



2013 Caribbean/Pacific Division Joint Meeting Abstracts

Abstracts presented at the joint meeting of the APS Caribbean and Pacific Divisions in Tucson, Arizona, June 17–19, 2013. 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: Ali, N., Ramsubhag, A., Farrell, A., and Jayaraman, J. 2013. Effect of application of *Ascophyllum* extract on the growth, disease incidence and yield of field tomatoes in Trinidad. (Abstr.) *Phytopathology* 103(Suppl. 3):S3.11. <http://dx.doi.org/10.1094/PHYTO-103-9-S3.11>

Effect of application of *Ascophyllum* extract on the growth, disease incidence and yield of field tomatoes in Trinidad

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Two field experiments were conducted to study the effect of *Ascophyllum* seaweed extract on the growth, disease incidence and yield of tomato. Tomato plants (Hybrid 61) were sprayed and or root drenched with an alkaline seaweed extract made from *Ascophyllum nodosum*. Treatments included foliar spray, root drench, spray+ drench of *Ascophyllum* extract, Chlorothalonil (fungicide-control) Chlorothalonil+seaweed extract (fungicide alternately sprayed with the seaweed, extract) and water control. Seaweed extract (0.2%) was applied every 15 days from transplanting until harvesting. Growth, yield and disease prevalence data were recorded 7 days after treatment applications. The results indicated that plants foliar sprayed with seaweed extract and chlorothalonil had 30% less mean disease severity of *Alternaria* blight. Plants foliar sprayed with the seaweed extract showed significant increase in plant height by 11%, and fruit yields by 34% when compared to control plants. In the second field trial, a higher rate (0.5%) *Ascophyllum nodosum* seaweed extract was foliar sprayed along with other treatments including, salicylic acid, chlorothalonil, seaweed extract + chlorothalonil and water control. The results indicated that the seaweed extract + fungicide treatment had less (33%) severity of *Alternaria* blight than control. Plant height and plant yields significantly increased by 24% and 65% respectively, when compared to control. It is hypothesized that the reduced disease incidence levels in treated plants might be due to induced resistance activated by elicitor compounds present in the seaweed extract. Stimulated plant growth might be due to the presence native plant growth regulatory compounds present in the seaweed extract. Experiments are ongoing to study the biochemical and molecular basis of possible induced resistance and induced plant growth mechanisms in tomato evoked by seaweed extract treatment.

Morphological and molecular identification of *Colletotrichum* spp. causing postharvest anthracnose in papaya fruits cv Maradol in Colima, Mexico

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Mexico is one of the main papaya producers worldwide, with approximately 17,000 ha of land under papaya cultivation; however, postharvest fungal

infections are common limiting factors of fruit quality. The objective of this study was to identify the fungal species of *Colletotrichum* involved in postharvest anthracnose on fruit. In July 2012, papaya fruits of the cv. Maradol were collected in Colima, Mexico. Fruit were maintained at 20°C and 85% relative humidity. During 15 days in storage, fruit were observed for anthracnose lesions, and small sections were placed on potato dextrose agar to isolate the infecting fungi. Pathogenicity tests with two representative isolates were performed on healthy fruit of cv. Maradol, which were injured in different areas and then inoculated with aliquots of spores or mycelial discs, with fruit maintained in a moist chamber at 25°C and 85% relative humidity to observe development of lesions. The identification of fungal species was performed by comparison of morphological characteristics (shape, color and diameter) of the conidia and fungal colonies, as well as through molecular identification by amplification and sequencing of the internal transcribed spacer regions (ITS). Based on the morphological and molecular analyses, two species of *Colletotrichum* were identified on papaya fruits from Colima, Mexico.

Monitoring and management of fungicide resistance in populations of *Alternaria* spp. from California pistachio orchards

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Pistachio growers continue to rely on effective fungicides to effectively manage *Alternaria* late blight, but the ability of the causal pathogens to develop resistance to single-site fungicides can eliminate much of the usefulness of available products from an entire chemical class. The succinate dehydrogenase inhibiting (SDHI) fungicide (carboxamide) boscalid was registered on pistachios in 2003 in the pre-packed mixture Pristine® along with the strobilurin pyraclostrobin and has been a cornerstone in *Alternaria* late blight management programs. However, the efficacy of Pristine® has been reduced due to the development of resistance. Recently, new SDHIs (fluopyram, penthiopyrad, fluxapyroxad) have been introduced into the marketplace. In the present study, we established the baseline sensitivities of *A. alternata* to the new SDHIs and established their cross resistance patterns with boscalid by testing their activities against a collection of boscalid-sensitive isolates and boscalid-resistant mutants originating from commercial orchards that have been exposed to boscalid. The results revealed different levels of cross-resistance (low, moderate, and high resistance) between boscalid and penthiopyrad and between boscalid and fluxapyroxad, but showed a lack of cross resistance between boscalid and fluopyram in *A. alternata*. Results from the 2012 monitoring studies with about 230 isolates collected in several pistachio orchards confirmed the widespread occurrence of boscalid resistance and the efficiency of fluopyram. However, monitoring of the variation in fluopyram sensitivity in *A. alternata* isolates from experimental orchards where Luna products had been used revealed the presence of isolates with moderate resistance to fluopyram. In addition, we established baseline sensitivities of *A. alternata* to the demethylation inhibiting (DMIs) fungicides (tebuconazole, propiconazole, and difeconazole),

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fludioxonil (phenylpyrrole) and cyprodinil (anilinopyrimidine) which are rotated or used in combination with SDHIs. The above data provides essential information for evaluating anti-resistance strategies and for estimating the risk of resistance developing in particular chemical groups.

Neofusicoccum parvum causing black spots on avocado fruit in Michoacán, Mexico

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Smooth, black circular spots were noted on the surface of avocado fruits in the spring of 2009 in orchards of Nuevo Parangaricutiro County in Michoacán, Mexico. This study was conducted during the winter of 2010 to ascertain the etiology of the disease. The ITS of the rDNA gene from representative isolates were Sanger sequenced in both directions and assembled. A maximum parsimony tree was constructed with the loci for six strains. *C. acutatum* was designated as the outgroup for construction of the tree. BLAST Nucleotide search of GenBank database showed 100% of maximum identity of *Neofusicoccum parvum*. A representative nucleotide sequence of this region was deposited in GenBank under the accession number JN203129. Strains of *N. parvum* produced mycelium and conidia typical for this specie. Pathogenicity tests were conducted with six avocado fruit cv. Hass by inoculating fruits at three evenly spaced locations on the fruit surface, by placing 5 µL of 1×10^6 conidia mL⁻¹ suspension. Conidia culture of 8-day-old was used. Inoculated fruit were maintained in a moist chamber at 25°C. Black lesions appeared on all sites four days post-inoculation. The pathogen was reisolated from black lesions of the inoculated fruit thus fulfilling Koch's postulates. *Neofusicoccum parvum* has been reported as a plurivorous and cosmopolitan pathogen causing diseases in several important hosts.

Effects of peanut seed treatments on plant stands and implications for disease management in Nicaragua

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Peanut (*Arachis hypogaea*) is a high value crop of increasing importance in Nicaragua, but obtaining optimum plant stands is sometimes a problem. The effects of seed treatments on plant stands and pod yield of peanut were evaluated in three field trials on cultivar GA-06G in 2011 and 2012. Vitavax 40SC (carboxin and thiram) and Trilex Star (captan, trifloxystrobin, metalaxyl and thiophanate methyl) were compared to nontreated seed in replicated field trials planted with 21 seeds/M. Plant stands at 14 days after planting (DAP) were 2.2, 8.3 and 10.0 plants/M, and pod yields were 2049, 3929 and 4395 kg/ha for the nontreated, Vitavax and Trilex Star treatments, respectively. Both treatments were significantly better than the nontreated seed ($P = 0.05$) but usually were not different from each other. Plant density studies showed optimum yields at 12–13 plants/M where stem rot (*Sclerotium rolfsii*) was not present, and 10–11 plants/M in fields with stem rot. Maximum economic return was at slightly lower populations due to savings in seed cost.

Identification of viruses in small-farm lily and dahlia cut flower crops in western Washington

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Virus surveys were conducted at 27 grower sites in 2011–2012 to identify viruses present at lily and dahlia farms that mainly supply cut flowers to local markets. Samples were collected from a total of 259 lily plants and 194 dahlia plants. Samples were tested individually by ELISA (lily and dahlia) and PCR (dahlia) for viruses. Approximately one third (36.3%) of the lily plants sampled were positive for a least one virus. Lily symptomless virus (LSV) was the most prevalent (32.4%) virus detected. The incidence of a potyvirus (7.3%) and Cucumber mosaic virus (CMV) (3.5%) were much lower. No Impatiens necrotic spot virus (INSV), Tomato aspermy virus (TAV), Tobacco ringspot virus (TRSV), or Tomato spotted wilt virus (TSWV) was detected in any of the lily samples. The overall prevalence of viruses in dahlias was much higher (80.9%). The most common virus was one or more of the caulimoviruses associated with dahlia mosaic: the endogenous plant para-retroviral sequence DvEPRS was the most prevalent (65.6%), followed by Dahlia common mosaic virus (33.5%) and Dahlia mosaic virus (11.9%). TSWV and TSV were each detected in 22.7% and 19.6% of the samples, respectively. All of the dahlia samples were negative for CMV, INSV, and potyviruses. These results suggest that viruses, particularly in dahlias are a widespread problem.

HYT A and HYT B effect on fungal pathogen growth in broccoli and lettuce – A biological fertilizer program

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HYT A, a liquid concentrate containing an ecosystem of naturally occurring soil-based microbes, in conjunction with HYT B, an L-amino acid complex extracted from natural sources produced by Agrinos Inc, was applied to Lettuce and Broccoli crops on the Central California Coast. HYT A and HYT B were applied at varying rates and were compared to both untreated plots and conventional fertilizer programs. No fungicides were applied and plants were not artificially inoculated. One week before broccoli harvest, the conventional treatment had significantly more *Alternaria* Leaspot (*Alternaria brassicae*) lesions than all other fertilizer treatments. Two days before lettuce harvest, the conventional treatment had significantly more Downy Mildew (*Bremia lactucae*) lesions than all other fertilizer treatments. The conventional fertilizer programs on both broccoli and lettuce produced higher disease response than the untreated check as well. The HYT A and HYT B fertilizer programs had plant height, width and total marketable yields comparable or greater than the conventional standard programs on both broccoli and lettuce crops.

Potato zebra chip disease: From ignorance to insight

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In the late 1990's, symptoms of an apparently new disease of potatoes were observed in Mexico. Affected tubers showed internal discoloration, often in stripes and streaks, and when processed into potato chips the symptoms were even more striking, and the name "zebra chip" (ZC) was adopted. Foliar symptoms included stunting, chlorosis, purpling, and leaf scorch. By the mid-2000's, symptoms were widespread on potatoes in the Lower Rio Grande Valley of Texas and ZC caused severe economic losses. In 2006–7, ZC was associated with the presence of the potato psyllid, *Bactericera cockerelli*. Although the symptoms could be maintained by grafting, no definitive pathogen was identified until 2008 when three research groups associated a new alpha proteobacterium with ZC. The bacterium, *Candidatus Liberibacter solanacearum* (Lso) (Ca. L. psyllaourous), was transmitted to potatoes by the psyllid. Within a year, numerous groups were using PCR for detection of the bacterium, and shortly thereafter real-time PCR methods for detection were developed. Currently, ZC and the psyllid occur in North and Central America and New Zealand. Diagnostic methods include variations of end-point and real-time PCR, and recently loop-mediated isothermal amplifications. We've come a long way since 2005, but many questions remain unanswered including alternate sources of Lso, overwintering and persistence of the psyllid, possible long-distance migration of the psyllid, introduction of the psyllid to new locations, genetic variants of the psyllid and Lso, incidence of Lso in various psyllid populations, improved diagnostic methods, and so on. In this presentation I will provide an overview of the history and summarize the current knowledge of zebra chip disease and psyllid research.

Botryosphaeriaceae associated with almond trunk cankers: A threat to the almond industry?

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Botryosphaeriaceae pathogens are known to cause band canker of almond. Isolations from orchards revealed several species of Botryosphaeriaceae within cankers associated with pruning wounds and wind-cracks within the scaffolds. These cankers enlarge throughout the year, girdling branches and causing eventual tree loss. Most orchards with high incidence of disease are close in proximity to riparian areas. Differences in canker incidence amongst almond varieties have been observed in the field: 'Padre' appeared with more damage than 'Butte' and 'Nonpareil' and 'Aldrich' showed more damage than 'Carmel.' Because wounds are the point of entry of wood pathogens, their management will be dependent upon time of pruning and reduction of wind-cracks by better tree structuring and proper tying. The objective of this research is to identify the disease inoculum present within orchards at the time of pruning. This information will be used in IPM programs by providing tree structuring recommendations to growers in order to reduce disease risks. Initial spore trapping data has found high inoculum of *Dothiorella iberica* along with other ascomycete fungi associated with November rainfall events within a sampled orchard near a riparian area. This pathogen was previously identified in grapes, avocados, and citrus within California, but has not been screened for pathogenicity on almond. Surveying, sampling, and spore trapping is planned to be continued in several orchards.

Tospoviruses a new threat to vegetable production in Puerto Rico

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Tospoviruses vectored by thrips affect important vegetables crops worldwide. In Puerto Rico vegetables are grown under irrigation mainly in the south of the island. In November 2012, tospovirus-like symptoms including chlorotic and necrotic lesions on leaves and stems were observed on tomatoes in Santa Isabel. In 2013, in two different tomato farms, tomato leaves exhibited discoloration and fruits appeared with necrotic ringspots in both Santa Isabel and Juana Díaz with an incidence of 50 to 60% in affected fields. Serological tests indicated the presence of a tospovirus and RT-PCR (Reverse Transcription – Polymerase Chain Reaction) was used to identify *Tomato chlorotic spot virus* (TCSV). Similar symptoms were also observed in peppers and jimsonweed, and TCSV was again identified by RT-PCR. Other vegetable crops with tospovirus-like symptoms have been subsequently observed and are currently being tested. Thrips control solely using systemic insecticides has not been successful in the tomato farms in Puerto Rico for tospovirus management. Thus, eradication of weed hosts may be an important alternative until resistant cultivars are available.

Supporting integrated pest management in the Western United States

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The Western Integrated Pest Management Center is one of four regional centers funded by the USDA to promote IPM principals by building multi-state partnerships and a communication network linking a diverse audience that includes researchers, growers, commodity organizations, regulators, environmental groups, pest control professionals and others. From our office located at UC Davis, we serve 14 Western states and territories stretching from the Northern Mariana Islands to Colorado. The Western IPM Center promotes IPM principals by providing funding, fostering connections, leveraging resources and creating publications. Our goal is to bring the right people together with the necessary resources to solve the important and emerging pest problems in the West. We fund multi-state work groups and special issues research; support the publication of Pest Management Strategic Plans and communicate through a regular newsletter and our website at www.wripmc.org.

A recombinant of Bean common mosaic virus induces necrosis in an I gene bearing line of common beans

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Bean common mosaic virus (BCMV) exists as a complex of strains distinguished based on reactions towards seven resistance genes known in common beans; seven BCMV pathotypes have been identified, numbered I to VII. However, pathotype VI was previously found associated almost exclusively with a distinct virus, *Bean common mosaic necrosis virus* (BCMNV) that induces necrosis in common bean cultivars with the *I* gene, conferring extreme resistance or immunity to all other BCMV pathotypes. These necrotic BCMNV isolates can be easily distinguished from non-necrotic BCMV isolates serologically, using antibody-specific serotyping. Serotype A is characteristic of BCMNV, and serotype B characteristic of BCMV. Here, we describe biological and molecular characterization of a new BCMV isolate, BCMV-RU1m, that is able to induce severe whole plant necrosis in bean cultivar Jubila that carries the *I* gene. Based on a biological characterization with twelve bean differential lines, this isolate was classified as belonging to pathotype VII, similar to a control isolate US10, and exhibited B-serotype. The whole genome of BCMV-RU1m was cloned and sequenced and determined to be *ca.* 10-kb excluding poly(A), with a single open reading frame. Most of the genome was 98–99% identical to the BCMV isolate RU1-OR (also pathotype VII). Analysis of the nucleotide sequence for BCMV RU1m revealed that it originated through a recombination event between BCMV RU1-OR and a yet unknown potyvirus. A *ca.* 1-kb fragment of an unknown origin in the RU1m genome may have led to its ability to induce necrosis in beans carrying the *I* gene. This is the first report of a BCMV isolate inducing *I* gene mediated necrosis in common beans.

Alternative compounds for management of Zebra chip of potato

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Zebra chip of potato (ZC) is a disease that is caused by the bacterium *Candidatus Liberibacter solanacearum*. Management practices have focused on chemical control of the potato psyllid, which vectors this bacterium. Other preliminary approaches for management of ZC have focused on plant resistance, planting dates, and biological control. From 2010 to 2012, the use of alternative compounds that may suppress pathogen development, delay the onset of symptoms, promote tuber production, induce a systemic resistance from the plant, or target plant health using plant nutrients, have been experimented in Springlake, Texas, in a commercial potato production farm. Compounds such as antibiotics, systemic acquired resistance (SAR) inducers, micronutrients, macronutrients, salts of phosphorous acids, and potassium phosphite, were some of the compounds tested in small plots in a randomized complete block design of four (2010 and 2011) or six replicates (2012). Treatments with individual compounds or in select combinations were back-packet sprayed biweekly at or around flowering. All plots, including the untreated control, also received applications of fertilizers, herbicides, fungicides, and insecticides consistent with producer production practices for that year, including insecticides against the potato psyllid. In 2010, streptomycin sulfate and streptomycin sulfate plus acibenzolar-S-methyl, had significantly higher yields for U.S. No. 1 tubers (4–18 oz.) than the control (grower conditions). In 2011, streptomycin sulfate plus SAver™ had significantly higher yields than the control for U.S. No. 1 tubers. In 2012, a treatment with a modified Keyplex® 1000DP (micronutrients) plus Renew™ (balanced nutrients), had significantly higher yields for U.S. No. 1 tubers. Zebra chip was present, although at low levels, in the experimental field plots and producer fields for all years. Further research trials will compare the best treatments, alone or in combination, and determine which may be useful against ZC.

A suite of sub-viral DNA molecules associated with Cotton leaf curl Gezira virus from the southwestern Saudi Arabia

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The whitefly transmitted *Cotton leaf curl Gezira virus* (CLCuGV), a monopartite begomovirus, is known to cause vein thickening and leaf curl symptoms. Okra leaves exhibiting similar symptoms were collected from Jazan area in Saudi Arabia. Total DNA extracted from these okra samples was used as template to enrich for circular DNA component by rolling circle amplification. The obtained clones were completely sequenced. The complete genome sequence showed the presence of near identical 2769-nt-long isolates of a begomovirus. Blasting the assembled nucleotide sequences in GenBank database revealed a sequence identity at 88–93% to previously reported strains of CLCuGV. In addition, we also cloned a defective betasatellite and two distinct alphasatellites. The betasatellite shared 60% nt identity with Cotton leaf curl Gezira betasatellite reported from the Nile Basin and sub-Saharan Africa. Nt sequence comparison revealed that one alphasatellite shared 88% nt identity with Cotton leaf curl Gezira alphasatellite (DNA-1 type), while the second satellite shared the highest nt identity (64%) with *Ageratum* yellow vein Singapore alphasatellite (DNA-2 type) from Oman and Singapore. The relatively low nt identities these molecules shared with previously reported molecules suggest that these satellites are probably not resulted from a recent introduction.

Field performance in Arizona cotton crops of a biological control product made of *Aspergillus flavus* AF36 coated onto roasted milo

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Aflatoxins, toxic and carcinogenic metabolites produced by *Aspergillus flavus*, frequently contaminate crops. Competitive displacement by atoxigenic *A. flavus* is the only proven method to reliably reduce aflatoxins in crops. The atoxigenic strain AF36 is typically applied on colonized, steam sterilized wheat grain. The traditional process of producing this formulation is slow, expensive and requires considerable capital. To meet increased demand, lower cost manufacturing was developed. The new formulation which consists of roasted milo coated with AF36 spores is less energy and capital intensive and can more rapidly be scaled. The present study sought to compare the traditionally formulated colonized wheat with roasted and coated milo under field conditions. Coated milo made with the new process was compared to wheat made with the traditional colonization method in four fields in each of two areas in Arizona. Each product was applied to about 8 acres in each field with plots separated at least by 100 m. Results indicate that coated milo and

the traditional wheat have similar production of spores, but the number of resident and sporulating grains were higher with coated milo (18 and 16) than with wheat (10 and 7). Displacement was also slightly better with coated milo than with wheat in both cottonseed (~95% to ~90%) and soil (~75% to ~70%), respectively. Displacement of aflatoxin producers was associated with reductions in crop aflatoxin content.

Corky bark disease of langsat in Hawaii

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Langsat (*Lansium domesticum* Corr.) is a fruit from South East Asia with an expanding niche market in Hawaii. The aim of this study was to identify the causative agent of the cankers on langsat trees in Hawaii. Symptoms similar to those found on rambutan (*Nephelium lappaceum*) and litchi (*Litchi chinensis*) were observed in an orchard located along the Hamakua Coast of Hawaii Island, approximately 6 miles north of Hilo. The cankers covered the branches and racemes, often resulting in little to no fruit production. Isolations from ascospores present in the cracks of the cankers resulted in pure cultures of the fungus. The fungus was identified as *Dolabra nepheliae* based on morphological characteristics and molecular analysis. Upon closer observations of the diseased samples, sections of corky bark contained at least two larval insects. The beetles were identified as *Corticicus* sp. (Coleoptera: Tenebrionidae) and *Araecerus* sp. (Coleoptera: Anthribidae) by the USDA-ARS Systematic Entomology Laboratory (Beltsville, MD). It is not known the extent to which either of the beetle species is associated with *L. domesticum* in Hawaii or if they play a role in the bark disorder. This is the first report of *Dolabra nepheliae* being found on langsat in Hawaii. Effective management practices should be established to avoid potential production losses or spreading the disease to alternative hosts.

Peduncular infection and seed transmission of the plant pathogenic fungus *Verticillium dahliae* in chile pepper

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Chile pepper (*Capsicum annum* L.) is a very important vegetable crop in southwestern United States, especially in the states of New Mexico, Arizona, and Texas. Chile growers in New Mexico encounter several serious pest and disease problems, one of which is *Verticillium* wilt caused by *Verticillium dahliae*. Seed transmission of *V. dahliae* has been proved in various crops like spinach, lettuce, and cotton, but has not been determined in chile pepper. The objective of this study was to assess peduncular infection and seed transmission of *V. dahliae* in chile. Pods of chile were collected from field-infected plants from Doña Ana and Luna Counties in southern New Mexico. Peduncles, seeds, and placenta tissues were removed from infected pods, surface-sterilized, and plated onto a *Verticillium*-selective medium. Colonies emerging from plated plant tissues including seeds were identified based on morphological features and through PCR using primers specific for *V. dahliae*. Incidence of peduncular infection was from 62.5 to 92.3% whereas the incidence of seed infection varied from 3 to 15.43%. Infection of placental wall tissue was between 55.5 and 80.6%. Confocal microscopy was also utilized to confirm the presence of the fungus in chile seeds. The emission spectra of the fungus in the seeds were also observed and were found to be at approximately 536 nm. This is the first study documenting seed transmission of *V. dahliae* in chile pepper. This study highlights the possible seed transmission method of *V. dahliae* in chile from the stem vascular system into the peduncles, placental wall tissue, and then into the seeds.

Doing more with less: Use of very young plants for biological indexing for graft transmissible pathogens of citrus

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With citrus budwood source trees, testing for the presence of graft transmissible pathogens (GTP) using biological indicator plants provides a high degree of confidence that any known and even unknown pathogens will be detected. Biological indexing of mother trees in citrus budwood programs is the cornerstone of the California citrus certification program. Here we report on the use of very young indicator plants, 10–12 weeks after sowing, for identification of GTP, and compare the results with the traditional method of indexing using 8–12 month old indicator plants. The use of very young indicator plants offers the advantages of requiring less greenhouse space, and the smaller plants allow for better visualization of symptoms. Cool temperature indexing can be accomplished in the summer time by placing racks of plants near evaporative cooling pads.

The role of carbon sources in relation to pathogenicity of *Sclerotinia sclerotiorum* on Valencia peanut

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Sclerotinia sclerotiorum, is a fungal pathogen with a broad host range that typically produces white mycelium on growth media. In New Mexico and Texas, darkly pigmented (SD) variants of *S. sclerotiorum* have been reported. In previous studies, stable mutants with white mycelium (SW) were derived using melanin inhibitors. SD isolates were pathogenic on peanut in contrast to SW isolates. Even when melanin was restored, SW isolates remained non-pathogenic. On potato dextrose agar-amended bromophenol blue (PDA-BPB), SD isolates produced oxalic acid (as indicated by a yellow halo within the medium), while SW isolates did not. Quantification by gas chromatography/mass spectrometry revealed a significant ($p < 0.002$) down-regulation of oxalic acid as well as many sugars. Based on these results, restoration of pathogenicity in SW by glucose or sucrose was studied. SD and SW isolates were transferred to complete glucose (CG), complete sucrose (CS) and PDA plates. Peanut plants were wounded at the cotyledonary axils, and inoculated by placing plugs from the CG, CS and PDA plates onto the wound sites. Plugs from each isolate on CG, CS, and PDA media were also transferred to PDA-BPB medium to test for oxalic acid production. After one week, plants inoculated with SD isolates on CG, CS and PDA were necrotic and died. Plants inoculated with SW isolates on CS and PDA showed no symptoms; however plants inoculated with SW on CG showed necrosis and death similar to inoculation with the SD isolate. All SD isolates on CG, CS and PDA along with SW isolates on CG produced a yellow halo after three days of incubation on PDA-BPB medium. These results suggest that glucose may play a role in the production of oxalic acid and thus in the pathogenicity of *S. sclerotiorum*.

Pathogenicity of *Pythium aphanidermatum* on papaya seedlings cultivar Maradol, and hybrids Sensation and Tainung

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In Mexico, papaya crops are grown on approximately 17,000 ha. *Pythium aphanidermatum* has been reported as a root rot pathogen in nurseries and field orchards. This study was conducted to evaluate the effect of *P. aphanidermatum* inoculation on papaya seedlings. The pathogen inoculum was increased on V8 agar and then inoculated on a substrate consisting of greenhouse peat moss and soil (vol 2:1) previously sterilized in autoclave. Four-week old papaya plants from the variety Maradol and hybrids Tainung and Sensation were used in the experiments. Prior to transplanting to the *P. aphanidermatum*-infested substrate contained in 750 g-volume pots, the roots of the plants were washed with sterilized water and then transplanted to the infested substrate. In the control treatment, non-infested substrate was used. Plants were maintained under a shade net with sufficient moisture in the substratum, and assessments of disease symptoms were conducted daily. Twelve days after inoculation, plants from the hybrid Tainung showed 70% of incidence of the disease; followed by the cultivar Maradol, with 60%, and the hybrid Sensation, with 40% of incidence. Symptom severity ranged from wilting to death of the plants. Small sections of roots and stem were collected from some of the infected plants and placed onto V8 agar or in sterile water. Sporangia of *P. aphanidermatum* were observed growing from the infected tissues placed in sterile water, and colonies typical of *P. aphanidermatum* developed on V8 agar.

Intimacy of Botryosphaeriaceae among temperate nut crops in California

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The Botryosphaeriaceae comprise a large family of fungi whose members are important pathogens of numerous agricultural, forest, and ornamental plants. Although reports of diseases caused by these fungi (under various names) date from the early nineteenth century, in the last two decades members of these fungi have been recognized as aggressive pathogens in a plethora of plant species. In California, several members of Botryosphaeriaceae have now been shown to cause devastating diseases of pistachio and almond, and recently were associated with canker and blight symptoms of walnut. *Botryosphaeria dothidea* was first reported on pistachio in 1984 (*Pistacia vera*), causing panicle and shoot blight of pistachio in an old orchard. It took about 14 years for this disease to spread and reach epidemic levels in California pistachios, and we know now that more than one species is involved, with *N. mediterraneum* being the most aggressive. Fortunately, pruning and repeated fungicide sprays have been very successful in managing the disease. In

almond (*Prunus dulcis*), a band canker was reported in the mid 1950's caused by *Botryosphaeria dothidea*. Recently, seven different species were reported to cause stem cankers or shoot blight in almond trees. There is no fungicide effective against the band canker syndrome of the disease, although cultural practices such as keeping the trunks dry helps reduce both the incidence and the severity of the disease. Ten species of Botryosphaeriaceae and two *Diaporthe* spp. have now been recovered from diseased tissues of walnuts (*Juglans regia*). Although the damage these species cause is not yet well characterized on walnut, the presence of such a group of fungi among nut crops, which are frequently planted next to each other, calls for a more comprehensive research effort in order to develop disease management tools to benefit all three California nut industries.

Reducing fungicide applications to control *Alternaria* late blight of California pistachios by spraying after early season 10-unit DSV thresholds

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Alternaria late blight of pistachio, caused by three species in the *A. alternata* complex: *A. alternata*, *A. tenuissima*, and *A. arborescens*, is a major disease in California orchards that have prolonged periods of dew and high relative humidity. Pistachio growers in the areas most prone to this disease apply as many as 5 sprays. In addition to the financial cost of such a spray program, reducing the number of fungicide applications should reduce the risk of developing fungicide resistance among *Alternaria* populations. This is necessary because resistance to strobilurins and the succinate dehydrogenase inhibiting (SDHI) fungicide boscalid was observed in *Alternaria* shortly after their registration on pistachios. Disease severity values (DSV) were calculated using the disease forecasting system, FAST, which was initially developed to identify periods when environmental conditions are favorable for tomato early blight development. Fungicides were applied when the 7-day DSV exceeded 10 or 14 units. Fewer sprays were required with the 14-unit DSV threshold, but unacceptable levels of disease developed. The 10-unit DSV predicted sprays were as effective as three or four calendar sprays ($P < 0.05$), but often required as many, and sometimes more sprays. Reducing sprays to just the first two times the 7-day 10-unit DSV threshold was exceeded was as effective ($P < 0.05$) as spraying after all three (2012) and all four (2011) 10-unit DSV predicted sprays, and was also as effective ($P < 0.05$) as three calendar sprays.

Laurel wilt: An unusual and exotic vascular wilt disease of avocado and other lauraceous host plants

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Laurel wilt (LW) has killed members of the Lauraceae plant family (Laurales, Magnoliid complex) throughout the southeastern U.S. Over 400 million native trees have been lost, and production of an important commercial crop, avocado (*Persea americana*), is impacted in (Florida, \$55 million yr⁻¹), or threatened (e.g. California, \$350 million yr⁻¹, and Mexico, the world's top producer). LW is caused by a fungal symbiont, *Raffaelea lauricola*, of an Asian ambrosia beetle vector, *Xyleborus glabratus*. LW resistance has been sought in avocado and redbay (*P. borbonia*), and numerous fungicides, insecticides and repellents have been tested. Direct and indirect means to detect LW have been investigated with an ultimate goal of developing sanitation measures to mitigate disease spread. Much has been learned about LW epidemiology. *X. glabratus* is attracted to volatiles produced by host and non-host plants, as well as *R. lauricola*. Despite its rapid acropetal and basipetal movement in inoculated xylem, low concentrations of *R. lauricola* are evident in LW-affected hosts. Lethal disease develops in avocado and redbay after inoculation with as few as 100 conidia of *R. lauricola*, and detection of the pathogen microscopically and via DNA analyses is difficult. Vascular function in avocado is reduced in affected avocado, and reduced hydraulic conductivity and increased tylose formation are correlated with increased wilting, foliar necrosis and sapwood discoloration. Since its arrival in the U.S., *R. lauricola* has moved laterally to several other species of ambrosia beetles, some of which might also serve as vectors.

A preliminary host-range study of two infectious full-length clones of *Beet severe curly top virus*

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Beet severe curly top virus (BSCTV) is one of three curtoviruses affecting sugar beet in dry desert climate of western U.S. BSCTV is a member of the genus *Curtovirus*, family *Geminiviridae*. It is a phloem-limited virus,

transmitted by leafhoppers, with a ca. 3.0-kb genome represented by a single-stranded circular DNA. In order to create a tool for convenient screening of the curly top-resistant sugar beet germplasm, we generated two full-length infectious clones of BSCTV (1.2 and 1.8 genomes) in a binary construct suitable for delivery into plants via agroinoculation. The infectivity of both clones was confirmed for three varieties of sugar beet, two varieties of tomato, and *Nicotiana benthamiana*. The time-course of BSCTV symptom development was compared to symptoms caused by another curtovirus, *Beet curly top virus* (BCTV, strain Logan). Infectivity of BSCTV and BCTV infectious clones was monitored through a combination of symptom observations, ELISA, and PCR tests.

The effect of an open-ended high tunnel production system on late blight of tomato in western Washington

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Not only is the coastal climate of WWA conducive for outbreaks of late blight on tomato, production is challenged by the region's short growing season. A 3-yr study near Mount Vernon in 2010–2012 compared naturally-occurring epidemics caused by *Phytophthora infestans* (US-11) in open-ended high tunnel (HT) vs. open field (OF) settings. Plots were arranged according to an RCB split-plot design. Main plots (HT, OF) were placed at the same location in the field each year, but subplots (cultivar) were re-randomized each year. Six cultivars in 2010 and 2011, and seven in 2012, were evaluated weekly for disease during the growing season. Onset of late blight was later in HT than OF plots in 2010 (2 Sep vs. 26 Aug) and 2011 (13 Sep vs. 2 Sep), but occurred on the same date (26 Jul) in 2012. Leaf wetness duration (hours) was less in HT vs. OF each year (857 vs. 1060; 598 vs. 998; 885 vs. 923, respectively). In spite of rescue foliar fungicide applications, late blight severity was 0.02% in HT compared to 37% in OF in 2010; total number of infected trifoliate leaves in HT vs. OF was 41 vs. 360 in 2011, and 86 vs. 407 in 2012. Cultivar reactions to late blight could not be differentiated in HT but 'Celebrity' and 'Oregon Spring' were among the most susceptible in the OF. Total tomato yield (t·ha⁻¹) was always significantly ($P < 0.0001$, $P = 0.0003$, $P = 0.0035$, respectively) greater in HT compared to OF: 36 vs. 10 in 2010, 47 vs. 18 in 2011, 33 vs. 7 in 2012. 'Oregon Spring', 'Stupice', and 'Glacier' were among the best performing for marketable yield. Open-ended high tunnels could be used to reduce leaf wetness, offering a new management strategy for late blight in the region.

Jefferson hazelnut meets eastern filbert blight in western Oregon

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The progression of eastern filbert blight (EFB) (*Anisogramma anomala*) symptoms in a 25A 'Jefferson' hazelnut (*Corylus avellana*) orchard planted near Newburg, OR in 2007 and 2008 was documented. This cultivar has a single dominant gene for resistance to EFB. Cankers were first observed fall 2010, averaged 7 cm and were characterized by longitudinal cracks, flat and/or slightly sunken areas on trunks. Some cankers had a few small stromata and viable ascospores were observed when stained with a 0.05% trypan blue in lactoglycerol. Characteristic mycelia were found in cankers without stroma when stained with 0.05% trypan blue. Only 1.8% of the trees planted in 2007 had cankers while 5% of the 2008 trees had cankers. Most of 143 marked cankers (90%) increased to an average of 19 cm during the 2011 growing season. Three cankers develop new stromata but most did not develop any. Although cankers were longer they appeared to have substantial callus formation indicating a strong wound healing response. Most cankers (75%) decreased to an average of 14 cm during the 2012 growing season. Several cankers (21%) had healed over completely while others could only be recognized by superficial cracks, indentations or scar tissue. No new cankers developed during the study. Resistant trees such as 'Jefferson' can be infected under high inoculum conditions and growers may wish to use fungicide applications during the spring of the first year of planting.

Caribbean pathways for invasive pathogens: Perspective and actions from Puerto Rico

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Invasive pest species are affecting world agriculture, forests and natural areas, causing billions of dollars of losses. With globalization and increases in trade

and movement of people, the frequency of species invasions has substantially grown in the last few decades. Puerto Rico is geopolitically located in a key strategic position and has the potential to host and play a crucial role in studies on “prevention and preparedness for invasive species”. Puerto Rico is located in a region where the probability of interception of new pests coming to the Americas and U.S. mainland is high, and where a proactive approach could be the front line for management of invasive species. In addition, Puerto Rico has its own agricultural interests and it, along with the rest of the Caribbean basin, would directly benefit from a U.S. offshore quarantine facility that provides research, appropriate training on target pests and potentially beneficial organisms. Currently, there is a joint initiative to establish a world-class Center for Excellence in Quarantine & Invasive Species. The Center is a collaborative effort among University of Puerto Rico (UPR), Puerto Rico Department of Agriculture and Department of Natural Resources, USDA APHIS PPQ, US Forest Service, Caribbean Food Crop Society, and other agencies.

Potyviriidae in cucurbits and wild hosts in Puerto Rico

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Potyviriidae causes important yield losses in watermelon, pumpkin, zucchini, melon and squash, and are so far the most frequent family of viruses reported in cucurbits in Puerto Rico. Sampling throughout the island (2006 to 2012) showed that PRSV (*Papaya ringspot virus*) and ZYMV (*Zucchini yellow mosaic virus*) were the most frequent virus species infecting cucurbit species. In addition, SqVYV (*Squash vein yellow virus*), has caused severe losses in watermelons. The presence of wild cucurbit species provides a constant source of inoculum to crops and vice-versa. A common wild cucurbit species in Puerto Rico, balsam pear (*Momordica charantia* L.) can be found everywhere on the island. West Indian gherkin (*Cucumis anguria* L.) and hedgehog gourd (*Cucumis dipsaseus* Ehrenb. ex Spach), are more frequently found at lower elevations. ELISA, immunostrip tests, RT-PCR and sequencing of a coat protein gene fragment were conducted to identify and characterize these viruses affecting cucurbits. Mechanical transmission to *Cucurbita moschata* ‘Waltham’ was conducted and symptoms were evaluated in order to select virus isolates to challenge resistant lines of *C. moschata*. The different virus isolates induced a wide range of symptoms to *C. moschata*, indicating their biological variability, which was confirmed by the genetic diversity of their sequences. Co-infections by multiple virus species or strains usually infect cucurbits and their potential interaction is an additional challenge to cucurbit breeding programs.

Effect of the green algae *Chlorella* on vegetative growth and production of sporangia by *Phytophthora capsici*

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Phytophthora blight, caused by *Phytophthora capsici*, is a serious disease of vegetable crops worldwide. In New Mexico, chile pepper (*Capsicum annuum*) growers face production constraints imposed by *P. capsici*. The disease is typically severe in flood-irrigated fields. Observations by some producers indicate that the incidence of Phytophthora blight appears to be reduced in field areas where algal growth is widely present. A study was conducted to determine the effect of the fresh water algae *chlorella* on *P. capsici*. Culture of the algae was obtained from the Algal Bioenergy Program at New Mexico State University. An isolate of *P. capsici* was grown for 5–7 days on V8-agar medium in the dark. Mycelium plugs were taken from the V8 agar medium and placed in 9-cm diameter petri dishes containing 25 ml sterile distilled water or algal cell suspension, and incubated in a growth chamber at 26°C under continuous light for 48 h. Plugs were then examined for mycelial growth and sporangial production. Transplants or seedlings established from direct seeding of a chile pepper cultivar susceptible to *P. capsici* were treated by root dipping or soil drenching with either algal cell suspension or water. Plants were inoculated with *P. capsici* 7 days after treatment. Generally, sporangia production was initiated earlier in algal cell suspension than in water, and production of sporangia in algal cell suspension was as high as or higher than in water. Symptoms of infection typically appeared first in plants treated with algal cell suspension. Results suggest that the green algae *chlorella* enhances sporulation by *P. capsici* and is unlikely to prevent plant infection.

Evaluation of selected biorational products for efficacy against fungal pathogens of Valencia peanut

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Organic peanut production is a vital sector within the peanut industry in the U.S. New Mexico is one of the leading states in organically produced peanut. Several challenges are encountered by organic peanut farmers, and include pest, disease, and soil fertility problems. Diseases caused by soilborne fungal and oomycete pathogens are limiting factors to peanut production. Whereas conventional growers have access to a wide array of management options, organic peanut growers are faced with few organically acceptable management tools to alleviate disease problems. This study focuses on evaluating biorational products for efficacy against three soilborne pathogens of Valencia peanut, *Sclerotinia sclerotiorum* (causal agent of Sclerotinia blight), *Sclerotium rolfsii* (causal agent of stem rot and pod rot), and *Rhizoctonia solani* (causal agent of pod rot). Selected biorational products included formulations containing bacterial and fungal microorganisms, and botanical extracts were tested in vitro using a modified agar well diffusion assay. Each formulation (100 ml) was dispensed into six 1-cm diameter wells in PDA around a mycelial plug of each pathogen. Radius of mycelium growth was measured after 48 h incubation at 25°C. Detached leaflets of Valencia peanut were treated by dipping in each formulation, and then inoculated with *S. sclerotiorum*. In general, very little inhibition was displayed on agar well diffusion assay, with the greatest growth inhibition exhibited by a formulation containing *Bacillus subtilis* QST713. Lesion area on detached Valencia peanut leaflets inoculated with *S. sclerotiorum* was variable among formulations, and none prevented leaflet infection. Further research is needed on evaluating additional biorational products in order to identify formulations efficacious against soilborne fungal pathogens in organic peanut production systems.

The Botryosphaeriaceae, a major player associated with tropical fruit diseases in Puerto Rico

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Worldwide, fungi in the family Botryosphaeriaceae have been associated with fruit rot, stem canker, dieback, gummosis, stem end rot and blossom blight in longan, rambutan and mango. During a disease survey from 2008 to 2013, diseased tissue of mango, rambutan and longan showing symptoms of fruit rot, inflorescence blight, rachis necrosis and leaf blotch were sampled at six orchards of rambutan and longan, and one orchard of mango germplasm in Puerto Rico. Diseased tissue were disinfested and plated onto APDA. Out of 1143 fungal isolates, 260 (22.7%) were morphologically classified as belonging to the Botryosphaeriaceae. Isolates were further identified using morphological parameters and by sequencing of the ITS1-5.8S-ITS2 region of the rDNA, and elongation factor (EF1- α). A total of 154 *Lasiodiplodia* spp., 96 *Neofusicoccum* spp. and 10 *Guignardia* sp. were identified from rambutan, longan and mango. Pathogenicity tests showed that *Lasiodiplodia theobromae* and *Neofusicoccum parvum* caused inflorescence blight and rachis necrosis, respectively. Two *Neofusicoccum* spp. were identified causing rachis necrosis and inflorescence blight in mango. In all cases, inflorescences turned brown and white mycelial growth was observed. *Lasiodiplodia* spp. caused leaf blotch, inflorescence blight and fruit rot. On leaves, necrotic spots and pycnidia were observed. Rachis necrosis, flower abortion and inflorescence blight were observed on inflorescences. Fruit rot and aril (flesh) rot were also observed. *Lasiodiplodia* sp. was also pathogenic to longan, causing inflorescence blight and fruit rot. Inflorescences turned brown and flower mummification was observed. The exocarp (peel) and endocarp (aril) turned dark brown, mycelial growth and pycnidia were observed on fruits. *Guignardia* sp. was only found as endophytic fungi in mango and rambutan tissue. Further DNA sequences analyses will speciate the remaining *Lasiodiplodia* spp., *Neofusicoccum* spp. and *Guignardia* sp.

Environmental factors influencing disease suppression by compost water extracts (CWE) under controlled conditions

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Compost potentially offers a sustainable approach to control disease caused by important soilborne pathogens, but variability in composition and efficacy has hampered application. As one example, factors that result in altered extracellular pH at the root tip can alter growth and development. Production of compost water extracts (CWE) provides a reproducible means to develop

homogeneous mixtures whose effects on growth, development, and disease development can be quantified under controlled environmental conditions. A growth pouch assay was used to measure root infection by *Fusarium solani* in pea (*Pisum sativum* L) seedlings, which normally develop local lesions within 24–48 h after inoculation with the fungal pathogen. When seedlings were co-inoculated with the pathogen and CWE, no infection occurred (Phytopathology 103:255). Protection was sustained when plants were transplanted from pouches into 4-inch containers of coconut coir in a greenhouse and watered with hydroponic nutrients. The purpose of this study is to explore the potential for using a growth pouch assay to predict ability of specific CWEs to suppress disease on specific crop plants in greenhouse conditions engineered to facilitate optimal compost-root interaction.

Evaluation of relative susceptibility of lettuce varieties to *Tomato spotted wilt virus* and *Fusarium wilt*

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In the lettuce production area of Central California, *Tomato spotted wilt virus* (TSWV) and *Fusarium wilt* have both been on increasing distribution and severity over the last 10 years. To address these disease problems in western Fresno County, trials were conducted to evaluate response of lettuce varieties or breeding lines to the pathogens. The trial site was in an area with a history of TSWV and it was naturally infested with *Fusarium oxysporum* f. sp. *lactucum*. On 17, 30 Aug and 13 Sep lettuce seed of 20 to 24 varieties were sown and sprinkle irrigated. Disease incidence was recorded at least 2 times for each planting date and five heads per plot were harvested and size and quality were recorded for the first two planting dates. Difference in TSWV and *Fusarium* susceptibility among varieties were apparent. Generally, the romaine-types were more tolerant of *Fusarium*, but there were also several iceberg types, such as Salinas, Sun Devil, Diamond back, Javolina, Sidewinder, Sharp Shooter, Sniper and Sunquest with lower levels of *Fusarium* wilt expression. Highest levels of TSWV incidence were present in the romaine types.

Fungicide resistance of two *Alternaria* species to azoxystrobin and boscalid in the Columbia Basin

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Early blight and brown spot of potato are caused by *Alternaria solani* (*As*) and *A. alternata* (*Aa*), respectively. Fungicides are extensively used for control of both diseases. Azoxystrobin and boscalid interfere with cellular respiration and resistance is expected in fungal populations due to the specific modes of action. Thirty-nine isolates of *As* and 55 of *Aa* were randomly selected from several hundred collected from the Columbia Basin of Washington between 2009–2011. Mycelial growth assays were then conducted on water agar (WA), WA + azoxystrobin (1.0 mg/mL) and WA + boscalid (0.5 mg/mL) to initially screen for fungicide resistance. Growth ratio (experimental/control mycelial growth) was calculated, where growth ratio > 0.5 was resistance and growth ratio ≤ 0.5 was sensitive. *As* had a large range in sensitivity and *Aa* did not vary in sensitivity to azoxystrobin. *As* resistance to boscalid may have increased in the Columbia Basin population as indicated by significant increase in mean growth ratio between 2010 and 2011. *Aa* showed no difference in growth ratios to boscalid between 2010 and 2011. A subsample of two isolates of *As* and three of *Aa* were selected to compare spore germination based on mycelial growth ratio. On media amended with azoxystrobin (0.01, 0.1, or 1.0 mg/mL) spore germination of *As* was the least at 1.0 mg/mL. Spore germination of *Aa* did not differ among the three concentrations. On media amended with boscalid (0.005, 0.05, or 0.5 mg/mL), spore germination of *As* did not significantly differ among the three concentrations whereas spore germination of *Aa* was lowest at 0.05 mg/mL.

Resistance to azoxystrobin and boscalid in the Columbia Basin dictates the need for improved control methods.

Monitoring program and training system to face the problem of the Soybean Cyst Nematode in Puerto Rico

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In 1998, the Soybean Cyst Nematode (SCN), *Heterodera glycines* race 2, was reported for the first time in Puerto Rico. The infection was detected in an experimental plot in the northern area of the island. Since its discovery, local and federal agencies have managed the problem with pesticides and cultural practices. Recently, a monitoring program was established to evaluate the incidence and its possible dissemination to other areas. Over fifteen hundred samples were collected in fields planted with soybean through the island. Samples were analyzed using a simple and easy method of cyst extraction developed at our laboratory. Samples showed high numbers of cysts in the same area that SCN was reported initially. In addition, other adjacent plots were also spotted as infested by the SCN. In other soybean fields, located at the northern and southern areas of Puerto Rico, SCN was not detected. Apparently, SCN had not been disseminated to other soybean fields. To avoid the potential spreading or new introduction of this nematode, ten workshops were given during the last year. These workshops were sponsored by the USDA-CSREES, TSTAR-161 project. Extension educators, specialists, staff of soybean seed companies, and federal and local agricultural inspectors had been trained in diagnosis methods and preventive control practices of SCN.

Role of *Sclerotinia sclerotiorum* isolate aggressiveness, multiple inoculations and post-inoculation evaluation dates for selecting white mold resistant common bean

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White mold (WM) is an important disease of common bean and partial resistance is found in the primary and secondary gene pools. The objectives of this study were to determine (i) the aggressiveness of four isolates of *Sclerotinia sclerotiorum*, (ii) the most appropriate post-inoculation evaluation date, (iii) the presence or absence of *Phs*, SMe1Em5.110 and SAU5.1360 markers, and (iv) the most resistant genotypes. Thirty one genotypes were evaluated in the greenhouse against ARS12D and ND710 isolates in Idaho and CO467 and NY133 in Colorado. One to three inoculations per plant were made beginning at the 5th internode and disease severity was recorded from 7 to 35 days post inoculation (DPI). Isolate ND710 (5.6) was more aggressive than ARS12D (5.0), and no significant differences were observed between CO467 (5.4) and NY133 (5.3). Evaluation at 28 DPI was optimum in Idaho, and score at 21 DPI (5.4) was significantly higher than at 7 DPI (3.6) in Colorado. ‘Chase’, ‘ICA Bunsí’, and USPT-WM-1 had susceptible scores (>6) in Idaho and intermediate (>4-6) in Colorado. A 195 and interspecific breeding line (IBL) VCW54 derived from *P. coccineus* had the lowest scores in both environments (4-5.1). SE153 (USPT-WM-1/CORN601//USPT-CBB-1/92BG-7) carried the *Phs* marker. A 195, G 122, PC 50, VA 19, SE152 (CORN501/G 122//A 195/VCW 55) and SE154 (VA 19/MO 162//A 195/G 122) had the *Phs* and SMe1Em5.110 markers. NY6020-4 exhibited the *Phs* and SAU5.1360 markers while SE155-9 (A 195/4/NY6020-4/92BG-7//MO 162/19365-25//ICA Bunsí/G 122) possessed all markers. None of the markers were detected in Middle American genotypes and IBL. Breeding line SE152-6 and pinto SE153-7 with 3.7 score followed by SE153-6, SE153-3, and VC13-5 (UI320²/PI439534) with 3.8 scores had the highest levels of resistance. Use of multiple isolates, delayed evaluations (21 or 28 DPI) and at least three inoculations were required for selecting highly WM resistant genotypes.