2014 Pacific Division Meeting Abstracts

Abstracts presented at the APS Pacific Division meeting in Bozeman, Montana, July 9–11, 2014. The abstracts are arranged alphabetically by the first author's name. Recommended format for citing division meeting abstracts, using the first abstract below as an example, is as follows: Batche, H., Valenzuela-Solano, C., and Hernandez-Martinez, R. 2014. Production of conidiospores in solid-state fermentation of *Trichoderma harzianum* and *T. asperellum* isolated from grapevine in Baja California. (Abstr.) Phytopathology 104(Suppl. 3):S3.179. <http://dx.doi.org/10.1094/PHYTO-104-11-S3.179>

Production of conidiospores in solid-state fermentation of *Trichoderma harzianum* and *T. asperellum* isolated from grapevine in Baja California

H. BATCHE (1), C. Valenzuela-Solano (2), R. Hernandez-Martinez (3)
(1) Department of Microbiology, Center for Scientific Research and Higher Education of Ensenada (CICESE), Ensenada, Mexico; (2) National Institute of Forestry, Agricultural, and Livestock Research (INIFAP), Ensenada, Baja California, Mexico; (3) Department of Microbiology, Center for Scientific Research and Higher Education of Ensenada (CICESE), Ensenada, Baja California, Mexico
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Trichoderma spp. are beneficial fungi that exhibits antagonistic behavior against plant pathogens. Due to its fast growth and excessive sporulation, a wide variety of substrates can be used to develop formulations for field trials. Grapevine trunk diseases fungi from Botryosphaeriaceae have been previously reported in Mexico. In an attempt to develop a control strategy, several *Trichoderma* isolates were obtained from field-grown grapevines and tested *in vitro*. *T. harzianum* MXT6 and *T. asperellum* MXTR0 were selected, for their antagonistic effect, to produce conidiospores in solid state fermentation. Rice, wheat, red millet grains or grapevine wood were combined with vermiculite and water (7:1:14). Autoclaved flasks of 130 ml were then inoculated with 10^5 spores, and grown under a 16-hour light/8-hour dark photoperiod at 28°C. At 7, 10 and 13 days, spores produced in 1 g of substrate were collected, 10 ml of 5% Tween20 in water was added, tubes were shaken at 120 rpm for 15 min and filtered through a 45 µm sieve. Recovered conidios were counted using flow cytometry with calcofluor 0.1%. Experiments were done by triplicate. In *T. asperellum* the highest yield (1.1×10^9 spores/g SD $\pm 6.3 \times 10^8$) was obtained in wheat, followed by red millet was 8×10^8 spores/g SD $\pm 1 \times 10^7$. For *T. harzianum* the highest production was also in wheat (1.4×10^9 spores / g SD $\pm 2.2 \times 10^8$) followed by red millet (1.2×10^9 spores/g SD $\pm 3.1 \times 10^8$). Produced conidiospores are being used in field trials. Since in grapevine wood *T. harzianum* and *T. asperellum* yields were 3×10^8 spores/g SD $\pm 8 \times 10^7$ and 5.2×10^8 spores/g SD $\pm 5.1 \times 10^7$ respectively and management of agricultural waste wood arising from grapevine pruning can reduce the source of infection of trunk diseases fungi, currently attempts to improve *Trichoderma* growth in this substrate are underway.

Transcriptomics and yeast-2-hybrid to identify *Bactericera cockerelli* genes important for *Candidatus Liberibacter solanacearum* circulative, propagative transmission

J. K. BROWN (1), T. J. Rast (1), T. W. Fisher (1)
(1) University of Arizona, Tucson, U.S.A.
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Ca. Liberibacter solanacearum (CLso) is the bacterial causal agent of the zebra chip and tomato vein-greening disease of potato and tomato, respectively. This fastidious bacterium is transmitted by the potato psyllid (POP) *Bactericera cockerelli* Sulc in a circulative, propagative manner. To identify and functionally characterize protein effectors involved in CLso invasion and establishment in the gut, and their presumed entry into the salivary glands, a combination of transcriptomic, proteomic, and yeast-2-hybrid (Y2H) analyses were employed. *In silico* profiling of expressed sequence tags (ESTs) from POP nymph and adult whole body, and adult midgut and salivary gland tissues identified transcripts (ESTs) and proteins with altered expression in response to CLso infection of whole insects and organs. Several of those transcripts were selected as bait for validation using the Y2H system, to detect protein-protein interactions. Those positive by Y2H were subjected to RNA-interference and qPCR to quantify the extent of knockdown. Emphasis has been on candidates of greatest interest and are those having a putative role in invasion, virulence, nutritional, and host immune responses that could facilitate CLso invasion/virulence, and multiplication and exit from the gut, circulation, and entry into the salivary glands.

The *Magnaporthe grisea* PTH11-Like G Protein-Coupled Receptor PLG1 is required for rice blast pathogenicity

K. D. CASTILLO (1), A. G. Tag (1), T. L. Thomas (1)
(1) Texas A&M University, College Station, TX, U.S.A.
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Magnaporthe grisea is the causative agent of blast disease, which destroys economically important crops such as rice, wheat and barley. Successful infection relies on the ability of the fungus to sense both environmental and host surface signals at different stages of the disease cycle. Surface sensing is carried out in part by G-Protein Coupled Receptors (GPCRs). This project is focused on the functional analysis of PLG1, a member of the *M. grisea* PTH11 class of GPCR-like proteins. Targeted deletion of *PLG1* generated a mutant that is unable to infect intact rice and barley plants, but is capable of invasive growth on wounded leaves. *M. grisea* requires a hard and hydrophobic surface to develop necessary infection structures. Infection-related morphogenesis assay using a hydrophobic membrane showed that germ tube hooking and apical swelling occurred in the $\Delta plg1$ mutant. However, the $\Delta plg1$ mutant strain developed melanized appressoria, the main infection structures at a lower frequency (~10%) compared to the wild-type 70-15 strain. When $\Delta plg1$ conidia were incubated on a hydrophobic membrane in the presence of inducers, either cAMP or 1,16-hexadecanediol (HDD), a significant increase in the formation of appressorial-like components was observed after 24 hours. With cAMP or HDD treatment of $\Delta plg1$ conidia on barley leaves, pathogenicity of wild-type level was not restored, indicating possible defects in the appressoria which developed in the presence of inducers. Overall, these results suggest that as a predicted receptor, PLG1 may not be necessary for penetration or invasive growth, but may function during the pre-infection process possibly by interacting with a ligand that enables the fungus to recognize the plant host or any suitable

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surface. Further characterization of PLG1 is being done to confirm its interaction with G proteins and identify its possible downstream targets.

A complex of *Botrytis* species associated with gray mold on peonies

G. A. CHASTAGNER (1), K. Coats (1), A. DeBauw (1), A. Garfinkel (1), P. S. Holloway (2)

(1) Washington State University, Puyallup, WA, U.S.A.; (2) University of Alaska, Fairbanks, AK, U.S.A.

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Gray mold, caused by *B. paeoniae* or *B. cinerea*, is the most important disease of peonies. These pathogens cause leaf spots and blights, reduce yields and can cause complete pre- and postharvest destruction of cut flowers. Although gray mold on peonies has been known for over 100 years, very limited information is available relating to the development of this disease. During 2012 and 2013, a survey of over 30 fields in AK and WA was conducted to better understand the prevalence of *B. paeoniae* and *B. cinerea* on this crop. *Botrytis* species were identified based on the ITS and G3PDH regions of sequenced DNA. The results indicate that there is a complex of *Botrytis* species on peonies in these states. In addition to *B. paeoniae* and *B. cinerea*, three additional species were found. This includes: *B. pseudocinerea* which is a newly-described species with natural resistance to fenhexamid fungicide; an unnamed species that is closely related to *B. calthae*, which has only been reported on marsh marigolds; and an unnamed species that is closely related to *B. convoluta*, a pathogen of rhizomatous iris. Given the immense variability in spatial and temporal distribution and fungicide resistance among species of *Botrytis*, the presence of previously unknown *Botrytis* species in the peony production systems warrants a thorough investigation into the population structure to provide growers with the appropriate tools to manage gray mold.

A multiplex RT-PCR assay for the detection and accurate identification of the complex strains and recombinants of Potato virus Y

M. CHIKH-ALI (1), S. M. Gray (2), A. V. Karasev (1)

(1) University of Idaho, Moscow, ID, U.S.A.; (2) USDA-ARS, Ithaca, NY, U.S.A.

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Potato virus Y (PVY) has a complex strain structure of nine strains and additional unclassified recombinants that vary in their genome structures, phenotypes and their economic importance. The strain composition of PVY differs depending on the geographic area and the set of predominant potato cultivars. The lack of the ability to identify specific PVY strains complicates any efforts to eradicate and control any specific strain. This led to deal with PVY as a virus complex when plan control program which in turn helped to build up the population of some significant and most damaging PVY strains in potato production areas. Multiple RT-PCR assays were developed but most of them were not comprehensive enough and focused on a certain fraction of PVY strains or were unable to differentiate certain strain types with similar genomic structures. In the current study, a multiplex PCR assay was developed in which a set of 12 primers targeting five recombination junctions of PVY genome were designed or implemented from previous studies. This RT-PCR assay was able to detect and identify fourteen strains and recombinants of PVY simultaneously including PVY^O, PVY^N, PVY^{NA-N}, PVY^Z (syn. PVY^{NTN-A}), PVY^E, PVY-NE11, PVY^{N-Wi}, PVY^{N-O}, PVY^{NTN-B}, PVY^{NTN-NW} (SYR-I, SYR-II and SYR-III) and other rare types like 261-4. The sophisticated primer set and multiple RJs targeted by this assay insured the flexibility and efficacy of this assay to differentiate some significant PVY strains such as PVY-NE11 and PVY^E even they were not specifically targeted when this assay was developed in the first place. The reliability of this multiplex RT-PCR assay was tested using well-characterized reference isolates represent PVY strains and recombinants. The effectiveness of this RT-PCR was confirmed when used for both conventional RNA extracts and immunocaptured (IC) virus particles from potato and tobacco samples makes it applicable and suitable for large-scale routine virus testing and typing. It was successfully used to study PVY populations in several countries with drastically different populations of PVY strains circulating in potato, including Japan and the United States.

Epidemiology of spinach downy mildew in coastal California

R. A. CHOUDHURY (1), S. T. Koike (2), K. V. Subbarao (3), S. J. Klosterman (4), N. McRoberts (1)

(1) University of California Davis, Davis, CA, U.S.A.; (2) University of California Cooperative Extension, Salinas, CA, U.S.A.; (3) University of California Davis, Salinas, CA, U.S.A.; (4) USDA ARS Salinas, Salinas, CA, U.S.A.

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Downy mildew pathogens thrive in humid and low light environments, and can disperse asexual spores aerially over long distances. A better under-

standing of the conditions that lead to disease might help to improve the timing of protective fungicide applications and to better deploy resistant crop lines. Spinach downy mildew, caused by the oomycete *Peronospora effusa*, is the most serious threat to fresh market spinach production in California. New pathogenic race types have overcome a series of resistant lines and threaten the long-term stability of the industry. A quantitative PCR (qPCR) method was used in conjunction with spore impaction samplers to monitor the amount of *P. effusa* present in the Salinas Valley. Four sites throughout the valley were monitored in 2013. In addition to spore trapping, weekly disease incidence ratings were performed using cluster sampling near the trap sites. In 2013, spore density increased exponentially over the course of the year as more spinach was planted. Autocorrelation analysis revealed weak periodicity in spore density numbers, possibly due to the life cycle of the pathogen. Noise in the spore trap data suggests that environmental fluctuations affect local spore density. A logistic regression was used to explore the connection between weather data and DNA copy number derived from the qPCR. Overall, the results of the logistic regression suggest that strong morning winds and cool afternoon and evening temperatures with low relative humidity increased the number of spores detected. Disease incidence ratings from the field closely matched spore trap density data for 2013. These findings increase our understanding of the epidemiology spinach downy mildew and can contribute to development of disease management strategies.

Screening carrot germplasm for resistance to *Xanthomonas hortorum* pv. *carotae*

C. E. CHRISTIANSON (1), S. S. Jones (1), L. J. du Toit (1)

(1) Washington State University Mount Vernon NWREC, Mount Vernon, WA, U.S.A.

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Xanthomonas hortorum pv. *carotae* causes bacterial blight of carrot and is readily seedborne. Genetic resistance is limited in commercial cultivars, and there has been little public research on screening for resistance. Carrot plant introduction (PI) lines (n = 66) from the United States Department of Agriculture (USDA) National Plant Germplasm System, two inbred lines from the USDA Agricultural Research Service, and 17 cultivars were screened for resistance to *X. hortorum* pv. *carotae* in a greenhouse in each of 2012 and 2013. Evaluations were based on the percentage of foliage that developed symptoms as well as population (CFU/g dry foliage) of the pathogen detected on a semi-selective agar medium in both trials. In 2012, foliar disease severity 6 weeks post-inoculation (wpi) ranged from 0 to 50% blighting (mean $\pm$ SE of $8.8 \pm 0.4\%$). The pathogen population ranged from 1.38×10^4 to 3.28×10^{11} CFU/g dry foliage ($8.16 \times 10^9 \pm 1.07 \times 10^9$ CFU/g) for individual plants. Eight putative resistant PI lines and five highly susceptible PI lines selected from the 2012 screening were evaluated again in 2013 along with an additional 2 PI lines, 12 cultivars, 2 inbreds, and 16 carrot wild relatives. In the 2013 screening, severity of foliar blighting 6 wpi ranged from 0 to 90% ($11.8 \pm 0.4\%$). The pathogen population ranged from 4.90×10^4 to 1.30×10^{11} CFU/g dry foliage ($1.00 \times 10^{10} \pm 5.29 \times 10^5$ CFU/g) for individual plants. PIs 418967, 432905, and 432906 were the most resistant based on the pathogen population detected. There was little resistance displayed by carrot wild relatives to *X. hortorum* pv. *carotae*. Resistance from selected PIs will be integrated into commercially-acceptable carrot inbreds (USDA ARS inbred lines A2566 and A0493) for release to public and private carrot breeding programs.

Monitoring prevalence of *Liberibacter solanacearum* and haplotypes of its insect vector *Bactericera cockerelli* in Idaho potato fields

J. DAHAN (1), B. D. Thompson (1), E. J. Wenninger (2), N. Olsen (2), A. V. Karasev (1)

(1) University of Idaho, Moscow, ID, U.S.A.; (2) University of Idaho, Kimberly, ID, U.S.A.

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The bacterium *Candidatus* *Liberibacter solanacearum* (Lso) is the causal agent of the zebra chip disease in potato (ZC), an emerging threat to potato production which reduces yields and tuber quality. This phloem-restricted bacterium can also infect a variety of *Solanaceae* species and close relatives. Based on ribosomal genes sequences, five haplotypes of Lso have been described, among which only 2 (A and B) are associated with ZC in potato. Infection of potato plants occurs through the psyllid *Bactericera cockerelli*, for which 4 different haplotypes have been described, presenting different preferential geographical repartitions in the United States. To monitor progression of ZC disease, large-scale surveys were conducted in Idaho during the 2012 and 2013 potato growing seasons by collecting ca. 1,200 potato psyllids each year. These psyllids were tested for the presence of Lso by PCR and the hapotype of the bacteria was determined; a subset of insects was haplotyped using the high-resolution melting (HRM) analysis. Whereas

26% of the psyllids were Lso-positive in 2012, Lso was hardly detectable in 2013 (2.8% positive psyllids). Moreover, whereas all of the 2012 tested samples were of A-type Lso, the first occurrence of the B-type Lso was detected during the 2013 season, constituting the first report of the presence of this Lso haplotype in Idaho. *B. cockerelli* populations were also slightly different, with the Central haplotype being prominent during 2012 and rare during 2013. These results highlight the dynamics of Lso and *B. cockerelli* populations and call for additional studies on the importance of interactions among psyllid and bacterium haplotypes with respect to ZC occurrence.

Environmental factors influencing airborne ascospore concentrations of ergot in perennial ryegrass and exploratory development of a predictive model

J. K. DUNG (1), S. C. Alderman (2), D. L. Walenta (3), P. B. Hamm (4)
(1) Oregon State University, Madras, OR, U.S.A.; (2) USDA-ARS, Corvallis, OR, U.S.A.; (3) Oregon State University, LaGrande, OR, U.S.A.; (4) Oregon State University, Hermiston, OR, U.S.A.
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Ergot, caused by the fungus *Claviceps purpurea*, is an important disease affecting perennial ryegrass (PRG) seed production in the Columbia Basin of eastern Oregon. The objectives of this study were to identify environmental factors that contribute to ascospore production and develop a model to predict ascospore production events. Airborne ascospore concentrations were monitored in one PRG field in 2012 and two PRG fields in 2013. Environmental data were compiled from a nearby weather station in Hermiston, Oregon. Non-parametric regression, univariate analysis, and box-and-whisker plots identified several trends in ascospore concentrations and environmental variables. Minimum air temperatures between 5 and 12°C, soil temperatures between 14 and 22°C, daily soil degree days between 6 and 11, and a mean dew point between 3 and 10°C were significantly ($P < 0.05$) correlated with peak spore production. Based on non-parametric local regression surfaces and distributions of environmental variables, point values were assigned to the following environmental variables: minimum air temperature (MinAirT), maximum, minimum, and mean soil temperature (MaxSoilT, MinSoilT, and MeanSoilT), soil degree days (SoilDD), and mean dew point (MeanDew). When used to predict the appearance of ≥ 1 ascospore/day, all environmental variables except MinAirT resulted in significant chi-square values and ranged in accuracy from 60 to 83%. All environmental variables resulted in significant chi-square values when used to predict the occurrence of ≥ 100 ascospores/day with an accuracy of 60 to 89%. An environmental favorability index (EFI) model was developed that included MaxSoilT, MinSoilT, SoilDD, and MeanDew. An EFI value of 2 predicted the occurrence of ≥ 1 ascospore with an accuracy of 81% while an EFI value of 3 predicted the occurrence of ≥ 100 ascospores with an accuracy of 90%. These results suggest that predictive models can be used to forecast ergot ascospore production events in PRG seed production systems of the Columbia Basin for improved control.

Western IPM Center grant program

J. FARRAR (1), M. Baur (1), S. Elliott (1)
(1) Western IPM Center, University of California Division of Agriculture and Natural Resources, Davis, CA, U.S.A.
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The Western IPM Center is one of four regional centers funded by the USDA to promote IPM principals by building multi-state partnerships and communication networks linking a diverse audience that includes researchers, growers, commodity organizations, regulators, environmental groups, pest control professionals and others. The Center promotes the application of IPM principals to pest-management problems in agriculture, communities and natural areas. The Center's goals are to improve the cost-benefit analyses of adopting IPM practices and to reduce the environmental and human health risks associated with managing pests. The Center grant program provides \$15,000 to \$30,000 one-year awards in four categories – project initiation, work groups, outreach and implementation, and IPM planning documents. Project Initiation proposals are to begin new IPM project ideas, such as proof-of-concept projects and preliminary experiments for data to support larger grant proposals. IPM Work Groups support bringing together diverse, multi-state groups to collaboratively address a regional IPM priority. Outreach and Implementation projects are to build on previous IPM research and development projects by providing outreach to stakeholders to encourage implementation of IPM practices. IPM Planning Documents projects are to support development of Pest Management Strategic Plans, IPM practices evaluations, and similar IPM planning documents. The RFA is typically released in the fall and is open to applications from universities, extension, state and local governments, commodity and other industry groups, and non-governmental organizations.

Proposed terminology for pseudosclerotial stages of *Monilinia vaccinii-corymbosi*

J. FLORENCE (1), J. Pscheidt (1)
(1) Oregon State University, Corvallis, OR, U.S.A.
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Mummy berry, caused by *Monilinia vaccinii-corymbosi*, is an economically important disease of blueberries throughout North America. From late summer until early spring, pseudosclerotia reside on or in the soil surface layer of blueberry fields. Environmental factors result in the coordination of apothecial production with vegetative bud break, the beginning of blueberry bush susceptibility. *M. vaccinii-corymbosi* then replaces blueberry fruit with a pseudosclerotium. A comparison of research throughout North America exposed contradicting terms and overlapping definitions for pseudosclerotial stages, while some stages were unnamed entirely. A developmental classification scheme was derived from observations of naturally conditioned pseudosclerotia over a one-year period in Corvallis, Oregon. The proposed pseudosclerotial stages include invasion, maturation, dormancy, germination, emergence, differentiation, and sporulation. Invasion occurs when mycelium of *M. vaccinii-corymbosi* invades the carpels of the berry. Invasion is followed by maturation, which is characterized by dehydration of infected berries turning shriveled, pinkish-purple and hardening. The pseudosclerotium then sheds the outer blueberry tissue during dormancy, revealing melanized hyphae. Germination occurs after pseudosclerotia accumulate chill hours (hours with a mean temperature $< 7.2^{\circ}\text{C}$) and form a stipe initial, or a hardened protrusion measuring < 5 mm. During emergence, the stipe initial continues to grow, between 5–15 mm, and has been referred to as a fundament. During differentiation, the hardened stipe elongates, becomes fleshy, and produces a tip indentation. Laboratory observations have shown that these stipes can elongate to a length of over 10 cm. Sporulation occurs with adequate degree-days (base 7.2°C) when the tip indentation furrows to become an apothecial cup, bearing mature ascospores that will infect the susceptible bud tissue of blueberry bushes. The standardization of these terms will allow researchers to better interpret results for their unique geographic location and climate and compare findings to other regions of the world.

Substrates from soybean and corn influence pathogenesis and growth of *Fusarium virguliforme*

G. FREED (1), D. Malvick (2)
(1) Washington State University, Pullman, WA, U.S.A.; (2) University of Minnesota, St. Paul, MN, U.S.A.
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Soybean sudden death syndrome (SDS), caused by *Fusarium virguliforme*, requires exogenous nutrients from crop residues or germinating seeds for pathogenicity. This study examined the role of inoculum rate, enhanced by various soybean and corn crop residues, to evaluate their combined influence on the pathogen and its capacity for early root infection, the driver of SDS severity. MN1606 and MN1410 seeds, moderately resistant and susceptible cultivars respectively, were planted in a soil mixture blending one inoculum rate (0, 10^1 , 10^2 , or 10^3 conidia/cc soil) with one of seven crop residue treatments (none, soybean seed, corn seed, sorghum seed, corn stalk, corn root, or soybean stem). Incidence of early root rot severity measured 15 dai corresponded directly to foliar disease severity assessed at 50 dai for all treatment combinations. Corn and sorghum seed treatments developed SDS symptoms at lower inoculum rates compared to the more cellulosic soil amendments of stems and stalks. Plants grown with no added substrate exhibited little or no disease severity. The effect of seed exudates was examined from four soybean cultivars and one corn hybrid. Seed exudates were collected at ten time points (0, 2, 4, 6, 8, 26, 32, 48, 62, and 72 hours) during germination. Filter-sterilized exudates were aliquoted into 96-well microplates and inoculated with 10^2 macroconidia of four *F. virguliforme* isolates. Fungal growth (OD) was assessed using a BiologTM plate reader. Exudates of MN1606 collected at the time of radicle emergence (26 hai) supported significantly ($p < 0.001$) more fungal growth compared to all exudates tested. Seed exudates released at the time of radicle emergence have a dramatic influence on SDS severity. Useful information about the source of exogenous substrates may be obtained through understanding the timing of the release of seed exudates during germination, which enhance the activation of *F. virguliforme* growth in the surrounding environment.

Loss of potato tuber yield and quality as a result of infection by *Potato virus Y* under western Washington growing conditions

D. A. INGLIS (1), B. Gundersen (1), C. Benedict (2), D. McMoran (3)
(1) WSU Mount Vernon NWREC, Mount Vernon, WA, U.S.A.; (2) WSU/Whatcom Co. Extension, Bellingham, WA, U.S.A.; (3) WSU/Skagit Co. Extension, Burlington, WA, U.S.A.
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Potato tubers with cracks in the shape of canoe-shaped crevices recently have been reported on some specialty potato cultivars in western Washington. Preliminary evidence indicates that a primary source of this defect is PVY-infected plants, although environmental conditions and herbicide injury also cause similar symptoms. A greenhouse study addressed whether cracked tubers can lead to PVY-infected plants. Four reps of three tubers of Chieftain with canoe-shaped cracks (alleged virus infected) were used as seed tubers, and planted in 3.8 L pots in potting mix on 12 Apr. Plants rated 4 wk later on 18 May, showed no virus symptoms. Leaves submitted to Agdia (Elkhart, IN) for general potato virus screen on 12 Jun, tested negative for six viruses including PVY, and all progeny tubers harvested on 18 Jun were free of cracks. A field study with PVY-infected Chieftain planted on 3 Jun addressed whether PVY-infected plants can lead to cracked tubers. Three each of healthy, suspect and symptomatic plants in four reps, were selected and flagged, and PVY presence/absence verified using Agdia ImmunoStrip test kits on 20 Aug, at 0, 92 and 100 percent, respectively. Tubers harvested on 29 Aug were kept separate and rated; those discolored, malformed and/or cracked were 12 for healthy, 41.5 for suspect, and 98.5 percent for symptomatic. Cracked tubers alone were 83.5 and 91.5 percent higher for suspect and symptomatic relative to healthy. Marketable tuber weight differed significantly ($P < 0.0001$) between healthy, suspect and symptomatic, and yield was reduced 63 and 99 percent for the two latter. Tuber malformation and cracking associated strongly with PVY infection, but not exclusively. A greenhouse grow-out of the progeny tubers and PVY strain identification are in progress.

Recombinant strains of *Potato virus Y* - a new problem in potato

A. V. KARASEV (1)

(1) University of Idaho, Moscow, ID, U.S.A.

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Over the past 10–15 years, recombinant strains of *Potato virus Y* (PVY) spread throughout the potato production states in the U.S. These recombinant strains are often associated with tuber quality problems, in addition to the yield reduction. Origins and evolution of these recombinant strains of PVY are not completely understood at the moment. Resistance to some recombinant and non-recombinant strains can often be found in commercial cultivars grown in the U.S., while some recombinants can overcome most of the resistance genes in potato known today. More research would be necessary to reveal driving forces behind quick changes in composition of PVY strains circulating in potato in North America, related to host reactions in different cultivars and differences in vector transmission efficiencies.

Foamy bark canker: A new disease on coast live oak (*Quercus agrifolia*) in California

S. C. LYNCH (1), D. H. Wang (1), P. F. Rugman-Jones (1), J. S. Mayorquin (1), R. Stouthamer (1), A. Eskalen (1)

(1) University of California Riverside, Riverside, CA, U.S.A.

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Since 2000, it has been well documented that fungal pathogens and insects have caused significant decline of oak species (*Quercus* spp.) throughout California. Declining coast live oak (*Quercus agrifolia*) trees have recently been found throughout urban landscapes in Los Angeles, Orange, Riverside, Santa Barbara, Ventura and Monterey counties. The fungal species *Geosmithia pallida* was recovered from necrotic plant tissues in association with the western oak bark beetle *Pseudopityophthorus pubipennis* (Coleoptera: Curculionidae: Scolytinae) on declining trees. Symptoms include branch dieback and ultimately tree death. Symptoms on the trunk and primary branches include wet discoloration seeping through pin-needle sized entry/exit holes. Peeling back the outer bark reveals phloem necrosis surrounding each entry/exit hole. As the disease advances, a reddish sap oozes from the entry/exit hole, followed by a prolific foamy liquid. Fungal colonies and conidia of *G. pallida* were additionally observed within the gallery walls produced by the beetle. The fungus and beetle were identified based on morphological characteristics and BLAST search comparisons in GenBank using amplified portions of the rDNA internal transcribed spacer (ITS) and mitochondrial COI genes. Pathogenicity tests were performed by inoculating 12 27.0 cm long detached coast live oak shoots with a spore suspension of *G. pallida* and sterile distilled water for controls. After four weeks of incubation in a moist chamber at 25°C, the lesion length produced by *G. pallida* averaged 8.3 cm and was significantly longer (ANOVA; $P < 0.0001$) than the control (average 0.4 cm). *G. pallida* was re-isolated from all the inoculated plants. The western oak bark beetle is a common secondary beetle native to California, and is not previously associated with a specific fungus. To our knowledge, this is the first report of *Geosmithia pallida* causing foamy bark canker in association with the western oak bark beetle on coast live oak in California.

Breeding for root lesion nematode resistance in Montana winter wheat

D. MAY (1), P. Bruckner (1), A. Dyer (1), J. Berg (1), D. See (2)

(1) Montana State University, Bozeman, MT, U.S.A.; (2) Washington State University, Pullman, WA, U.S.A.

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Root lesion nematodes (*Pratylenchus* spp.) present a serious challenge to dryland wheat production worldwide. Development of resistant cultivars would provide great economic benefit to growers. From 2012–2013, a set of backcross lines (MT08185/MT08184/Persia 20) was screened twice for resistance to *P. neglectus*. Progeny and parent lines were grown in infested soil for 16 weeks. Nematodes were then extracted from roots of individual plants and counted to obtain per plant final populations. ANOVA results from the 2013 screen showed significant differences in mean *P. neglectus* populations among lines ($p < 0.01$). The median final population of susceptible parent MT08184 was an estimated 4.0 times greater than that of resistant parent Persia 20. A 2013 field trial in the absence of root lesion nematodes indicated reductions in grain yield, volume weight, and protein were not associated with resistance. Identification of QTL for resistance to root lesion nematodes will facilitate marker-assisted introgression of resistance genes in a backcross-breeding program. Single marker analysis of 104 genome-wide SNPs was performed to identify genomic regions associated with resistance to *P. neglectus*. The analysis identified putative marker-trait associations on chromosomes 1A, 1D, 2B, 5B, 5D, 7A and 7D (all $p < 0.05$). These potential QTL regions will be tested in analysis of phenotypic and genomic data obtained from 200 BC₁F₃ (MT08185/MT08184/Persia 20) progeny, and 228 F₂ progeny derived from a separate cross (Persia 20/McNeal).

Functional microclimate size in powdery mildew epidemiology and impacts for forecasting and disease control

B. MCGUIRE (1), W. D. Gubler (1)

(1) UC Davis, Davis, CA, U.S.A.

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Powdery mildew causes major damage to wine grapes in California, and is one of the most expensive diseases to treat (1, 3, 4). Typical treatment consists of sulfur applications throughout berry development. The Gubler-Thomas model is used to estimate powdery mildew risk based on temperature and moisture changes, in order to identify intervals in which to treat for disease (2). The model cannot predict risk without accurate weather information and these weather station costs are often prohibitive to many growers. These growers instead use calendar-based fungicide sprays or risk estimated from regional weather stations—these methods typically involve more frequent sulfur application and can be inaccurate (5, 6). Virtual weather networks can interpolate data from existing, observing weather stations and the data is freely available online offering an alternative to in-field weather stations to determine powdery mildew risk and make recommendations. This study examines the success of virtual weather networks in calculating risk as compared to an observing weather station in ten vineyards in Lodi, California and the subsequent recommendations these risk-estimation methods have on powdery mildew control. Observing stations (Adcon Telemetry) were assembled and deployed in each of the 10 sites. Virtual models did not include output from the observing stations in their calculations. Results indicate that in some sites nearer to water, the virtual station weather network indicates lower risk than the observing station, though in dry areas the virtual and observing indices converge over 80% of the time, giving essentially identical application recommendations. Evidence further suggests that humidity with little percent change throughout the day contributes to powdery mildew growth, that high temperatures may not limit growth as much as previously thought, and that certain microclimate features, even furrow irrigation, may be enough to significantly change local humidity to increase powdery mildew propagation.

Efficacy of various potential biological control agents for the control of *Fusarium euwallaceae* – a symbiotic fungus of the Polyphagous Shot Hole Borer (*Euwallaceae* sp.)

F. NA (1), D. Wang (1), M. Twizeyimana (1), J. Mayorquin (1), A. Eskalen (1)

(1) University of California Riverside, Riverside, CA, U.S.A.

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Polyphagous Shot Hole Borer (*Euwallacea* sp.) is an invasive species of ambrosia beetle newly introduced to California that are able to colonize a wide range of host trees in California and transmit a species of pathogenic fungus. Among the host trees are avocado (*Persea americana*), an important commercial crop in California, and California sycamore (*Platanus racemosa*), a common landscape tree. The wide distribution of host plants and the current lack of effective disease control strategies has resulted in uncontrolled proliferation of the beetle-fungus complex in California. The species of symbiotic fungus is the pathogen *Fusarium euwallaceae*, which causes *Fusarium* dieback and is used by the beetles as their sole source of food. In this study, potential

biological control agents were evaluated for their effectiveness in inhibiting the growth of *Fusarium euwallaceae* in vitro. Bacteria and fungi were collected from xylem of disease-resistant members of known host species by obtaining and surface sterilizing cylindrical cores of xylem, then grinding the xylem in water to make a suspension. The suspension was incubated for one day in room temperature then spread onto fungal and bacterial media to cultivate and isolate endophytic fungi and bacteria. The fungi and bacteria were screened for their ability to inhibit *F. euwallaceae* by simultaneously culturing them with mycelial plugs of the *Fusarium* on the same nutrient agar plates. Zones of inhibition between the endophytes and *F. euwallaceae* were noted and rated depending on the sizes of the zones. Commercial biological control agent *B. subtilis* QST713 (CEASE®), as well as 13 isolates of bacteria obtained from xylems of *Persea americana* and *Platanus racemosa*, were found to inhibit *F. euwallaceae*. Further screening of additional endophytes from other susceptible hosts, the endophytes' effectiveness against other beetle-associated fungi, and greenhouse studies evaluating the potential biological control agents in planta are currently underway.

Problems confirming zebra chip disease in potatoes

C. NISCHWITZ (1), B. Rhoads (1), M. McCullough (1), C. Dhiman (1)
(1) Utah State University, Logan, UT, U.S.A.

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Zebra chip (ZC) disease is an important disease of potatoes and has been reported from every state bordering Utah but not in Utah. In February 2013, a Yukon Gold potato sample from storage with symptoms resembling zebra chip disease was sent to the Utah Plant Pest Diagnostic lab. Total DNA was extracted from one potato and PCR was conducted with primer sets specific for zebra chip OA2/OI2c and ZCF/OI2c and the Qiagen Taq PCR Core kit. All the samples were negative. Another diagnostic lab could not confirm the samples either. However, positive controls were positive. We then used primers Lso TX16/Lso TX 23, developed in Texas, in combination with New England Biolabs Phusion reagents. The sequence of the 383-bp band obtained from the symptomatic potato sample matched a Candidatus *Liberibacter solanacearum* sequence from New Zealand 99%. When using the Lso TX 16/Lso TX 23 primer set with the Qiagen PCR Taq Core kit only the positive control (potato psyllid) produced a band, but the potato sample did not. Our results indicate that the specific combination of primers and reagents is critical for confirming zebra chip disease in potato samples and must be considered before samples are dismissed as negative.

Management of blueberry replant disease caused by *Mesocriconema ornatum* in the early establishment phase of growth

J. P. NOE (1), P. M. Brannen (1), W. T. Holladay (1), G. B. Jagdale (1)
(1) University of Georgia, Athens, GA, U.S.A.

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Blueberry (*Vaccinium* spp.) in Georgia has a value in excess of \$250 million per year. Blueberry replant disease (BRD) is an increasing problem as growers are replacing older plantings. Symptoms of BRD, associated with *Mesocriconema ornatum*, include poor growth, yellowing, stunting, and severely reduced yields in replanted areas. Field studies were undertaken to determine the efficacy of preplant soil treatments for management of BRD in the early establishment phase of growth. In 2011, six treatments including preplant fumigation with methyl bromide/chloropicrin, 448 kg/ha (Mebr), 1,3-dichloropropene at 89 (T10), and 266 (T30) kg/ha, preplant solarization, controls with plastic mulch, and nontreated controls were applied with 6 replicates of each treatment at two BRD sites. In 2013, two years after treatments were applied, plant-parasitic nematode population densities and blueberry growth parameters were estimated. Soil treatment with T10, T30 and Mebr reduced *M. ornatum* population densities to 3, 9, and 30/100 cm³ soil, respectively, compared with 836/100 cm³ soil in the nontreated plots ($P \leq 0.05$). Estimated blueberry shoot volume was 1019 cm³ × 10³ in plots treated with T10 as compared to 481 cm³ × 10³ in the nontreated control plots. Identification of preplant management options for blueberry replant disease that have positive effects lasting through the early establishment phase of blueberry growth is critical to continued production on replant sites.

Reference genes validation and cutinase relative expression in response to heat shock on the fungus *Lasiodiplodia theobromae*

M. PAOLINELLI-ALFONSO (1), C. E. Galindo-Sánchez (2), R. Hernández-Martínez (3)

(1) Department of Microbiology, Center for Scientific Research and Higher Education of Ensenada (CICESE), Ensenada, Baja California, Mexico; (2) Department of Marine Biotechnology, Center for Scientific Research and Higher Education of Ensenada (CICESE), Ensenada, Baja California, Mexico; (3) Department of Microbiology, Center for Scientific Research and Higher Education of Ensenada (CICESE), Ensenada, Mexico
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L. theobromae is a phytopathogenic fungus, reported as the most aggressive pathogen on grapevine among the Botryosphaeriaceae, and with higher incidence in warmer regions. Transcriptomic response of some fungi exposed to heat-stress is similar to the observed during plant infection, suggesting that pathogenic factors are induced with high temperatures. The aim of this work was to study the *in vitro* expression of the pathogenic factor cutinase in response to heat shock. To achieve this, total RNA was isolated from the fungal mycelium collected at 10 or 120 min after heat shock (1 h at 42°C) grown in Vogel's salts with or without 1% grapevine wood (GW). Controls non-exposed to heat stress, and two biological replicates per condition were considered. qRT-PCR was done to amplify gene fragments of actin (*Act*), glyceraldehyde-3-phosphate dehydrogenase (*Gapdh*), b-tubulin (*Tub*), elongation factor-1a (*Ef*), and cutinase (*Cut*). The quantification cycle obtained for each target/sample combination was used to evaluate the stability of the reference genes *Act*, *Gapdh*, *Tub* and *Ef*, through the GeNorm, NormFinder and Bestkeeper applications. The pair *Tub/Ef* was the best strategy to normalize the cutinase expression in all the conditions evaluated. The cutinase was overexpressed at 120 min after heat-shock, when the fungus was grown in the presence of GW but not in its absence. Cutinase degrades cuticle, the main barrier that covers the aerial part of the plants. Although it has been reported that *L. theobromae* infection occurs mainly through pruning wounds, the capacity to breakdown the cuticle is highlighted by the cutinase expression in the evaluated conditions, suggesting that adhesion and penetration of non-wounded tissue may occur after heat stress events.

Chemical control of the wheat curl mite, vector to *Wheat streak mosaic virus*, on spring and winter wheat

C. POL (1), M. Burrows (1), Z. Miller (1)

(1) Montana State University, Bozeman, MT, U.S.A.

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In the Great Plains region of the United States, *Wheat streak mosaic virus* (WSMV) is the most widespread virus of wheat. This pathogen has become a global problem, spreading to Australia and Argentina in the last decade. No known chemical control options exist for the virus vector, the wheat curl mite (WCM, *Aceria tosichella* Keifer). To further understand WCM management, spring wheat (*Triticum aestivum* cv. Chateau) was infested with viruliferous WCM in the growth chamber and treated. Field plots of spring wheat and winter wheat (*Triticum aestivum* cv. Genou) were planted and treated after a 3 week period of viruliferous mite infestation. Treatments included Organic Materials Review Institute (OMRI) approved products, common wheat insecticides and acaricides, testing 18 modes of action. The population growth rate for growth chamber plants was measured, comparing mite number applied and mite number after treatment. Carbamate and Organophosphate chemicals reduced the population growth rate of WCM under growth chamber conditions compared to the control plants ($p = 0.01$ and $p < 0.0001$, respectively). "Lorsban 4E" exhibited the greatest potential, decreasing WCM growth rates on average so that a population of 1000 WCM would be reduced to no mites in the span of 11 days, versus 58 days with no treatment (p -value < 0.0001). Preliminary data from spring wheat plots in the field (testing nine modes of action) indicated little reduction of virus presence compared to untreated control plots.

Genetic diversity of the NE-11 strain of *Potato virus Y*

A. QUINTERO-FERRER (1), K. J. Evans (1), A. V. Karasev (1)

(1) University of Idaho, Moscow, ID, U.S.A.

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Strain NE-11 of *Potato virus Y* (PVY) is a recombinant, composed of three parental sequences, PVY^N, PVY^{NA-N}, and NE-11 proper. During 2012 Othello, WA, trials two isolates of PVY were collected and classified as NE-11 using serological profiling and molecular typing by RT-PCR. Both isolates induced vein necrosis in tobacco. They were subjected to whole genome sequencing and subsequent recombination analysis. A novel recombinant pattern was revealed distinct from the original NE-11 isolate. Specifically, the two isolates from Othello had a shorter NA-N segment than the original NE-11, ca. 600 nt versus ca. 800 nt, respectively. Phylogenetic analysis of individual sections coming from different parental genomes confirmed the distinct evolutionary origin of the original NE-11, on one hand, and a group of NE-11 isolates collected later in Idaho and in Othello, on the other hand. Clade placement of the NE-11 isolates coming from different states suggested possible geographic origins of NE-11 isolates.

Risk period of *Wheat streak mosaic virus* movement on green bridge hosts in Montana

N. B. RANABHAT (1), M. E. BURROWS (1), F. D. Menalled (1), Z. J. Miller (1)

(1) Montana State University, Bozeman, MT, U.S.A.

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Diversity and availability of alternative hosts in the field is one of the most challenging aspects of managing *Wheat streak mosaic virus* (WSMV) as these hosts can act as a “green bridge” for WSMV. Understanding the risk period of its vector (wheat curl mite, WCM, *Aceria tosichella* Keifer) infestation and WSMV infection is crucial for management. We compared the probability of WCM infestation and WSMV infection in six different green bridge hosts: pre- and post-harvest volunteer wheat (*Triticum aestivum* L.), corn (*Zea mays* L.), fall and spring emerged downy brome (*Bromus tectorum* L.), and green foxtail (*Setaria viridis* (L.) P. Beauv.). WCM and WSMV presence on trap plants were monitored weekly for thirteen weeks from August 2013 to October, 2013. The probability of mite infestation varied significantly by week ($P < 0.0001$) and treatment ($P = 0.0021$). However, the risk of WSMV infection was not significantly different with treatment ($P = 0.15$) but was significantly different by week ($P < 0.0001$). The probability of WCM infestation on trap plants (mean=77%) and WSMV infection (mean=50%) were observed between mid-August to mid-September. During this period, there was no difference in WCM infestation with treatments compared to control (bare ground). The probability of WSMV infection on a WCM infested plant was 79%. The risk of WSMV infection was mainly driven by WCM immigration. Our results show that escaping the peak risk period of WCM infestation and WSMV infection by adjusting seeding date is a practical management option for WSMV.

Sclerotia production by *Verticillium dahliae* in vitro as affected by selected fungal and bacterial microorganisms

S. SANOGO (1), M. Lytle (1), S. T. Diaz (2), D. Hartman (3)
(1) New Mexico State University, Las Cruces, NM, U.S.A.; (2) Mizkan Americas, Inc., Deming, NM, U.S.A.; (3) Hartman Farms, Deming, NM, U.S.A.
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Verticillium dahliae is a fungal pathogen that attacks a broad range of host plants including chile pepper in New Mexico. The persistence of this pathogen has been well documented in the literature, and sclerotia play a central role in the survival of this pathogenic. This study is part of a project aimed at evaluating the efficacy of biorational formulations on sclerotia formation by *V. dahliae*. In experiments conducted in vitro, *V. dahliae* was paired separately with the following fungal and bacterial microorganisms: *Trichoderma atroviride*, *T. harzianum*, and *T. virens*, *Clonostachys rosea* and two bacterial species including *Bacillus subtilis*. Agar plugs with sclerotia of *V. dahliae* or segments of stem from chile plants infected by this fungus were placed in suspension of various commercial products containing fungal or bacterial microorganisms and incubated at room temperature (22–25°C) for 14 days on a rotary shaker. In simultaneous pairings, mycelium growth was reduced by more than 95% by all *Trichoderma* species and microsclerotia production by *V. dahliae* was not observed. Recovery of *V. dahliae* was least when plugs or stem segments were treated with suspension of commercial products containing *T. atroviride*, *T. harzianum*, and *T. virens*. Management schemes that minimize formation of sclerotia should reduce the inoculum potential of *V. dahliae* in soil.

Efficacy of mycorrhizal seed treatment and in-furrow application of fungicides for control of *Phytophthora blight* in chile pepper

S. SANOGO (1), P. A. Lujan (1), S. Kundu (1), R. Nelson (1), V. Hernandez (2)
(1) New Mexico State University, Las Cruces, NM, U.S.A.; (2) Biad Chili Co, Las Cruces, NM, U.S.A.
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Phytophthora blight, caused by *Phytophthora capsici*, is a major disease of chile pepper in New Mexico. A study was conducted in a chile producer's field near Las Cruces, NM, to compare the efficacy of mycorrhizal seed treatment and in-furrow application of fungicides for control of *Phytophthora blight*. Chile pepper seeds were treated with a mycorrhizal product, a *Glomus intraradices*-based commercial formulation. Seeds not treated with the mycorrhizal product served as control. Seeds treated and not treated with formulated *G. intraradices* were planted with in-furrow application of the fungicides mefenoxam and azoxystrobin on 25 March, 2013. Plot size was 8 rows in width. Percentage of seed germination was similar and plant stand was comparable across all treatments until the appearance of symptoms of *Phytophthora blight*. Disease incidence was assessed on 8 August, 2013 and 9 September, 2013 by examining 8 rows (15 m long) or 4 rows (30 m long) in each plot. In either case, the area observed had 400 plants. There was a trend of lower disease incidence in plots planted to seeds treated with formulated *G. intraradices* than in plots planted to seeds that were untreated. Seed treatment with mycorrhizae may be combined with soil treatment with fungicides to reduce the incidence of *Phytophthora blight* in chile pepper.

Activity of three species of *Trichoderma* against *Phytophthora capsici*, causal agent of *Phytophthora blight* in chile pepper

S. SANOGO (1), O. Guerrero (1), M. Lytle (1), P. A. Lujan (1), B. Bailey (2)
(1) New Mexico State University, Las Cruces, NM, U.S.A.; (2) USDA-ARS, Beltsville, MD, U.S.A.
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Three *Trichoderma* species, *T. atroviride*, *T. harzianum*, and *T. virens* were evaluated for activity against *Phytophthora capsici*, a major pathogen of several crops including annual and perennial crops. Plate assays were conducted by pairing separately an isolate of each *Trichoderma* species with an isolate of *P. capsici* on potato dextrose agar at 25°C in the dark. There was no indication of antibiosis. Typically, isolates of the *Trichoderma* species grew faster than *P. capsici* and therefore overgrew *P. capsici*. In growth chamber and greenhouse studies, seedlings of a chile pepper cultivar susceptible to *P. capsici* were dipped for 30 min in conidial suspension of an isolate of each *Trichoderma* species prior to transplanting, or soil with established plants was drenched with conidial suspension of each *Trichoderma* species. Three to seven days after treatment, plants were inoculated with either mycelium plugs or zoospore suspension of *P. capsici*. None of the three *Trichoderma* species prevented plant infection by *P. capsici*. However, symptom development was generally delayed by few days in plants treated with *Trichoderma*, which survived longer than plants not treated with *Trichoderma*. Formulations containing *Trichoderma* may serve as a component for the development of an efficient integrated management of *Phytophthora blight*.

***Rhodococcus* sp. associated with abnormal ‘UCB1’ pistachio rootstock in California and Arizona**

R. S. STAMLER (1), J. Kilcrease (1), J. J. Randall (1)
(1) New Mexico State University, Las Cruces, NM, U.S.A.
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An association of *Rhodococcus* sp. with clonally propagated ‘UCB-1’ (*Pistacia atlantica* × *P. integerrima*) rootstock was characterized for the first time using multiple approaches. In the last three years, a large number of clonally-propagated ‘UCB-1’ rootstocks newly planted in California and Arizona orchards exhibited symptoms that included shortened internodes, stunted growth, swollen lateral buds, bushy/bunchy growth pattern, and twisted roots with virtually no lateral branching. In California, many of these trees also had stem galls arising from lateral buds. Only 30% of symptomatic rootstocks were successfully t-budded and within one to two years many trees exhibited unusual bark cracking around the bud-union. The percentage of these abnormal rootstocks within affected orchards varied from 8% to 63%. Symptomatic ‘UCB-1’ trees from fields and nurseries were analyzed using PCR amplification, DNA sequencing, selective culture techniques, and microscopy. PCR amplification generated non-target amplicons from symptomatic field and nursery samples that were 99% similar to *Rhodococcus* sp. 16S rDNA. PCR and sequence analysis of the chromosomal virulence locus *vicA* resulted in amplicons of the expected size that were 99% similar to *R. fascians*. PCR amplification of the *fas-1* virulence gene resulted in amplicons of the expected size and sequence matched the pFID188 virulence plasmid, associated only with pathogenic strains of *R. fascians*. Electron microscopy of symptomatic trees revealed signs histological abnormalities and unidentified microbes. Confocal microscopy identified evidence of bacteria and biofilm formation on symptomatic leaves. Bacterial cultures on selective D2 media resulted in the growth of orange colored, gram-positive colonies that were *vicA* and *fas-1* PCR positive. Pathogenicity testing and Koch's postulates are currently being performed to determine if the *Rhodococcus* sp. isolated from ‘UCB-1’ rootstock is responsible for any/all of the observed symptoms.

***Desbotaca arida*: A culturable mycorrhizal ascomycete with a wide host range including *Brassica* from desert habitats**

M. Stanghellini (1), M. MOHAMMADI (1), L. Stowell (2)
(1) University of California, Riverside, CA, U.S.A.; (2) PACE Turf, LLC, San Diego, CA, U.S.A.
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During the course of investigations on the indigenous nature of *Mono-sporeascus cannonballus* and *Olpidium bornovanus* in desert soils of Southern California, we discovered a new mycorrhizal fungus. This fungus, tentatively named *Desbotaca arida*, is a culturable ascomycete and was associated with roots of diverse plants, including *Brassica* host plants (*Plantago ovata* and *Brassica tournefortii*). The fungus forms abundant, spherical to pear-shaped spores (50–120 µm in diameter), either singly or in large clusters, on multinucleate, septate hyphae (6–17 µm in diameter). Spores are beige to cream-colored, thin-walled, and contain a dense, granular cytoplasm. Air-dried spores germinate rapidly (4–6 hr) upon rehydration and the fungus

grows optimally at 35°C. Significant increases in plant growth responses were recorded on broccoli and *Lolium perenne* sown in autoclaved desert soil inoculated with pure cultures of the fungus. The fungus produces siderophores in iron-deficient minimal media and tested positive for acid and alkaline phosphatases, nitrate reductase and catalase. The fungus has an ITS sequence similarity most closely related (81%) to Wilcoxinia, an ectomycorrhizal ascomycete on conifers and deciduous trees.

Occurrence of apothecia of *Oculimacula acuformis* and *O. yallundae* on wheat stubble in the U.S. Pacific Northwest

D. I. VERA (1), T. D. Murray (1)

(1) Washington State University, Pullman, WA, U.S.A.

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Oculimacula yallundae (OY) and *O. acuformis* (OA) are the causal agents of eyespot disease in wheat. Apothecia of OA but not OY have been reported in WA (Walla Walla County). Seventy-three harvested wheat fields in eastern WA, northern ID and northeastern OR (PNW) were surveyed for apothecia of OA and OY during Jun and Oct 2012 and 2013. About 100–150 stem bases (straw) were collected along transects in each field. Straws with intact lower leaf sheaths were examined for apothecia using a stereo microscope and a species-specific qPCR assay was used for species determination. Apothecia were found in 19% of the fields surveyed. Apothecia of OY and OA occurred alone in 35% of the fields, and together in 28% of the fields. Apothecia were greatest in samples from WA (22%), followed by ID (21%) and OR (8%). More apothecia were found in spring (27%) compared to fall (10%). Presence of OY apothecia was confirmed in OR (Umatilla County) and WA (Adams, Asotin, Columbia and Whitman Counties); OA was confirmed in ID (Benewah County) and WA (Walla Walla and Whitman Counties). This is the first report of *O. yallundae* apothecia occurring in commercial wheat fields in the PNW, and the range of *O. acuformis* is expanded to include ID. The occurrence of OA and OY apothecia in commercial fields in spring and fall

demonstrates that sexual reproduction of both species is occurring and may be important in contributing inoculum to the disease cycle.

***Verticillium dahliae* infects specific rotation crops of potato in the Columbia Basin, WA**

D. L. WHEELER (1), D. A. Johnson (1)

(1) Washington State University, Pullman, WA, U.S.A.

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Crop rotation with specific crops is being investigated to control *Verticillium* wilt, caused by *Verticillium dahliae* (*Vd*); however inconsistent results suggest that specific rotation crops may be infected by *Vd*. Inoculations and field surveys were used to test the hypothesis that selected rotation crops of potato are asymptotically infected by *Vd*. Mustards, grasses, Austrian winter pea, peppermint and potato were planted in soil infested with 8 isolates of *Vd* from various hosts, both mating types (*MAT1*-1 & *MAT1*-2), and different multilocus haplotypes. Crops were grown to senescence and stems, seed, roots and soil were assayed for *Vd* on semi-selective media. At least one *Vd* isolate was detected from all crops. More colony forming units (CFU)/g of stem ($P<0.05$) were detected from 4 of 7 crops inoculated with the *MAT1*-1 *Vd* isolate than the *MAT1*-2 isolates. Fewer CFU/g of stems were detected from the grasses inoculated with 7 of 8 isolates than potato, Austrian winter pea and arugula. Differential infection of crops by *Vd* suggests that rotation crops should be screened against representative *Vd* isolates for infection and microsclerotia production before planting. Results were validated by collecting stem samples of rotation crops grown in fields with a history of *Verticillium* wilt of potato in central WA. *Vd* was detected in stems of crops from 4 of 6 fields and stem incidence varied from 0% in arugula, 2% in millet and wheat and, 6–63% in white and brown mustards. Results suggest that specific rotation crops can be differentially and asymptotically infected by *Vd* from different hosts and mating types.