). The rainy season began in June and thus was adults; immediately the first application was made, dividing the orchard into three sections. Block I sprinkled with *B. bassiana* 1x10¹¹ CFU/L (0.40%, equivalent to 3.5 g.i.a./L); Block II was applied *M. anisopliae* 1x10¹¹ CFU/L (0.40%, equivalent to 3.5 g.i.a./L); Block III *B. bassiana*+*M. anisopliae* 1x10¹¹ CFU/L (0.195% + 0.195%, equivalent to 1.75 + 1.75 g.i.a./L). Four applications were made with intervals of 21 days. For the second year of testing the application of management strategies in validation were repeated. For the first year was quantified highly a significant impact on removing adults with the three strains of biopesticides validated; for the second year adult populations was insignificant, and the in third year neither infested branches or adults were detected in the foliage.

Brown root rot of avocado in Australia

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Phellinus noxius is a wood rotting fungus of a wide range of woody plant species in natural tropical ecosystems. However, in disturbed areas or where orchards or plantations have been established under a monoculture, it causes brown root rot and tree death in several horticultural and forestry hosts resulting in severe economic loss. *Phellinus noxius* impacts productivity of avocado in tropical and sub-tropical growing regions in Australia, that is, all production zones in Queensland, and northern New South Wales. The disease spreads by root to root contact along the row and current management relies on removal of infected trees and installation of root barriers to prevent spread of infection. We have been working on several aspects of the problem. We confirmed that remnant roots (2-4 cm in diameter) from dead trees could harbour the fungus for more than 4 years, causing young replants to die when their roots became infected after contact with the old infested roots buried in soil. Glasshouse inoculation trials demonstrated that avocado and macadamia were very susceptible but passionfruit, citrus and mango were less susceptible, however, more work is necessary to determine whether these species may be suitable options for replanting into infested sites. While there are currently limited practical eradication options, our preliminary glasshouse work has identified some promising disinfestation treatments.

Peruvian phytosanitary certification system for avocado (*Persea americana*) Hass variety destined to international market

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In the last years, the agricultural Peruvian exports had a sustained growth due to a coordinated work between the agro-exporter private sector and the Ministry of Agriculture and Irrigation through the National Service of Agricultural Health- NSAH. Through this, many projects and investment programs have been promoted in order to support and improve the agricultural health in Peru.

For the specific case of Hass avocado, Peru developed a research to demonstrate that this fruit, under Peruvian conditions, is not a host of fruit flies; these results allowed the successful access of the fruit to the North American market in 2011 and in this year 2015, the access to Chinese and Japanese markets.

In order to ensure the compliance of the phytosanitary requirements of the importer country under the international regulations, SENASA has a specific regulation for the phytosanitary certification of Hass avocado destined to be exported, this includes the different phytosanitary measures that should follow the exporter, before and during the export process; mainly with process such as the certification of the production places, packing houses, guidelines for the transport and move of the export fruit, phytosanitary inspection of each shipment and the sealing of all the containers for ensuring the identity and integrity of the export shipments.

In the last campaign, Peru has exported approximately 170,000 tons to the international market, being the main markets from United Kingdom and United States; and since this year, the markets from China and Japan. However, SENASA is in a permanent paperwork with many Asian countries and Latin American countries in order to spread out the destinations of this Peruvian fruit, which in the last years is still increasing in hectares, throughout the whole country.

Fusarium dieback, an emerging exotic disease / pest complex causing dieback throughout agricultural, urban, and wildland landscapes in Southern California

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The polyphagous shot hole borer (PSHB) is an invasive ambrosia beetle that forms a symbiosis with multiple fungi. Together, they cause fusarium dieback (FD), a pathogen/insect complex that affects trees in agriculture, ornamental landscapes, and native forests in California. PSHB was first reported on black locust in California in 2003 but there were no records of fungal damage until 2012, when *Fusarium euwallaceae* was recovered from the tissues of several backyard avocado trees infested with PSHB in Los Angeles County. Since early 2012, FD has been confirmed on more than 139 species of tree in landscape and urban forest in Los Angeles, Orange, San Bernardino and San Diego counties. The objective of this study was to identify, characterize fungal species associated with the PSHB in California. Beetles and larvae from infested plants were collected from eight different tree species including *Platanus ramosa*, *Acer negundo*, *Persea americana*, and *Ricinus communis* in Los Angeles county. The head and the abdomen of the beetle or larva were individually macerated in tube containing 200 µl of sterile water. Tubes were vortexed and a suspension of 50 µl was spread onto potato dextrose agar (Difco) amended with 0.01% tetracycline hydrochloride (PDA-test). The relative abundance of fungal species associated with PSHB in different hosts was determined by counting colony-forming units (CFUs) of each fungal species identified within the head and abdomen of ten beetles each from eight different hosts. *F. euwallaceae*, *Graphium* sp. and *Acremonium* sp. were most frequently recovered from the head of the female beetles. No fungus was recovered from the male heads. *Graphium* sp. was recovered at higher frequencies than *F. euwallaceae* from the larvae. These data suggest the beetle carries more than one fungal species, and this beetle-disease complex potentially may establish in a variety of plant communities locally and worldwide.

■ Towards commercialisation of avocado rot prediction

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A survey of 32 avocado orchards throughout the three major growing regions of New Zealand was conducted in the 2012/13 season to validate the prediction of fruit rots at harvest. The model predicted 80% and 82% of the variation in the data for the Whangarei and Bay of Plenty districts, respectively. In the 2014/15 season leaves were sampled from 100 trees in two avocado orchards. DNA was extracted from leaves and a qPCR analysis of fungal populations was conducted. The variability in the distribution of qPCR crossing thresholds (Ct values) in each orchard was determined using spatial analysis and GPS coordinates. An optimal sampling strategy was designed to maximise the robustness of using Ct values to predict fruit rots at harvest on avocados harvested from individual orchards.

■ Systematic orchard survey to determine regional and seasonal variation of fruit quality in Michoacán avocados

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A survey was conducted to identify the most common quality issues for avocados grown in three climatic zones of Michoacán. Twelve representative orchards were selected, and fruit were harvested at c. 2 week intervals throughout the season. Dry matter was determined at each harvest. Fruit were stored for 28 days at 5.5°C, then ripened at 20°C. Internal and external disorders were assessed when fruit were eating ripe. The two major quality issues were postharvest rots and vascular browning. Orchard factors that affected these disorders and that could ameliorate them will be discussed.

■ Conditional non host status avocado fruit var. Hass (*Persea americana*) to *Anastrepha fraterculus* and *Ceratitis capitata* (Diptera: Tephritidae) in Peru

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The objective was to determine the status of non-host of avocado var Hass. *Persea americana* Miller with commercial maturity and possible mechanisms of resistance to “fruit flies” *Anastrepha fraterculus* (Wiedemann) and *Ceratitis capitata* (Wiedemann). Studies were conducted in four commercial fields avocado, located in Ancash and Lima, in 2009 and 2010. The natural population density of “fruit flies”, was determined using McPhail and Jackson traps and sampling of natural hosts fruits. To determine infestation of “fruit fly” in avocado, various tests were performed such as: harvested fruit and exposed on the ground; avocado fruit from field and packing was sampled during the harvest season. Forced oviposition tests were performed, exposing fruits to gravid female of “fruit flies” in confined environments, covering an entire tree with a mesh cage of 4 m. high by 4.5 m. diameter and enclosing an entire branch with sleeves of 1.5 m long by 1.0 m. diameter.

As results no infestation was reported in avocados sampled of field (3750 fruits) and packing (1600 fruits), as well as 4800 avocados placed on the ground. 7200 avocado fruits were used in the experiment of forced oviposition cages, which were not infested and in sleeves, were used 600 fruits, no reported larvae or pupae, however, *C. capitata* eggs were found in 5 intact fruits and 10 punctured, but were encapsulated by callous tissues and died (resistance mechanisms).

Based on these results, USDA published the final rule, concluding that: mature green fruit “Hass” avocado peruviana is not a pathway, and is a conditional non-host for *A. fraterculus* and *Ceratitis capitata*.

■ Mirids produce pre-harvest spotting on avocado fruits, affecting sales

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The appearance of a specific type of skin spots on pre-harvest fruit is seriously impacting upon avocado production in Colombia. These skin spots imply a loss of profits for the producer, who may not be able to sell the fruit at all (especially in the case of export markets) or may be forced to sell it at a lower price, due to its appearance. These irregular spots are caused by Mirids that attack fruits in early stages of development by injecting toxins that damage the fruit's skin. As well as attacking the fruit skin, Mirids may also attack the fruit peduncle, causing early fruit drop, also implying a loss of earnings for farmers. These specific spots had been erroneously attributed to fungi (*Pseudocercospora purpurea*, *Sphaceloma perseae* and *Colletotrichum gloeosporioides*) due to humidity conditions in adult crops. Because laboratory tests proved the presence of these and other fungus, such assumptions seemed reasonable, but the recommended responses did not resolve the problem. General and specific field observations reveal that these spots are actually caused by Mirids and that the fungi develop after the insect attacks (similarly to the case of *Monalonion velezaengelii*). The initial symptoms of the insect attack are transparent protuberance on the fruit's skin surface with multiple points of penetration in a small area, though without impact on the fruit's pulp. As the fruit grows, the protuberance turns into large irregular spots, damaging the appearance of the fruit. Preliminary studies identified the insect as from the genus *Hyaliodes* sp. (Hemiptera: Miridae). This genus is reported as a possible controller of avocado lacebug (*Pseudacysta perseae*), but field evaluations found that these insects, in immature states and even as adults, attack avocado fruits in twilight times.

■ Challenges of growing avocado's in subtropical South Africa

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In South Africa, avocados comprise a large portion of the subtropical fruit industry. Because the South African avocado industry is export-orientated, emphasis is placed on ensuring high fruit quality standards. One of the most serious threats to the maintenance of these standards are pre- and post-harvest diseases such as *Pseudocercospora purpurea* (Cercospora spot), *Colletotrichum gloeosporioides* (anthracnose) and various species in the Botryosphaeriaceae group (stem-end rot).

For more than 20 years research has been conducted at Westfalia Fruit Estate to develop effective control measures for these avocado diseases. Westfalia is situated in the hilly subtropical area of the Limpopo province, where most of the country's avocados are grown. The average rainfall is between 800mm and 1300 mm, but in some years, it exceeds 1800 mm during the warm summer months. These warm, wet conditions contribute to a very favourable environment for avocado fruit diseases to develop. It is therefore crucial to have an adequate pre-harvest spray programme in place for this purpose. Control of these pathogens focuses mainly on inoculum reduction and prevention of latent infections.

Currently diseases are controlled by high volume pre-harvest fungicide applications. Recent research has focused on reducing the volumes of fungicides applied to orchards by exploring new formulations and new application methods. It is thought that ultra-low volume application technologies, such as TracFog and/or Electrostatic spray systems could reduce spray volumes, run-off and the amount of fungicide applied per hectare compared to traditional commercial practices using hand guns or mist blowers.

Molecular characterization of species of *Colletotrichum* spp associated with avocado anthracnose in the central region of Michoacan

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Colletotrichum spp. are species of fungi associated with damage to branches, flowers and fruits, avocado related symptom known as anthracnose. In recent years, is considered one of the most important pathogens that limit crop production in Mexico since significantly affect fruit quality and business performance. The previous record support a thorough study of the species involved to lay the foundation to a regionalized management according to the species. For this research was conducted to determine the diversity of the species involved in production regions in Michoacan. Fruit orchards in five site the central region of Michoacán, which were taken according to the surface of each site, were collected, they were taken to the laboratory where isolations and purification of them were made, yielding ninety-six isolates of *Colletotrichum*. They were then grouped according to each region and proceeded to perform DNA extraction through crop potato dextrose agar developed. The DNA from each isolate was processed through PCR with the gene gliceraldehído dehidrogenase-3-phosphate (GAPDH). The sequences obtained were analyzed with the software MEGA5.22 to compare with the species belonging to *Colletotrichum* complexes deposited in the GenBank. Phylogenetic analysis showed that there is great diversity in the insulation, and pooled within complex of *Colletotrichum gloeosporioides*, *Colletotrichum acutatum* and *Colletotrichum boninense*, the four haplotypes were only twenty two isolates were selected to be representative for the reactions with the complementary genes were actin, calmodulina, β -tubulina and the ITS region, which are still under study.

Integrated analysis of early *Phytophthora cinnamomi* infection in tolerant and susceptible avocado rootstocks

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Phytophthora root rot is arguably the most devastating disease affecting avocado production worldwide, - resulting in necrosis of feeder roots, tree dieback and severely decreased fruit yield. The causal agent, *Phytophthora cinnamomi* Rands, is a ubiquitous oomycete plant pathogen reported to have a host range of over 3000 species. Avocado rootstocks harbouring tolerance for *P. cinnamomi*, together with treatments of phosphite salts, are currently the only effective control strategies practiced by commercial farmers. However, the mechanisms conferring tolerance to infection in rootstocks are still poorly understood, thus confounding the development of improved breeding programmes. This study aimed to compare tolerant and susceptible avocado rootstocks on both morphological and molecular levels, through various early time points, - during which infection was effectively established, using scanning electron and epifluorescent microscopic techniques, gene expression data and nested qPCR. From 3 hours post inoculation (hpi), the magnitude and rate of *P. cinnamomi* zoospore encystment and germination was significantly higher in the susceptible rootstock than that of the tolerant rootstock. Simultaneously, cell wall fortification was observed in both rootstocks, with that of the tolerant composed primarily of callose and that of the susceptible rootstock exclusively of lignin. Expression analysis of three defence-related genes, - PR5, PAL, and *Endochitinase*, substantiated findings that the early molecular reactions are crucial in establishing tolerance to *P. cinnamomi* thus producing a significantly lower overall pathogen load only after 24 hpi, determined by nested qPCR. The results of this study corroborate those of similar studies indicating that chemotaxis and cell wall fortification play a vital role in the ability of *P. cinnamomi* to effectively establish infection and thus need to be addressed in the rootstock selection process. An improved understanding of the mechanisms conferring tolerance in various avocado rootstocks will facilitate improved screening methods and more effective use of phosphite treatments.

Effective host-delivered RNA silencing targeting *Phytophthora cinnamomi*

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Plant diseases may be controlled by RNA silencing of essential pathogen genes. It has been proposed that this process can be induced by uptake of pathogen-specific, host-produced small RNAs. We have designed and engineered hairpin RNA constructs targeting essential genes in *Phytophthora cinnamomi*, an oomycete pathogen of global economic significance that affects more than one thousand host species. Soaking of the mycelium in dsRNA produced from the constructs targeting these genes resulted in the arrest of mycelial growth of *P. cinnamomi* on culture media. Furthermore, transgenic *Arabidopsis thaliana* carrying the hairpin constructs produced small interfering RNAs (21-24nt) that were specific for the targeted pathogen genes. Proof of concept for host delivered RNA silencing-based resistance to *P. cinnamomi* was demonstrated by increased root length and growth in all transgenic *A. thaliana* lines compared to wild type under heavy *P. cinnamomi* inoculum loads. This concept can now be applied to Avocado rootstocks, where root rot disease caused by *P. cinnamomi* is a major constraint. This strategy may also have broader applications for control of other fungal diseases using host delivered RNA silencing.

Chemical and biological control in avocado trees with symptoms of wilt in Matanguaran Mpio. In Uruapan, Michoacán, Mexico

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The wilt of the avocado tree could take place at any development's phase of the plant, wilt and foliage's loss, usually; the tree's avocado's wilt doesn't present any new shoots. It shows dieback branches phenomenon and root necrosis. The main researching objective was to evaluate four biological products and five chemical ones against associated fungi with the wilt of the avocado tree. We selected trees with symptoms of the disease symptoms and we worked with them in four sampling processes of root, two of them were before rainy season and the other two in dry season. We washed and weighed these sampling roots and put them on a PDA medium, to verify the presence of fungi. We applied Commercial products based on fungi and bacteria used for biological control: Bactiva + E.M (efficient microorganisms). 25g; Natucontrol + E.M. 100 g; Spectrum-L + E.M.; S-mic-0 bac + E.M. 1 L diluted in 100 L of water and under an absolute control. We applied this four times, two of them were in rainy season and the other two dry season. For chemical control Fosfimax® is applied 30 mL; Fosfimax® + Biorradicante 15 mL; ; Aliette® 7.5 g; Nutriphite® 15 mL; Ridomil® 7.5 mL en 15 L of water every two months and under an absolute control. The parameters that were measured to assess disease control were root weight and length of buds (SPAD) chlorophyll content. The best treatments were: Aliette®, Ridomil®, Fosfimax®, Nutriphite® and Nactucontrol.

Population distribution of "Red Spider" *Oligonychus* sp. (Acari: Tetranychidae), on avocado trees (*Persea americana* Miller) var. Hass in Lima, Peru

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The distribution of the "avocado red mite" *Oligonychus* sp on avocado trees has been studied trying to improve the sampling methodology and phytosanitary management. This investigation was conducted in avocado trees of the variety Hass in an area of three hectares in the town Cañete (Lima), between May 2013 and March 2014. During this period, were registered the agronomic practices developed, pesticides use and environmental conditions. In each tree, 12 sections were taken as follows: *per stratum* (high, intermediate and low), of the tree avocado for per cardinal point (north, south, east and west), Samples were taken every 15 days, using the leaf as the sample unit and counting the number of mites present (individuals and eggs). A total of two leaves were observed in each of 24 sections, making a total of 24 leaves for each tree and 240 for each sampling date. Avocado Red Mite populations were present from February to June, but the most populations were found in the west tree stratum and the canopy exterior. Significant differences in pest population density are time of the year, tree stratum and developing stages; information that will help establish appropriate evaluation and management strategies.

■ Expression analysis of putative RXLR genes from *Phytophthora cinnamomi*, during *in vitro* growth and avocado roots infection

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Phytophthora root rot remains one of the most devastating diseases of avocado around the world. The causal agent, *Phytophthora cinnamomi* Rands, is a very successful pathogen with a host range exceeding 3000 plant species. This hemibiotroph employs a complex set of pathogenicity genes to infect the roots and avoid detection by the plant surveillance system. *Phytophthora* species secrete hundreds of effector proteins that act on the plant host. They manipulate host cell structure and function, thereby facilitating infection and/or triggering host defense responses. We have previously identified 46 putative RxLR genes from the germinating cyst transcriptome of *P. cinnamomi* using Illumina sequencing. In the current study, fifteen RxLRs were selected based on the presence of the signal peptide, homology to other RxLRs and positive amplification of the gene fragments. Root material from a susceptible avocado cultivar (R0.12) was inoculated with three *P. cinnamomi* isolates. Infected roots were harvested at different time points for RNA extractions and cDNA synthesis. Gene expression analysis compared the expression profiles of RxLR genes from the *in vitro* life stages of *P. cinnamomi* and from *P. cinnamomi* infected root material, using the Bio-Rad CFX96 instrument. RxLR transcripts were expressed in the *in vitro* life stages (sporangia, zoospores, and germinating cysts) as well as in planta. The expression data were normalized to mycelia set at 1.0. Expression patterns differed significantly between individual RxLRs. Two RxLR transcripts (RxLR 3 and 5) were highly expressed in sporangia. RxLR 5 was down regulated in *P. cinnamomi* when in planta at 3,6,9,12,24 and 48 hpi. In contrast, RxLR 7 was not up regulated *in vitro* but highly up regulated in planta at all time points. This study has shed some light on putative RxLR genes that could be involved during avocado root infection by *P. cinnamomi*.

■ The effects of alternate food and host plant on the conservation of plant feeding predatory mites for pest control in avocado

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Alternate food for beneficials are important components in the conservation biological control tool box for sustaining and enhancing the populations of omnivorous predators. For generalist and specialized pollen feeding predatory mite species, belonging to the family Phytoseiidae, methods have been developed for outdoor cropping systems using gramineous windborne pollen provisioning cover crops. For example our studies have demonstrated the efficacy of Rhodes grass for the augmentation of *Euseius scutalis* and the subsequent prevention of outbreaks of *Oligonychus perseae*. A more innovative approach now being conceived is to plant hedges as windbreaks cloned to flower from late spring through autumn to serve both as a source of windborne pollen and as a reservoir for predators. An additional trait of some of these phytoseiid species is their ability to feed directly from the plant. Accordingly a new group of phytoseiids with plant cell piercing abilities has been proposed, including the generalist genera *Kampimodromus*, *Typhlodromus*, *Typhlodromalus* along with the specialized pollen feeders *Euseius*, *Iphiseius* and *Iphiseiodes*. The uptake of plant fluids by species suspected of plant feeding has been demonstrated using systemic dyes, systemic pesticides and radioactive isotopes. Here we present video documentation of plant feeding by phytoseiids, a proposed mechanism for cheliceral penetration, and plant damage following phytoseiid plant feeding. The ability of these predators to feed from the plant could explain how these predators can survive on their host plant when food is scarce but also emphasizes the importance of host plant compatibility.

■ Investigating soilborne nectriaceous fungi associated with black root rot in avocado

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Black root rot, associated with a complex of fungal *Calonectria*, *Ilyonectria*, *Cylindrocladiella* and *Gliocladiopsis* spp., is considered to be a severe disease of young avocado trees, with symptoms including black necrotic roots, tree stunting, and leaf drop prior to tree death. More than 50 isolates of Nectriaceous fungi have been isolated from symptomatic avocado roots from mature trees and nursery stock across Australia, and from other Lauraceae species in natural environments. Gene sequencing of ITS, β -tubulin and histone 3 loci have assisted with identification of several isolates, however it is likely that new species will be described as recent phylogenetic studies have re-classified and separated some of these genera into new clades. Glasshouse pathogenicity tests with 'Reed' avocado confirmed that two isolates of *Ca. ilicicola* were severely pathogenic, causing wilting or seedling death in 63% of seedlings 5 weeks after inoculation whereas none of the mock, *Cylindrocladiella* or *Gliocladiopsis*-inoculated seedlings wilted or died in that time. Seedling height and leaf, stem and root mass were also reduced significantly ($P < 0.001$) by *Ca. ilicicola*, while *Cylindrocladiella* and *Gliocladiopsis* were confirmed as not pathogenic. Inoculation of 'Hass' avocado fruit with *Ilyonectria* and *Ca. ilicicola* produced necrotic lesions in 100% of wound-inoculated sites. *Ca. ilicicola* isolated from avocado produced necrotic lesions in 58% of non-wounded sites, while *Ilyonectria* isolates from avocado and *Ca. ilicicola* from papaya did not produce any lesions at these sites. Test fungi were successfully re-isolated from the necrotic roots and fruit of selected plants.

■ Development of a nucleic acid based membrane hybridization test for the detection of ASBVd

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As an addition to its wide portfolio of viroid tests, Agdia has developed a nucleic acid hybridization RNA dot blot test to detect avocado sunblotch viroid (ASBVd) in leaves of avocado trees. The test is specific to ASBVd and does not cross-react with pospiviroids or other members of the Asunviridae family. The test has a sensitivity of at least 6 pg of ASBVd RNA, which translates to 7×10^7 genome copies. The extraction methodology is a simple one, which utilizes Ames Buffer. The extract is applied directly to the membrane without need for further purification. Sampling methodologies will be discussed.

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■ A multi target approach to fruitspotting bug management

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Fruitspotting bugs (FSB) *Amblypelta nitida* Stål and *Amblypelta lutescens lutescens* (Distant) (Hemiptera: Coreidae) are major native pests in subtropical and tropical horticultural crops in Australia and a key pest in avocado.

The pest is known to cause significant damage to more than 25 different tree fruit and nut crops and some vine fruits through the coastal and sub-coastal areas of Queensland, northern New South Wales, the Northern Territory and NW Western Australia.

Current management is repeated applications of broad-spectrum insecticides, which is not sustainable in the long-term. This study, which started in March 2011 is looking at an integrated approach.

- 1. Chemical control:** A number of insecticides have been evaluated in laboratory and field trials. We are working towards availability of new chemicals for farmers.
- 2. Monitoring:** Monitoring of FSB has been a challenge, but is crucial to strategic management. Two different monitoring tools have been investigated.
 - Trap crops:** Investigation of small hedges of highly attractive host plants as a monitoring tool. A technique and monitoring protocol for *A. nitida* has now been developed.
 - Pheromone traps:** Pheromones have been investigated for both *Amblypelta* spp. A use pattern and a prototype of trap for *A.l. lutescens* have been developed, and commercialisation of the trap is underway.
- 3. Biological control:** Different natural enemies of FSB have been investigated including three species of egg parasitoids of FSB to improve our understanding of their biology and ecology. Their potential as a biological control agent is being evaluated in small scale trials
- 4. Area Wide Management (AWM):** In order to allow management beyond farm level an area wide scale strategy is being developed and trialled with a pilot group of farmers.

As this study is finishing in April 2016 outcomes of different components to date and long-term benefits to industries involved will be presented.

■ Optimizing phosphonate uptake in Shepard avocado

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Phytophthora root rot (PRR), caused by the pathogen *Phytophthora cinnamomi*, is the most significant disease of avocados worldwide and requires cultural and chemical management to prevent widespread tree decline or death. PRR cannot be controlled, but must be actively managed. Potassium phosphonate, the recommended chemical, is systemic and after application travels to the most actively growing part of the tree. To effectively manage PRR, phosphonate must reach the roots, therefore timing applications to when roots are actively growing is essential.

Phosphonate root levels were monitored monthly in five cv. Shepard avocado orchards in north Queensland, Australia, in 2012/13. Trees in five orchards received the farmer's standard phosphonate trunk injection program of one or two injections annually. Three orchards received additional monthly foliar phosphonate treatments. Tree phenology (root, leaf, flower and fruit growth) and root phosphonate levels were monitored to determine the effects on phosphonate movement to and accumulation in the roots.

The samples confirmed that foliar phosphonate applied at, or shortly before, periods of peak root flushing is highly effective in increasing avocado root phosphonate levels in healthy trees. In these orchards, the key periods were March to June and mid-November to early December. Results also indicated that more than one phosphonate treatment (either by injections or foliar sprays) is required to maintain adequate phosphonate levels in the roots all year round. The study also reinforced the importance of root phosphonate monitoring to achieve successful PRR management.

■ Collaborative research on tree decline. Old problem, new approach

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Tree decline continues to impact avocado production worldwide, with biotic and environmental factors combined with cultural practices contributing to the severity of this impact. New Zealand has three main avocado growing regions, each with unique climatic and soil conditions which are thought to influence the occurrence, severity and cause of tree decline. A five year program has been established to identify risk factors in the different growing regions and what management practices should be employed to mitigate them. Working groups in each region, made up of growers, industry professionals and science staff, are working together to develop and implement a diagnosis process, decide on remedial actions and monitor any changes in orchard health.

Diagnostic work conducted in the Far North region points to a relationship between soil moisture management, Phytophthora presence and tree health. Amelioration actions have included facilitating the accumulation of leaf litter, increasing sprinkler coverage to increase the wet area of soil and modifying irrigation strategies based on more effective soil moisture monitoring. A visual tree health classification along with leaf chlorophyll, yield, and fruit size is being used to monitor trees, with changes in environmental factors also being monitored.

At the completion of this programme a practical diagnosis process will be available for growers and industry professionals to assess tree health risk factors in their orchards. Accompanying this will be region specific knowledge about remedial actions. The cross industry working group structure also presents an exciting collaboration to tackle future research questions in a comprehensive way.

■ Transcriptomic analysis of avocado Hass fruit (*Persea americana* Mill.) in the interaction system avocado fruit-chitosan-*Colletotrichum*

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Chitosan besides presenting an antifungal effect, also acts as elicitor which is capable of stimulating the defense mechanism of the plant that induced host resistance. Currently there is few data regarding the expression genes related to the defense system of the fruit during its interaction with chitosan. The objective of this research was performed transcriptome analysis of genes regulated by the action of low molecular weight chitosan in the system interaction Fruit-Chitosan-*Colletotrichum*. The study samples were obtained from fruits treated with chitosan, inoculated with the fungus, chitosan-treated and inoculated with the fungus and a control. The samples were taken of fruits in intermediate maturity (between physiological maturity and consumption maturity) at different times (0, 1, 6, 9 and 24 h post-inoculation treatment). The RNA obtained from each condition at different set times, was sequenced using the platform HiSeq 2000 ILLUMINA. The alignment of the sequences was carried out using the program RSEM taking as reference a transcriptome from var drymifolia. To identify differentially expressed genes by maximum likelihood analysis was performed ($P < 0.05$) using the R statistical with EdgeR library. The sequencing results showed 313 million reads of length of 100 bp (format pair-end). Alignment 73% on average was obtained, for each of the conditions with reference transcriptome. Expression profiles showed that in the fruit-chitosan system there are a greater number of differentially expressed genes, compared to fruit-pathogen system. The analysis of the categorization of differentially expressed genes compared to the control shows that there is a large number of metabolic processes regulated by chitosan. The regulation of various metabolic processes induced chitosan triggers greater resistance from the host, thus preventing the spread of phytopathogen.

■ Effects of root rot caused by *Phytophthora cinnamomi* in the five most used avocado rootstocks of Chavimochic irrigation

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In the Chavimochic Irrigation there are about 7500 hectares of Hass avocado grafted on different rootstocks like Zutano, Lula, Degania, Ashdot and TopaTopa. Zutano and Lula cover more area with about 70%, and root rot caused by *Phytophthora cinnamomi* is the main root disease problem in avocado crop. The objective of this study was to know the behavior of the five most used rootstocks in Chavimochic against the Root Rot caused by *P. cinnamomi*. Seeds of Zutano, Lula, Degania, Ashdot and TopaTopa were planted in 7 lt capacity bags with sterile substrate. Once the plants had 30 cm high, were inoculated with *P. cinnamomi* growth in 35 g of sterile wheat. Each rootstock had an uninoculated control. Parameters of Root length, stem diameter, dry weight and percentage of healthy root were evaluated on three different occasions. It was found that all rootstocks were infected by *P. cinnamomi*, however it was the rootstock Zutano showed statistical differences ($\alpha = 0.05$) in various parameters. Zutano has showed better root length, dry weight and percentage of healthy root. The Sensitivity Index (IDS) was calculated with the percentage of healthy root and it was found that Zutano was the best pattern, followed by Lula, Degania, Ashdot and finally, TopaTopa as the most sensitive. Zutano is a susceptible rootstock but its high capacity of root production, and vigor, enabled him to better respond to *P. cinnamomi* under the conditions of this experiment.

■ *Bruggmanniella perseae* (Diptera: Cecidomyiidae) emerging plague for the Mexican avocado and beneficial organizations associated

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Mexico is the largest producer of avocados in the world and Michoacán is the first nationally, hence the importance of updating from damaging pests and beneficial organisms. The aim of this paper is report for the first time to Mexico to *Bruggmanniella perseae* as a pest of avocado in Michoacan, Mexico and simultaneously notify the associated beneficial organisms. The research was conducted in an orchard avocado Hass located in the town of Ziracuaretiro, Michoacán. Samples of suspicious fruits from 0.2 to 20 mm in diameter were taken, which were revised in the laboratory. With a 73.5% infestation in misshapen fruit and the presence of one or two insect larvae: in 16.74% of the trees in the orchard, the presence of *Bruggmanniella perseae* (Diptera Cecidomyiidae) was identified. Among them, associated cecidómidos larvae, immature parasitoid wasps were found in 44.73% of trees belonging to the genera *Galeopsomyia* sp. (Eulophidae), *Torymus* sp1. *Torymus* sp.2 (Torymidae) and one species of Eulophidae undetermined. In all samples within the fruits with presence or absence of the parasitoid, cavities colonized by a white fungus hyphae, identified as *Colletotrichum acutatum* Simmonds were observed. The fact of not being informed as a pest of avocado in Mexico, involves risk, since the measures for management could be deficient. The importance of the report is meant as an emerging pest for cultivation in a region where the avocado is one of the first economic sectors.

■ Isolation, identification and pathogenicity of fungi associated with the wilt of the aguacatero in Michoacan, Mexico

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The wilt of the avocado tree (*Persea americana*) is one of the main diseases of root, it causes defoliation, drying of branches and in some cases a "quick" tree death and as a side effect great economic losses. In Michoacan, it occurs in approximately 5% of the cultivated area. The objective of the present research was to isolate, identify and apply tests of fungi pathogenicity associated with the rot root of avocado trees with wilt symptoms. We collected roots of diseased trees for the isolation and identification of micro-organisms, as a result we obtained healthy avocado Creole breed Mexicana plants to apply the Koch's postulates, in vitro and in greenhouse. In a PDA medium, 27 were obtained to know. Fungi genres were selected by its incidence and most important were selected for pathogenicity tests: *Fusarium oxysporum*, f. *sambucinum*, f. *moniliforme*, f. *tabacinum*, f. *solani*, f. *sporotrichioides*, *Verticillium* sp., *Cylindrocarpum* sp., *Cylindrocladium* sp., *Verticicladium* sp., y *Phytophthora cinnamomi*, at concentrations of 1×10^6 and 1×10^9 conidium, in 200 mL of water to each plant. The symptoms were at 4th and 14th days in vitro and from 7th to 52th days in the greenhouse, confirmed Koch's postulates. It is concluded that several fungi involved in this disease act in different time and different places in the rhizosphere of the plant.

■ Minimal effective Fluazinam fungicide dose in combined applications with *Trichoderma atroviride* to control avocado white rot

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Previous studies of our team revealed the possibility of combined use of Fluazinam (a contact fungicide) and the biocontrol agent *Trichoderma* given the compatibility between them and their effectiveness *in vitro* against *Rosellinia necatrix*, the causal agent of avocado white root rot. However, the minimum dose of Fluazinam (0.05%) tested *in vivo*, presented similar efficiency than its combination with *Trichoderma* spp., suggesting the possibility of further reducing the concentration of fungicide.

In this paper combinations of fungicide at much reduced dose (0.001%) and *Trichoderma atroviride*, were applied into the soil containing avocado plants cv. Topa-Topa and inoculated with four isolates of *R. necatrix* representatives of different groups of virulence.

The combined treatments were effective for all four pathogen isolates and significantly differed from the inoculated controls. Additionally, in two of them (Rn400 and Rn320) no significant differences were found between the combined and individual treatments with *Trichoderma* or Fluazinam, respectively. This suggests the possibility of an exclusive use of *Trichoderma atroviride* to control infected plants with Rn400 isolate, or to decrease the fungicide dose (below 0.001%) in combined treatment with the antagonist, for Rn320 isolate.

The overall the results obtained *in vivo* demonstrate the effectiveness of combining control of *Trichoderma atroviride* with the low dose (0.001%) of Fluazinam as it delayed the onset of wilt symptoms and significantly reduced the area under the curve of epidemic progress the disease.

■ Pathogenic fungi associated with branch dieback of avocado in Southern Spain

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Fungi as *Neofusicoccum parvum*, *N. australe* and *N. luteum* have been identified in avocado branches affected by dieback in California. *N. parvum* was also found affecting fruit in Mexico. *Lasiopodia theobromae* causes dieback in avocado orchards of Perú. Various species of Botryosphaeriaceae (*N. australe*, *N. parvum*, *Diplodia* spp.) have also been described in almond crops in Spain.

An increased incidence of dieback on branches in several avocado orchards in Southern Spain has been observed in 2013 and 2014. Disease symptoms include a rapid collapse of the branches in spring and summer, following by desiccation and death. A redness of the affected areas inside the branches that progresses to dark brown is observed.

Surveys in different orchards were conducted from April 2013 to September 2014, sampling affected branches to make isolation process in laboratory. The fungal isolates were sequenced and identified to species level.

A wide range of fungal species belonging to the genus *Neofusicoccum* was found, including *N. parvum* (42%), *N. australe* (17%), *N. luteum* (15%) and *N. mediterraneum* (2%), *Colletotrichum gloeosporioides* (22%) and *Lasiopodia theobromae* (2%). Currently, artificial inoculations of six months-old avocado plants cv. Topa Topa with these isolates to test their pathogenicity are underway.

■ Identification of Crinkler (CRN) genes in *Phytophthora cinnamomi*

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Phytophthora cinnamomi Rands is one of the most devastating plant pathogenic oomycetes affecting avocado. Studies involving the identification of pathogenicity genes and their transcriptional changes will give insight into the molecular mechanisms employed during root infection. This is important to aid in the development of effective treatments against this pathogen. Illumina transcriptome sequencing of the germinating cysts of *P. cinnamomi* revealed more than 2000 genes with homology to pathogenicity related genes in other *Phytophthora* species. Further analysis identified 22 Crinkler (Crinkling and Necrosis; CRN) genes that have been shown to be involved during host infection in other *Phytophthora* species. Phylogenetic analysis was done and a subset of CRN genes was selected for quantitative-RT PCR. Susceptible avocado roots were inoculated with a zoospore suspension and harvested at 3 and 12 hours post-infection (hpi). RNA was extracted followed by cDNA synthesis using oligo-dT primers. Data was analysed using the Bio-Rad CFX Manager Software Version 1.5, and expression data was normalised using two endogenous control genes from *P. cinnamomi*, β -Tubulin and glyceraldehyde-3-phosphate dehydrogenase (GAPDH). Expression patterns of CRN genes changed over a time course in *P. cinnamomi* when analysing infected avocado roots. Some CRN genes showed an increase in expression from 3 to 12 hpi. This data forms an important resource for future studies that will provide insight into the role of specific CRN genes in oomycete pathogenicity.

■ Effect of avocado sunblotch viroid (ASBVd) on the postharvest quality of avocado fruits from Mexico

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The effect of Avocado sunblotch viroid (ASBVd) on the postharvest quality was studied in avocado (*Persea americana* Miller) Hass fruits from five classes: healthy fruits from healthy trees (C1), asymptomatic fruits from asymptomatic but infected trees (C2), asymptomatic fruits from symptomatic trees (C3), symptomatic fruits severity rating of 1 (C4) and symptomatic fruits severity rating of 2 (C5) harvested in 2011 and 2012. The postharvest parameters evaluated were: firmness, color, weight loss, dry matter, mineral and oil content. C4 and C5 were significantly different (Tukey, $p=0.05$) in delayed ripening, less dark coloration and less weight loss compared to C1, C2 and C3. Firmness in C4 and C5 was category III and category IV on the eighth day, while C1, C2 and C3 reached it on the sixth day. Coloration in C1, C2 and C3 was black on the eighth day, whereas in C4 and C5 remained 75% obscure. Weight loss in C4 and C5 was 1.4 g/day whereas in C1, C2 and C3 was 2 g/day. Dry matter, mineral and oil content were similar in the five classes. ASBVd affected the postharvest quality of symptomatic fruits. Asymptomatic fruits satisfied the international quality standards.

■ Conditions detected in avocado orchards to develop canker dieback caused by Botryosphaeriaceae species in Chile

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Species of Botryosphaeriaceae family in Chilean production of Hass avocado can cause canker dieback in young and old avocado trees, which affect trunk and branch, producing damage and tissue death. The infection begin at the vascular tissue and is spread systemically to others healthy parts of the plant, altering the water and nutrients distribution. This condition can affect the accumulation and availability of reserves, which are mainly located on the trunk and branches, necessary for fruiting the following season; thus, canker dieback can reduce productivity of the orchard. Also, this disease produced in preharvest can generate latent infections inside the fruit, causing rot that affects the normal development of fruit during postharvest.

A prospective research is been developed in Chilean Hass avocado orchards, from Illapel (31° 37'S) until Melipilla (33°33'S). Some of the data being registered to study its relationship with the disease are orchard age, climatic variables, soil chemical and physical features, irrigation management, and abiotic stress problems, such as drought, salinity, extreme temperatures, wind and mechanical damage. Other variables that are being take into account are pruning and girdling managements, pest and other pathogens that could raise host susceptibility.

So far, we have found that there are abiotic factors that predispose to damage avocados trees and fruits by this complex of fungi. Symptoms have been detected in most of the visited orchards, but the incidence and severity depend heavily of pre and postharvest handling.

■ Control Antracnosis *in vitro* applying low and medium molecular weight chitosan in Hass avocado fruits (*Persea americana* Mill.)

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The high production avocado Hass in Mexico is often reduced because large losses postharvest insomuch as the avocado fruit is susceptible to attack by phytopathogens including *Colletotrichum* sp, causal agent of anthracnose. Is important to explore new ways to combat postharvest fungi given that the application of chemical fungicides can be harmful to the environment and consumer health. A promising alternative in controlling postharvest diseases is the application on the fruits of chitosan films, which is a naturally occurring biopolymer friendly to the environment and non-toxic. In this study the antifungal activity in vitro of low and medium molecular weight chitosan on two strains of *Colletotrichum* sp., isolated from Hass avocado fruits was evaluated. Chitosan solutions at 0.1, 0.5, 1.0, 1.5 and 2.0% (w/v) were used to assess mycelial growth and inhibition of mycelial growth, sporulation and germination of spores every 24 h for 9 days. In vivo trials were also carried out by inoculating avocado fruits with *Colletotrichum* sp (1x10⁶ spores/mL) and then the fruits were immersed in low molecular weight of chitosan solution (1.5% w/v). The incidence of disease, physiological weight loss, firmness and dry matter of fruits were evaluated after 12 days of storage at 25 °C. In vitro results showed that low and medium molecular weight chitosan solution at from 1% inhibited more than 90% of mycelial growth and sporulation and germination of conidia *Colletotrichum* sp. were significantly reduced. Application of chitosan on avocado fruits reduced physiological weight loss, kept firmness and decreased disease incidence. The application of chitosan film on avocado fruits is a plausible alternative to control *Colletotrichum* sp and preserve avocado quality parameters during its storage at room temperature.