2016 Pacific Division Meeting Abstracts

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Development of products for management of broad host-range gall forming phytopathogens

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Crown gall and leafy gall disease caused by phytopathogenic *Agrobacterium tumefaciens* and *Rhodococcus*, respectively, are a significant, costly, and chronic problem in the ornamentals nursery industry. Crown gall has been particularly troublesome in crops that are clonally propagated. Losses are due to the lack of effective chemicals for control of diseases caused by these bacteria. In an attempt to provide growers with viable options for disease prevention, we embarked upon a program to test various botanical extracts for use against *A. tumefaciens* and *Rhodococcus*. We investigated the antibacterial activity *Achillea millifolium* (yarrow), *Withania somnifera* (ashwagandha), *Artemisia absinthium* (wormwood), *Centaurea cyanus* (bachelor's button), *Matricaria recutita* (chamomile), *Taraxacum officinale* (dandelion), *Celtis boninensis* and *Strobilanthus cusia*. Extracts of these plants were prepared and tested using agar diffusion and micro-dilution assays. We found that a number of extracts from these plants have good antimicrobial activity against *R. fascians*, indicating their potentials to be used as green alternatives to synthetic agrochemicals for treating plants. The present results encourage further effort to investigate plant-derived bioactive compounds that may possess good antibacterial properties against these gall-forming plant pathogens.

Bull's-eye rot management: Understanding the disease cycle of *Neofabraea* spp. occurring on apples grown in the U.S. Pacific Northwest

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In the U.S. Pacific Northwest, bull's-eye rot of pome fruit, caused by *Neofabraea* spp., is a major quarantine concern. Of the four fungi causing this disease, *Neofabraea perennans* and *Neofabraea kienholzii* are common in north central Washington. In addition to fruit decay, *N. perennans* causes perennial canker and dieback of apple trees. Currently the over-seasoning strategies of *N. kienholzii* have not been elucidated. The objectives of this research were to determine the relative capacity of *N. kienholzii* to induce cankers in comparison with *N. perennans*, determine the timing of canker induction and fruit infection by either fungus, and to evaluate the efficacy of various fungicides for control of bull's-eye rot. Based on the findings from this research, *N. kienholzii* is capable of inducing cankers however, cankers were generally smaller than those induced by *N. perennans*. Canker induction by both species was prevalent in October compared to other inoculation periods ($p < 0.01$). Fruit infection was observed at each inoculation period, however disease incidence was greatest near the end of the fruit growing season (October, $P < 0.0001$). Among those examined, thiophanate-methyl, thiabendazole and pyrimethanil were the only chemicals found to provide effective bull's-eye control ($P < 0.0001$), yet due to modes of action and current use by industry, resistance to these fungicides is considered high risk.

Surprising revelations in the biology of crown and root rot pathogens of wheat

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Temperature and moisture have profound influences on the activity of *Fusarium culmorum*, *F. pseudograminearum*, *Rhizoctonia solani* AG-8 and *R. oryzae* causing crown and root rots of wheat respectively, in the dryland wheat production area of the Pacific Northwest region of the United States. This study determined the effects of temperature and water potential (a measure of soil moisture) on the mycelial growth, dry weight and spore germination of these wheat pathogens on potato dextrose agar and broth adjusted to different osmotic and matric potentials (–0.13 to –10 MPa) with sodium chloride, potassium chloride, and polyethylene glycol (PEG-8000), and incubated at temperatures ranging from 4 to 35°C. Linear mycelial growth of both *Fusarium* spp. was optimal at 20–25°C and –1 to –3 MPa. Dry weight gain showed similar trends in *F. culmorum*, but *F. pseudograminearum* showed variation in optimum temperature in response to shift in water potential. Optimum temperature for spore germination ranged from 10–30°C for *F. culmorum* and 15–30°C for *F. pseudograminearum* with some decline at extreme temperature and drier ranges. *Rhizoctonia solani* AG-8 was more restricted for optimal growth, while growth rate of *R. oryzae* declined less compared to AG-8 with lower water potential and higher temperature. This

study contributes to the knowledge of the biology and epidemiology of these pathogens, and will be used in predicting their potential distribution under future climate scenarios.

Assessing the sanitary status of certified grapevine mother blocks in Washington State

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Maintaining virus-tested grapevines in certified nurseries is recognized as the first line of defense in preventing the spread of viruses through the planting stock. Towards this objective, a collaborative project between Washington State University, WSDA Plant Services Program and grapevine nurseries was initiated to systematically examine the sanitary status of certified mother blocks in Washington State. In 2015, we tested samples from white-fruited cultivars of *Vitis vinifera*, since these cultivars do not express visual symptoms. A composite sampling strategy was adopted to collect and process grapevine samples for virus indexing. A total of 1,226 composite samples, representing 4,919 individual grapevines, from seven white-fruited cultivars were tested by RT-PCR/PCR for the presence of *Grapevine leafroll-associated virus 3* (GLRaV-3) and *Grapevine red blotch-associated virus* (GRBaV). The results indicated that nearly 12% of composite samples were positive for GLRaV-3. None of the 1,226 composite samples tested positive for GRBaV. Total RNA isolated from about 10% of composite samples were analyzed by next-generation sequencing (NGS). The NGS data revealed the presence of sequences aligning with GLRaV-3, *Grapevine rupestris vein feathering virus*, *Grapevine Rupestris stem pitting-associated virus* and two viroid species (*Hop stunt viroid* and *Grapevine yellow speckle viroid 1*), and the absence of sequences specific to GRBaV.

Potential seed transmission of *Pyrenopeziza brassicae* and *Mycosphaerella capsellae* in brassicas in the Pacific Northwest U.S.A.

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Pyrenopeziza brassicae and *Mycosphaerella capsellae* are new pathogens to the Pacific Northwest U.S.A. that cause light leaf spot and white leaf spot, respectively, on many species in Brassicaceae. Cabbage (*Brassica oleracea*) and mustard (*B. juncea*) plants were inoculated separately at pod set with *P. brassicae* and *M. capsellae* to produce naturally infested seed lots. Symptoms developed on the leaves and pods of plants inoculated with *P. brassicae*, and only on the leaves of plants inoculated with *M. capsellae*. Incubating infested seed on NP-10 agar medium for 3 weeks at 4°C followed by 3 weekly microscopic examinations resulted in detection of *P. brassicae* on $12.5 \pm 2.3\%$ of the mustard seed and $0.4 \pm 0.5\%$ of the cabbage seed. *M. capsellae* was not detected on seed. When the *P. brassicae*-infested mustard seeds were planted under misters in a greenhouse, 1.6% of 1,000 seedlings developed light leaf spot (20% seed transmission rate from the 12.5% infested seeds). In April 2016, *P. brassicae* was found on wild *B. rapa* (bird's rape mustard) plants and *B. juncea* cover crops in Skagit, Snohomish, and Whatcom Counties in Washington; and *M. capsellae* was detected on wild *B. rapa* in Skagit and Whatcom Counties. Koch's postulates confirmed pathogenicity of the *P. brassicae* isolates, and β -tubulin DNA sequencing confirmed the species identification. This is the first report of *P. brassicae* and *M. capsellae* in Washington State, a critical region of brassica seed production.

Complete genome sequencing of *Zucchini yellow mosaic virus* causing mosaic in cucurbits in Trinidad

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Zucchini yellow mosaic virus (ZYMV) was recently noticed in pumpkin and squash in Trinidad with severe mosaic and yellowing symptoms in leaves and deformation and color alterations in the fruits. The symptoms were noticed at all growth stages of the crop and a maximum disease incidence of 74% was recorded in the dry season during 2014 and 2015. Leaf samples were collected from pumpkin (5 sample) and squash (5 sample) showing severe symptoms from farmer's fields representing different cropping zones of Trinidad. Total RNAs were extracted from all the leaf samples. cDNA was synthesized with 1 µg of RNA and PCR was carried out with coat protein primers ZYMVCP-F1/ZYMVCP-R1 to confirm the presence of virus. For sequencing of the whole genome, 10 pairs of overlapping primers targeting the entire genome of ZYMV was designed and PCR conditions were optimized. PCR amplifications were performed and the resulting amplicons were cloned into pGEM®-T vector and sequenced by Sanger sequencing. All the sequences were analyzed using various different bioinformatics tools including blast, Clustal W and Mega 6 software and complete genome of ZYMV (~9590 bp) was obtained for the Trinidad isolates. The blast analyses proved that the Trinidad isolate is a new strain of ZYMV since which showed more than 6.0% variability with other reported isolates. The closest relationship of Trinidad isolate was found with NAT isolate (EF062582) from Israel with 94.1% nucleotide identities followed by 94.0% identities with SEO4T (Slovakia), AG (Israel) and H (Czech republic) isolates. The lowest nucleotide identities of 80.6% was obtained with ZYMV13PREP from Reunion Island. From this study a new strain of ZYMV causing severe mosaic in cucurbits was identified in Trinidad.

Identification and pathogenicity of *Fusarium* species associated with crown rot of pistachio in California

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Only a few *Fusarium* species are known to cause crown and root rot diseases in woody plants. During recent surveys in California, crown rot symptoms and decline of pistachio (*Pistacia vera*) trees were observed in orchards located in Merced and Fresno Counties. Twenty-seven *Fusarium* isolates were isolated in culture medium from symptomatic bark and wood tissues. Fungi were identified based on morphological characteristics and comparison of DNA sequences of the internal transcriber spacer region (ITS) of the rDNA, translation elongation factor 1- α (TEF), and the second largest subunit of the RNA polymerase 2 (RPB2) with reference sequences. Phylogenetic analyses using maximum parsimony of the various sequence data sets allowed the identification of *Fusarium solani*, *F. oxysporum*, *F. equiseti* and *F. proliferatum*. Two separate lineages were also detected within each of the *F. solani* and *F. oxysporum* clade. To determine the pathogenicity of the various *Fusarium* isolates, 1-year-old detached twigs of *P. vera* cv. 'Kerman' as well as 'UCB-1' (*P. atlantica* $\times$ *P. integerrima*) and *P. integerrima* rootstocks were inoculated with representative isolates of the four *Fusarium* species. After one month incubation in the laboratory, lesions produced in twigs inoculated with the different *Fusarium* isolates were significantly longer than those formed in the agar-only inoculated control. This study suggests that *Fusarium* species may act as crown rot pathogens of pistachio in California.

Relative abundance of potato psyllid haplotypes in potato fields during 2012 to 2015, and incidence of *Candidatus Liberibacter solanacearum*

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The potato disease Zebra Chip (ZC) has been linked to infection of plants by the bacterium *Candidatus Liberibacter solanacearum* (Lso). Five haplotypes (hapA to hapE) of Lso have been described so far in different crops, with only hapA and hapB being associated with ZC in potato. Both haplotypes are vectored and transmitted to a variety of solanaceous plants by the tomato/potato psyllid, *Bactericera cockerelli* (Šulc). Four psyllid haplotypes have been identified: Northwestern, Central, Western and Southwestern. Here, psyllid samples collected in Idaho potato fields from 2012 to 2015 were used to clarify spatial and temporal patterns in distribution and abundance of psyllid and Lso haplotypes. A shift from hapA toward hapB of Lso was revealed during these four seasons, indicating possible evolution of Lso populations in Idaho fields. Although we confirmed that Western psyllids were the most

abundant by far during the four seasons of observation, we also observed changes in abundance of other haplotypes, including increased diversity of psyllid haplotypes during 2015. South-central Idaho exhibited more diversity in psyllid haplotypes than Southwestern Idaho. Seasonal changes observed for the Northwestern and Central haplotypes could potentially be linked to psyllid migration and/or habitat changes.

Development and validation of a quantitative PCR assay for the detection of *Claviceps purpurea sensu lato* ascospores

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Claviceps purpurea sensu lato is an important seed replacement pathogen of grasses grown for seed. Microscopic methods that are used to detect and quantify *Claviceps* ascospores captured by spore traps are usually not rapid enough to inform fungicide application decisions within an ergot IPM program. The objectives of this project were to develop and validate a quantitative PCR (qPCR) procedure to detect and quantify *C. purpurea sensu lato* ascospores caught by spore traps. A SYBR Green qPCR assay was developed that amplified a 96 bp region of the *C. purpurea sensu lato* genome. Specificity of the primers was confirmed against 41 *C. purpurea sensu lato* isolates collected from perennial ryegrass, Kentucky bluegrass, barley, rye, smooth brome, and cordgrass and 11 isolates of six other *Claviceps* species. The qPCR reactions were >97% efficient and melt curve analysis confirmed that the primers generated a single product. Ct values were significantly related to DNA quantity ($R^2=0.99$; $P=0.0002$) and the number of spores ($R^2=0.99$; $P=0.0004$). The assay was applicable for samples containing 1 pg to 10 ng of DNA or 4 to 40,000 spores. Results from qPCR and microscopic examination of 34 spore trap samples were correlated ($r=-0.68$; $P<0.0001$) and the qPCR assay detected spores on four samples from which they were not observed using microscopy. This assay provides a rapid and sensitive means for detecting and monitoring *C. purpurea sensu lato* ascospores captured by spore traps.

Scaling up: The effect of plot size and initial inoculum on dispersal gradients of *Puccinia striiformis* f. sp. *tritici*

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How the spatial scale of dispersal studies affects the measured dispersal gradient has been a question of great importance in disease ecology. This question can help address the appropriateness of extrapolating an observed dispersal gradient beyond the scope of plots used in an empirical study, which is of great interest for modelers of dispersal and epidemic spread. To address this question, we combined a small-scale dataset (up to 1.5 m) and a field-wide dataset (up to 103.6 m) of the primary disease gradients of *Puccinia striiformis* f.sp. *tritici*, the causal agent of wheat stripe rust. The small- and large-scale dispersal studies were conducted from 2012 to 2014, and 2002 to 2003, respectively, under similar growing conditions. The two datasets were each normalized by the number of lesions at a common observation distance, 0.914 m from the source. The two datasets were then merged, log-transformed, and fit by the modified inverse-power distribution $y=a+(x+c)^b$, with a best-fit c found by iteration, resulting in a slope of $y=0.22+(x+0.12\text{ m})^{-2.32}$, with a b within the 95% prediction interval of those fit to local and field-wide dispersal gradients of *P. striiformis* separately, as well as the dispersal gradients of several other aerially dispersed organisms. Additionally, the proportion of small- and large-scale lesions as a function of distance in m were fit by maximum likelihood estimation of the Modified Pareto probability mass function, a discrete form of the associated probability density function: $y=7.51*(1+x/0.41)^{-4.08}$. These results suggest it is possible to extrapolate the results of a dispersal study to better understand dispersal at a larger scale, given that the study systems remain constant.

Expression profiling of pathogenesis-related protein genes in wheat resistance to the stripe rust pathogen (*Puccinia striiformis* f. sp. *tritici*)

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Interactions of the stripe rust pathogen (*Puccinia striiformis* f. sp. *tritici*) with wheat plants activate a wide range of host responses. Among different defense responses associated with termination of a pathogen is the expression of particular pathogenesis-related (PR) protein genes. The objective of this study was to identify PR protein genes involved in wheat resistance to stripe rust. The expression levels of eight PR protein genes were quantitatively evaluated at 0, 24, and 48 hours post inoculation (hpi) and 7 and 14 days post inoculation (dpi) in near isogenic line of wheat with resistance gene *YrTr1* and a recombinant inbred line with *YrExp2* using races PSTv-4 (incompatible) and PSTv-37 (compatible). Analysis of quantitative real time PCR (qRT-PCR) revealed that PR protein genes appear to be upregulated and downregulated differently at different time points during the infection process between the wheat lines. Majority of the PR protein genes, *PR1.2* (2.1), *PR2* (5.8), *PR3* (10.25), and *PR5* (16.18) were upregulated at 48 hpi in the incompatible reaction of *YrExp2*, whereas in the *YrTr1* line, *PR1.2* (9.5), *PR2* (7.5), *PR3* (8.0), *PR9* (5.2), and *PR10* (6.4) were upregulated at 24 hpi. *PR4* was upregulated at all time points in *YrExp2*. The PR protein genes were significantly ($P>0.05$) higher in the incompatible interactions than the compatible interactions for both lines. The results provide useful information for understanding wheat resistance against stripe rust.

Pest Management Strategic Plans improve investment in integrated pest management in Pacific Northwest hops

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Pest Management Strategic Plans (or PMSPs) identify pest-management practices and needs in a crop or setting. Developed by researchers, extension specialists and growers, they document pest-management research, regulation and education priorities. Plans are then used several ways. State and federal agencies use them to understand pest management practices and needs, and as evidence of stakeholder involvement and priorities. Researchers, extension specialists and growers use them to direct research, promote best pest-management practices and leverage funding. On average, a dollar invested in a PMSP generates \$20 in additional funding. The Western Integrated Pest Management Center compared PMSPs for Pacific Northwest hops published in 2008 and 2015 as a case study to show the impact and changes that PMSPs can make. The 2008 PMSP identified disease and other pest research, regulatory and educational needs, and generated over \$3 million for researchers and extension specialists to address those priorities. The 2015 PMSP documented improvements that funding helped bring about: broadened use of mechanical control of mildews and the targeted control of powdery mildew during the early stages of cone development (resulting in an estimated yield increase worth \$2 million.) PMSPs can be developed for other crops to prioritize research and leverage funding to address diseases and pests in Western agriculture, and expand IPM practices that protect human and environmental health.

Impact of integrated pest management (IPM) in California

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Integrated pest management (IPM) is sometimes described as a method to reduce pesticide use. This has resulted in questions about the success of IPM in relation to continued use of pesticides in agriculture. IPM is more correctly described as a method to reduce economic, human health and environmental risks from pests and pest management strategies. Therefore, total pounds of pesticide applied is a mis-measure of IPM impact. Instead, IPM should be evaluated in regards to economic, human health and environmental risks from pests and pest management strategies. In California from 1995 to 2014, total pounds of pesticides declined by 8% but the value of agricultural productivity increased by 142%. The reduction in pesticide

application has not been uniform. From 2000 to 2014 there were significant declines in the use of pesticides known to cause reproductive toxicity (71% decrease), cholinesterase-inhibiting pesticides (60% decrease), potential ground water pollutants (72% decrease) but there were increases in the use of potential carcinogens (18% increase) and toxic air contaminants (15% increase). Pesticide residue data indicates the percentage of food samples with pesticides residues that exceed tolerances are 1.0% or less. USGS data indicates that pesticide in agricultural watersheds are decreasing. California air monitoring data documents no or low concentrations of pesticides, except for one sample which exceeded target screening levels for 1,3-dichlorpropene.

Seedling reactions of Mexican wheat varieties and advanced lines to four races of *Puccinia striiformis* f. sp. *tritici*, the stripe rust pathogen

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Stripe rust of wheat is a disease that during the 21 century has increased in importance in many regions of the world, including Mexico. Because selection for stripe rust resistance was not an important criterion in Mexico, little is known about resistance in most of its commercial varieties. The objective of this work was to evaluate stripe rust resistance in a historical series of 188 bread wheat varieties (BWV) and 28 durum wheat varieties (DWV) released in Mexico between 1950 and 2009, and 29 bread-wheat advanced lines (BWAL). Seedling tests were performed by inoculating the wheat genotypes with races PSTv-4, PSTv-14, PSTv-37, and PSTv-40. Results showed that 41 genotypes (16.7%) were resistant to PSTv-37, 63 (25.7%) resistant to PSTv-40, 98 genotypes (40%) resistant to PSTv-4, and 127 genotypes (51.8%) resistant to PSTv-14. Among different wheat genotypes, DWV had the highest percentage (17.9%) of resistant genotypes to the four races, followed by BWAL (13.8%) and BWV (1.06%). Trigo F2012 and Don Carlos "s" were the only BWV with resistance to all four races, whereas in DWV Oviachic C65, Yavaros C79, Aconchi C89, CEVY OROC2008, and Anatoly C2011 were resistant to all races. Of the BWAL group, Colibri and one line each from the following crosses were also resistant to the four races: Colibri//Finsi, Colibri//Kronstad, and Colibri//Berkut.

Double Nickel biofungicide efficacy on root health for young grape in the San Joaquin Valley

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Double Nickel LC™, a broad spectrum biofungicide containing *Bacillus amyloliquefaciens* D747, was tested in a three-year longitudinal study on newly planted Primitivo variety wine grapes. Root Knot Nematode (*Meloidogyne* sp.) related damage did negatively affect newly established vines that were untreated when planted into medium textured soil artificially infested above threshold. Double Nickel injected into drip irrigation at 1 qt/a in spring and 2 qt/a in fall applications resulted in significantly fewer Root Knot Nematode counts in soil compared to the untreated plots. A reproduction factor was calculated for the population increase between spring and fall counts, and control based on this population response to treatment was 74% compared to the untreated in year 3, and 30% improvement over standard of Telone II® applied PPI. Trunk girth, soluble sugar in juice and canopy senescence were all positively affected by Double Nickel treatments. Trunk diameters were nearly 2mm wider on treated vines compared to the untreated, and after three years, Double Nickel treated vines had 15% larger diameter trunks than untreated. Leaf senescence was significantly more advanced in Double Nickel treated vines in year 1 and 2, but juice had a lower sugar content compared to other treatments. When fruit was produced in years 2 and 3, fruit production trended higher from vines treated with Double Nickel compared to the untreated and standard.

Reduced sensitivity to potassium phosphite in *Phytophthora* species and its implication for the management of *Phytophthora* brown rot of citrus

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Phytophthora citrophthora (*Pc*) and *P. syringae* (*Ps*) are the major species causing brown rot of citrus fruit in California. Potassium phosphite (PP) is used as a preharvest treatment and is the only registered postharvest fungicide to manage this disease. We evaluated in vitro toxicity of PP against 40 isolates each of *Pc* and *Ps* from 29 orchards. The majority of isolates (77.5% of *Pc* and 90% of *Ps*) were sensitive to PP with mean EC₅₀ values for mycelial growth of 7.8 and 18.6 µg/ml, respectively. The remaining isolates had mean EC₅₀ values of 92.0 (range 50.8 to 251.8 µg/ml) and 120.8 µg/ml (range 97.9 to 141.6 µg/ml), respectively. Preharvest applications with labeled rates of PP were not effective in reducing brown rot of orange when inoculated with a less sensitive isolate (EC₅₀ = 69 µg/ml) of *Pc*, but reduced decay by 88% as compared to the control when a sensitive isolate was used. In postharvest studies, 10-s in-line drench applications at 54°C with 4,000 µg/ml PP reduced brown rot of orange fruit inoculated with sensitive or resistant (EC₅₀ = 186 µg/ml) *Pc* isolates by 90% or 0%, respectively. Dip treatments for 5 s at 54°C were more effective and resulted in a 100% or 90% reduction in decay for the two isolates, respectively. Therefore, postharvest PP can still be very effective in reducing brown rot caused by less-sensitive isolates when applied in heated solutions. New, highly effective preharvest fungicides with different modes of action are in development.

Susceptibility of weedy hosts to *Verticillium dahliae* isolates

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Verticillium wilt, caused by *Verticillium dahliae*, is a persistent disease of dicotyledonous crops due to a wide host range and survival in soil. Some *V. dahliae* isolates exhibit increased aggressiveness on a specific plant host while retaining a wide host range. It is known that weeds serve as hosts for *V. dahliae*, but unknown if weeds could serve as inoculum sources for aggressive *V. dahliae* isolates from crop hosts. The goal of this research was to quantify *V. dahliae* microsclerotia from twelve weeds grown in potting mix infested with one of eight aggressive *V. dahliae* isolates from crop hosts. All twelve weedy hosts were infected by at least one *V. dahliae* isolate, although the number of microsclerotia from some infections was low (< 5 microsclerotia/g dry plant). Black nightshade and litchi tomato provided greater inoculum density of the *V. dahliae* potato isolate than any other isolate ($P \leq 0.0055$) in each of two years. Interestingly, a *V. dahliae* isolate from tomato was observed to have greater inoculum density in large crabgrass and wild oats ($P \leq 0.0055$) in one year. These observations indicate that black nightshade growing during and in-between potato crop rotations may increase the inoculum density of the potato-aggressive isolates of *V. dahliae*.

Effect of temperature on virulence of fungal isolates collected in mycofloristic survey of *Camassia quamash*

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Populations of *Camassia quamash*, an herbaceous perennial, often dominate native wetlands, and the bulb is transplanted into mitigation sites. Refrigeration may reduce bulb losses due to storage rot. Objectives of this work were to determine the fungi in *C. quamash* bulbs and seed; and to test for pathogenic fungal taxa at two temperatures among the isolates recovered. *Camassia quamash* seeds were obtained from GRIN W6 45020 (USDA-ARS National Plant Germplasm System). Fungal isolates were obtained from bulbs at three locations: a forest wetland meadow, a garden and a wetland prairie. Bulb tissue was excised from the apical tip, basal plate, leaf scale and terminal bud then plated onto agar, as were seeds. Candidate isolates for pathogenicity testing on *C. quamash* bulbs were selected: three *Penicillium* spp., two *Fusarium* spp., one *Trichoderma* sp. and one *Botrytis* sp. Bulbs were surface disinfested and wounded (1 mm × 4 mm). Inoculum (1.5 × 10⁶/1 ml) was pipetted into the wound. Mock-inoculated controls with water agar were used. Prepared bulbs were incubated at either 5C or 23C. Rate of lesion expansion (mm/wk) was recorded at the end of the incubation period. *Botrytis* Cq23 exhibited significantly higher ($p < 0.001$) virulence among all isolates tested. Temperature effect was highly significant ($p < 0.001$) for *Botrytis* Cq23, *Fusarium* Ta9C and *Trichoderma* Tr25D. Prior reports of these taxa on *C. quamash* bulbs appear lacking. *Trichoderma* is rarely reported as a pathogen.

Strain specific resistance to *Potato virus Y* (PVY) in potato efficiently reduces the prevalence of the PVY^O strain under semi-field conditions

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Potato virus Y (PVY) is a serious threat to potato production due to the negative effects it has on tuber yield and quality, in particular, due to induction of potato tuber necrotic ringspot disease (PTNRD), typically associated with recombinant strains of PVY. Recombinant strains have spread in the U.S. for the past several years, although the reasons for their continued spread remain unclear. To address the shift in strain abundance, screen-house experiments were conducted and revealed that three of the four most popular potato cultivars grown in the Columbia Basin of Washington and Oregon, Russet Burbank, Ranger Russet, Alturas, and Umatilla Russet, exhibited strain-specific resistance against PVY^O, which resulted in selective multiplication of recombinant strains of PVY under semi-field conditions. Five weeks after inoculation, the recombinant strains PVY^{N-Wi} and PVY^{NTN} were significantly more prevalent than the non-recombinant strain PVY^O. The negative selection against the non-recombinant PVY^O strain is likely caused by the presence of the *Ny_{1br}* gene identified in potato cultivars in laboratory experiments.

Testing the efficacy of commercially-available compounds for managing *Agrobacterium tumefaciens*

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Crown gall disease, caused by *Agrobacterium tumefaciens*, remains a costly problem for growers of plants used for ornamental purposes. *Agrobacterium* requires a wound for infection and spreads easily through common pruning and propagation practices. Currently there are no known treatments—biological or chemical—that effectively prevent infection by *A. tumefaciens* throughout its host range. The objective of this study was to test commercially available products for their ability to challenge symptom development in gall-prone plants, with the ultimate goal of helping the nursery industry to better manage the spread of crown gall. Selection of products for *in planta* assays was guided through *in vitro* growth inhibition assays using the Kirby-Bauer disk-diffusion method. Active ingredients with *in vitro* efficacy included phosphorus acids, peroxides, benzoic acid, and oxytetracycline. Biocontrols were also selected for *in planta* assays and included *Bacillus subtilis*, *Streptomyces lydicus*, and extract of *Reynoutria sachalinensis*. *Rosa* and *Leucanthemum* were wounded and inoculated with a mix of *A. tumefaciens* isolates. Biocontrols were applied three days prior to inoculation, and chemical treatments were applied immediately after inoculation. All treatments were continually applied at weekly intervals until development of symptoms. Preliminary results suggest none of the trialed products are effective in preventing infection.

Impacts on plant health of complex pathogen communities

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Root pathogen communities are often a complex collection of agents whose interactions with regards to plant health are little understood. To explore the impacts of these interactions on plant health, a study was conducted to document the interactions among five rhizosphere-inhabiting pathogens in-field: *Cochliobolus sativus*, *Fusarium pseudograminearum*, *Rhizoctonia solani*, *Pythium ultimum*, and *Penicillium claviforme*. Interactions among pathogens were observed by their effect on plant health. Plant health was measured as seedling emergence, mid-season vigor, plant height, and yield, and was observed in response to inoculations with all single, pairwise, four-pathogen and five-pathogen combinations. Four significant antagonistic relationships were observed among all pairwise combinations of *C. sativus*, *P. claviforme*, and *P. ultimum*, affecting emergence and vigor ($P < 0.05$, all). Conversely, synergism was observed between *F. pseudograminearum* and *R. solani* that significantly reduced seedling emergence ($P = 0.002$). This synergism was also apparent in the community inoculations (emergence $P = 0.017$, vigor $P = 0.005$). Interactions within complex root pathogen communities of wheat involve dynamic processes that largely favor antagonism and improved plant health. All interactions among pathogens were measured through indirect effects (i.e., plant health). Work is ongoing to establish whether these interactions are reflected in changes in pathogen populations.

Diversity of *Cytospora* species associated with fruit and nut crop canker diseases in California

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Cytospora species are important pathogens of many hardwood tree species causing severe canker and dieback symptoms. In the fruit and nut crops, *Cytospora* infections have often been considered secondary to freeze, sunburn and other injuries. Identification of *Cytospora* to the species level is made difficult by limited taxonomic studies and sequence data availability. While morphological and phylogenetic studies have been conducted on important host such as *Eucalyptus*, little work has been done to address the diversity and pathogenicity of *Cytospora* species associated with the fruit and nut crops in California. This study aims to identify the principal species associated with canker diseases in woody perennial crops, and investigate their distribution and host range. Isolates were collected from cankers in almond, pistachio, cherry, plum, peach, olive, quaking aspen and various cottonwoods. Multi-locus sequence analyses of the ITS and β -tubulin genes combined with morphological analyses revealed several new species of *Cytospora*. Similarities between isolates from riparian areas (cottonwoods) and from agricultural hosts (*Pistacia vera*) suggest that riparian areas may serve as a source of inoculum of these pathogens as they spread in wet weather conditions. Pathogenicity tests were performed on almond and pistachio to assess the ability of the isolates to cause disease in these hosts.

Evidence that tuber cracking in potato can be caused by *Potato virus Y*

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Specialty potatoes for fresh market must meet high quality standards. “Cracking” is a defect that occurs when tubers split while growing, and sometimes can be caused by fluctuating soil moisture. To determine if an association exists between cracked tubers and *Potato virus Y* (PVY), the following was done using cv. Chieftain. Field-grown plants were flagged for mosaic. At harvest, healthy plants had significantly fewer ($P = 0.05$) cracked tubers than plants mildly or highly symptomatic. During the subsequent winter grow-out of progeny from the PVY negative and positive plants, 0% and 22–32% of tubers, respectively, were cracked. Tuber cracking in screenhouse and field trials planted to both cracked and certified virus-free nuclear tubers was not altered by either constant or alternating, nor high or low irrigations. Instead, the main factor leading to cracked progeny tubers was planting cracked seed tubers from PVY positive plants. Sampling at seasonal time points in a field trial yielded cracked tubers 63 days after planting. When cracked tubers from PVY^{N-Wi} positive plants were cut with a sterile knife at sprouted eyes to obtain sap, 25% of plants grown from recipient nuclear seed tubers cut with the same knife had mosaic symptoms, tested positive for PVY^{N-Wi}, and averaged 3.8% cracked tubers—the control source and recipient tubers remained healthy and PVY negative. PVY needs to be among the disease differentials when diagnosing cracked potato tubers.

Nitrogen fertilization increases powdery mildew, arthropod pests, and nitrate accumulation in hops

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Nitrogen fertilization may exacerbate or suppress various plant diseases depending on the pathosystem and form of nitrogen applied. Studies were conducted during 2014 and 2015 to quantify how nitrogen fertilization rate influences powdery mildew on hop (caused by *Podosphaera macularis*), as well as arthropod pests, yield, and nitrate accumulation in cones. In a commercial hop yard in Washington State in 2015, the incidence of leaves and cones with powdery mildew in plots that received 90 to 270 kg nitrogen per hectare was increased 15% and 30%, respectively, late in the season with the highest fertilization rate. Powdery mildew did not develop in plots in Oregon in either year, but the abundance of two spotted spider mites and defoliation from hop looper increased with increasing nitrogen fertilization. In both Washington and Oregon, yield of cones and bittering acids was maximal with intermediate rates of nitrogen. Nitrate levels in cones, a negative quality factor in hops, increased concomitantly with nitrogen fertilization rate. These early results indicate it may be possible to moderate powdery mildew, multiple arthropod pests, and nitrate accumulation in cones without negatively impacting yield, by avoiding excessively high rates of nitrogen fertilizer.

Monitoring *Phytophthora infestans* sporangia using volumetric trapping

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Late blight, caused by the oomycete *Phytophthora infestans* (Mont.) de Bary, is a devastating disease in potato and tomato and causes yield and quality losses worldwide. The US-23 strain appears to tolerate warmer and dryer climates and consequently threatens the Canadian Prairie commercial and seed potato industries. The best management practice for controlling this pathogen relies on proactive fungicide application on a regular basis. A volumetric spore trap network was established during the growing seasons of 2014 and 2015 in Alberta and real-time data provided growers with a 10–14 day advanced warning that the pathogen was present in the environment in advance of late blight development. A density of 1–10 sporangia per cubic meter of air for three consecutive days was the threshold that predicted an infection in the field. Volumetric air sampling combined with spore sticky traps mounted onto an unmanned aerial vehicle (UAV) was successfully used to triangulate late blight hotspots. This enabled rouging and pretreatment to reduce pathogen pressures and eradicate the late blight pathogen from fields. The *P. infestans* genotype US-23 dominated late blight pathogen populations in Alberta and was usually observed first on tomatoes. The prevailing westerly winds and decomposing compost piles of backyard gardeners influence the dispersal of *P. infestans* sporangia near commercial potato growing regions in Alberta, Canada. This volumetric spore trapping network may be used for risk-based decision making in order to reduce the number of proactive fungicide treatments required during the growing season and provide economical and environmental benefits.

Changing epidemiology of *Potato virus Y* in potato, and the role of strain specific resistance in facilitating the spread of recombinant strains

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Since 2002, recombinant strains of *Potato virus Y* (PVY) spread throughout the potato production areas in the U.S. Some of these recombinant strains are associated with tuber quality losses, in addition to the yield reduction. The driving forces behind the emergence and spread of these recombinant strains of PVY are not completely understood at the moment. Resistance to non-recombinant and some recombinant strains can be found in commercial cultivars grown in the U.S. However, certain recombinants can overcome most of the resistance genes in potato known today, and these are the ones the most prevalent in potato at the moment. Field studies and model experiments suggest that the currently observed prevalence of specific recombinant strains of PVY may be related to strain specific resistance to PVY exhibited in the most popular potato cultivars. More interactions between breeding programs and plant virologists involved in PVY studies are needed to control PVY in potato.

Investigating motility and persistence of PBTS *Rhodococcus* spp.

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Rhodococcus fascians is a bacterial phytopathogen with a large host range consisting of both monocot and dicot plants. It can exist as an epiphyte or gain entry into the host at which point it may cause economically significant damage in crops. Symptoms of *R. fascians* include stunting, shortened internodes, leafy galls, leaf deformation, and altered root morphology. A disease outbreak named Pistachio Bushy Top Syndrome (PBTS) in the U.S. was caused by two *Rhodococcus* spp. related to *R. fascians*. Research on these isolates includes understanding the mechanisms of motility and their persistence in soil. Experiments involving PBTS isolates include in vitro assays combined with microscopic imaging to examine the growth and potential movement of the bacteria. The PBTS *Rhodococcus* isolates were also used in plant assays on both ‘UCB-1’ trees and *Nicotiana benthamiana* plants. Both plant species developed symptoms that included stunting and leaf malformations and bacterial colonization and movement was assessed by microscopy. Survival of the pathogen in soilless potting medium was assessed under greenhouse conditions. Overall the data from these assays suggest that *R. fascians* persists at least three months in potting medium under greenhouse conditions. This information will help both growers and researchers better understand how to manage diseases caused by these pathogens.

Water stress exacerbates the severity of *Botryosphaeria* dieback in grapevines infected by *Neofusicoccum parvum*

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Botryosphaeria dieback (causal fungus *Neofusicoccum parvum*) is a detrimental grapevine trunk disease, causing internal wood degradation, killing shoots, and reducing yields. We examined the interactive effects of drought and *N. parvum* infection, common vineyard stresses, on wood-lesion development. Woody stems of potted *Vitis vinifera* ‘Cabernet Sauvignon’ were inoculated after wounding (IW). Control plants were either non-inoculated-wounded (NIW) or non-inoculated-non-wounded (NINW). At 2 weeks post-inoculation (WPI), water stress was imposed on half of the plants. Water use was estimated weekly for non-stressed plants to provide 20% of this volume to stressed plants. Leaf water potential was maintained, through weekly monitoring, at > -8 bars for non-stressed and < -13 bars for stressed plants. To test the specificity of host-based markers of infection, developed previously for *N. parvum* in non-stressed plants, asymptomatic leaves were collected for RNA extraction at 2 WPI (before water stress), and at 8 and 12 WPI (6 and 10 weeks post-stress, respectively). IW-stressed plants had the most severe levels of wood lesions. At 2 WPI, none of the markers were differentially expressed among treatments. By 12 WPI, seven markers showed higher expression, but only in stressed plants, regardless of inoculation treatment. Two markers showed consistent overexpression at 8 WPI in IW plants that were stressed and non-stressed, suggesting their specificity to *N. parvum* infection.

Characterization of somatic recombinant isolates of *Puccinia striiformis*, the stripe rust pathogen

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Natural population studies of *Puccinia striiformis* (the causal agent of stripe rust on wheat, barley, and grasses) have indicated that somatic recombination plays a possible role in the pathogen variation. To study somatic recombination, susceptible wheat or barley plants were inoculated with mixed urediniospores of paired isolates of *P. striiformis*. Possible recombinants were isolated from progeny spores through a series of inoculations of

wheat or barley genotypes. They were compared with the parental isolates on the set of 18 wheat and/or 12 barley genotypes used to differentiate races of the wheat and barley stripe rust pathogens, respectively, for virulence changes. The isolates were tested with 51 simple sequence repeat and 144 single-nucleotide polymorphism markers for genotype changes. From 68 possible recombinant isolates obtained from nine combinations of isolates based on virulence tests, 53 were proven by markers. Various types of recombinants were determined, including lost virulence from both virulent parental isolates, gained virulence from both avirulent isolates, combined virulences from both parents, and inherited virulence from one parent and avirulence from another. Marker data indicate that most recombinants were produced through chromosome re-assortment and cross-over after the hybridization of two parental isolates. The results demonstrate that somatic recombination is a mechanism by which new variants can be generated in the fungus.

Development of *Puccinia striiformis* f. sp. *tritici* mutants for avirulence characterization

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Puccinia striiformis f. sp. *tritici* (*Pst*) is an obligate biotrophic fungus causing stripe rust of wheat. Mutation is considered as a major mechanism of the pathogen variation, but has not been experimentally determined. The objectives of this study were to develop and characterize *Pst* mutants generated by mutagenesis. Urediniospores produced from a single-uredinium isolate of race PSTv-18, which has the highest number of avirulence genes, were treated with ethyl methanesulfonate (EMS) at concentrations of 0.02M and 0.03M for 6, 7, and 8 minutes. The treated spores were grown on wheat genotypes susceptible to the wild type isolate and screened on 50 genotypes of wheat and barley with various genes for resistance to stripe rust. Potential mutants isolated from the screening experiment were tested for virulence patterns on the set of 18 wheat *Yr* single-gene lines and 18 simple sequence repeat (SSR) markers. Virulence tests showed that 20 mutant isolates were different from the wild type. Changes of avirulence to virulence were observed for resistance genes *Yr1*, *Yr2*, *Yr6*, *Yr8*, *Yr9*, *Yr10*, *Yr31*, *Yr43*, *Yr44*, *YrSP*, *YrTr1*, *YrTye*, and genotypes Paha and those with *YrA+* and *Yr39+*. SSR markers showed that 18 of the 20 mutants were different from the wild type. Both virulence and SSR marker data showed that EMS mutagenesis was able to generate multiple-site mutants. These mutants are useful for further characterizing avirulence genes and understanding the pathogen evolution.

Virulence characterization of *Puccinia striiformis* f. sp. *tritici* in the U.S. for the past 48 years using the *Yr* single-gene differentials

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Puccinia striiformis f. sp. *tritici* causes wheat stripe rust, an important disease in the U.S. Races of the pathogen were identified using various numbers of wheat differential cultivars from 1968 to 2009 and using a set of 18 *Yr* single-gene lines since 2010. To characterize races and identify virulence changes of the pathogen, 908 isolates were randomly selected from the collections of 1968–2009 in the U.S. and tested on the new differential set. The virulence patterns were compared with races identified with the new differential set in 2010–15. A total of 184 races were identified, including 96 races detected in 1968–2009 but not in 2010–15 and 13 races detected only after 2010. The number of races identified in each year had a trend of increasing, ranging from 5 in 1986 to 41 in 2010, and the number of virulences per race also increased over the years. Most races developed from previously existing races through single-step mutation, but some races formed through virulence recombination. Multiple introductions of races were detected. Virulences to *Yr1*, *Yr6*, *Yr7*, *Yr17*, *Yr32*, *Yr44*, and *YrExp2* were detected throughout the 48 years. Virulences to *Yr10* and *Yr24* were first detected in 1970, *Yr27* in 1971, *Yr43* in 1974, *YrSP* and *YrTye* in 1972, *YrTr1* in 1975, and *Yr8* and *Yr9* in 1999. No virulences were detected for *Yr5* and *Yr15*. The results are useful in understanding the pathogen evolution in virulence and utilization of effective resistance genes for control of the disease.

A novel *Fusarium* pathogenic to common rose mallow (*Hibiscus moscheutos*) is a sister taxon to *Fusarium buharicum*

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Hibiscus moscheutos cultivars are highly desirable ornamentals. First detected in 2012, a novel *Fusarium* species was diagnosed as causing crown rot on several cultivars of *H. moscheutos* in Washington State. To complete Koch's postulates, pathogenicity trials were conducted on *H. moscheutos* cultivar Luna Rose in a greenhouse in the winter of 2014–2015, using two isolates recovered from symptomatic crowns (NRRL 66179, NRRL 66182) and one isolate from a symptomatic root (NRRL 66184). Negative controls were mock-inoculated with sterile water. Trials were repeated in a growth room in the summer of 2015. Crown rot developed at the point of inoculation in each treatment plant; the negative controls remained healthy. Isolates recovered from symptomatic crown tissue, but never from controls, were identified as the novel *Fusarium* sp. employing morphological data and partial translation elongation factor 1- α (*TEF1*) sequences, thus completing Koch's postulates. Phylogenetic analyses of partial *TEF1*, DNA-directed RNA polymerase II largest (*RPB1*) and second largest subunit (*RPB2*) nucleotide sequences placed the novel *Fusarium* sp. within the *F. buharicum* species complex and sister to *F. buharicum*. The latter species was reported to cause stem lesions on kenaf (*H. cannabinus*) in Iran and crown rot of cotton in Uzbekistan and Russia. Therefore, the available data suggests these pathogens might specialize on members of the Malvaceae.

Historical survey of cherry viruses in Oregon

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Sweet cherries (*Prunus avium*) rank in the top three fresh market fruit commodities in Oregon. Despite a major focus on sweet cherry viruses in Washington state, a low priority ranking was given to virus diseases by cherry growers in Oregon. Due to potential economic harm, an investigation was initiated to determine viral presence in Oregon cherry production areas. Published data was compared to digital and physical queries for viral diagnoses of *P. avium* samples assessed at the Oregon State University (OSU) Plant Clinic from 1956 – present. Additionally, the OSU Herbarium *Prunus* sp. collection (oldest sample dated 1882) was visually inspected for commonly associated viral symptoms, such as mosaics, ringspots, line patterns or enations. *Cherry twisted leaf-associated virus* was diagnosed by the OSU Plant Clinic in cherry samples from the Willamette Valley and The Dalles regions. *Cherry green ring mottle virus* was diagnosed in cherry samples from the Willamette Valley and *Tobacco ringspot virus* (TRSV) was found in cherry samples from the Grand Ronde Valley. Each of these viruses were previously unpublished as occurring in Oregon. OSU Plant Clinic records allowed for specific cherry growing regions to be identified, as opposed to only state or Pacific Northwest designations. Of the 373 *Prunus* sp. vouchers surveyed in the OSU Herbarium, one, *Prunus emarginata* from Douglas County, OR, displayed possible leaf mosaic symptoms. Finding the presence of viruses, such as TRSV and the others, allows for more effective disease management of existing orchards or establishment of new orchards in specific cherry growing regions.

Diversity of mycelial compatibility groups of *Sclerotium cepivorum* causing white rot in Oregon

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White rot, caused by the fungal pathogen *Sclerotium cepivorum*, is one of the most important diseases of *Allium* crops worldwide. Significant economic losses occur when *S. cepivorum* infests an *Allium*-growing area, partially because the pathogen can survive for >20 years in an infested field. The Oregon Department of Agriculture certifies *Allium* crops as free from white rot by inspecting 100% of the plants in each field. From 2007 to 2015, 92 isolates of

S. cepivorum were collected from 72 infested fields (2,519 acres) in five counties from a total of 458 garlic fields (12,804 acres) in ten counties around the state. Genetic diversity of the *S. cepivorum* isolates was examined by identifying the isolates' mycelial compatibility groups (MCG). Four MCG (MCG1, MCG2, MCG3, and MCG4) were identified among isolates. The majority of isolates (88%) belonged to MCG3, followed by MCG1 (6%), MCG4 (5%), and MCG2 (2%). The greatest diversity was observed in Jefferson County, where 3 MCG groups were detected. These results indicate that there is little genetic diversity in Oregon's *S. cepivorum* population.

Chokecherry and sweet cherry are infected by two host-specific *Podosphaera* species

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Powdery mildew of sweet cherry caused by *Podosphaera prunicola*, is a major problem in the cherry growing regions of Washington State. It is not known whether the powdery mildew that infects sweet cherry is same species as the one that infects chokecherry. Morphological features of both powdery mildew species were compared, the chasmothecial appendages of the sweet cherry powdery mildew were arranged equatorially, whereas the chasmothecial appendages of the chokecherry powdery mildew were fasciculate. Based on this major difference, it was determined that the powdery mildew that infects chokecherry is *Podosphaera tridactyla* and the powdery mildew that infects sweet cherry is *Podosphaera prunicola*. Isolates of *P. tridactyla* and isolates of *P. prunicola* formed separated clades, in a phylogenetic tree generated with ITS and 28S rDNA sequences. In cross inoculation experiments, *P. tridactyla* did not infect sweet cherry and *P. prunicola* did not infect chokecherry, supporting the presence of host specificity among species. Morphological, phylogenetic and pathological evidence support the presence of two different species of *Podosphaera* that infect chokecherry and sweet cherry in Washington State.

Transcriptome-wide mining of potato genes targeted by *Potato virus Y*-derived viral small interfering RNAs

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Potato virus Y (PVY) is an economically important pathogen of potato. The existence of biologically distinct strains that differ in their symptoms in potato complicates disease management efforts. The molecular mechanisms underlying the potato-PVY interactions are largely unknown. Plants respond to viral infection by accumulating small RNAs that mediate targeted degradation of the viral RNAs. We previously characterized virus-specific, small interfering (vsi) RNAs for PVY-N, PVY-NTN and PVY-O in potato cv. Russet Burbank. The three strains exhibited diversity in absolute number, copy number and hotspots for vsiRNA in their respective genomes. To elucidate the silencing effect of the vsiRNAs on host genes, we mined, *in silico*, the potato transcriptome and identified transcripts for their propensity for strain-specific vsiRNAs. Target transcripts represented KEGG pathways in plant hormone signaling, plant-pathogen interaction, RNA transport and, the GO terms in defense response and response to stress and were validated by qRT-PCR. These findings suggest that the virus targets and perturbs host plant defenses. Differential targeting of host genes was exhibited by the three strains and may partly account for their differential behaviour in potato. These clues on virus-host interactions can be applied in developing novel strategies for disease management.

The current status of nepoviruses in Washington vineyards

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Diseases caused by nematode-transmitted viruses (nepoviruses) are recognized as one of the serious impediments to the long-term sustainability of vineyards in Washington State. Previous studies have shown the presence of *Grapevine fanleaf virus* (GFLV) and absence of its nematode vector (*Xiphinema index*) in Washington vineyards. However, the significance of nepoviruses has been elevated with a recent report of the occurrence of *Tobacco ring spot virus* (TRSV) in a grower vineyard. In 2015 season, a limited survey of vineyards using virus-specific diagnostic assays revealed the presence of GFLV and TRSV in three wine grape (*Vitis vinifera*) cultivars in geographically separate vineyards. RNA1 and RNA 2 of TRSV was determined to be 7,519 and 3,927 nucleotides, respectively, and showed maximum similarity with corresponding sequences of TRSV-SK strain from South Korea. Using cucumber baiting assays, the spread of TRSV from symptomatic grapevines to healthy cucumbers by soil-inhabiting nematodes was demonstrated. Using morphological characteristics and molecular analysis of genomic DNA, *X. rivesi* was identified in soils collected from TRSV-affected vineyard. The detection of TRSV by RT-PCR in nematodes suggested the potential of *X. rivesi* as a vector of the virus. Studies on impacts of TRSV on fruit yield and quality in a wine grape cultivar showed that symptomatic grapevines produced significantly less fruit with low amounts of sugars compared to non-symptomatic vines.

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

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To date, only a few diseases are known to affect pistachio in California. Nevertheless, recent changes in weather patterns and prolonged drought have increased plant stress, thus potentially reducing plant tolerance to diseases. During the year 2015, we conducted surveys in California to detect possible new or emerging diseases of pistachio. Cankers in trunks and branches as well as dieback symptoms of mature pistachio trees were observed in several orchards of the San Joaquin Valley. Canker symptoms consisted of brown to dark brown vascular discoloration, from which isolation of putative fungal pathogens was attempted. Isolated fungi were identified by sequencing and NCBI BLAST of the partial internal transcribed spacer region (ITS). Preliminary sequence analyses revealed species of *Cytospora*, *Colletotrichum*, and *Phoma*, *Diaporthe ambigua*, *Neofusicoccum mediterraneum*, *Phaeoacremonium mortoniae*, and *Schizophyllum commune* in cankers. Pathogenicity tests on detached pistachio twigs were conducted in the laboratory to determine the ability of the various fungal species to cause diseases. Results indicated that all these species were pathogenic to pistachio, producing typical cankers. Among all, *Cytospora* species appeared to be the most virulent. Further detailed studies are being conducted to complete the identification of these fungal pathogens and better understand their biology and distribution in California pistachio.

Exploration of wheat root phenotyping for *Rhizoctonia* resistance

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Current screens for *Rhizoctonia* resistant wheat incorporate both field trials and greenhouse assays, but pathogen populations in the field are difficult to reproduce from year to year. Using three wheat genotypes having enhanced root growth in the presence of *R. solani* AG-8, we are exploring endogenous

early seedling root growth phenotypes as predictors of *Rhizoctonia* resistance in Pacific Northwest wheat lines. One genotype is the ethyl methanesulfonate mutant Scarlet-Rz1, in which resistance is inherited as a single dominant or co-dominant allele; two other genotypes, SYN 172 and SPCB 3104, are synthetic or synthetic-derived wheats in which resistance is conferred by multiple quantitative trait loci. In all genotypes, resistance was correlated to higher total root length relative to that in the corresponding susceptible genotypes Scarlet and Louise (WinRHIZO, Regents Instruments). However, other root growth traits that can be monitored within 14 days at low cost are also of interest. Preliminary experiments with susceptible Scarlet and resistant Scarlet-Rz1 indicate that resistant roots emerge more slowly but grow more quickly after emergence than susceptible roots. This trend is not observed in resistant and susceptible synthetic genotypes. Other preliminary experiments indicate that wheat seedling roots grow well in the gellan gum-based clear medium GelZan (Phytotech Labs). When combined with 360° imaging (Ortery Technologies), seedling root architecture can be assessed in three dimensions and quantified using GiaRoots software. Three pathogen-based approaches will enable a comparison of resistant and susceptible genotypes with and without pathogen: 1) browning of seedling roots after exposure to the pathogen in a ground oat substrate; 2) seminal root angle of seedling roots grown against the walls of transparent pots; and 3) architectural traits of mature roots in soil, including lateral root number, branching angle and root hair number, quantified using the CI-600 *in situ* root imaging system.

Predictive models for tospoviral proteins involved in virion assembly and host defense suppression

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Tospoviruses (*Tospovirus*:*Bunyaviridae*) cause significant losses to a wide range of agronomic and horticultural crops worldwide. To engineer virus resistance, target proteins and motifs with critical functions have to be characterized. *Tomato spotted wilt virus* was used to understand the structure of the nucleocapsid (N) and silencing suppressor (NSs) proteins coded by the viral small RNA. We used several state of the art 3D modeling algorithms, MULTICOM, ITASSER, ROBETTA and CONFOLD to predict the tertiary structures, and pairwise ranking method APOLLO and TM-SCORE to rank the resulting models. A total of 31 and 27 complete sequences of the N and NSs genes, respectively, of all known tospoviruses were aligned. At least 8 conserved residues of the N protein and 9 in the NSs protein were identified. The N protein models predicted were built based on the Orthobunyavirus templates. All models showed β -sheets, consistently located near the amino terminus, and several α -helices. For the NSs protein, no *Bunyaviridae*-based proteins were available. The modelers selected several unrelated templates to build the NSs protein predictions. The predictions made by all three servers were similar to the N protein, whereas, it was less so for the NSs protein. This is the first attempt to predict the 3D structure of any tospoviral NSs protein. Our results form the basis for further work on structure-function relationships of tospoviral proteins.

Diverse mycoviral sequences in the sweet cherry powdery mildew fungus (*Podosphaera prunicola*) revealed by next-generation sequencing

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Fungal viruses (mycoviruses) are frequently found in several plant pathogenic fungi. This study was undertaken to examine mycoviral sequences in the fungus *Podosphaera prunicola*, the causal agent of powdery mildew disease in sweet cherry. Double-stranded RNA preparations of the fungal conidia and mycelium scraped from the infected cherry leaves were analyzed by next-generation sequencing (NGS). The quality filtered NGS reads were assembled *de novo* into contigs using the CLC Genomics workbench 8.5.1 software and subjected to BLASTn and BLASTx analyses against sequences available in public databases. The results showed the presence of distinct mycovirus-like sequences. Four of these sequence contigs, ranging in size between 1,915 and 2,334 nucleotides (nt), contained a single open reading frame (ORF), potentially encoding a protein with similarity to the RNA-dependent RNA polymerase of viruses in the family *Partitiviridae*. Three additional sequence contigs, ranging in size between 1,736 and 2,104 nt, contained a single ORF encoding a putative protein similar to the capsid protein of viruses in the family *Partitiviridae*. Another contig of 11,449 nt containing four putative ORFs showed similarity to the genome of an unclassified mycovirus, *Macrophomina phaseolina tobamo-like virus*, in the family *Virgaviridae*. These preliminary results suggest that the fungus causing powdery mildews in cherries may contain diverse mycovirus populations.

Prevalence of Grapevine red blotch-associated virus in British Columbia

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Grapevine (*Vitis vinifera* L.) is an important crop in British Columbia (BC) contributing over CAD \$2.1 billion into the Canadian economy. BC, with over 10,000 acres, is the second largest grape-growing region in Canada with the majority of commercial vineyards located in the Okanagan Valley. Grapevine red blotch-associated virus (GRBaV) was first observed in New York in 2012. Since then, it has been reported to occur in wine-grape cultivars as well as free-living *Vitis* spp. from several grape-growing regions in the U.S.A. Negative impacts associated with GRBaV infection in wine-grapes, including reduced yield and poor fruit quality could pose a threat to the sustainability of the grape and wine-industry. To determine the current status of GRBaV in BC vineyards, field surveys were conducted during both 2014 and 2015 growing seasons. In total, 205 vineyard blocks were surveyed in BC. Overall, 2,196 random composite samples (10,980 vines) and 229 single targeted vines were tested for the presence of GRBaV by PCR using virus specific primers. GRBaV was detected from 34 (1.5%) and 26 (11.3%) composite and targeted samples, respectively, indicating a low incidence of this virus in BC vineyards. Phylogenetic analysis based on the full-length genome of eight representative isolates from BC indicated the presence of mixed genetic variants in BC vineyards. This work provides the foundation for further studies to investigate the role played by GRBaV on grapevine health and will assist the development of management strategies in BC.

Discovery of grapevine Pinot Gris Virus and current status of other less-common grapevine viruses in British Columbia

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Grapevine (*Vitis vinifera* L.) hosts the widest variety of pathogens of any woody perennial crop and at least over 70 different viruses have been found to infect grapes. Among them, less-common viruses, including *Grapevine fanleaf virus* (GFLV), *Grapevine fleck virus* (GFkV), *Arabis mosaic virus* (ArMV), and/or the recently discovered *Grapevine Pinot gris virus* (GPGV) have been shown to affect vine health by reducing vine growth and fruit quality. To understand the current status of these grapevine viruses in British Columbia (BC), surveys were conducted between 2013 and 2015 growing seasons. Both random-composite samples and single-targeted vines were collected in different grape-growing regions of BC and tested for the presence of GFLV, GFkV, and ArMV by DAS-ELISA and/or RT-PCR. GFLV was detected in 17 out of 188 (9%) blocks surveyed and in 25 out of 1,220 composite samples (2%). GFkV was detected in 231 out of 788 (29.7%) of the composite samples collected and no positives were detected for ArMV from 998 composite samples. Additionally, single tube RT-PCR using specific primers to the coat protein (CP) gene detected GPGV in one sample showing severe chlorotic mottling and deformation of the leaves. GPGV isolate from BC was confirmed by completing the full genome sequence. This study represent the first report of GPGV in BC, which underscores the importance of rigorous virus testing for monitoring the occurrence and/or introduction of less-common viruses in commercial vineyards.

Developing a wheat germplasm with linked genes *Yr64* and *Yr65* for resistance to stripe rust

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Stripe rust, caused by *Puccinia striiformis* f. sp. *tritici*, is one of the most important wheat diseases worldwide, and resistance is the best control strategy. In a previous study, two genes, *Yr64* and *Yr65* originally from durum wheat conferring resistance to all tested *Pst* races, were mapped to different loci, but linked in the short arm of chromosome 1B. The objective of this study was to pyramiding *Yr64* and *Yr65* into a single common wheat line. A cross was made between common wheat lines of *Yr64* and *Yr65* selected from previous studies. A line was selected from the F_4 progenies to make a cross with 'Avocet S' based on its infection type (IT) 0 that was different from IT 2 on both *Yr64* and *Yr65* lines. Among 336 F_2 plants from the new cross tested with race PSTv-11 in the greenhouse, 5 plants were susceptible and the remaining 331 plants were resistant. Testing 100 F_3 lines in the greenhouse with the same race resulted in homozygous susceptible lines from susceptible F_2 plants and separated homozygous resistant lines from segregating lines derived from resistant F_2 plants. The results confirmed the linkage of the two genes and indicated that the selected line had two genes. Genotyping the F_4 line, *Yr64* and *Yr65* lines, and Avocet S with molecular markers flanking the two genes supported the presence of both genes in the selected line. The new line with linked *Yr64* and *Yr65* should be useful for developing wheat cultivars with durable resistance to stripe rust.

Effectors from *Puccinia* suppress plant host defense responses

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Plants employ several defense systems to guard against infection. In order to successfully infect the host, pathogens secrete a number of proteins that attenuate host defenses. Here we report nine effector proteins from cereal rust pathogens, *Puccinia graminis* and *Puccinia striiformis* that suppress the plant hypersensitive response (HR) when expressed transiently in *Nicotiana benthamiana*. Of the twenty putative effector proteins selected and expressed in *N. benthamiana* using *Agrobacterium*-mediated transient transformation, nine proteins, *Shr1* – *Shr9* (Suppressors of Hypersensitive Response), were found to suppress R gene mediated hypersensitive response. These proteins differentially interfere with R gene Pto(Y207D), and effector-R gene combination GPA/RBP-1 and ATR13/RPP13 mediated HR. Expression analysis of these nine suppressors showed that they may be required at all stages of rust infection. In addition, *Shr7* reduced production of reactive oxygen species (ROS) triggered by flg22, a common PAMP elicitor. Furthermore, delivery of *Shr7* into wheat leaves via *Pseudomonas* type-III secretion system suppresses non-specific HR induced by *Pseudomonas syringae* pv. *syringae* DC3000. To our knowledge the present report constitutes the first evidence for the presence of plant defense suppressors in *Puccinia*. Overall, these studies reveal that fungi, much like bacteria and oomycetes, use suppression of host defenses as a strategy to infect plants.

Utilizing field surveys, variety trials and indicator species analysis to diagnose a soil borne disease complex in winter squash

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A novel late-onset, soil borne disease is threatening winter squash produced in Oregon's Willamette Valley. Anecdotal evidence from growers and extension personnel suggest that total winter squash yield has fallen by 50% due to this disease. In 2014 and 2015, field surveys were conducted to determine the presence and diversity of fungi associated with commercial squash plants. A total of 2300 fungal isolates were subjected to DNA extraction and sequencing of *ITS* and *TEF1 alpha* genes. *Fusarium oxysporum* (26% of isolates) and *Plectosphaerella cucumerina* (21% of isolates) were consistently associated with diseased plants, as well as *Fusarium solani* and *Setophoma* spp. Cucurbit variety trials also were conducted and rated (1 to 4) for disease severity in roots, crowns and vascular tissue. Disease was most severe on winter squash (average rating 2.4) followed by watermelon, pumpkin and cucumber (ratings of 2.0, 1.9 and 1.3, respectively). Utilizing indicator species analysis, comparisons of isolated fungal communities confirmed *F. oxysporum* to be more prevalent in diseased fields compared to healthy fields ($P \leq 0.001$); however, *P. cucumerina* and *F. solani* were significantly associated ($P \leq 0.001$) with diseased fields on smaller-sized organic farms. Additional experiments on pathogenicity and host range of collected fungi are underway to better understand this disease complex.

Emerging root-infecting pathogens of marihuana (*Cannabis sativa*)

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Legalized cultivation of marihuana is encouraging the development of previously unreported plant diseases, of which root rot pathogens are an emerging threat to the industry. During 2013–2015, plants with symptoms of stunted growth, wilting and yellowing of foliage were sampled. Affected plants had brown discolored roots and evidence of root rot. Isolations from surface-sterilized tissues on various agar media revealed the following which were identified from ITS1-ITS4 rDNA sequence comparisons: *Pythium dissotocum* and *P. myriotylum*, and *Fusarium solani* and *F. oxysporum*. Pathogenicity tests were conducted on rooted cuttings grown in a hydroponic nutrient solution by adding pathogen inoculum (agar plugs or spore suspensions) and maintaining plants in a growth chamber set at 25 C and a 16-hr photoperiod for 10 days. Alternatively, rooted cuttings were inoculated directly with a mycelial plug placed on the main root and incubated in a moist environment for 7 days. Roots were examined for discoloration and root rot symptoms and rated on a scale of 1 (no symptoms) – 4 (extensive browning and rot). Re-isolations were made from diseased roots. All four pathogens caused symptoms of root discoloration and rotting to varying degrees. The most aggressive species were *P. dissotocum* and *P. myriotylum*, followed by *F. solani* and *F. oxysporum*. These root-infecting pathogens have the potential to spread extensively once introduced into a commercial growing facility.

Alternative management practices of *Pratylenchus penetrans* in red raspberry in the Pacific Northwest

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In recent years, red raspberry (*Rubus idaeus*) planting longevity in the Pacific Northwest has declined significantly. This decline has been partially attributed to severe pressure from the plant-parasitic nematode, *Pratylenchus penetrans*, which has been shown to contribute to reduced raspberry plant vigor, yield, and economic returns. Several commercial fumigant products are effective at suppressing *P. penetrans* populations, but efficacy is short-lived compared to the potential longevity of a perennial production system like florican red raspberry. This research was designed to test alternatives to chemical fumigation that have potential to suppress *P. penetrans* and enhance soil quality in red raspberry field production, in addition to maintaining commercial fruit yields. In one experiment, a two-year study was established in fall of 2014 evaluating the effects of nine cover crops planted in the alleyways of an established raspberry field in northwest, Washington. In the spring of 2015, *P. penetrans* populations in raspberry roots were highest in plots seeded with a perennial ryegrass mix, although this ground cover supported the lowest *P. penetrans* numbers in its own roots. A similar trend was observed in the fall of 2015. There were no significant differences in raspberry fruit yield among treatments the first summer after establishment. In a second experiment, a two-year study was established evaluating pre-plant treatments of brassicaceous seed meal (BSM) and chemical fumigation with or without root inoculum removal in a commercial red raspberry field in northwest Washington. The treatments were BSM + inoculum removal, full rate metam sodium, full rate metam sodium + inoculum removal, and half rate metam sodium; all were applied once at the beginning of the study. The first summer after planting, no significant differences in vegetative growth were observed among treatments. In the fall, *P. penetrans* populations were highest in raspberry roots planted in soil treated with BSM.

Effect of culture filtrates from four *Trichoderma* species on sporangia and zoospore production, and mycelial growth by *Phytophthora capsici*

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Sporangia and zoospores are important for the dispersal of *Phytophthora capsici* and other species of *Phytophthora*. Reduction in the production of these propagules, and in the vegetative growth of *P. capsici*, may lead to a reduction in inoculum potential and thereby result into a decrease in the incidence and severity of *Phytophthora* blight. Research was conducted to assess the effect of four species of *Trichoderma* (*T. asperellum*, *T. atroviride*, *T. harzianum*, and *T. virens*) on asexual reproduction and vegetative growth of *Phytophthora capsici*. Sporangia production and zoospore release were monitored on 1-cm mycelium plugs from a 5–7 day-old culture of an isolate of *P. capsici* placed in 25 ml conidia suspension (105 conidia/ml) of *Trichoderma*, and incubated at 27°C for 72 h. Additionally, mycelial growth of *P. capsici* was evaluated on 1-cm mycelium plugs placed in filter-sterilized (0.22 µm) culture filtrate of *Trichoderma* grown in potato dextrose broth (PDB) or in PDB amended with casamino acid for 5 days under agitation on a rotary shaker at room temperature (22–25°C). Sporangia production was abundant in the presence of all *Trichoderma* species, with 75 to 100% of the plugs showing sporangia production greater than 100 sporangia per microscopic view. However, with *T. virens*, 75 to 100% of plugs showed sporangia production at less than 50 sporangia per microscopic view. Zoospores were released in the presence of all *Trichoderma* species. There was a drastic reduction in growth of *P. capsici* in culture filtrate of *T. virens* on potato dextrose broth amended with casamino acids. Sporangia production and zoospore release were not negatively affected by the *Trichoderma* species examined in this study. Results also suggest that culture filtrates of *Trichoderma* contain factors that may affect the growth of *P. capsici*.

First report of *Urocystis camassiae* causing smut of Camas (*Camassia quamash*) in Idaho, United States

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Camas (*Camassia leichtlinii* and *C. quamash*; Asparagaceae) is a traditional native root vegetable important to the Native American tribes in western North America. Smutted stems and leaves of camas were observed and collected in Nez Perce County, Idaho, U.S.A., in June of 2015 in a wet meadow located at 46.1597 N, 116.7982 W. Only several plants in a population of more than a hundred were infected (less than 5% incidence). Lead colored, blister-like sori covered by the epidermis developed on infected stems and leaves, and gradually ruptured exposing the blackish brown, powdery mass of spore balls. Spore balls were subglobose, to ovoid, 22–30 × 20–50 µm, brown, composed of (1) 2–5 spores completely surrounded by a layer of smooth, hyaline-yellowish sterile cells. Teliospores were subglobose or ovoid, 11–15 × 12–17.5 µm diam., reddish brown with smooth ca. 1 µm thick walls. To confirm the morphological identification, specimens of *Urocystis* on *Camassia* from the United States National Fungus Collection (Herbarium BPI), including the type specimen on *C. quamash* from British Columbia, were examined and compared with the one from Idaho. Since there are no sequences of *U. camassiae* deposited in GenBank, the specimen from Idaho was used for the DNA extraction. NL1 and NL4 primers were used to amplify the large subunit ribosomal RNA gene region (LSU). The LSU sequence of *U. camassiae* showed 99.7–99.8% similarity with three other species of *Urocystis* (*U. carcinodes*, *U. ficariae*, *U. floccosa*) previously deposited in GenBank. A representative specimen was deposited in the WSU Mycological Herbarium. Camas smut is quite rare and has previously been reported only from British Columbia, Indiana, and Oregon. This is the first report of *U. camassiae* causing smut of camas in Idaho.

Using DNA extracted from soil to quantify inoculum densities of multiple soilborne pathogens in long-term cropping systems trials

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Root pathogens in rainfed field crops are typically studied in short-term trials having a focus on only a few pathogens. Studies rarely compare treatments in long-term trials where pathogens may have attained a state of equilibrium. Four experiments at the Columbia Basin Agricultural Research Center near Pendleton, OR are among the oldest field trials in North America; annual cereals (84 yr), wheat-fallow rotations with either residue and fertilizer treatments (84 yr) or tillage and fertilizer treatments (75 yr), and a wheat-pea rotation with tillage treatments (52 yr). Inoculum densities of 17 fungal and nematode pathogens were quantified during two years using DNA extracted from 500 g soil samples. Treatments strongly affected DNA concentrations (pg/g of soil) of *Bipolaris sorokiniana*, *Drechslera tritici-repentis*, *Fusarium culmorum*, *F. pseudograminearum*, *Gaeumannomyces graminis* var. *tritici*, *Helgardia* spp., *Phoma pinodella*, *Pratylenchus neglectus*, *P. thornei*, *Pythium* spp., and *Rhizoctonia solani* AG-8. Two of the pathogens had not been previously detected at this location. Positive and negative correlations occurred between species of *Pratylenchus*, between *Fusarium* and *Gaeumannomyces*, and between *Pratylenchus* and either *Fusarium*, *Gaeumannomyces*, *Pythium*, or *Rhizoctonia*. The paper at <http://dx.doi.org/10.1094/PDIS-09-15-1020-RE> describes this research and proposes that disease management can be improved by using DNA technologies to study pathogen dynamics in soil.

Western flower thrips can transmit *Tomato spotted wilt virus* from infected tomato fruits

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Tomato spotted wilt virus (TSWV) and other thrips-vectored tospoviruses have long been known to spread via plant propagation material. Global dissemination of tospoviruses has also been linked to transport of thrips-infested and virus-infected horticultural products through trade. The role of tomato fruits transported across state and national borders has not previously been examined as a means of virus spread or as a source for thrips acquisition of virus. Tomato fruits displaying symptoms consisting of chlorotic and necrotic rings, irregular blotches, fruit deformation and discoloration were observed for sale in several grocery stores in eastern Washington State. Many of these symptomatic fruits tested positive for TSWV in lateral flow immunoassays. First instar western flower thrips (*Frankliniella occidentalis*) larvae acquired TSWV from infected tomato fruits and transmitted the virus as adults to *Emilia sonchifolia* plants. Symptomatic *E. sonchifolia* plants were confirmed positive for TSWV by lateral flow immunoassays and sequence analysis of a portion of the nucleocapsid gene. These results have important implications in the dissemination of TSWV (and likely other tospoviruses) to new geographic areas by human-assisted transport of infected tomato fruits. Additionally, infected tomato fruits discarded by grocery stores could serve as a virus source for local thrips vectors to spread extrinsic virus isolates to susceptible plant species in the vicinity.

Classification powdery mildew fungi in Viet Nam and potential in bio-control by indigenously endophyte bacteria *Bacillus amyloliquefaciens* BA1

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Studies from the Viet Nam-Japan co-operation project funded by Ministry of Science and Technology (MOST) of Viet Nam in years 2013–2015 on classification powdery mildew fungi have already showed that there are 4 genus powdery mildews fungi (*Erysiphe*, *Golovinomyces*, *Podosphaera*, *Microdium*) with 9 species damaging in 11 host plant families with total of 27 main crops and other sub-host plants in Viet Nam. Improved management methods are needed to prevent yield losses and reduced agricultural product exports. Especially, the objection on finding out the new bio-product for prevention and suppression powdery mildew has priority over conventionally chemical methods due to its safety to animal, environment and human health as well. End of year 2013, endophyte bacteria *Bacillus amyloliquefaciens* BA1 was isolated from leaves of para-rubber tree. This endophyte has high activity in catalyzing chitin, beta-glucan and cellulose but its antagonistic activity against powdery mildew fungi is unknown. After tests in laboratory,

it is suggested that antibiotic compounds possibly found in cultured solution of *B. amyloliquefaciens* as inturin and surfactin may play a role in inhibition the germination of powdery mildew conidia. Later, it is asked whether bio-product BA1 made from this endophyte with 3.7×10^8 CFU/ml may have effect in bio-control powdery mildew fungi on several main crops in natural conditions of Viet Nam. As being expected, results from field trials with twice treatments by BA1 at 7% concentration in a period of 7 days proved a significant decrement in severity index of powdery mildew on mandarin and soybean obtained after spraying 14 days being 62.1% and 60.0%, respectively. Consequently, indigenously endophyte bacteria *B. amyloliquefaciens* BA1 may be used for making a bio-product with potential in control powdery mildew fungi disease on several main crops and plants in Viet Nam.

Dynamics of colonization of pome flowers by biocontrol strains of *Aureobasidium pullulans*, a yeast that effectively suppresses fire blight

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Aureobasidium pullulans is used as a biocontrol for fire blight protection in apple and pear. We assessed floral colonization of *A. pullulans* in orchards near Lakeport CA, and Medford and Corvallis OR. Blossom Protect, a mix of *A. pullulans* strains CF10 and CF40, and its acidic companion, Buffer Protect, were sprayed at 80% bloom. In late bloom, flowers were sampled, washed individually and dilution-plated onto potato dextrose agar. Filamentous yeasts resembling *A. pullulans* were selected and isolated into pure culture. *ITS*, *Br2*, and *Elo2* PCR-primers were used to confirm *A. pullulans*, while strain specific primers, *SCAR6* and *SCH3 RAPD*, were used to distinguish CF10 and CF40 from environmental strains. Overall, 1273 of 1414 isolates (90%) were *A. pullulans*, but only 28% were confirmed as CF10 or CF40. Incidence of CF10 or CF40 was higher after Blossom Protect treatment (46% of flowers) compared to no treatment (22%). On non-treated trees, most *A. pullulans* isolates (77%) were apparently environmental. Questions arise after contrasting the outstanding biocontrol efficacy of Blossom Protect with results of this effort: e.g., is *A. pullulans*' ability to suppress fire blight strain specific?, and does the acidic buffer recruit environmental *A. pullulans* to flowers? This study also is the basis of the recommendation to treat every row with Blossom Protect as the secondary flower-to-flower movement of CF10 and CF40 appears limited.

First occurrence of *Pseudomonas syringae* pv. *syringae* causing lesions on pumpkin fruit in WA, U.S.

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In Summer 2015, 'Cinnamon Girl' pumpkin grown in the Skagit Valley of western WA exhibited into sunken lesions. Small sections of fruit tissue were excised, macerated with sterile distilled water, and the macerate was plated onto NBY agar. Two colonies from fruit were purified in culture. DNA was extracted from each isolate, and the 16S rDNA region amplified and sequenced. Sequences exhibited 96–99% similarity with *Pseudomonas syringae* sequences (*PS*) in GenBank. Four-mo-old fruit were injected with a suspension of bacterial cells at a concentration of approximately 1×10^8 CFU/mL. Sunken lesions were observed after 4 days on fruit. To assess the *PS* pathovar, three housekeeping genes, *rpoD*, *gyrB*, and *gltA* were amplified and sequenced. Sequences were aligned with reference isolate sequences acquired from the PAMDB database, and an NJ analysis was conducted. The three pumpkin isolates clustered with sequences from *PSS* and *P. syringae* pv. *aptata* (*PSA*) isolates. Pathogenicity testing on 'Red Ace' beet seedlings, a host of *PSA*, revealed that none of the three WA isolates caused lesions on the beet seedling leaves, thus the pumpkin isolates were not *PSA*. *PSS* previously has been isolated from cucurbits. However, this is the first time *PSS* has been reported on pumpkin in WA.

Young vine decline DNA-microarray: A fast, specific, sensitive, and multiplexing diagnostics tool to assess the health status of grapevine nursery propagation material

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The young vine decline complex (YVD), associated with the grapevine trunk diseases black foot and Petri disease, is caused by a wide range of taxonomical unrelated fungi and it is responsible for substantial untenable economic losses to the grapevine industry worldwide. YVD fungi are known to occur in nursery propagated material and thus, detection prior to planting is critical to assure longevity of newly established vineyards. A YVD-DNA-microarray based on the reverse dot-blot hybridization and with capability to simultaneously detect 34 YVD fungi has been recently developed. The objectives of this study were i) to implement this diagnostic tool to evaluate the health status of grapevine propagated material and ii) to compare this technique against currently used detection tools for fungal pathogens at the nursery level. Two hundred ready to plant vines (grafted and self-rooted) were provided by two nurseries. For each plant, total DNA was obtained from roots, rootstock basal end, graft-union, and scion and processed with the DNA-microarray. The DNA-microarray successfully detected and identified several YVD fungi and showed *Phaeomoniella chlamydospora* to be the most prevalent YVD pathogen followed by *Cadophora luteo-olivacea*, *Phaeoacremonium minimum*, and *Ilyonectria* spp. Among all different plant parts, YVD pathogens were detected primarily in the basal end of the rootstock. The DNA-microarray was shown to be a much faster and a more accurate, and sensitive detection tool than other techniques used in the nursery such as traditional plating or single PCR.

Pomegranate dieback in California caused by *Lasiodiplodia gilanensis*

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The fruit and juice of Pomegranate (*Punica granatum* L.) have been shown to be an important source of nutrients and antioxidants, which has increased its popularity among consumers in the past few years. Pomegranate production in California has significantly increased during the last decade and over 33,000 acres are currently planted primarily in the Southern San Joaquin Valley. During the summer of 2009, decline of young pomegranate trees was observed in several orchards in Riverside County. Symptoms were characterized by lack of spring growth and overall dieback expressing throughout the entire tree or in one to several branches. Sunken areas were observed on trunks and branches of diseased trees and cross sections revealed cankers in the wood. A total of 25 symptomatic young trees (3- to 5-year-old) cv. 'Wonderful' were collected from five different orchards to determine the canker causal agent/s. White fluffy and fast-growing fungal colonies that became dark-green with age were consistently isolated from the cankered tissue. Morphological characters along with DNA sequence analyses of three gene regions (*ITS1-5.8S-ITS2*, β -tubulin and *TEF-1 α*) allowed the identification of *Lasiodiplodia gilanensis*. Koch's postulates were completed in 2 year-old pomegranate trees cv. 'Wonderful' planted at the UC Davis Plant Pathology Field Station. Twelve months after inoculation, *L. gilanensis* isolates showed to be highly virulent producing cankers of up to 21 cm in length. This study reports for the first time the botryosphaeriaceous fungus *L. gilanensis* as the causal agent of cankers and consequent dieback of pomegranate trees in California.

Variation of telial formation in the *Puccinia striiformis* f. sp. *tritici* population

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The wheat stripe rust pathogen, *Puccinia striiformis* f. sp. *tritici*, produces uredinia and telia on wheat plants. Telial formation after urediniospore production may contribute to the aggressiveness and fitness, and therefore affect the pathogen survival and disease epidemics. Significant increase in telial quantity and earliness of formation has been observed in fields. To determine differences in telial formation among the pathogen populations,

percentage of telial formation (proportion changed from uredinia) were observed on susceptible wheat plants 40 days after inoculation with urediniospores for 1002 isolates collected from 2013 to 2015 throughout the U.S. under controlled greenhouse conditions. Overall, 76% of the isolates were able to produce telia, ranging from 1 to 90% with an average of 20%. For different years, 79%, 52%, and 94% of the isolates produced telia with an average of 25%, 7% and 33% telial formation in 2013, 2014, and 2015, respectively. Differences in telial formation were observed among race groups, regions of collection, and months of sampling. Telial formation was more related to specific races than the numbers of virulence factors. Generally, isolates from the western U.S. produced few telia than those from the eastern U.S. Isolates collected in earlier season had low telial formation than those collected in the later season. The results may be useful for predicting the relative aggressiveness and fitness of races in the pathogen population.

Grape berry colonization and biological control of *Botrytis cinerea* by indigenous vineyard yeasts

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Botrytis bunch rot, caused by *Botrytis cinerea*, is one of the most important grape diseases in wet environments. Our specific objectives were to evaluate indigenous yeast strains for colonization ability of grape berries and antagonistic activity against *B. cinerea* *in vitro* and on wounded berries. In a previous study, fifty indigenous yeasts strains of 16 genera, isolated from Washington vineyards, were tested for suppressive activity. In the current study, colonization of eleven strains of *Aureobasidium pullulans*, *Candida saitoana*, *Curvibasidium pallidicorallinum*, *Metschnikowia chrysoperlae*, *Mets. pulcherrima*, *Meyerozyma guilliermondii*, *Saccharomyces cerevisiae* and *Wickerhamomyces anomalus* was quantified. Rapid and stable growth was observed for all yeast strains from 2 to 10 days after inoculation, with mild to moderate discoloration of the skin and internal tissues around the wound. *Aureobasidium pullulans* P01A006 showed effective inhibitory against nine *B. cinerea* isolates *in vitro*. The most extensive antagonism *in vivo* was shown by seven yeast strains, which consistently reduced symptom development and sporulation of *B. cinerea*. Strains of *Mets. pulcherrima* significantly controlled *Botrytis* bunch rot *in vivo*. Our findings indicate that indigenous yeasts that might contribute to the unique flavors of Washington wine could provide effective control against *B. cinerea*.

Pyramiding stripe rust resistance genes on wheat chromosomes 2B, 4B, and 7B

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Stripe rust, caused by *Puccinia striiformis* f. sp. *tritici*, is one of the most damaging diseases of wheat. The disease can be controlled by developing resistant cultivars. To develop wheat lines with two resistance genes on same chromosomes, crosses were made with wheat lines carrying linked resistance genes. Progeny lines with potentially pyramided resistance genes were selected through screening 165 to 726 F₂ plants and F₂₋₃ lines in each bi-parental cross for resistance to stripe rust, and the selected lines were further screened using molecular markers flanking each gene. F₆ lines advanced through single seed descent were developed and evaluated in fields in consecutive 4–5 years for confirming resistance and further selected based on desirable plant types. A total of 14 new wheat germplasm lines were developed, including 2 winter and 12 spring wheat lines, each carrying two linked resistance genes. The pyramid genes and their genetic distances (cM) in the spring lines are *Yr5/Yr53* (35.6) on chromosomal arm 2BL; *Yr50+Yr62* (27.1) on 4BL; and *Yr52+Yr59* (5.4), *Yr59+YrPI182103* (5.8), *Yr59+YrZh84* (6.0), *YrZh84+YrPI182103* (6.4), *Yr39+YrPI182103* (9.6), *Yr67+YrPI182103* (7.5), *Yr52+YrZh84* (12.2), *Yr39+Yr52* (31.2), *Yr39+Yr59* (41.1), and *Yr52+YrPI182103* (6.1) on chromosome 7BL. The two winter lines have resistance genes *YrZh84+YrPI182103* and *Yr50+Yr62*. These lines are useful for facilitating incorporation of pyramided resistance genes in linkage into wheat cultivars.

Stability and fitness advantages of metalaxyl-resistant isolates of *Pythium ultimum*

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Pythium damping-off of chickpea is an important disease, and has been traditionally managed through seed treatment using the fungicide metalaxyl. However, *Pythium* populations in the U.S. Pacific Northwest have developed resistance to this fungicide and metalaxyl is no longer effective. Conventional approach to managing fungicide resistance is to stop using the fungicide to let the resistant population disappear due to resistance-associated fitness cost. To evaluate this approach for managing the metalaxyl-resistant *Pythium*, we investigated the stability and saprophytic fitness of metalaxyl-resistant *Pythium* isolates. The stability of the metalaxyl-resistance trait was studied over 10 generations, and the relative competitiveness of the metalaxyl-resistant isolates was compared with that of metalaxyl-sensitive isolates for growth rate. Results showed that metalaxyl-resistant isolates consistently grew faster in terms of colony diameter (4.9 cm/day) and mycelia dry weight (24 mg/day) than metalaxyl-sensitive isolates (3.6 cm and 19 mg/day, respectively). The metalaxyl-resistant isolates remained resistant after 10 generations of sub-culturing in the absence of metalaxyl, as indicated by ED50 values. Because of the fast growth rate and stable resistance trait of the resistant isolates, simply stopping using metalaxyl is not a viable solution. More active management approaches such as using alternative fungicides are required to manage the metalaxyl-resistant *Pythium*.

Clonality within disease foci and field populations of *Sclerotinia sclerotiorum* causing basal stalk rot in sunflower seed crops in central Washington

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Sunflower seed production in the Columbia Basin of central Washington has grown from 20 ha in 2008 to >2,000 ha in 2015. White mold, caused by *Sclerotinia sclerotiorum*, is the main disease affecting this crop. Basal stalk rots are caused by direct infection from soilborne sclerotia, whereas mid-stalk and head rots are caused by ascospores. To assess the genetic diversity and population structure of *S. sclerotiorum* in sunflower seed crops, the fungus was isolated from basal stalk infections of sequentially infected plants (disease foci) in a field near Ephrata, WA (92 isolates from 26 foci) and a field near Odessa, WA (73 isolates from 26 foci). Genotyping the isolates with 8 simple sequence repeat markers revealed 28 and 19 multilocus haplotypes in the Ephrata (EP) and Odessa (OD) populations, respectively, with 7 common to both fields. A chi-square test of the observed frequency of isolate pairs from adjacent plants with the same haplotype compared to the expected frequency at random revealed that the spatial aggregation of haplotypes within disease foci was highly significant ($P = 10^{-34}$ and 10^{-32} for the EP and OD populations, respectively). Both populations were highly clonal, and analysis of molecular variance indicated no genetic differentiation between the populations ($P = 0.98$). The results suggest infection of adjacent plants within white mold foci in sunflower seed crops is likely from sclerotia of the same isolate or mycelium from the same sclerotium.

Evidence that specific rotation crops infected by *Verticillium dahliae* in Washington State do not serve as reservoirs for the mating type, *MAT1-1*

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Verticillium dahliae causes yield losses of a wide range of economically valuable wild and domesticated plants worldwide. The clonal reproductive model used to explain *V. dahliae* population structure has been challenged by evidence of recombination; however, both mating types requisite for typical recombination are not detected in the 1:1 ratio expected for a sexually reproducing organism. We tested the hypothesis that asymptomatic rotation

crops serve as reservoirs for the underrepresented mating type, *MAT1-1*. Fourteen rotation crops of potato and mint were collected from 21 fields in the Columbia Basin of Washington State. *Verticillium* spp. were detected from rotation crop stems sampled from 8 of 21 (38%) fields and the incidence of infected stems ranged from 1 to 63%. Recovered isolates were identified as *V. dahliae* and *V. isaacii* with morphological criteria and, for 2 isolates from each field, multilocus sequence typing using actin (*ACT*), elongation factor 1 alpha (*EF1-a*), glyceraldehyde-3-phosphate dehydrogenase (*GPD*), and tryptophan synthase (*TS*) sequence data. Consistent with the predominantly clonal reproductive model proposed for *V. dahliae*, the mating types of all isolates from rotation crops were *MAT1-2*. The absence of *MAT1-1* in rotation crops together with accumulating evidence of recombination support the presence of nonmeiotic forms of recombination or other, not yet sampled, hosts in ancestral and/or contemporaneous cryptic sexual recombination.

Adaptation to qualitative and quantitative host resistance by *Podosphaera macularis* in the Pacific Northwest

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Hop powdery mildew (caused by *Podosphaera macularis*) is an important disease of hop. Disease management is achieved with a variety of tactics, with host resistance being a favored method. However, durable resistance is challenging to identify because of the continuous adaptation of *P. macularis*. In 2012, powdery mildew developed in fields containing cultivars with the resistance factor R6 in Washington and Idaho. By 2013, R6-virulent strains of the fungus were reported in Oregon, becoming widespread in 2014. Concurrently, reports of powdery mildew on the cultivar Cascade, which is believed to possess quantitative resistance, have increased since 2012. Both R6-virulent and Cascade-derived strains of *P. macularis* are positive for the mating type *MAT1-1* and are able to overcome resistance genes Rb, R3, R5. R6-virulent strains also infect cultivars reported to possess R4 and R6, whereas virulence on R4 and R6 is rare in Cascade adapted strains. However, the latter demonstrate specialization to Cascade unlike R6-virulent strains. Surveys of commercial hop yards during 2012 to 2015 document that powdery mildew is now prevalent on cultivars possessing R6 resistance throughout the Pacific Northwest while powdery mildew on Cascade appears to be limited to Washington and Idaho at present. While resistance genes R1 and R2 remain effective in the Pacific Northwest, new sources of resistance and strategies for deployment are needed for the future.

Molecular mapping of stripe rust resistance genes in spring wheat line W18

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Stripe rust, caused by *Puccinia striiformis* f. sp. *tritici*, is one of the most important diseases of wheat worldwide, and is best controlled by growing resistant cultivars. Spring wheat line W18 is highly resistant to stripe rust. To map the resistance gene(s), W18 was crossed with Chuanyu 12, and 189 F₁₀ recombinant inbred lines (RILs) were developed. The tests of the RILs with races PSTv-37 and PSTv-40 at seedling stage under controlled greenhouse conditions indicated that W18 had two different genes for resistance to these races, respectively. The parents and RILs were also phenotyped in randomized field experiments at Chengdu, China from 2011 to 2013, and at Pullman and Mount Vernon, Washington in 2014. The RILs were genotyped with 700 simple sequence repeat (SSR) markers. The phenotype and genotype data mapped two major genes for stripe rust resistance, one in chromosome 1B near the centromere and the other in the long arm of chromosome 5D. The 1B gene was detected with race PSTv-37 in the greenhouse seedling test and the field tests in Chengdu and Mount Vernon, whereas the 5D gene was detected with race PSTv-40 in the greenhouse seedling test and in the fields at Pullman and Mount Vernon. The 1B gene is likely *Yr26*, whereas the 5D gene is new because its chromosomal location is different from previously reported *Yr* genes. The genes and closely linked markers identified in this study are useful for developing wheat cultivars with stripe rust resistance.

Suppressor of RNA silencing from wheat stem rust fungus, *Puccinia graminis*

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Plants use RNA silencing to defend against viral infections. To successfully infect plants, viruses encode proteins, called silencing suppressors (VSRs), which suppress RNA silencing as counter-defense strategies. Like viruses, bacteria and oomycete plant pathogens also have evolved to suppress RNA silencing to cause disease. Here, we report that one gene encoded effector-like protein from wheat pathogen *Puccinia graminis* suppresses RNA silencing in plants. This gene was induced in infected wheat leaves and includes two alleles which display 5 bp difference. The proteins encoded by the two alleles were found to partially suppress cell death caused by Pto (Y207D) when inoculated 24 hours prior to the *Agrobacterium* strain carrying Pto (Y207D). Furthermore, transient expression of this gene in *Nicotiana benthamiana* enhances plant susceptibility to a virus, Impatiens Necrotic Spot Virus (INSV). Our results suggest that pathogens use RNA silencing suppression as a universal strategy to cause disease.

Towards construction of genetic linkages for mapping virulence genes in *Puccinia striiformis* f. sp. *tritici*, the wheat stripe rust pathogen

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Puccinia striiformis f. sp. *tritici*, the wheat stripe rust pathogen, is a dikaryotic, biotrophic, and macrocyclic fungus. To determine the genetics of virulence and construct linkages for mapping and cloning virulence genes, we developed a segregating population of 120 isolates by selfing isolate 08-220 on barberry under controlled greenhouse conditions. The progeny isolates were phenotyped on a set of 32 wheat *Yr* single-gene lines and genotyped with 30 simple sequence repeat (SSR) and 23 single nucleotide polymorphism (SNP) markers. The progeny isolates were all avirulent to resistance genes *Yr5*, *Yr10*, *Yr15*, *Yr24*, *YrSP*, *YrTr1*, *YrCV*, *Yr45*, *Yr53*, and *Yr32*; and virulent to *Yr29* and *Yr41*, indicating that these avirulence or virulence loci were homozygous. Segregation of the 1:3 (avirulence : virulence) ratio was observed for the virulence loci corresponding to *YrAvS*, *YrA*, *Yr1*, *Yr6*, *Yr7*, *Yr8*, *Yr9*, *Yr25*, *Yr26*, *Yr27*, *Yr28*, and *Yr35*; the 1:15 ratio for *Yr21*, *YrTye*, *Yr44*, *Yr43*, and *Yr31*; the 7:9 ratio for *Yr17* and *YrExp2*; and the 9:7 ratio for *Yr2*. The ratios indicated that virulence can be controlled by one or two genes with different modes of inheritance and interactions. Linkages were found among virulence loci corresponding to *YrA*, *Yr1*, *Yr6*, *Yr7*, *Yr8*, and *Yr9* and marker *SNP340*. More markers are being identified for constructing more linkage maps. The results are useful for studying the host-pathogen interaction and improving control strategies using host resistance.

Presence of *Citrus psorosis virus* with watermelon ‘moon rings’ in Washington State

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Watermelon fruit with concentric ringspots, referred to as ‘moon rings’, have been observed in many parts of the world. While suspected of viral etiology, especially of a tospovirus or a potyvirus, none of them could be detected. In western Washington State, watermelon fruit with ring spots on the rind have been observed for at least 15 years. Symptoms appear late in the season as fruit start maturing. Recent testing for several tospoviruses and potyviruses by serological methods did not provide evidence of the presence of any of the tested viruses. We used next generation sequencing of the total

RNA extracted from the symptomatic portions of the rind followed by de novo assembly of the resulting sequences. BLASTX analysis to the non-redundant protein sequence NCBI database showed a major class of contigs representing the genome of *Citrus psorosis virus* (CPsV, genus *Ophiovirus*, family *Ophioviridae*). Several minor contigs had sequences representing a wide range of other virus families. CPsV is an important pathogen of citrus worldwide. The host range of CPsV is relatively narrow and is believed to be limited to Citrus species and their hybrids such as grapefruit, mandarin, orange and tangerine, lemon, pomelo and lime. The role of CPsV in the induction of 'moon rings' symptoms in watermelon remains to be investigated. Further research is underway to test additional samples and perform transmission experiments.