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Comparison of three RNA extraction methods for RT-qPCR citrus diagnostics

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High quality RNA (i.e. purity, concentration, & integrity) is required for reliable RT-qPCR in routine citrus diagnostics. Citrus tissues are known to have high levels of polyphenols and polysaccharides that can affect RNA quality and inhibit RT-qPCR. We compared three RNA isolation methods: TRIzol[®], a phenol-chloroform based method; Qiagen Plant RNeasy Mini Kit, a silica column-based method; and MagMAX-96 Viral RNA Isolation Kit (MME-96), a magnetic-bead based high-throughput method, utilizing citrus viruses and viroids maintained in the Citrus Clonal Protection Program (CCPP) disease bank (e.g. tristeza, psorosis, exocortis). We assessed the RNA purity and concentration with spectrophotometry (260 & 280 nm) and the RNA integrity with RT-qPCR targeting the citrus gene Nad5. All methods produced RNA with acceptable purity (260/280: 1.8-2.5). TRIzol[®] yielded the highest concentrations (507.56 ± 135.62 ng/uL, n = 32), however it required serial dilutions in order to remove RT-qPCR inhibitors and effectively detect the targeted citrus pathogens. Based on the Nad5 Cq values, the RNA integrity for the Qiagen kit was variable (18.76 ± 1.48 , n = 32). MME-96 provided high quality RNA, with uniform concentrations (61.49 ± 12.79 ng/uL, n = 32), purity (260/280: 2.30 ± 0.19 , n = 32) and consistent integrity (Nad5 Cq: 21.88 ± 0.8 , n = 32). Therefore, MME-96 has become the preferred high-throughput RNA isolation method for thousands of samples in CCPP's quarantine and certification programs.

Molecular characterization of Citrus tatter leaf virus isolates and their detection using a novel RT-qPCR assay

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Citrus tatter leaf virus (CTLV) is threatening citrus production because it induces decline of citrus propagated on citranges, a commonly used rootstock worldwide, due to its tristeza resistance properties. The full-length viral genome of 12 CTLV isolates derived from diverse geographical locations preserved at the Citrus Clonal Protection Program (CCPP) since 1958, was identified by next generation sequencing technologies. A phylogenetic analysis was performed using the 12 CCPP CTLV isolates and available sequences of other capilloviruses in the GenBank. The CTLV isolates were clustered into 4 distinct phylogenetic groups (I-IV), related to virus isolates from Japan/China (I), Germany/India (II), Taiwan/China (III) and Florida (IV). An RT-qPCR assay for the detection of CTLV was designed based on the conserved regions identified by a multiple sequence alignment of all available full virus genome sequences (12 CCPP isolates & GenBank). The capacity of this RT-qPCR assay in detecting CTLV was assessed using multiple virus isolates and the viral load, reproducibility and repeatability was also determined. The Cq values for CTLV detection ranged from 20.88 to 33.25 with a mean of 23.37 (± 2.72 , n = 22; E = 93.4%, $R^2 = 0.9968$). This robust RT-qPCR assay can be used as a diagnostic tool for citrus germplasm programs as well as for citrus surveys to ensure the availability of CTLV-tested materials for citrus propagation and disease management programs, respectively.

An assessment of plant parasitic nematodes associated with Montana's wheat and barley fields

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Nematode surveys have identified significant populations of *Pratylenchus neglectus* and *Tylenchorhynchus* spp associated with wheat production in Montana, but a comprehensive survey of plant parasitic nematodes in the state has not been conducted. To address this need, surveys were conducted in 2015 and 2016 across eleven economically important wheat and barley producing counties in Montana. For this, sixty-seven fields were sampled, involving 20 soil subsamples for each field; in addition, ten plants were sampled. Each field location was recorded with GPS. Soil and root inhabiting nematodes were extracted via several established methods. Classical morphological and molecular identification were performed to identify plant parasitic nematodes to genus and species. Nine genera were identified: *Aphelenchus*, *Haplolaimus*, *Helicotylenchus*, *Heterodera*, *Paratylenchus*, *Pratylenchus*, *Tylenchorhynchus*, *Tylenchus*, and *Xiphinema*. Important species identified from these genera include *Pratylenchus neglectus*, *Heterodera filipjevi* and *Helicotylenchus vulgaris*, in addition to *Tylenchorhynchus clarus* and *Tylenchus parvus*. Species frequency, species relative frequency, species density, species relative density, coefficient of similarity, and Simpson's diversity index were calculated. This work provides the first comprehensive assessment of plant parasitic nematodes for Montana's small grain production.

The Citrus Clonal Protection Program-CCPP

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The Citrus Clonal Protection Program (CCPP) is an industry, university, and government collaborative program. The CCPP provides a safe mechanism for the introduction and distribution of citrus varieties from around the world into California and via the National Clean Plant Network (NCPN) to the whole U.S.A. This mechanism includes disease diagnosis and pathogen elimination followed by maintenance and distribution of citrus budwood for scion and rootstock propagation. The CCPP operates at three facilities. The Rubidoux Quarantine Facility (RQF) is used for citrus therapeutics and biological diagnostics of newly introduced varieties. The UCR Laboratory is used for high-throughput diagnostics and research for the characterization of graft-transmissible pathogens of citrus. The Foundation Block Facility (FBF) at the UC Lindcove Research & Extension Center maintains 1,000 trees representing over 350 citrus varieties for distribution and trueness-to-type evaluation. In a five-year period (2012–2016), RQF received 173 introductions, therapied 190 accessions, and performed 2275 biological tests for the quarantine release of 65 citrus varieties. The UCR lab performed over 135,000 diagnostic tests and characterized the causal agents of diseases such as vein enation and yellow vein. Finally, the FBF distributed 214,840 buds to 1,016 growers, nurseries, scientists, and citrus enthusiasts.

Encouraging grape growers to prevent trunk diseases through collaborative research with social scientists, extension specialists, and stakeholders

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Every vineyard in California eventually is infected by causal fungi of the grapevine trunk-disease complex (Esca, Botryosphaeria-, Eutypa- and Phomopsis diebacks). The wood infections cannot be eradicated without costly removal and retraining. Yield losses accumulate over time until the vine is either unproductive or dead. Preventative practices have been shown in research trials to minimize infection, but adoption is limited. Understanding grower decision-making about prevention can help reduce disease incidence. Our in-person survey of California table-grape growers asked about their usage and perceptions of two preventative practices: delayed pruning and pruning-wound protectants. To compliment survey results, we simulated economic benefits of adopting preventative practices in a young healthy Crimson Seedless vineyard (years 3 and 5) and in a mature vineyard in year 10, after symptoms appear. In spite of acknowledging the impacts of trunk diseases, 70% of growers use neither practice. Of growers who use a practice, >50% incorrectly adopt in mature vineyards, missing proven benefits from earlier adoption. Of growers with negative perceptions of cost-efficacy, 80% either do not adopt or do so incorrectly in mature vineyards. Although preventative practices adopted in years 3 and 5 are more effective than in year 10, they take up to three years to pay for themselves, since the initial yield losses avoided by prevention are small. Our study brought new insights to disease management, helping us shape outreach materials in economic terms, which resonate more clearly with growers than infection data presented by plant pathologists.

Fluopyram provides effective control against *Anguina pacifica* in annual bluegrass

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The Pacific shoot-gall nematode (*A. pacifica*) parasitizes annual bluegrass (*Poa annua*) on golf course putting greens from coastal Monterey peninsula to Humboldt County. The diagnostic symptom is a cavity-containing gall at the grass shoot base that contains various juvenile stages, adults and eggs of the pathogen. Under severe disease pressure, putting greens become patchy and bumpy from turf loss. No effective nematicide is registered in California turf. During 2014 at a heavily infested putting green at the Pebble Beach Golf Links we evaluated the novel nematicide fluopyram (0.195 or 0.39 oz/1,000 ft²) in comparison to the local standard of biweekly

Neemix 4.5 (a.i. azadirachtin, 9 oz/1000 ft²) in 4 × 6 ft plots. The experimental layout was a complete randomized block design with 4 replications. Only fluopyram improved turf appearance within 6–8 weeks. After 4 months, the nematode's population in the soil was reduced and shoot galls on new growth were almost absent. In contrast, Neemix 4.5 plots remained as heavily galled as the non-treated control. Similar trials during 2015/16 at Pasatiempo Golf Club in Santa Cruz, Links at Half Moon Bay, Links at Bodega Harbour, and Pajaro Valley Golf Club in Watsonville confirmed that 1–2 applications of fluopyram provided season-long protection against damage caused by *A. pacifica*. In 2016 fluopyram received US EPA registration as the turf nematocide Indemnify; registration with California's DPR is pending.

Baseline sensitivities of new fungicides for *Phytophthora cinnamomi* causing avocado root rot in California

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Phytophthora root rot (PRR), caused by *Phytophthora cinnamomi* (*Pc*), is the most destructive disease of avocado worldwide. Currently, only mefenoxam and potassium phosphite (PP), are registered to manage this disease in the United States. Four new Oomycete-specific fungicides, ethaboxam, fluopicolide, mandipropamid, and oxathiapiprolin, all with different modes of action (MOA), were evaluated against *Pc* *in vitro*. Baseline sensitivities for inhibition of mycelial growth of 70 isolates from different avocado growing areas of California were determined using the spiral gradient dilution method for the new fungicides and compared to mefenoxam. *In vitro* toxicity of PP against *Pc* isolates was also tested. EC₅₀ values for ethaboxam, fluopicolide, mandipropamid, oxathiapiprolin, and mefenoxam were 0.016 to 0.069, 0.046 to 0.331, 0.002 to 0.012, 0.0001 to 0.0007, and 0.023 to 0.139 µg/ml, respectively. EC₅₀ values for PP were 12.9 to 583.9 µg/ml, indicating a wide range of sensitivity in the *Pc* population. Initial baseline studies for PP are lacking. In other pathosystems, PP was not effective in managing disease caused by isolates of *Phytophthora* with similarly high EC₅₀ values. The high activity of the new fungicides against *Pc* supports their development as treatments to control PRR of avocado. Their different MOAs allow for effective fungicide resistance management. Greenhouse trials using these fungicides against PRR on avocado have also yielded promising results.

The California Citrus Nursery Disease Testing Program

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Since 1962, the California Department of Food and Agriculture (CDFA) registration program for citrus nurseries included mandatory testing for tristeza and voluntary testing for psorosis and citrus viroid diseases. In May 2010, following the June 2009 workshop “Meeting the Challenge of the Asian Citrus Psyllid in California Nurseries”, CDFA developed the “Citrus Nursery Stock Pest Cleanliness Program” that included mandatory testing for Huanglongbing (HLB), tristeza, psorosis and citrus viroid diseases. In May 2012, a high throughput nucleic acids extraction protocol from citrus and RT-qPCR universal detection of citrus viroids, developed by the Citrus Clonal Protection Program, with industry, federal and state funds, was submitted to the CDFA. In Sept. 2012, CDFA issued the “QC 1354, Permit For PCR Protocol For Viroid Testing” and in Jan. 2014, issued the “NO. QC 1388, Permit For PCR Protocol For Virus Testing”. Since HLB has never been detected in CA nurseries, and tristeza and psorosis have only been detected sporadically the viroid infection rate was used as an indicator for the success of the new program. In the 2004–10 period, the voluntary program had tested ~2,700 samples, and the viroid infection rate was 4.3–9.5%. Since 2010, the new program has tested over 13,600 samples reducing the viroid infection rate to 0.5–0.8%. California's citrus nursery testing program is a success story of the collaborative power of industry, university and regulatory agencies.

Thousand cankers disease of walnut: Current research and status in California

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Thousand cankers disease (TCD) is an emerging disease responsible for dieback and death of black walnut in western states and now widespread in English walnut and its rootstocks in California orchards. TCD results from infection by *Geosmithia morbida* following attack by the walnut twig beetle (WTB, *Pityophthorus juglandis*), which aggregates and transmits the pathogen. The multiple infections cause cankers that coalesce to girdle and kill branches and stems. Various *Juglans* species used in walnut rootstock breeding differ in their susceptibility to *G. morbida* and attractiveness and suitability for WTB. Genetic analyses of *G. morbida* populations suggests that the pathogen forms a large panmictic group in California walnuts. When combined with the collection history and population genetic analyses of WTB, these data suggest that *G. morbida* and WTB are not newly associated, and that the pathogen and vector are not recently introduced to California. TCD management in orchards must include the capacity to determine which host trees are likely to become infected and the ability to detect and deter the vector. Male WTB produce a hemiterpene alcohol to attract conspecifics, and this pheromone is used in lures to trap and monitor WTB populations. Results of experiments to identify host VOCs that attract or repel WTB and efforts to improve the lure for monitoring beetle populations and develop a repellent for disrupting WTB aggregations will be presented.

Field-testing of pruning-wound protectants for trunk-disease management in California table grapes

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Grapevine trunk diseases are a leading cause of vine death. Protectants have been shown to minimize infections of pruning wounds in winegrapes, but have not been tested in tablegrapes. In a Scarlet-Royal vineyard post-pruning we tested four tractor-applied protectants vs. water as the non-treated control: Luna Experience (Fluopyram + Tebuconazole, Bayer), Pristine (Pyraclostrobin, BASF), a mixture of Topsin (Thiophanate-methyl, United Phosphorus) and Rally (Myclobutanil, Dow AgroSciences), and Serifel (*Bacillus amyloliquefaciens*, BASF). With rain post-pruning, three spurs on each of eight vines per treatment were inoculated with causal fungi of trunk diseases Phomopsis dieback (*Diaporthe ampelina*), Botryosphaeria dieback (*Neofusicoccum parvum*), Eutypa dieback (*Eutypa lata*), and Esca (*Phaeomoniella chlamydospora*). Half the vines were re-treated and re-inoculated 22 days post-pruning, to test re-application. Pristine, Topsin + Rally, and Luna significantly reduced *N. parvum* infection by 87, 80, and 71%, respectively. Recovery of *D. ampelina* was significantly reduced from 22% (non-treated spurs) to 1% and 0% with 2nd applications of Pristine and Topsin + Rally, respectively. Low recovery of *E. lata* and *P. chlamydospora* from non-treated spurs meant no significant effects, but lower recovery rates were found with Pristine and Serifel, respectively. Our findings suggest protectants reduce infection, although one product may not protect equally well against all pathogens.

Probe-based multiplex real-time PCR as a diagnostic tool to distinguish two distinct *Fusarium* spp. associated with *Euwallacea* spp. in California

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Fusarium Dieback (FD) is a vascular disease resultant from inoculation of fungal mutualists (*Fusarium* spp., *Graphium* spp., and *Paracrematium pembeum*) vectored by two distinct invasive ambrosia beetles (*Euwallacea* spp.) named Polyphagous Shot Hole Borer (PSHB) and Kuroshio Shot Hole Borer (KSHB), and is effecting a wide range of woody plant species in California. *Fusarium euwallaceae* and *Fusarium* sp. associated with PSHB and KSHB, respectively, were exploited to detect and distinguish the morphologically indistinguishable ambrosia beetles through diagnosis of their fungal symbionts from field samples using a novel real-time PCR assay. Hydrolysis probes and primers sets were designed from polymorphic regions of the β -tubulin gene distinct from each other as well as other related Fusaria including *Fusarium ambrosium*, *Fusarium solani*, and *Fusarium oxysporum*. The multiplex assay was shown to be effective in detecting the target species from DNA extracts originating from pure culture, infested wood, female beetle heads, and spores suspended in sterile water; with a sensitivity of detection as low as 20 fg of genomic DNA from pure culture. This assay provides rapid identification of symbionts associated with PSHB and KSHB from field samples in new and existing locations, which allows us to track disease progress of these two populations in California.

Organic acid sanitizers for re-circulating fungicide solutions that are used in postharvest management of fruit decays

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Under the Food Safety Modernization Act, fruit packinghouses must sanitize recirculating fungicide solutions to ensure that human bacterial pathogens do not accumulate and cause foodborne illnesses to consumers. Typically, packinghouses use oxidizers such as sodium hypochlorite or peroxyacetic acid for sanitation. These may be incompatible with certain fungicides and produce odors that may irritate workers. To identify alternative, highly effective, and odorless sanitizers, we tested two organic acids: lactic acid (LA) and citric acid (CA). Both are designated Generally Recognized as Safe and exempt from tolerance in the United States. The acids were tested in vitro and in fruit assays against a non-pathogenic surrogate strain of *Escherichia coli* (K-12). In solutions of azoxystrobin, fludioxonil, imazalil, or natamycin contaminated with 2×10^6 CFU/ml *E. coli*, 2000 μ g/ml LA or CA killed all bacteria within 24 h. The addition of 100 μ g/ml of the food-grade surfactant sodium dodecylbenzenesulfonate (SDBS) improved efficacy of 2000 μ g/ml LA and CA against *E. coli*, killing all bacteria within 1 h. When applied to *Penicillium digitatum*-inoculated lemons, flood treatments of heated (48°C) fungicides containing 2000 μ g/ml LA or CA + 100–110 μ g/ml SDBS were similarly effective as compared to fungicide-only treatments. No phytotoxicity was observed with any treatment. Thus, acidification is an alternative sanitation method to oxidation for recirculating fungicide solutions.

Candidatus Liberibacter asiaticus: Conserved effectors and the citrus response

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Huanglongbing (HLB) is one of the most destructive citrus diseases and has caused tremendous damage to the citrus industry worldwide. It is caused by the Gram-negative bacterial pathogen *Candidatus Liberibacter asiaticus* (CLAs). In the United States, HLB has been detected in Florida, Texas, and California. In order to gain greater insight into CLAs biology, we have initiated genome sequencing and comparative analyses of CLAs from different geographical regions. CLAs strains from Texas, Florida, California, and China have been sequenced. CLAs contains the Sec secretion system, through which a variety of secreted effectors (SEs) may be

secreted into the phloem. We have mined genome sequences to identify core SDEs. Data will be presented on the identity and conservation of the CLas effector repertoire as well as the citrus response to CLas infection. Collectively, this research has provided insight into how CLas manipulates citrus as well as a more detailed understanding of CLas genetic diversity.

Grower fungicide use patterns for control of gray mold and powdery mildew of strawberry in California

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Most foliar fungicide applications in California strawberry production are for the prevention of gray mold and powdery mildew, caused by *Botrytis cinerea* and *Podosphaera aphanis*, respectively. The objective of this study was to summarize fungicide use patterns within the major strawberry fruit-production regions using data reported to the California Department of Pesticide Regulation. Requests for pesticide use information were submitted to five counties where 96% of California strawberry production acreage is located. Thirty-two fall-planted farms comprising 1,960 acres (6% of CA acreage) were queried for applications of fungicides labeled for gray mold and powdery mildew during 2016. Fungicides labeled for gray mold and powdery mildew were applied an average of 15.0 and 17.6 times at an average interval of 11.7 and 11.2 days, respectively; the season lasting an average of 186 days. The most popular fungicides labeled for gray mold in descending order were Captan (7.2 average applications/season), Switch (2.3), Pristine (1.8), and Elevate (1.4), while Microthiol Disperss (6.8 average applications/season), Switch (2.3), Rally (1.8), and Pristine (1.8) were the most popular fungicides labeled for powdery mildew. Average application rates were often near the maximum labeled rate. Rotation between modes of action was broadly employed except for fungicides containing captan or sulfur. This information helps us understand reported fungicide resistance patterns.

Understanding the role of citrus-associated microbes in Huanglongbing infected trees in Florida

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Animals and plants are known to harbor a wide variety of microbes that are important for maintaining proper functions and health. The goal of this project was to identify microbes or consortia of microbes, including bacteria and fungi, associated with citrus that can protect trees against Huanglongbing (HLB). *Candidatus Liberibacter asiaticus* (CLas) infected citrus trees with and without (survivor trees) typical HLB symptoms were identified in Florida, where HLB is ubiquitous. In 2016 and 2017, stem, leaf, and root samples were collected from 215 infected trees and the disease ratings (1-5) were recorded. Samples were utilized for culture-independent and culture-dependent analyses and microorganism identification. The bacterial and fungal hyper variable rRNA internal transcribed spacer (ITS) was sequenced from cultures and citrus tissues using Illumina MiSeq. We found correlations between the disease rating and CLas titers as well as identified some microorganisms that negatively and positively correlate with CLas. These results provided important insights for understanding the structure and composition of the microbial communities associated with CLas infected citrus in the context of HLB development and changes to the communities over time. Future studies include the identification of citrus microbial communities of trees in California and Texas with two different levels of HLB disease pressure.

Adapting to water insecurity: Using alternative sources of irrigation water and balancing reduced water use with root disease risk

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With increasing water insecurity, nursery crop growers are searching for irrigation strategies to mitigate production constraints and costs. Use of recycled water and sensor-driven deficit irrigation networks can reduce water inputs, but grower adoption is hindered by disease risk concerns. The objectives of this study were to (i) establish whether recycled irrigation water at a cooperating nursery was a source of oomycete pathogens and (ii) test the effects of Deficit Irrigation (DI) on root disease in commercial greenhouse crops. Pond water analyses identified *Phytophthora litorale*, *Phytophthora hydropathica*, *Ph. cryptogea*, *Pythium oopapillum*, and *P. aff. diclinum* as the predominate species in recycled water. *P. oopapillum*, *P. aff. diclinum* and *Ph. cryptogea* were highly virulent to chrysanthemum seeds and seedlings (90% damping off), and caused root rot on mature chrysanthemum and geranium (63 and 50% of plants respectively). When commercially grown poinsettias were inoculated with the root rot pathogen *Pythium aphanidermatum* and placed under saturated, mild and moderate DI treatments, disease incidence was significantly lower under saturated and mild deficit conditions, compared to the moderate deficit treatment ($P = 0.05$). These studies indicate that recycled water is a source of diverse pathogens, which may be more severe under extreme deficit, highlighting the need for more in-depth studies to mitigate risks associated with necessary changes in water use.

Identifying grapevine microbial endophytes for control of Pierce's Disease

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Pesticides are an effective management strategy, but as food security, pesticide resistance and environmental health become prominent issues in our society, it is imperative that innovative and alternative methods are implemented to create sustainable agriculture. Xylella

fastidiosa (Xf), the causative agent of Pierce's Disease (PD) of grapevine is caused by a xylem-dwelling bacterium that reduces California's grape yield and has no cure. The overarching goal of our project is to identify both prophylactic and curative measures for PD by utilizing the grapevine microbial communities. The rationale is that in commercial vineyards, vines under PD pressure are affected by Xf while others show no or few symptoms of the disease (a.k.a. escape vines). We hypothesized that the differences in the vines' microbiome influence disease phenotype. The goals of our project are to (i) study microbial community dynamics in PD-escaped and -infected grapevines, (ii) evaluate a single organism-based approach for PD management and (iii) identify genes and bioactive molecules responsible for the beneficial characteristics. Metagenomic tools and culture-independent methods were utilized to reveal beneficial organisms that mitigated PD. In complement, *in vitro* and *in planta* assays were developed to evaluate the culturable organisms for both their anti-Xf properties and for their plant growth promoting attributes.

Antifungal activity of volatiles emitted by bacteria isolated from the avocado rhizosphere

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The Polyphagous Shot Hole Borer and the Kuroshio Shot Hole Borer (*Euwallacea* spp. nr. *forficatus*) are invasive beetles that vector a disease called Fusarium Dieback, caused by fungi that the beetles inoculate inside host tissue and utilize as a nutrition source. Fusarium dieback affects avocado (*Persea americana* Mill.) and many other tree species throughout California and has recently been reported in Tijuana, Mexico. Its expansion, if not controlled, could affect avocado production and the health of many forest tree species. Research efforts need to consider biocontrol as an option since the use of pesticides is restricted for avocado export and consumption. Rhizosphere bacteria were isolated from four healthy avocado trees and four avocado trees with symptoms of Fusarium dieback. The obtained bacterial isolates were tested *in vitro* against *Fusarium solani*, a close relative of the causal agent of Fusarium dieback, *Fusarium euwallaceae*. Fungal and bacterial isolates were cultured on separate Petri dishes before being combined, to avoid direct contact and assess antifungal effects from volatiles emitted from the bacterial isolates. After 7 days, 7 of the 45 tested bacterial strains inhibited the growth of *Fusarium solani* by more than 20%. The inhibiting isolates were identified using 16S rDNA gene sequencing as *Pseudomonas* spp. and *Bacillus* spp. Future work is aimed at the characterization of the volatile compounds produced by the bacterial isolates with antifungal activity, to determine the mechanisms behind the antagonism.

Monitoring grapevine trunk diseases in young and mature vineyards in California wine regions

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California vineyards eventually succumb to one or more trunk diseases (Esca, Botryosphaeria-, Eutypa-, and Phomopsis diebacks). Symptoms appear years after the fungal trunk pathogens infect pruning wounds, thus growers often neglect preventative measures until widespread infection. We compared trunk-pathogen communities in young apparently healthy (2 to 5 yr) and mature diseased vineyards (12 to 15 yr) via spore traps to determine if young and mature vines are at similar infection risk. In three young and three mature vineyards each in Napa and Lodi, ten petroleum jelly-coated glass slides were placed on vine cordons. Traps were collected after rain events during the 2014–15 to 2016–17 dormant seasons. Trapped spores were suspended, filtered and plated on PDA, putative trunk pathogens subcultured, and species identified by ITS. There were only three detection events in 2014–15, the driest, but 66 in 2016–17, the wettest recorded. We detected seven Botryosphaeria- and seven Phomopsis-dieback pathogens, but no Esca pathogens. The one Eutypa-dieback pathogen was *Diatrype stigma*. The most virulent Botryosphaeria-dieback pathogen identified was *Neofusicoccum parvum*, but *Diplodia seriata* was most common. *Diaporthe chamaeropsis* was the most common Phomopsis-dieback pathogen, with only one instance of *D. ampelina* (syn *Phomopsis viticola*). Later-season recovery was higher. Pathogen communities were depauperate but present in young vineyards. Thus, preventative measures for trunk diseases in young vineyards are justified.

Interaction of grapevine phenolic compounds and wood-colonizing fungi that cause grapevine trunk diseases

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The grapevine trunk-disease complex is characterized by taxonomically unrelated Ascomycete pathogens that cause internal wood cankers, often as mixed infections in the same vine. *Neofusicoccum parvum* and *Diplodia seriata* (causal fungi of Botryosphaeria dieback) and *Eutypa lata* (causal fungus of Eutypa dieback) cause similar wood symptoms, but literature on the woody cells they colonize, their food substrates, and mechanism(s) of damage to host cells/tissues is fragmented. We examined the role phenolic compounds, which have been shown to increase in response to wounding and infection, and have anti-microbial activity in other woody hosts. Woody stems of potted grapevines were inoculated and stem phenolics were measured over a 3 month period. Plants infected with *N. parvum* had less phenolic accumulation than plants infected with *D. seriata* or *E. lata*, as well as wounded-only controls. The resveratrol dimer ϵ -viniferin accumulated significantly less in *N. parvum*-inoculated plants compared to the other treatments. The effects of classes of phenolic compounds (hydrolyzable tannins, catechins and procyanidins, flavanols, and stilbenoids) were tested on the fungi *in vitro*, to determine whether they are inhibited by concentrations found in grapevine wood. Both Botryosphaeria dieback pathogens removed the phenolic compounds gallic acid, rutin, and piceid from amended media, presumably to use as a carbon source, more than *E.*

lata. *N. parvum* removed greater amounts of these phenolics than *D. seriata*. It is possible that the reduced phenolic accumulation, especially in *N. parvum*-inoculated plants, was associated with utilization of phenolics by the pathogen for an energy source or some other purpose.

Multiple new pathogens revealed in a survey of peony diseases in the United States

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Peonies (*Paeonia* spp.) are popular ornamentals in temperate regions of the United States. Limited refereed information is available on the frequency and distribution of plant pathogens associated with peonies and first reports that include Koch's postulates are scarce. Surveys to identify *Botrytis* spp. on peony from 2013–2015 in the Pacific Northwest revealed a wide diversity of fungal plant pathogens on samples, many of which had not been reported before in the United States or in the state in which the samples were collected. We previously reported the discovery of *Pilidium concavum* and *Mycocentrospora acerina* on peony in the United States, however, trials had not yet been done to confirm pathogenicity. In 2016, we expanded our surveys to include other regions of the United States to collect non-*Botrytis* pathogens of peony. From 2013–2016, our surveys spanned 11 states. Potential fungal pathogens were isolated from diseased tissue and identified using both morphology and sequence analysis. The results of our survey identified additional states in which *P. concavum* is infecting peonies and several new host-pathogen-state combinations, such as *Sclerotinia sclerotiorum* in Alaska and *Sclerotium rolfii* in Washington. The pathogenicity of these fungal pathogens were confirmed by completing Koch's postulates. The information from our survey can be utilized regionally and nationally to aid in diagnosis of peony pathogens for better informed disease management strategies.

Effects of new Oomycete-specific fungicides on tree health, fruit yields and Phytophthora root rot of citrus

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Phytophthora root rot (PRR) of citrus, caused by *Phytophthora parasitica* and *P. citrophthora*, is an important disease of citrus in California. PRR is especially damaging for newly planted trees and can lead to a significant decline in tree growth and fruit production compromising orchard establishment. In previous greenhouse and field studies, the new fungicides ethaboxam, fluopicolide, mandipropamid, and oxathiapiprolin showed high activity against PRR. A navel orange orchard was established and inoculated with *P. parasitica* in fall 2013 to determine the effects of the fungicides on tree health and productivity in 2015 and 2016. All treatments, except mefenoxam, increased trunk size, tree canopy (based on image analysis), and crop yield as compared with the untreated control. A second navel orange field trial was established and inoculated with *P. citrophthora* in summer 2016 to determine minimum usage rates of the fungicides. PRR incidence and *P. citrophthora* soil populations were determined in fall 2016. Significant reductions in root rot incidence and soil populations were observed in treated trees using lower rates as compared to the control. For oxathiapiprolin, even rates 8-fold lower than those in the first trial were highly effective. Once registered, these new soil fungicides will allow the first rotation program with four distinct modes of action. This should reduce the potential for resistance development and losses from PRR in California citrus.

Development of a rapid nanopore diagnostic system (RNDS) for plant pathogens of wheat

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Disease diagnostics can be time consuming and labor intensive. This is particularly true for root and crown pathogens, which are often difficult to distinguish symptomatically. Rapid nanopore sequencing, available through Oxford Nanopore Technologies, has tremendous diagnostic potential. These nanopore sequencers have the capacity to sequence 250 bases per second per nanopore. This program's goal is to use this sequencing technology to develop a rapid nanopore diagnostic system (RNDS) for fungal pathogens of wheat and barley. An initial one-hour sequencing test using a MinION sequencer for a bulked sample containing amplified ITS regions from 14 different wheat pathogens was conducted. It yielded 1.6 million base reads and detected and distinguished 10 fungal ITS sequences. Further assessment of this technology based on additional amplicon choices are in the works. The preliminary performance of RNDS as it relates to pathogen identification and amplicon choices will be discussed. In the future, the program seeks to develop barcoding protocols that will enable several diagnostic samples to be run concurrently, and strategies for the generation of quantitative data that would dramatically enhance the assessment of pathogen impacts on crop health. Based on preliminary results, RNDS has the potential to transform the field of modern plant diagnostics by providing a rapid and egalitarian tool for pathogen identification.

Pre- and post-harvest management of *Xanthomonas fragariae* to overcome trade barriers of California strawberries

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Angular leaf spot (ALS), caused by *Xanthomonas fragariae* (Xf), is an important disease of strawberry and has impacted export of California fruit due to quarantine restrictions in some export markets. Management is difficult and limited to the use of planting material free of latent Xf infections, preharvest applications of copper bactericides that can be phytotoxic, and proper irrigation practices. Postharvest fumigation with methyl bromide is effective against quarantine insect pests of strawberry, but does not reduce or eradicate

bacterial populations from infected plant material without causing significant phytotoxicity. In our studies, propylene oxide was identified as a potential commercial fumigant of strawberries with activity against *Xf* in infected tissues. In fumigation chamber trials, 2- to 4-h treatments at ≥ 214 $\mu\text{g/ml}$ consistently reduced bacterial populations by 2 to 5 log units and eradicated the pathogen using ≥ 355 $\mu\text{g/ml}$, with no phytotoxicity to leaves, sepals, or fruit under commercial storage practices. In greenhouse and field trials with applications before inoculation, the new bactericide amino-thiadiazole was highly effective by itself or when mixed with kasugamycin or copper and reduced ALS incidence and severity to low levels. Together, these pre- and post-harvest treatments could be registered as a systems-wide approach to overcome trade restrictions for California strawberries.

The California Strawberry Industry in a post-methyl bromide era: A pathological perspective

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Dec 31, 2016 marked the end of a 17-year phase-out of the fumigant methyl bromide (MB). During those years, over \$200 million was spent searching for products or practices that would be equally effective to MB for controlling soilborne pests. Although some alternatives show efficacy, no replacements were identified. As MB use decreased, soilborne diseases have increased. In fruit production fields, growers are facing increased disease pressure from *Macrophomina* charcoal rot, *Fusarium* wilt and *Verticillium* wilt. The emergence of *Macrophomina* and *Fusarium* is directly attributed to the phase-out of MB and in the case of *Macrophomina*, has occurred simultaneously in other countries such as Chile, Spain and Israel. In response to these challenges, growers are abandoning pathogen-infested soils while breeding programs have made resistance to soilborne diseases a primary objective. Anaerobic soil disinfestation has shown impressive efficacy against some soilborne diseases when conditions are optimum. Benchtop production in soilless medium avoids soilborne disease and is under evaluation in all California strawberry production regions. Many other products and practices are entering the void left by MB. Controlling soilborne diseases post-MB is likely to integrate several tactics, including host plant resistance, field sanitation, crop rotation and other practices yet identified.

Integrated control of citrus huanglongbing (HLB) with trunk injection of plant activators and antibiotics

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Huanglongbing (HLB) or greening is a devastating disease of citrus worldwide and no cost-effective control measure is currently available in Florida. Plant activators represent environment friendly compounds capable of inducing resistance against many plant pathogens. In this study, nine plant activators and three antibiotics were evaluated in 3 field trials for their potential for HLB control by trunk injection of young and mature sweet citrus trees. Results showed that 2–4 trunk injections of several activators including salicylic acid, oxalic acid, acibenzolar-S-methyl and potassium phosphate provided significant control of HLB by suppressing Las titer and disease progress. Trunk injection of penicillin, streptomycin and oxytetracycline hydrochloride resulted in excellent control of citrus HLB. In general, antibiotics were more effective in reduction of Las titer and HLB symptom expressions than plant activators. These treatments also resulted in increased yield and better fruit quality. Injection of both salicylic acid and acibenzolar-S-methyl led to significant induction of PR-1 and PR-2 genes. Meanwhile, injection of either potassium phosphate or oxalic acid resulted in significant induction of PR-2 or PR-15 gene expression, respectively. These results demonstrated that trunk injection of plant activators and antibiotics can be a viable component for effective disease management program.

High-throughput RNA extraction and purification for citrus diagnostics

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High quality RNA is required for the detection and identification of viral pathogens of citrus with RT-qPCR assays. The California Citrus Clonal Protection Program (CCPP) developed a semi-automated, robotic, high-throughput and economical RNA extraction and purification procedure optimized for citrus tissues. The system utilizes SamplePrep's Cryo-station, Geno Grinder 2010 and the Applied Biosystems' MagMAXTM Express-96 (MME-96) along with a modified 5x MME-96 Viral RNA Isolation Kit. The quality of RNA extracts was defined by concentration, purity, and integrity. RNA concentration and purity were determined by spectrophotometry at 260 and 280 nm. The RNA concentration (260 nm) of the tested samples ranged from 10.48 to 172.4 ng/ μl with the mean of 71.81 (± 30.96 , $n = 4307$) and purity (260/280 nm) ranged from 1.03 to 3 with a mean of 2.21 (± 0.24 , $n = 4307$). RNA integrity was analyzed by RT-qPCR targeting the mRNA of the NADH dehydrogenase (Nad5) citrus gene. Nad5 Cq values ranged from 15.40 to 28.50 with an average of 19.84 (± 2.30 , $n = 558$). For the past seven years the CCPP has used this RNA extraction method to process over 21,000 citrus samples from budwood sources, orchards, nurseries, and germplasm introductions safeguarding the California citrus industry against diseases, such as, tristeza, psorosis, exocortis and cachexia.

Evidence of active movement of the causative agents of Pistachio Bushy Top Syndrome

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Rhodococcus fascians is a bacterial phytopathogen capable of infecting a wide range of plant species, with the capacity to cause economically significant damage in crops upon establishment of endophytic growth. Symptoms include stunting, shortened internodes,

leafy galls, leaf deformation, and altered root morphology. The disease outbreak Pistachio Bushy Top Syndrome (PBTS) in the US was caused by two *Rhodococcus* spp. related to *R. fascians*. The following research focuses on understanding the mechanisms of motility utilized by these isolates. Experiments involving the PBTS isolates include *in vitro* assays combined with microscopic imaging to examine the growth and potential movement of the bacteria when exposed to different substrates. The PBTS isolates were used in plant assays on both 'UCB-1' pistachio trees and *Nicotiana benthamiana* plants where symptoms were observed. Microscopy allowed for assessment of bacterial movement and colonization. *N. benthamiana* was grown *in vitro* and inoculated to observe the development of the plant-pathogen relationship. The data from these assays suggest that the PBTS isolates are capable of active movement. Microfluidic assays are underway to observe motility under conditions subject to pressure and flow as well as *in vitro* chemotaxis assays. This information will help both growers and researchers better understand how to manage disease caused by these pathogens.

TrunkDiseaseID.org: A molecular database for grapevine trunk pathogen identification

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Grapevine trunk diseases limit vineyard production and longevity around the globe. Trunk diseases have traditionally been distinguished based on causal agents (trunk pathogens) and etiologies (Botryosphaeria-, Eutypa-, and Phomopsis diebacks, and Esca). However, mixed infections are common in vineyards and often confound accurate pathogen diagnosis. The diversity of fungal assemblages of trunk pathogens and other wood-colonizing fungi span four classes in the Pezizomycotina (Ascomycota) and 10 genera in the Hymenochaetales (Basidiomycota). Morphology-based species identification is largely untenable because of considerable overlap in colony and/or spore characteristics, or lack of sporulation in culture. DNA-based searches of uncured, public molecular databases can lead to erroneous identifications. We introduce the new molecular database TrunkDiseaseID.org, populated with rDNA ITS sequences from over 250 isolates (pathogens and saprobes). Secondary molecular barcodes (TEF1- α and beta-tubulin) are also included for delineating closely related/cryptic species because the ITS barcode is inadequate for species-level identification for some members of the Botryosphaeriaceae, Diatrypaceae, and Hypocreales. Currently, no such comprehensive, curated database exists for grapevine wood-colonizing fungi. In addition to accurate ITS and secondary barcode sequences, this database provides a scientific reference, origin, and ecological status (pathogen, saprobe, or unknown) for each isolate, to aid diagnosticians in communicating results and recommendations to growers.

Citrus leaf microbiota as a prediagnostic tool in HLB management

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Candidatus *Liberibacter asiaticus* is a phloem-limited bacterium that causes Huanglongbing (HLB), a deadly disease of citrus. Infection of trees with *CLas* leads to characteristic symptoms (including asymmetrical yellowing of leaves) that allow HLB diagnosis. However, it is known that *CLas*-infected trees can become infectious prior to formation of these symptoms. This has prompted the quest for tools that are pre-diagnostic, i.e. allow a preliminary diagnosis before symptoms appear. The use of *CLas*-specific PCR as a pre-diagnostic tool is problematic, given the typically sparse and spotty distribution of *CLas* bacteria in infected trees. Here, I will describe the progress on a research project that is grounded in the observation that *CLas*-infected and uninfected trees differ in the microbiota that colonize their root and leaf surfaces. We seek to exploit these differences and identify foliar bacterial and/or fungal taxa that can be used as pre-diagnostic biomarkers for *CLas* infection. I will describe our pipeline of sampling, processing, data generation and data analysis, and present preliminary results from sampling efforts in California, Texas and Florida. I will discuss the challenges and ways forward with this microbiota-based approach, as well as the possible mechanisms that underlie the differences in microbiota between healthy and diseased trees.

Virulence and molecular characterization of *Puccinia striiformis* f. sp. *tritici* mutants generated using ethyl methanesulfonate

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Puccinia striiformis f. sp. *tritici* (*Pst*) is an obligate biotrophic fungal pathogen causing stripe rust, one of the most important diseases of wheat worldwide. Mutation is considered as one of the major mechanisms causing changes in the pathogen population, but experimental evidence is limited. To study the effect of mutation on the pathogen changes, we developed 33 potential mutants by treating urediniospores of *Pst* race PSTv-18, avirulent to all of the 18 *Yr* single-gene differentials, with ethyl methanesulfonate (EMS). These isolates were characterized through virulence testing on a set of 18 wheat *Yr* single-gene lines and with 19 simple sequence repeat (SSR) and 48 single-nucleotide polymorphism (SNP) markers. Only one isolate was identified as a contaminant by comparing the phenotypic and genotypic data with those of isolates routinely used in the greenhouse tests. The combination of the virulence and molecular data clearly identified 32 isolates as mutants. The most of the mutants had more than one avirulence gene and more than one marker locus changed, indicating that EMS is able to cause mutation at multiple genome sites. Further studies will be carried out to sequence the mutants, determine changes in the genome structure, and identify avirulence genes. The information of difficulty to mutate the avirulence genes to *Yr5* and *Yr15* supports the usefulness of these genes in breeding for relative long-lasting and high level resistance to stripe rust.

Molecular mapping of stripe rust resistance QTL in Pacific Northwest winter wheat cultivar Madsen

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Stripe rust, caused by *Puccinia striiformis* f. sp. *tritici*, is one of the most devastating diseases of wheat in the world. Wheat cultivar Madsen has the combination of all-stage resistance and high-temperature adult-plant (HTAP) resistance to stripe rust. To map its resistance quantitative trait loci (QTL), Madsen was crossed with susceptible cultivar Avocet S, and 156 recombinant inbred lines (RILs) were developed. The RILs and parents were tested with races PSTv-37 and PSTv-40 in seedling stage in the greenhouse and in adult-plant stage in the fields of Pullman and Mount Vernon, WA in 2015 and 2016 under natural infection of the pathogen. The RILs were genotyped with single-nucleotide polymorphism (SNP) markers from genotyping by sequencing and the 90K Illumina iSelect wheat SNP chip. A linkage map comprising 1,348 SNP loci was constructed. QTL analysis identified three genes for all-stage resistance on chromosomes 1AS (*QYrMad.wgp-1AS*), 1BS (*QYrMad.wgp-1BS*) and 2AS (*QYrMad.wgp-2AS*) and two QTL for HTAP resistance on 3B (*QYrMad.wgp-3B*) and 6B (*QYrMad.wgp-6B*). *QYrMad.wgp-2AS* was the most significant QTL, explaining 16.03 - 71.23% phenotypic variation depending upon the race or environment. *QYrMad.wgp-6B* was consistently detected in all field experiments. Based on the chromosomal locations, *QYrMad.wgp-2AS* was identified as *Yr17* and *QYrMad.wgp-1AS* as a novel QTL. The results are useful for developing wheat cultivars with durable resistance to stripe rust.

Search of a biorational alternative program to chemical soil fumigation for control of wilt diseases in chile pepper in New Mexico

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Chile pepper (*Capsicum annuum* L.) is an important economical and ethnobotanical vegetable crop in New Mexico and in southwestern U.S. Soilborne pathogens causing wilt diseases are major constraints to chile production in New Mexico. Chemical fumigants such as KPAM and chloropicrin have been used for controlling soilborne pathogens. Owing to regulatory, environmental, and efficacy concerns, the chile growers' community is geared towards the use of biorational products (beneficial microorganisms or botanical extracts) for chile wilt control. In a series of field trials, two *Bacillus*-based formulations (*B. amyloliquefaciens* strain d747 and *B. subtilis* QST713) reduced the incidence of Verticillium wilt (*V. dahliae*) and Phytophthora blight (*P. capsici*) when applied in combination. Further reduction was observed when *Bacillus*-based formulations were used in conjunction with *Streptomyces*-based (*Streptomyces lydicus*) or Trichoderma-based formulations. In another field trial, mustard seed meal (*Brassica juncea*, Ida Gold), applied before bed shaping, in conjunction with fumigation with KPAM reduced chile wilt by 84 to 90% compared to control (KPAM alone). A new framework for wilt disease control is proposed and necessitates evaluation by vegetable growers and includes two programs. In one program, bioactive crop residues are applied in the fall, followed by soil treatment with formulations containing *Bacillus* (BBF), *Streptomyces* (SBF), and Trichoderma (TBF) from planting to pod initiation. The other program calls for the planting of a fall cover crop with soil treatment with BBF, SBF, and TBF, followed by cover crop incorporation in early spring and application of BBF, SBF, and TBF from planting to pod initiation.

Chemical management of Shot hole borer (*Euwallacea* spp.) and Fusarium Dieback, an invasive pest-disease complex in California sycamore

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Shot hole borer-Fusarium Dieback (SHB-FD) is an invasive pest-disease complex threatening hardwood trees in urban landscapes, wildlands, and avocado groves throughout California. Current management strategies for SHB-FD rely on the removal of infested trees to reduce beetle populations; however, these methods are not sufficient for large-scale land management given the broad host range and rapid spread of the shot hole borers. To investigate the use of several fungicides and insecticides for the control of SHB-FD, *in vitro* screens of 13 pesticides were performed to find candidates for a year-long study at three regional parks in Orange County, CA using *Platanus racemosa* with varying degrees of SHB-FD infestation. *In vitro* pesticide studies revealed that triazoles and strobilurins generally had lower EC₅₀ values for all fungal symbionts tested. In the field study, emamectin benzoate treated trees were found to have significantly reduced shot hole borer attacks compared to untreated trees in addition to trees treated with debacarb in combination with carbendazim, metconazole, tebuconazole, and propiconazole in combination with emamectin benzoate. No significant reduction in SHB attacks was observed for trees treated with bifenthrin, propiconazole, thiabendazole, or *B. subtilis* (QST713). This study provides evidence for the efficacy of several pesticides for use as part of an integrated pest management strategy for the control of SHB-FD in California.

Comparing methods for inducing root rot of *Rhododendron* with *Phytophthora cinnamomi* and *P. plurivora*

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Root rot, caused by *Phytophthora cinnamomi* and *P. plurivora* in containerized *Rhododendron*, can cause significant losses in the nursery industry. Studies commonly use a 48 h flooding event to stimulate root infection. While flooding rarely occurs in container nurseries, plants may sit in a shallow puddle of water when irrigated too frequently and drainage is poor. Nursery transplant practices can damage roots and may predispose plants to more rapid disease progression. A greenhouse study was conducted comparing disease development on 1.5-year-old *R. 'Boule de Neige'* plants inoculated with *P. cinnamomi* or *P. plurivora*, then either flooded (48 h, then maintained at container capacity) or placed in a saucer kept permanently full of water. The effect of low vs. high transplant damage was also evaluated. The experiment was a full factorial RCBD with 10 replicates of each treatment. Plants were evaluated weekly for symptom development. Regardless of water or damage treatment, plants inoculated with *P. cinnamomi* were more chlorotic and had a greater number of wilted and dead plants by week 12 than noninoculated controls or plants inoculated with *P. plurivora*. Noninoculated and *P. plurivora*-inoculated plants were similar, with few symptoms of chlorosis or wilt, and no dead plants. Results suggest that both methods of inducing high substrate moisture (flooding vs. saucer) are effective for inducing root rot symptoms in plants inoculated with *P. cinnamomi*.

Identification and pathogenicity of fungal species associated with canker diseases of pistachio in California

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A survey was conducted during 2015 and 2016 in 40 pistachio orchards throughout the San Joaquin Valley of California to investigate the occurrence of canker diseases. Cankers and dieback symptoms were observed rather commonly in > 20 years-old pistachio orchards. Symptoms of canker diseases included brown to dark brown discoloration of vascular tissues, wood necrosis and branch dieback. Isolations from the cankered tissues revealed the following fungal species: *Neofusicoccum mediterraneum*, *Cytospora* species, *Diaporthe ambigua*, *Schizophyllum commune*, *Phaeoacremonium mortoniae*, *Colletotrichum karstii* and *Phoma fungicola*. Isolates were identified by phylogenetic analyses of the internal transcribed spacer (ITS) region of rDNA and morphological studies. An *in vitro* pathogenicity test using detached pistachio twigs (cv. Kerman) was conducted in the laboratory to determine the ability of the various fungal species to produce cankers following one month incubation in moist chambers. A second pathogenicity test was conducted in the field using two to three year-old branches of 15-year-old pistachio trees cv. Kerman inoculated with a selection of 22 isolates. The length of cankers produced in inoculated branches was recorded after five month incubation. Results indicated that all fungal species were pathogenic to pistachio, producing wood cankers and discoloration with *Cytospora* species and *N. mediterraneum* being the most virulent and widespread canker pathogens of pistachio.

Streamlining Citrus Clonal Protection Program (CCPP) citrus diagnostics using multiplex qPCR

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The Citrus Clonal Protection Program (CCPP) is the citrus germplasm introduction and distribution program for the state of California (CA). The CCPP is currently using over 23 individual PCR assays for the detection of all known graft-transmissible pathogens of citrus, prior to the release from quarantine of a new citrus variety. These assays are currently being consolidating into high throughput, multiplex, quantitative real time qPCR based assays to streamline the CCPP diagnostic pipeline while increasing specificity, efficiency and reducing cost by saving time and labor. These multiplex qPCR assays will detect all citrus pathogens, endemic or exotic to CA, mandated in the USDA/CDFA approved CCPP protocols. So far, we have developed qPCR assays for citrus pathogens such as: *Citrus tristeza virus* (CTV), *Citrus psorosis virus* (CPsV), *Citrus leaf blotch virus* (CLBV), *Candidatus Liberibacter* species (CLspp.), *Spiroplasma citri*, citrus tatter leaf virus, *Citrus vein enation virus* (CVEV), citrus yellow-vein associated virus (CYVAV), as well as 7 species of citrus viroids. Currently, several multiplex qPCR assays have been in different stages of development for the simultaneous detection of pathogens such as a) CTV, CPsV and CLBV (Osman *et al.* 2015, *J. Virol. Meth.* 220: 64–75), b) several species of citrus viroids (Vidalakis & Wang 2013, US Pat. 20130115591, Osman *et al.* 2017, *J. Virol. Meth.* 245: 40–52), c) CLspp. and *S. citri* (validation), and d) CVEV and CYVAV (design).

Epidemiology and spatio-temporal distribution of grapevine viruses in British Columbia

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Grapevine leafroll-associated viruses (GLRaVs) associated with grapevine leafroll disease (GLD) are known to cause economic losses to grape production worldwide. The majority of GLRaVs are vectored by one or more species of mealybugs (*Pseudococcidae*) and/or scale insects (*Coccidae*). We tested for the presence of four GLRaVs (GLRaV-1; -2; -3 and -4) as well as the newly identified *grapevine red blotch virus* (GRBV) from composite samples collected throughout all grape-growing regions of British Columbia (BC). Disease prevalence and genetic variability of field populations of GLRaVs and GRBV from BC vineyards were recorded and partial heat-shock protein-70 homolog (HSP70h) gene sequences of selected GLRaVs and full-length genomes of GRBV isolates were characterized. Our results indicated GLRaV-3 to be the most widespread (23.5%) followed by GLRaV-2 (6.9%), GLRaV-1 (2.9%), and GLRaV-4 (2.1%). PCR-based diagnostic tests for GRBV resulted in 1.6% positives, indicating its low incidence in BC vineyards. Significant differences were observed in the relative distribution of GLRaVs among regions and age of vines in established vineyards. Surveys and molecular

characterization of partial cytochrome oxidase (COI) gene for potential insect vectors revealed the presence of *Pseudococcus maritimus*, *Parthenolecanium corni* and other *Pulvinaria* spp. Spatio-temporal distribution of GLRaV-3 in four vineyard blocks during 2013–2016 growing seasons indicated disease spread rates of up to 19%. Regional differences in the relative incidence of GLRaVs in BC vineyards underline the importance of sustainable virus management practices.

Pseudosclerotial survival of *Monilinia vaccinii-corymbosi* (mummy berry disease) in western Oregon

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Blueberry losses in Oregon due to mummy berry (*Monilinia vaccinii-corymbosi*) have been estimated to be as high as 36 or 100% in conventional and organic fields, respectively. Pseudosclerotia (mummies) overwinter on the soil and produce primary inoculum (ascospores) in apothecia in early spring. It is not known if mummies survive more than 1 year. Foliar applications of Actinovate (*Streptomyces lydicus*) are moderately effective as a preventative treatment but of unknown efficacy with regards to apothecial suppression. Round wire mesh corrals (15 cm in diameter) each containing 100 mature mummies in direct contact with the soil were placed between ‘Bluetta’ blueberry bushes on 24 Sep 2015. Mummies were drenched with 500 ml Actinovate AG (2 tsp/gal water) or 500 ml water per corral in the fall of 2015. Treatments were arranged in a randomized complete block design replicated 24 times. Another set of 48 corrals with no drench treatments was also placed between bushes in the fall of 2015. Open, sporulating apothecia were not observed in any of the treatments in the spring of 2016. A total of 45 apothecia were observed in 16 of 48 nontreated corrals on 26 Mar 2017. A total of 23 apothecia were observed in 10 out of 24 water drenched corrals but only 2 apothecia were found in 1 out of 24 Actinovate treated corrals. Pseudosclerotia can survive and produce apothecia 2 years after contact with the soil surface and may be suppressed when treated with Actinovate.

Screening of U.S. mini-core for resistance against Sclerotinia blight using the detached leaflet and whole plant inoculation methods

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Peanut is a high-value leguminous crop and is grown in several states of the U.S.A. including New Mexico. Among the four market-type cultivars (Valencia, Spanish, Runner, and Virginia), Valencia is the most cultivated in New Mexico. *Sclerotinia sclerotiorum* causes an annual loss of more than \$200 million in USA. Host-plant resistance is most economic and effective method to control soilborne diseases, including Sclerotinia. Ninety-five accessions of US peanut mini-core collection were evaluated for resistance to Sclerotinia using detached leaf and whole plant inoculation method in controlled conditions on a scale of 0 to 4. Measurements on disease score, disease incidence, and disease severity index were taken. The disease score ranged from 2.7 to 4, disease incidence was 100%, and disease severity ranged from 86–100%. Accessions were categorized as highly susceptible, susceptible, partially resistant, and resistant based on the disease severity index and disease score. None of the accessions using detached leaf were found as resistant; however, PI 268586, PI 268696, PI 356004, PI 372305, and PI 429420 had lowest average disease score, 2.7, while based on whole plant inoculation method, PI 319770, PI 323268, PI 259658, PI 337293, PI 200441 had lower disease score index (86% to 90%).

New reports of downy mildew found on ornamental and native hosts in the western states

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The destructive capacity of downy mildews is significant and well known, but information on the taxonomy, host range, means of over-seasoning, diversification, and distribution of these organisms is often inadequate. There exists a need for increased documentation of downy mildews to facilitate study of this economically important group. To that end, we report here on downy mildews apparently new to the Western states, confirmed by the Oregon State University Plant Clinic (using morphology and, when possible, ITS sequencing) in 2015 and 2016. *Peronospora mesembryanthemi*, new to the US, was found on *Aptenia cordifolia*, occurring widespread in the landscape over several counties in California; and on *Delosperma cooperi* in a Washington nursery. *Peronospora* cf. *pulveracea* was found on *Helleborus × lividus*, a new host, in Washington. A novel species of *Peronospora* was discovered in Oregon on the native *Penstemon acuminatus*, a plant considered for use in restoration planting after fire. A previously unreported *Peronospora* sp. was detected on *Gambelia speciosa*, a native plant from California. These pathogens were responsible for 30–100% losses of affected plant material. When possible, specimens were deposited with the OSU Herbarium. Records of new finds, including physical samples in herbaria, will enrich the resources with which mycologists have to work.

Molecular confirmation of homothallism in *Claviceps purpurea*

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Claviceps purpurea causes ergot, a seed replacement disease of grasses that is of particular importance when the grass is grown for seed production, forage, or as livestock feed. Although the fungus has long been considered self-compatible (homothallic) based on inoculations with individual homokaryotic spores, previous mating-type studies using PCR-based assays have only detected either the *MAT1-1-1* or *MAT1-2-1* idiomorph in *C. purpurea*. The objective of this study was to modify an existing PCR-based assay using primers specific to the mating-type idiomorphs of *Clavicipitaceae* to confirm either the absence or presence of *MAT1-1-1* and/or *MAT1-*

2-1 genes in *C. purpurea*. Thirty-four single-spore isolates of *C. purpurea* were evaluated in this assay, with both mating-type genes detected in 24 isolates. Sequencing of PCR amplicons from eight representative isolates further confirmed the homothallic condition. For the remaining isolates only the *MAT1-1-1* ($n = 9$) or *MAT1-2-1* ($n = 1$) gene was detected; however, it is possible that further optimization of the PCR conditions or primers would allow for the detection of both mating-types in all isolates assessed in this study. Nonetheless, to our knowledge this is the first detection of both mating-types within the same isolate of *C. purpurea*, confirming homothallism in this economically important fungus.

Bactericide use in the fight against HLB disease in Florida

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A three-year project was conducted under a Cooperative Research and Development Agreement (CRADA) involving USDA-ARS¹ and AgroSource² to evaluate the effectiveness of currently registered temperate tree fruit agricultural bactericide formulations used as foliar sprays on the citrus greening (HLB) infected citrus trees in Florida. Experiments were performed using either individual or combination treatments with FireLine™ (oxytetracycline hydrochloride) and FireWall™ (streptomycin sulfate). Both formulations are products of AgroSource. Initial greenhouse and lab work demonstrated that systemic uptake and movement of the active ingredients in these bactericide formulations was significantly improved with the use of certain commercially available adjuvants. The adjuvants providing the most significant uptake improvement depended on the bactericide being used. Subsequent commercial field trials at 17 different treatment blocks and a total of 1,615 trees were conducted in citrus growing regions across south central Florida (Indian River, Central Ridge and Peace River regions). Trees included grapefruit as well as Valencia and Hamlin sweet oranges ranging in age from three to twelve years with varying levels of infection and tree health. Replicated trials were conducted using a randomized complete block design with trees blocked for disease severity and initial CLas titer. Sprays were conducted at a 100 gallons/acre equivalent using 1.5 lbs./acre of FireLine™ 17 WP and 11 oz./acre of FireWall™ 50 WP. The first year treatments were not started until August (late summer) but in the subsequent two years, trees were treated over the entire growing season. In the final growing season, an alternating combination of three sprays for each bactericide (6 treatments per tree), or a treatment of three tank mix sprays per tree were identified as the best strategies and other treatment regimens were stopped. During the first year, an average of a 57% drop in bacterial titer in leaves was observed across all treatments and citrus types with similar effects of either bactericide alone or when used in combination. Significant improvements in plant health parameters were observed and increased every year the trees were treated demonstrating a consistent improvement in tree health as a result of the treatments. After the last season of treatment all citrus and all treatments showed a statistically significant 7% improvement in health. In the last years of the study, there was a statistically significant 17% improvement in harvest yield across all citrus types and treatments in comparison to untreated controls. At the level of the whole grove, there was a demonstrable shift in maximum per tree yield and a pronounced increase in yield across poor- and medium-yielding trees as a result of bactericide treatments.

Management of bull's eye rot using preharvest and postharvest fungicides

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Neofabraea perennans and *N. kienholzii* are major causal pathogens of bull's eye rot in apple in Eastern WA. These fungi cause significant economic loss to the Washington State apple industry and have been listed as quarantine pathogens. Previous experiments indicate that fungicide treatments containing thiabendazole and thiophenate methyl provide effective bull's eye rot control, however use of this treatment is not recommended due to the potential for resistance development in populations of *Penicillium* spp. Hence, the objective of this research was to screen newer commercially available fungicides, possessing multiple active chemistries, for bull's eye rot control. Fungal sensitivity and disease control efficacy were tested *in vitro* and in orchard trials, respectively. Results from these trials with *Neofabraea* spp. isolates suggest that fungicide containing fluopyram and trifloxystrobin may inhibit *Neofabraea* spp. *in vitro* at a level equivalent to that of thiabendazole. *Neofabraea* spp. isolates resistant to preharvest fungicide difenoconazole and cyprodinil, and postharvest fungicide pyrimethanil were identified in the trial. Field trials utilizing preharvest sprays and postharvest drenches indicate that preharvest applications two weeks before harvest with fungicide containing fluopyram and trifloxystrobin is most effective in controlling postharvest bull's eye rot in storage.

Mating types A1 and A2 of different *Phytophthora* spp. occurring in ornamental nurseries in Mexico

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The presence of both mating types of different *Phytophthora* species in the same field has been reported in Mexico. Nevertheless, information regarding the existence of A1 and A2 within the same nursery is scarce. The objective of this study was to determine if both mating types A1 and A2 could be present in the same ornamental nurseries located in three states in Mexico, Mexico City, Michoacan and Morelos. *Phytophthora* isolates were obtained from 36 ornamental nurseries among plants with wilt symptoms in Mexico, during 2015. Both mating types were found in six nurseries. Furthermore, in three cases they were detected within the same plant, *Capsicum annum*, *Petunia* × hybrid sp. and *Rhododendron* sp. The isolates obtained from these three hosts were identified as *P. capsici* (A1 and A2), *P. drechsleri* (A1 and A2) and mating type A1 of *P. capsici* and A2 from *P. cinnamomi* respectively. Oospore formation was investigated on these hosts; therefore mating types A1 and A2 were inoculated together and separate on healthy plants of *Capsicum*

annuum, *Petunia* × hybrid and *Rhododendron* sp., depending on the host from which they were isolated. Type A1 and A2 of *P. capsici* and *P. drechsleri* were pathogenic on *Capsicum annuum* and *Petunia* × hybrid respectively, resulting in increased virulence when both mating types were inoculated together. No symptoms were observed on *Rhododendron*. Oospores formed *in planta* on *Capsicum annuum* and *Petunia* × hybrid. This finding suggests that sexual reproduction is occurring in ornamental nurseries.

Grapevine red blotch disease, an emerging problem for wine grape production in the U.S.

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Grapevine red blotch disease was first noticed in 2008, in the premium wine grape production areas of the North Coast in California. Diseased grapevines produce fruit with less sugar, increased acidity, lower anthocyanins, and suffer from delayed maturity, resulting in huge economic impact on wine quality. A ssDNA virus, *Grapevine red blotch virus* (GRBV, Genus: *Grablovirus*; family *Geminiviridae*), was found in infected grapevines by deep sequencing. Subsequent laboratory tests by private and public virus testing laboratories showed that the virus was present in grapevines exhibiting leafroll-like symptoms in both US and Canadian vineyards. Foliar symptoms appear in late summer through early fall as red blotches often accompanied by red primary and secondary veins on basal leaves of red-fruit cultivars. In some cultivars, the whole leaf blade may turn red or develop necrotic margins. Herbarium specimens collected in the 1940's and wild grapevines were also found infected by GRBV. The virus is known to spread in vineyards and three-cornered alfalfa hopper (*Spissistilus festinus* (Say); family: Membracidae) was able to transmit GRBV under laboratory conditions. Significance of this species and other membracids as vectors under natural conditions is not known yet. Wide distribution of GRBV and its economic impact on wine grape production have made grapevine red blotch disease an emerging and potentially serious problem in the US.

Insights into shoot infection biology of *Fusarium circinatum* in *Pinus radiata* and the role of wounding in pitch canker epidemiology

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Fusarium circinatum is widely regarded as a necrotrophic pathogen of pines that infects shoot tissue through mechanical or insect-mediated wounds, causing girdling lesions that result in death of infected branches. However, recent studies of *Pinus radiata* seedlings indicate that the fungus may colonize shoot tissue without a wound. In this study, *F. circinatum* colonized 100% of *P. radiata* seedling stems and 70% of mature tree branches that were not wounded. Hyphae were observed beneath the epidermis of non-wounded shoot tissue within 14 days following inoculation. In both seedling stems and branches of mature trees, most infections of non-wounded tissue did not induce symptoms under low nutrient conditions. Fertilizer additions at a rate of 200 and 400 PPM N increased the incidence of symptomatic infections in non-wounded tissue by ~40% compared to plants treated at 20 PPM N. In surveys of native *Pinus radiata* stands in California, *F. circinatum* was recovered from 7.5–100% of healthy-looking seedlings (2–3 years old) across three of the four stands surveyed, indicating that asymptomatic infections are not an artifact of artificial inoculation conditions. These results suggest that wounding is not a requirement for shoot infection under all circumstances and that *F. circinatum* can grow within shoot tissue without causing symptoms. The importance of wounding in this pathosystem may be overestimated due to the asymptomatic nature of intact tissue infections.

Two citrus viruses encode silencing suppressors to suppress posttranscriptional gene silencing

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Viral proteins have been known to have multiple functions in virus-host interactions. In this study, we identified the coat protein (CP) and movement protein (MP) of Citrus tatter leaf virus (CTLV) and P0 of Citrus vein enation virus (CVEV) as suppressors of host antiviral RNA silencing. CTLV CP acts as a local suppressor of RNA silencing while the MP as a systemic suppressor and CVEV uses the P0 as a local and systemic silencing suppressor. When the *Potato virus X* infectious vector harboring CTLV CP, MP, or CVEV P0 was inoculated on *Nicotiana benthamiana*, all three constructs promoted viral infection and symptom development, likely through their RNA silencing suppression activities. Mass spectrometry-based immunoprecipitation proteomics, identified both CTLV CP and MP interacting with components of the host ubiquitin-proteasome pathway and probably hijacking it to degrade host silencing pathway proteins. CVEV P0, which possessed an F-box motif, interacted with the Skp and Cullin to form SCF complex. This complex is a multi-protein E3 ubiquitin ligase complex catalyzing the ubiquitination of proteins destined for proteasomal degradation. We also observed that the P0 triggered robust hypersensitive response-like cell death in *N. benthamiana* probably through gene-for-gene interaction. P0 deletion assay showed that the first 140 amino acids at the N terminal and the last 40 amino acids at the C terminal have important role in host recognition leading to cell death.

Developing a model system for studying the interactions between *Vitis vinifera* and *Eutypa lata*

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Eutypa dieback of grapevine limits vineyard productivity worldwide. The causal fungus *Eutypa lata* infects vines through pruning wounds and causes a chronic wood infection that develops as a wood canker. Foliar symptoms can take several years after infection to

appear, which along with the perennial nature of the host can make the study of plant-pathogen interactions and the identification of resistant cultivars a challenge. Here we compared three infection assays for evaluating levels of resistance to *Eutypa dieback* among genetically-diverse cultivars (Black Corinth, Carignane, Husseine, Merlot, Muscat Hamburg, Palomino, Peloursin, Primitivo, Thompson Seedless). Using the standard assay, callusing dormant cuttings and inoculating immediately before rooting in pots, plants developed stem lesions and foliar symptoms after 12 months, with Primitivo and Merlot as the most resistant cultivars. Primitivo and Merlot were also most resistant using the second assay with mist-propagated, rooted, green cuttings; stem lesions, but no foliar symptoms, developed after 4 months. The third assay, with detached, dormant cuttings, produced foliar symptoms within only 5 weeks, but no stem lesions, and confirmed Primitivo and Merlot as the most resistant cultivars. This rapid assay will allow a deeper understanding of the molecular mechanisms of fungal virulence and grapevine resistance in this pathosystem. It also opens new perspectives for studying other grapevine trunk diseases and rapidly testing control strategies.

Biology and control of *Neofabraea* leaf spot and twig dieback, a new threat to the olive oil industry in California

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California produces more than 95 percent of the olives grown in the nation. In 2014, the bearing acreage for olives was 37,000 acres with a total of 94,000 tons of olives produced at a value of \$72.9 million. While these figures indicate a growing industry, olive oil production could be challenged by the recent discovery of an emerging disease in olive orchards. This new disease, namely *Neofabraea* leaf spot and twig dieback, was first detected during the winter of 2016 in super-high-density oil olive orchards in San Joaquin and Glenn Counties. Affected tree canopies revealed numerous leaf spots and cankers on shoots and twigs developed at wounds, caused mainly by mechanical harvesters. As the disease progressed into the spring, trees suffered severe defoliation of entire rows causing substantial yield loss. Two species, namely *Neofabraea vagabunda* and *N. kienholzii*, were found to be consistently associated with the disease and Koch's postulates were completed. Based on field observations and pathogenicity studies, the cultivar Arbosana appeared to be highly susceptible to the disease, whereas cultivars Arbequina and Koroneiki were fairly resistant. Fungicides trials were conducted in the field to determine the efficacy of various chemical products to mitigate the disease. Several fungicides reduced the incidence of the disease by 60 to 80 percent. Management strategy guidelines are now being developed and implemented to limit further spread of the disease.

Citrus Clonal Protection Program (CCPP) and National Clean Plant Network (NCPN): A model system for prevention of citrus diseases

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Pathogen-tested, true-to-type propagative materials are the basis of a successful citrus industry similarly to other vegetatively propagated crops. The Citrus Clonal Protection Program (CCPP) has its roots in the 1933 discovery of the virus nature of the psorosis disease by Dr. Fawcett, Citrus Experiment Station, Riverside, CA. That discovery triggered the establishment of the Psorosis Freedom Program in 1937. In 1957, the Citrus Variety Improvement Program, renamed to CCPP in 1977, was established after the request of the CA citrus industry. In 2005, 3 USDA agencies, APHIS, ARS, and NIFA, agreed that a national network is required to support programs generating, preserving and distributing clean propagative materials for specialty crops. The National Clean Plant Network (NCPN) was authorized by the US Congress in the 2008 Farm Bill and became permanent in 2014 with the mission of "providing high quality asexually propagated plant material free of target plant pathogens and pests that cause economic loss." Incorporation of citrus into NCPN began in 2007 and the charter for the Citrus Clean Plant Network (CCPN) was adopted in 2010. CCPN currently has 10 centers, including CCPP, in 9 US areas, CA, FL, AZ, TX, LA, AL, HI, MD, & PR. In a typical year, CCPN centers conduct over 75,000 diagnostic tests, distribute over 600,000 units of clean materials, perform therapeutics on 100s of accessions, maintain 100s of foundation plantings, and foster various extension programs.

Influence of reactive oxygen species on induction of the Rcs phosphorelay in *Pantoea stewartii*

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Pantoea stewartii subsp. *stewartii* (*Pnss*) causes Stewart's wilt in sweet corn, which is characterized by seedling wilt and water soaked (WS) lesion formation. These WS lesions have high levels of the reactive oxygen species (ROS), H₂O₂ and superoxides. The transcription factor OxyR plays a large role in protecting this phytopathogen from H₂O₂ exposure; its absence compromises exopolysaccharide (EPS) production and increases sensitivity to H₂O₂. The promoter region of the operon containing genes encoding components of the Rcs pathway, an environmental sensing pathway, that regulates EPS production, contains a conserved OxyR binding site. This operon also contains two *rtx* toxin genes, one of which encodes a RTX protein involved in WS lesion formation. We hypothesize that exposure to ROS in WS lesions stimulates expression of the RTX/Rcs operon and serves as a mechanism for the bacteria to sense ROS in their environment to stimulate the production of a protective EPS matrix and enter into biofilm mode, which aids in xylem colonization. Sublethal *in-vitro*, exposure to paraquat and H₂O₂ increased induction of expression of this Rcs/RTX operon. Linking expression of the RTX/Rcs operon through sensing of ROS via OxyR may be one mechanism *Pnss* uses to transition from the WS lesion phase to the xylem phase of plant colonization.

Efficacy of anaerobic soil disinfestation in reducing plant-parasitic nematodes under nursery conditions

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Plant-parasitic nematodes threaten sustainability of specialty crops. In California, 85% of the walnut acreage is infested with *Pratylenchus vulnus*. Infestations in deep soil are problematic when producing nematode-free nursery stock. Pre-plant soil fumigants are being legislatively phased-out or restricted due to environmental and human health concerns. A potential alternative, anaerobic soil disinfestation (ASD), has been used in annual and perennial crops for suppression of fungal and bacterial pathogens. In ASD, carbon-rich substrates are incorporated into the soil before plots are covered with impermeable film, and soil moisture held near saturation levels for 3 to 6 weeks. On August 15, various C-sources: rice bran, molasses, tomato pomace or mustard seed meal amendments at 20.2 ton per ha were tested for ASD. After incorporation, plots were covered with a plastic film and water was applied at 150 l per m² for soil saturation. A drench with water or Telone EC (250 ppm a.i.) served as controls. Anaerobic conditions developed within 2 days after all amendments at 15-cm and 46-cm depths. On September 20, anaerobic levels were greater under all C-sources than in Telone and water controls. On November 9, average numbers of *P. vulnus* per 250 cm³ of soil in the ASD treatments (0.05 to 0.4) were like those in the Telone EC treated plots (1.9), all lower than the water controls (18.6). ASD is promising as a preplant treatment of nematode infested nursery soil.

Secretome characterization and correlation analysis reveal putative pathogenicity mechanisms in stripe rust fungus *Puccinia striiformis* f. sp. *tritici*

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Stripe (yellow) rust, caused by *Puccinia striiformis* f. sp. *tritici* (*Pst*), is a destructive disease of wheat. The pathogenicity and molecular mechanisms that *Pst* uses to infect and interact with wheat are not clear. In this study, we characterized the *Pst* secretome to identify avirulence (*Avr*) candidates and effectors, the proteins or other molecules secreted from a pathogen to modulate plant immunity for infection. The secreted protein (SP) gene regions are more variable than non-SP regions, reflecting that *Pst* has developed diverse genes for interacting with host genes. *Pst* has a large portion of species-specific SP genes, many of them are presence/absence polymorphic. The secretome has a class of superoxide dismutase 5 (SOD5)-homologs proteins, suggesting antioxidant defense as a strategy for *Pst* to suppress host basal immunity. We sequenced seven *Pst* isolates and characterized them, together with seven previously sequenced isolates, for their avirulent/virulent profiles on 18 *Yr* single-gene lines. Correlation analysis of the virulence phenotypes with genomic structure variations identified *Avr* candidates corresponding to five *Yr* genes. One *Avr* candidate showed presence/absence polymorphism, suggesting that gene loss may be a mechanism responsible for *Pst* virulence changes. Our study indicates that SP-coding regions are interesting targets for further studying the evolutionary history of *Pst* population and the co-evolutionary arms race between *Pst* and wheat.

Characterization of *Alternaria* species causing Alternaria leaf spot of cotton in southern New Mexico

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Alternaria leaf spot (caused by *Alternaria* spp.) is one of the most common foliar diseases of cotton (*Gossypium* spp.) and occurs in most cotton-growing regions of the world. Leaf spot, resembling *Alternaria* leaf spot, occurs in cotton in New Mexico. However, there has been no previous systematic research on the disease. This study was conducted to determine the extent (prevalence and incidence) and the etiology of leaf spot of cotton in southern New Mexico. Fourteen cotton fields were evaluated in October and November 2016 when plants were at late growth stage. All fields exhibited leaf spot symptoms. Average disease incidence was 100% in each of 13 fields, and 30% in one field in Rincon. However, defoliation was more than 50% in this latter field. Average disease severity across all fields was between 22 to 87%. For identification of the causal agent, 28 isolates (two from each field) were characterized based on morphological features and through PCR using universal primers ITS4/ITS5. All 28 isolates were identified as *A. alternata*, exhibiting olive green colonies with predominant white margins. Optimum mycelium growth was observed at temperatures between 20°C to 30°C. Cotton plants inoculated with selected isolates of *A. alternata* displayed symptoms similar to those found under field conditions. This is the first report of *A. alternata* on cotton in southern New Mexico.