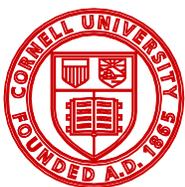


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[1] Interactions between grapevine and bacterial pathogens *Agrobacterium vitis* and *Xylella fastidiosa*

Tina Wu¹, Lingyun Hao², Desen Zheng², and Thomas J. Burr²

¹Iowa State University, Ames, IA 50011. ²Cornell University, Dept. of Plant Pathology and Plant-Microbe Biology, Geneva, NY 14456.

Two bacterial diseases that impact grapes are Pierce's Disease caused by *Xylella fastidiosa* (*Xf*) and Grape Crown Gall Disease caused by *Agrobacterium vitis*. Motility is one key feature in *X. fastidiosa* pathogenicity. A *pil-chp* operon of *Xf*, homologous to known *Escherichia coli* chemotaxis genes, includes *pilJ* and *pilG*. The role of these genes on motility in grape sap is examined using microfluidic chambers and micro concavity slides. *pilG* shows less motility than wildtype *Xf*. For *A. vitis*, F2/5 is a non-tumorigenic strain that inhibits grape crown gall formation. Understanding F2/5 and its mechanism of grape tumor inhibition (GTI) may lead to development of a biological control. Four genes identified to affect GTI in F2/5 include *soxR*, *oxyR1*, and *msrA* and *msrB*. Mutants of *oxyR1*, *msrA* and *msrB* demonstrated reduced GTI. *soxR* mutant showed loss of GTI, indicating it positively regulates GTI by F2/5. All mutants exhibited increased sensitivity to hydrogen peroxide but not to menadione, suggesting that the tolerance of oxidants may be involved in GTI by impacting survival of F2/5. Further research is needed to determine how the sensitivity of these genes to oxidants are associated with F2/5 GTI activity.

[2] Identifying a Major Source of *Iris Yellow Spot Virus* (IYSV) in New York Onion Cropping Systems

Emily Justus¹, Ashely Leach², Marc Fuchs³, Brian Nault²

¹Dept. of Entomology, Purdue University, West Lafayette, IN 47907. ²Dept. of Entomology, ³Dept. of Plant Pathology and Plant Microbe Biology, Cornell University, New York State Agricultural Experiment Station, Geneva, NY 14456

Iris yellow spot virus (IYSV) is an economically important pathogen of onions that is transmitted by *Thrips tabaci*, a common onion pest. In 2006, IYSV was detected in many New York commercial onion fields. Managing IYSV will require a thorough understanding of its epidemiology. There are three potential sources of IYSV inoculum: volunteer onions, imported transplant onions, and perennial weed hosts. To identify which source of IYSV may be significant in initiating epidemics, *Lisianthus* sp., an indicator plant for IYSV, was placed near each potential source and tested weekly for IYSV using DAS-ELISA. As of late July, none of the *Lisianthus* sp. had tested positive. Additionally, imported transplant onions were sampled and tested for IYSV using DAS-ELISA and confirmed by RT-PCR. Roughly 7,000 plants consisting of 7 cultivars (Bradley, Brandt, Festival, Highlander, Moondance, Red Sky and Red Defender) were examined. Transplant samples revealed that Moondance, Highlander, Red Defender, and Red Sky had high percentages of infected plants, 74, 51, 46 and 41%, respectively. Festival was the only cultivar testing negative for the pathogen. Results suggest that imported onion transplants from Arizona may be the most significant source of IYSV epidemics in New York.

[3] Sensory Evaluation and Instrumental Modeling: Applying Food Science

Maxwell Holle¹, Dr. Jessie Usaga², and Dr. Olga I. Padilla-Zakour²

¹Monmouth College, Monmouth, IL 61462. ²Cornell University, Department of Food Science, Geneva, NY 14456

Reduction of Sodium in Ranch Dressing using NuTek 14510

Over consumption of sodium is known to lead to health detriments. Potassium has been targeted for replacing sodium through substitution of KCl for NaCl. This isn't a simple substitution because potassium chloride possess a metallic bitter taste at certain concentrations. NuTek Salt Co. has developed a potassium chloride substitute (14150) in order to replace salt and/or MSG in ranch dressing. Ten formulations were developed and a sensory evaluation was performed on the best two (selected by NuTek). Sensory evaluations, a triangle and a preference test, indicated that there was no perceived difference and no significant preference between a standard ranch dressing and the ranch dressing made with 14510. The 14510 can be used to reduce sodium and MSG in ranch dressing.

Evaluating Ascorbic Acid for Commercial UV Pasteurization Modeling

UV pasteurization has been of particular interest to small juice producers due to its cost and effectiveness. The presence of UV absorbing chemicals interferes with the UV pasteurization. The UV absorbing chemical ascorbic acid is present in many juices. The absorption coefficients for varying ascorbic acid concentrations were determined and related to their flow rates. Using this relationship, pilot plant trials were conducted using commercial juices in order to evaluate whether the flow rate could be predicted from the absorptivity. Commercial juices were inoculated with *E. coli* ATCC 25922 to determine the log reduction after UV treatment. The model was able to predict flow rate, but 5 out of the 8 juices tested were not in compliance with the 5 log reduction required by the Federal Juice HACCP Regulation.

[4] Evaluation of Pea Varieties for Root Rot Resistance Utilizing a Soil Indexing Bioassay in the Greenhouse

Jenna Platt¹, George Abawi², Kundan Moktan², James Choi²

Auburn University, Department of Crop, Soil and Environmental Sciences, Auburn, AL 36830¹
Cornell University, New York State Agricultural Experiment Station, Geneva, NY 14456²

Root rot diseases are major production constraints to pea production in New York State, impacting yield quantity and quality. The primary soilborne pathogens known to cause root rot diseases on peas are *Fusarium solani* f. sp. *pisi*, *Pythium ultimum*, *Rhizoctonia solani*, and *Thielaviopsis basicola*, individually or in various combinations. The use of resistant varieties is the most cost-effective measure for controlling these root diseases of peas. Thus, we have been evaluating the reaction of commercially available and promising varieties of peas to root rot pathogens under field and greenhouse conditions. This study was conducted to evaluate the reaction of 23 pea varieties to root pathogens utilizing a soil indexing bioassay in the greenhouse and a naturally infested soil with a history of severe root rot incidence. None of the varieties tested were found to be resistant, but a number of these varieties showed tolerance to the root pathogens of peas as suggested by their lower root rot severity ratings, initial appearance of foliar disease symptoms, and plant fresh weight. The varieties with the lowest root rot ratings were Grundy, Ricco, Hudson and Seminis 0969QH. The predominate fungi isolated from infected pea roots and confirmed by DNA profiles were *Fusarium* spp.

[5] Characterizing an Interspecific Apple Population (*Malus domestica* x *Malus fusca*) for Plant Architecture and Leaf Lobing

Silas Childs¹ and Susan K. Brown²

¹West Virginia University, Department of Plant and Soil Sciences, Morgantown, WV, ²Department of Horticulture, NYSAES Cornell University, Geneva, NY

Tree architecture is important to apple production for its affect on tree spacing and fruit quality. In certain genetic backgrounds, the dominant columnar (Co) gene is suppressed, making phenotyping difficult. Leaf lobing is an ornamental trait that is important in crabapple breeding but is highly variable in some plants. A better understanding of leaf lobing and its transmission is needed to effectively breed for ornamental crabapples with leaf lobing. The lobed leaf trait in *Malus* has not been explored and may serve as a model to explore leaf development in plants. This study analyzed progeny from a cross between a columnar apple selection (NY152) and *Malus fusca*, a wild species with lobed leaves. Individuals were phenotyped for leaf lobing abundance, architecture, and several other traits, with an emphasis on characteristics appearing to segregate with either lobing or architectural traits. While lobed leaves occurred on 95% of the progeny, 40% of the lobed progeny only had 1 to 4 lobed leaves. Leaf venation was correlated strongly with leaf lobing and leaf length was correlated with tree architecture, leaf serration, and stipule length. Analysis of correlated traits has provided ways to identify phenotypes and has helped explain variability among leaf types.

[6] Temporal changes in concentrations of airborne inoculum in vineyards and effects of light quality on conidial production in *Erysiphe necator*.

Alexander Best¹, David Gadoury² and Robert Seem²

¹Tuskegee University, Tuskegee, AL 36088. ²Cornell University, Dept. of Plant Pathology and Plant-Microbe Biology, Geneva, NY 14456.

Prior work has demonstrated that conidiophore production is light-independent, but conidiation is light-dependent in the grapevine powdery mildew pathogen *Erysiphe necator*. My objectives were to determine the specific wavebands responsible for control of sporulation and document the patterns of sporulation under vineyard conditions as they relate to time after sunrise. Inoculated Chardonnay leaves bearing colonies ready to sporulate were incubated in (i) daylight, (ii) darkness, (iii) red light, or (iv) blue light. No sporulation occurred in darkness, but sporulation was abundant in daylight. Marginal and sparse sporulation occurred in both the red and blue light treatments. In field experiments, there was a clear peak in airborne inoculum dose over 9 consecutive days between 11:00 AM and 16:00 PM. The peak in concentration was correlated with time after sunrise, but not temperature, wind speed, rainfall, or RH, and reflected the expected time of release after sunrise based upon our prior lab studies, and from vineyard studies in Washington. Sparse sporulation in both the red and blue light may indicated a threshold effect of light. Repeating the study using narrow band pass filters or narrow band Light Emitting Diodes may clarify the role of specific wavebands of light in control of sporulation.

[7] *TAC1* and *LAZY1* Regulate Branch Angles in Connection with Gravity, Light, and Auxin

Allison Brown*, Courtney Hollender, Chris Dardick

USDA-ARS Appalachian Fruit Research Station, Kearneysville, WV 25430

Plant architecture has a significant impact on orchard productivity. Ideal tree architecture allows for mechanical harvesting and enables high planting density. The *TAC1* and *LAZY1* genes play an important role in determining branch angles. This was investigated using plum trees as well as *Arabidopsis thaliana* (*At*). Plants with a non-functional *tac1* gene display narrow branch angles, while plants with mutant *lazy1* genes have wide branch angles. *tac1* and *lazy1* mutants respond differently to gravity and light. When *At* plants were rotated 90°, *tac1* mutants responded faster, whereas *lazy1* mutants were delayed compared to wild type in terms of reorientation to vertical growth. When grown under high light, *lazy1* and *tac1;lazy1* double mutant branches grew downwards, away from the light. The altered branch angles in *tac1* and *lazy1* mutants may be a result of improper localization of auxin, a plant growth hormone. To study this, *tac1* and *lazy1* mutant *At* lines containing auxin reporter genes, including the DR5 promoter driving GUS, were developed to visualize the localization of auxin. Altogether, the data show that *TAC1* and *LAZY1* genes control plant architecture by regulation of auxin in response to light and gravity stimulation.

[8] Investigation of factors involved in DMI and QoI fungicide practical resistance in *Malus X domestica* ‘Empire’, ‘Jonagold’ ‘McIntosh’ and ‘Cortland’

Katrin A. Ayer¹ Sara M. Villani², and Kerik D. Cox²

¹Hobart and William Smith Colleges, Department of Biology, Geneva, NY 14456. ²Cornell University, Department of Plant Pathology and Plant-Microbe Biology, Geneva, NY 14456

Apple scab, caused by *Venturia inaequalis*, is one of the most devastating fungal diseases of apple, severely limiting fresh market production in the northeastern United States. Practical resistance to demethylation inhibitor (DMI) and quinone outside inhibitor (QoI) fungicides used to control apple scab is becoming increasingly prevalent in *V. inaequalis* populations throughout the region. Although sensitivity of *V. inaequalis* populations to these fungicides highly impacts their performance, other factors including cultivar and inoculum potential may also influence fungicide efficacy in the field. A fungicide efficacy trial was conducted at an ‘Empire’/‘Jonagold’ orchard and a ‘McIntosh’/‘Cortland’ orchard, both located in Geneva, NY, to determine the effects of cultivar and inoculum potential on practical resistance. Both locations have a history of resistance to DMI and QoI fungicides. For each cultivar, the incidence and severity of apple scab symptoms on leaves and fruit was determined for four fungicide programs (untreated check, Topguard, Inspire, and Flint WG). Additionally, apple scab lesion development on leaves and fruit for each cultivar and fungicide treatment was determined by evaluating lesion size and number of conidia produced per lesion. Preliminary data suggests that both cultivar susceptibility and inoculum potential are important factors of fungicide performance in the field.

[9] Rapid diagnostic and detection strategies for management of tomato late blight

Martha A. Sudermann^{1,2}, Zachariah R. Hansen², and Christine D. Smart²

¹Saint Olaf College, Northfield, MN 55057. ²Cornell University, Dept of Plant Pathology and Plant-Microbe Biology, Geneva, NY 14456.

Late blight, caused by the oomycete *Phytophthora infestans*, is a recurring disease that threatens tomato and potato crops. *P. infestans* was responsible for the Irish Potato Famine and continues to devastate potato and tomato crops on a large scale. This provides a strong impetus for the development of reliable and effective management strategies. A need exists for detection and diagnostic tools that can be used in the field. Loop-mediated isothermal amplification (LAMP) with hydroxynaphthol blue dye has the potential to be a diagnostic tool for *P. infestans* because it is rapid, does not require a thermocycler, and results can be observed visually. A series of LAMP assays were conducted to test the feasibility of using LAMP for *P. infestans* diagnostics. The limit of detection of *P. infestans* was found to be 0.1 pg/ μ L when using DNA isolated from pure cultures. *P. infestans* was also detected in DNA extracted from late blight lesions on tomato leaves. The LAMP assay demonstrated a high level of specificity when conducted with other oomycetes, as well as unrelated fungi and bacteria. However, these primers could not differentiate *P. infestans* from high concentrations of *P. nicotianae* DNA. The ongoing project of using roto-rod spore traps to detect *P. infestans* sporangia in air samples continued. All quantitative PCR results from spore trap samples collected from March to July 2014 were negative. This was in line with the late blight occurrence map. LAMP and the use of spore traps prove to be promising tools for the diagnosis and detection of late blight and *P. infestans* in the field.

[10] Pollinators of strawberries continue to use resources from wildflower strips after strawberry bloom

Ellie McCabe^{1,2}, Heather Connelly², and Gregory Loeb²

¹University of Wisconsin Madison, Madison, WI and 53706. ²Cornell University, New York State Agricultural Experiment Station, Geneva, NY 144562.

Planting wildflower strips has been recommended to attract more native pollinators to flowering crops in order to increase yields. These strips are designed to provide season-long food and habitat for native bees and have been found to increase bee abundance and diversity. However, it is unknown in many systems if the bees benefiting from the wildflower strips are the same bee species providing pollination services to the crop. In strawberries, bee pollination is important for the commercial value of the fruit. In this study, we compared the pollinator communities of strawberries and the wildflowers planted in adjacent strips in order to better understand how wildflowers strips affect crop production. Of the 18 species of bees caught on strawberry blooms, six species (33%) were also found visiting plant species included in wildflower strips, which bloom after strawberry. Of the six, three bee species (17%) were found only on *Penstemon digitalis* and three species (17%) were found only on *Coreopsis lanceolata*. There was no difference in the average number of visits by bees between these two wildflower species. Our results suggest a diversity of later blooming wildflowers is important in conserving wild bee communities, many of which provide pollination services to strawberry.

[11] Silencing Suppressors Improve *Grapevine Fanleaf Virus Infection in Planta*

Jesus Banderas¹, Marc Fuchs²

¹University of California Davis, Davis, CA 95616. ²Cornell University, Department of Plant Pathology and Plant-Microbe Biology, New York State Agricultural Experiment Station, Geneva, NY 14456

Grapevine fanleaf virus (GFLV) is an economically important virus of grapevines that causes reductions in yield and fruit quality. GFLV has a bipartite RNA genome. To study virus-host interactions, binary vectors containing one of the two cDNAs of GFLV RNA1 and RNA2 from two different strains, F13 and GHu, have been developed for use in agroinfiltration of *Nicotiana benthamiana*, a systemic host. Systemic infection is achieved *in planta* following agroinfiltration, but the efficacy of this system can be low. To improve the rate of GFLV infection in *N. benthamiana* following agroinfiltration, different combinations of RNA1 and RNA2 cDNAs from the two GFLV strains were tested in conjunction with one of four RNA silencing suppressors: 2b of *Cucumber mosaic virus*, P19 of *Tomato bushy stunt virus*, P24 of *Grapevine leafroll-associated virus 2*, and HC-Pro of *Tobacco etch virus*. Preliminary data suggest that P24 is the best candidate for increasing the rate of systemic GFLV infection. The identification of a silencing suppressor that interferes with antiviral plant defenses and improves the efficacy of the GFLV agroinfiltration delivery system has implications in the design of a new binary vector that carries, for example, both a viral cDNA and P24.

[12] Novel Pest Management: Release of Insects Containing a Dominant Lethal Gene

Devan George¹ Tim Harvey-Samuel^{2,3} Michael Bolton^{2,4} Anthony Shelton⁵

¹University of New Hampshire, Durham, New Hampshire, 03824. ²Oxitec Ltd, 71 Milton Park, Oxford OX14 4RX, United Kingdom. ³University of Oxford, Oxford, United Kingdom. ⁴University of East Anglia, Norwich NR4 7TJ, United Kingdom. ⁵Cornell University, New York State Agricultural Experiment Station, Geneva, NY 14456.

A Sterile Insect Technique known as Release of Insects Containing a Dominant Lethal (RIDL) is being developed and tested for diamondback moth (DBM) pest management. DBM is a major pest of crucifer crops annually causing ca. US\$4-5 billion in damage globally. RIDL insects contain a lethal transgene, which when expressed causes the activation of a lethal positive feedback loop in ♀. When reared in the lab, RIDL moths are fed tetracycline, which represses the positive feedback loop. Tetracycline is then removed from the diet before release, ensuring a ♂ only release. RIDL ♂ will then mate with wild ♀, producing ♀ and ♂ progeny with the RIDL gene. Unable to find tetracycline in the wild, the ♀ progeny will die off, and the ♂ will mate with wild ♀, passing on the gene. We will conduct two trials, the first involves releasing RIDL ♂ and wild type (WT) ♂ and ♀ into six field cages. The suppression potential of RIDL ♂ will be evaluated by determining the proportion of RIDL to WT progeny. An open field release of 10,000 ♂ DBM will compare the flight behavior and field persistence of RIDL and WT moths.

[13] Evaluation of Crucifer Interspecific Hybrids for use as Natural Food Colors

Rebecca Reeve^{1,2}, Alexandra Bennet², Didier Socquet-Juglard², Phillip Griffiths²

¹Oregon State University, Department of Horticulture, Corvallis, OR 97331. ²Cornell University, Department of Horticulture, New York State Agricultural Experiment Station, Geneva, NY 14456

Natural food colors are of increasing interest in the food industry as a replacement for artificial colors that are becoming unavailable due to government regulations. Anthocyanins are synthesized in berries, vegetables, and other crops. Acylated anthocyanins are more stable as a source of natural colors and are found in high levels in Crucifer vegetables. A relative increase in concentration of acylated anthocyanins per gram of product coupled with a decrease in odor/sulfur compounds could be obtained through interspecific crossing to create new breeding lines/cultivars selected for high levels. Red-hearted radish breeding lines (*Raphanus sativus*) were used as female parents based on internal coloration/ low odor and crossed with different morphotypes of *Brassica oleracea* (cabbage, kale, cauliflower, brussels sprouts, and kohlrabi) that have high levels of diacylated anthocyanins. Crosses were performed and either allowed to develop naturally into seed, or embryos were rescued via tissue culture. Selection of interspecific plants was based on phenotype/leaf morphology and high internal anthocyanin concentration in swollen hypocotyls/stems. Phenotypes of the interspecific hybrids were intermediate to the parents. Future assessment of factors influencing levels of diacylated anthocyanins and lower sulfur levels will allow for cost efficient production and commercialization of natural colors for the food industry.

[14] Genetic diversity of the *MQ8* locus, a major QTL conferring apple fruit acidity

Timothy Batz¹, Kenong Xu²

¹California State Polytechnic University Pomona, CA 91768. ²Cornell University, Dept of Horticulture, Geneva, NY 14456

Acidity is a major contributor to apple fruit quality. Apples with titratable acidity (TA) of 0.30-1.00% are acceptable for flesh consumption, whereas those with a TA beyond this range are either too tart or flat. Breeding apple varieties of optimum acidity levels is challenging as seedlings bearing low acid apples often account for >25% in the breeding populations. Malic acid is the predominant organic acid in mature fruit and largely determines acidity levels. The major QTLs (quantitative trait loci) that control fruit acidity are *Ma* (*malic acid*) and *MQ8* (*Malic QTL8*), which are located on chromosome 16 and 8, respectively. The *Ma* QTL has been recently isolated and characterized, but *MQ8* remains to be understood. The difficulty has been that the effect of *MQ8* is detectable only in 'Fiesta', an uncommon apple variety not available in the USDA's *Malus* collection. To identify the gene(s) underlying *MQ8*, a population genomics approach is taken to investigate the genomic region harboring *MQ8*. DNA was isolated from 470 *Malus* accessions in two sets and was fingerprinted with two markers linked to *MQ8*. The data reveal a tremendous diversity in these *Malus* accessions. Interestingly, there are 16 apple accessions of identical genetic patterns as 'Fiesta'.

[15] A performance comparison of depositional decay and drift related to sprayer louvre adjustment in Vineyard canopies

Kasia Taylor², Andrew Landers, Tomas Palleja Cabre², Kian Kenyon-Dean², and Pam Adams²

¹Mansfield University, Mansfield, PA 16933. ²Cornell University, Dept of Entomology, Geneva, NY 14456

The persistence of hazardous drift accompanied by the economic loss of pesticides is a continuous problem among growers. An experiment was conducted using *V. vinifera* cv. Vignole grapevines at NYSAES, Cornell University, Geneva, New York. An analysis was performed to compare the differences in deposition and drift associated with louvre adjustment, single and double-sided spraying and decay based on leaf location within the canopy. There were two trials each held on June 26th and July 21st respectively. Deposition concentrations were evaluated using Tartrazine dye and a multidetection microplate reader. Drift from the sprayer was determined using coverage on water sensitive cards at various distances (every 46cm) to the right of the target row. Results show that in the first trial the louvre half open gave better deposition because it matched the airflow requirement of the canopy. In the second trial double-sided and single-sided with louvre fully open have similar results because the increase in air was needed for liquid penetration into the canopy. And in all cases as expected, double-sided spraying with the louvre half open gave the best deposition, single-sided depositional decay occurred from the left (nearest to sprayer) to the right side of the canopy by 50%, and the louvre half open had increased drift at ground level due to louvre design.

[16] Effects of pH and tomato sap on biofilm formation for the vascular pathogen *Clavibacter michiganensis* subsp. *michiganensis*

Chase R. Crowell^{1,2}, Matthew A. Tancos², and Christine D. Smart²

¹Rhodes College, Memphis, TN 38112, ²Cornell University, Dept. of Plant Pathology and Plant-Microbe Biology, Geneva, NY 14456

Clavibacter michiganensis subsp. *michiganensis*, the causal agent of bacterial canker of tomato, is a seed-borne Gram-positive pathogen that inflicts considerable damage internationally. *C. michiganensis* subsp. *michiganensis* travels systemically throughout the xylem forming biofilm-like structures, and spreads laterally into the parenchyma cells when high titers ultimately macerate the vasculature. Our preliminary studies demonstrated that *C. michiganensis* subsp. *michiganensis* exhibited some method of motility, yet historically it has been characterized as being non-motile. Therefore, our study was looking into the possible effect of pH on motility and biofilm formation for *C. michiganensis* subsp. *michiganensis*. Vectors were designed (pGEM-T Easy) to knockout genes putatively involved in motility or biofilm development including: *chpE*, *nagA*, *expA*, *wcmN*, and *tadC*. Biofilm assays were conducted using extracted tomato sap and/or xylem mimicking media at the pH of 5.0, 5.5, 6.75, or 8.0. The preliminary data demonstrates that a mixture of 50% tomato sap and xylem mimicking media at a pH of 5 exhibited the greatest biofilm formation potential, which supports our hypothesis that the natural acidity of tomato xylem fluid (pH 5.0-5.5) acts as a spatial selection force *in planta* for *Clavibacter michiganensis* subsp. *michiganensis* colonization.

[17] Analysis of Columnar (*Co*) Candidate Genes in Apple

Thomas Jordan¹, Kenong Xu²

¹ SUNY College of Nanoscale Science and Engineering, Albany, NY, 12203. ² Department of Horticulture, NYSAES, Cornell University, Geneva, NY 14456.

Columnar apple trees have narrower branch angles and shorter internodes than standard trees, and bear fruit almost exclusively on spurs along the trunk and major branches. The gene underlying this phenotype is known as *Columnar* (*Co*). *Columnar* is dominant over standard and *Co* is localized to chromosome 10. It is reported that a transposable element insertion into the *Co* region is the genetic cause for the columnar phenotype, but the actual biological mechanism caused by the insertion is unknown. To help elucidate the mechanism, we analyzed the expression patterns of genes near the insertion. Leaves, main shoot tips, and branch tips were collected from columnar and standard individuals in a population segregating for both phenotypes. PCR primers designed to confirm the presence of the insertion were tested on DNA extracted from the leaves. The results showed the insertion is perfectly correlated with the columnar phenotype. RNA extracted from both main shoot and branches was reverse transcribed and tested for differential expression of three genes. A bHLH transcription factor gene showed no differential expression, and is therefore not responsible for the columnar phenotype. A 2OG-Fe(II) oxygenase gene showed inconclusive patterns of differential expression. An auxin response gene seemed more strongly expressed in columnar individuals and may cause the phenotype.

[18] Do golf course fungicide applications suppress beneficial soil biota?

Louisa Rogers¹, Huijie Gan², and Kyle Wickings²

¹Whitman College, Walla Walla, WA 99362, ²Cornell University, Dept of Entomology, Geneva, NY 14456

Fungal pathogens constantly threaten golf turfgrass, and as a result, course managers often rely heavily on fungicides. While fungicides are intended to reduce fungal pathogens, they may also suppress symbiotic and saprophytic fungi. Unintended shifts in fungal biomass and activity could have downstream effects on fungivorous arthropods. This study aimed to determine the effects of fungicide applications on golf turfgrass soil biota by quantifying differences in: 1) root colonization by arbuscular mycorrhizal fungi (AMF), 2) AMF spore density, 3) activity of extracellular microbial enzymes involved in carbon and nutrient cycling, and 4) collembolan and oribatid mite density between fungicide-treated golf courses, untreated golf courses, and an undisturbed grassy meadow. AMF colonization of roots was significantly lower in fungicide-treated turfgrass, though spore abundance was not affected. Microbial enzyme activity was not sensitive to fungicide application, but several enzymes varied with site. Six collembolan and oribatid taxa were found across the sites sampled. Oribatid mites were denser in the meadow than in one golf course, and tomoceridid collembolans were found exclusively in the meadow. Suppression of the AMF symbiosis could decrease turfgrass water and nutrient uptake in the long run, but the full ecological effects of AMF inhibition merit further exploration.

[19] Further Investigations of Acute-Cold Disease Suppression in the Grapevine Powdery Mildew Pathosystem.

Billy Weldon^{1,2}, David Gadoury², Jason Londo³, Lance Cadle-Davidson³

¹Butler University, Indianapolis, IN 46208. ²Cornell University, Dept. of Plant Pathology and Plant-Microbe Biology, Geneva, NY 14456. ³USDA-ARS Grape Genetics Research Unit, Geneva, NY 14456.

Prior work on powdery mildew of grapevine (*Erysiphe necator*) in European wine grapes (*Vitis vinifera*) demonstrated that acute overnight low temperature events (2-8 °C) induced a defense response in young susceptible leaves (3rd leaf); a response we call cold shock. My objectives were (i) to investigate this response in older (ontogenically resistant) leaves, (ii) to quantify changes in stress- and defense-related compounds in cold treated grape leaves, and (iii) to assess the responses of genes involved in pathogen penetration defense in Arabidopsis, in the context of acute cold treatments. Selected detached Arabidopsis leaves were exposed to cold treatments (4 °C) for 4 hr, inoculated 4 or 24 hrs later, and then germling development was rated. Results indicate a significant reduction in powdery mildew penetration in the samples inoculated 24hr post-cold shock. A significant reduction in secondary hyphal growth was reconfirmed in immature grape leaves, and we also observed a similar significant inhibition in the hyphal growth on mature leaves. HPLC quantification of salicylic acid levels in *V. vinifera* leaves exposed to acute cold was inconclusive due to insignificant readings for the positive controls, indicating a need to revisit the protocol for purifying leaf tissue for HPLC analysis.

[20] Variation of phenotypic characteristics in populations of *Sclerotinia sclerotiorum* causing white mold of snap bean in New York

Jenna K. Van Bruggen^{1,2}, Amara R. Dunn², Christine D. Smart², and Sarah J. Pethybridge²

¹Calvin College, Grand Rapids, MI 49546. ²Cornell University, Dept of Plant Pathology and Plant-Microbe Biology, Geneva, NY 14456

White mold, caused by *Sclerotinia sclerotiorum*, is one of the most economically damaging diseases of snap bean (*Phaseolus vulgaris*) and other vegetables. The goals of this project were to compare aggressiveness on snap beans among different isolates of *S. sclerotiorum*, and test for resistance to a commonly-used fungicide. Sclerotia were also conditioned to carpogenically germinate for the production of ascospores for use in field trials quantifying fungicide efficacy. Seventeen isolates were used in detached pod and leaf assays which quantified aggressiveness by measuring lesion size. The reproducibility of results between replicates from the pod assay was higher than using leaves. Moreover, in the leaf and pod assays, results were similar for some of the isolates. Significant variation in aggressiveness between isolates was also found. Of the isolates (n = 8), all were sensitive to the fungicide thiophanate-methyl at 10 mg/L based on a mycelial growth assay. Ascospore production and sclerotial conditioning was also conducted, and ascospores were used for inoculum of this year's field trial.

[21] Effects of Streptomycin and Kasugamycin on Epiphytic Bacteria in the Apple Phyllosphere

C. Miller¹, K. Bekoscke², S. Villani², and K. Cox²

¹Department of Chemistry, William Smith College, ²NYSAES, Department of Plant Pathology and Plant-Microbe Biology, Cornell University

Apple trees in bloom are highly susceptible to Fire Blight, a bacterial disease caused by *Erwinia amylovora*, and a major problem for the NY apple industry. Streptomycin is the most commonly used and most effective antibiotic to control fire blight. Unfortunately, streptomycin resistant strains of *E. amylovora*, which are widespread on the west coast and in Michigan, have recently been discovered in NY. The antibiotic kasugamycin has been shown to effectively manage fire blight, but it is not registered for commercial use in New York State. Our objective was to investigate how streptomycin and kasugamycin affect epiphytic bacteria in the apple phyllosphere. Trees were treated with either Agri-Mycin (streptomycin) or Kasumin 2L (kasugamycin) in sets of 0, 3, or 5 sprays on a seven-day schedule with four replicates per treatment. By collecting leaf samples, a subset of the epiphytic bacteria population was surveyed to identify changes caused by each antibiotic. PCR was used to identify the bacteria and whether or not it contained the most common mechanism of strep resistance found on the east coast, the *strA/strB* gene pair. Our results demonstrate that kasugamycin resistance is not present in the orchard and kasumin could reduce populations of streptomycin resistant bacteria. Future studies will focus on the effects of alternating kasugamycin and streptomycin for treatment of fire blight to mitigate the development of antibiotic resistance in NY orchards.

[22] Evaluation of fungicide resistance of *Botrytis cinerea* isolates from Long Island and comparison of *B. cinerea* isolation using two different media

Charlie Garcia^{1,2}, Megan Hall², and Wayne F. Wilcox²

¹California State University, Fresno, Fresno, CA 93740. ²Cornell University, Plant Pathology and Plant-Microbe Biology, NYSAES, Geneva, NY 14456

Gray mold (caused by *Botrytis cinerea*) is a major disease of wine grapes (*Vitis vinifera*). The first experiment examined the fungicide resistance of 42 *B. cinerea* isolates obtained from 10 Long Island vineyards, by assaying mycelial growth on media amended with various concentrations of boscalid, cyprodinil, trifloxystrobin, iprodione, and fenhexamid. All fungicides reduced growth at their highest concentration; however, with the exception of iprodione, there was no statistically significant dose response at the lower concentrations. Overall, the results of this study were inconclusive. The second experiment sought to compare the isolation of *B. cinerea* from “blossom trash” (necrotic floral parts remaining within the developing grape clusters) using either potato dextrose agar amended with antibiotics (PDA+) or a complex *Botrytis* Selective Media (BSM). Not only did BSM yield a higher frequency of isolations, but it was easier to view the *Botrytis* growing from tissues plated on this medium in order to make the assessment. Despite the more complicated media-making process, BSM is the preferred choice for evaluating the infection of grape blossom trash with *Botrytis*.

[23] Persistence of Entomopathogenic Nematodes in New York Apple Orchards and their Effectiveness in Controlling Plum Curculio

Emily Pennock^{1,2}, Tessa Lessord², and Art Agnello²

¹The Pennsylvania State University, University Park, PA 16802. ²Cornell University, Dept. of Entomology, Geneva, NY 14456.

Plum Curculio, *Conotrachelus nenuphar*, is a significant pest of apples, causing damage to fruitlets by oviposition. Even using insecticides, growers can sustain 0.5 to 3% yield loss due to these weevils. When studies showed entomopathogenic nematodes (EPNs) to be highly effective in controlling alfalfa snout beetle populations, researchers began to investigate nematodes as a biocontrol for Plum Curculio. In 2012, two species of EPNs, *Steinernema feltiae* and *S. carpocapsae*, were introduced into apple orchards. The persistence of these nematodes and their effectiveness against Plum Curculio was evaluated by placing Plum Curculio larvae into plots inoculated with nematodes in 2012, plots inoculated this summer, and plots that were not inoculated. Due to slow larval production, the experiment is still in progress; therefore the data is, as of yet, inconclusive. That being said, current data indicated diminished numbers of nematodes in the plots inoculated in 2012 as compared to those inoculated this year, although that number has building steadily after a slump in the 2012 season. Adult weevil emergence has been comparatively high in the inoculated plots, though fruit damage has been lower in these rows. Further research must examine soil characteristics and fauna to understand the apparent lag in EPN activity.

[24] Comparison of beetle feeding preferences in an interspecific *Salix* hybrid

Brenden Armstrong^{1,2}, Fred E. Gouker², Gregory M. Loeb³, Lawrence B. Smart²

¹Dept. of Horticulture, Kansas State University, Manhattan, KS 66502

²Horticulture Section, School of Integrative Plant Science, ³Dept. of Entomology, Cornell University, Geneva NY 14456

Imported willow leaf beetle (*Plagioderia versicolora* Laicharting) and Japanese beetle (*Popillia japonica* Newman) damage shrub willow (*Salix* spp.) destined for bioenergy production by defoliating them during feeding, which could lead to yield losses. Caged feeding trials in greenhouses were conducted to compare preferences among five genotypes of shrub willow, *Salix koriyanagi* clone ID SH3, *Salix purpurea* clone ID 95058, and three progeny of those two clones in the family 99239. Field-captured imported willow leaf beetle and Japanese beetle adults were released onto potted plants arranged in free choice and no-choice experiments to assess the genotypic differences of leaf damage caused by herbivory. The amount of foliage consumed among genotypes was not significant with regards to the imported willow leaf beetle experiments, whereas there were significant differences in the amount of herbivory by Japanese beetles among genotypes, with preference for SH3 and 99239-020, and little feeding on 95058. Differences in feeding preference of different beetles on the same willow genotypes warrants further study of the mechanisms of resistance.

[25] Characterization of Arabidopsis Mutants Compromised in Non-host Resistance against Grapevine Powdery Mildew

Breanne Kisselstein^{1,2} Rajtilak Majumdar³ Lance Cadle-Davidson^{2,3}

¹Rochester Institute of Technology, Rochester, NY 14623 ²Cornell University, New York State Agricultural Experiment Station, Geneva, NY 14456 ³USDA-ARS Grape Genetics Research Unit, Geneva, NY 14456

Grapevine Powdery Mildew is caused by *Erysiphe necator*, a fungal pathogen that reduces yield and quality of grape products worldwide. Identification and transfer of durable, broad-spectrum resistance to commercially-relevant cultivars is a high priority in grapevine. Functional validation of resistance genes through a transgenic approach in grapevine takes a long time. Arabidopsis provides a comparatively fast model system, but *Erysiphe necator* does not infect Arabidopsis. Our goal was to identify Arabidopsis mutants susceptible to *Erysiphe necator*, and thereby provide a heterologous expression system to test candidate resistance genes against grapevine powdery mildew. Our results suggested that two Arabidopsis mutants (*Pen 3-1/Sid 2-1* double mutant and *Pen 2-1/Pad 4-1/Sag 101-2* triple mutant) are compromised in non-host resistance against *Erysiphe necator* under both *in vitro* and *in vivo* conditions. On these mutants, *Erysiphe necator* penetrated leaf cells to form functional haustorial feeding structures followed by a network of secondary hyphae. Transformation of these Arabidopsis mutant backgrounds with candidate grapevine resistance genes is currently underway in order to more fully understand grape powdery mildew resistance gene function.

[26] Characterizing antifungal mechanisms by assessing cellular viability

Emily M. Mishina¹ Abigail B. Snyder² John J. Churey² Randy W. Worobo²

¹University of Hawaii, Manoa, Honolulu, HI 96821. ²Cornell University, New York State Agricultural Experiment Station, Geneva, NY 14456²

Today, while most bacterial pathogens are understood, there are few available antimicrobials against fungal growth. Mechanisms of many antifungals target the fungal membrane but also interfere with consumer cellular processes.

The widely used cheese preservative natamycin is an antifungal polyene, a category of compounds known for causing cell death by membrane-pore formation. Recent studies suggest that membrane-permeability may be a secondary effect of the ergosterol targeting mechanism. Similarly, the broad-spectrum antifungal polyene amphotericin B is commonly used drug in clinical applications, but has also been shown to induce alternative modes of action for fungal cell death besides membrane permeabilization, which has been observed at higher concentrations.

Cellular viability responses of model pathogenic organism *Candida albicans* were assessed in the presence of amphotericin B and natamycin at MIC (3.91 µg/ml; 15 µg/ml, respectively), 0.5 x MIC, and 4 x MIC. An initial 0.3-0.4-log reduction of *C. albicans* was observed upon exposure to both antimicrobials at MIC and half-MIC concentrations.

These preliminary data will contribute to the characterization of mechanisms for new antifungal agents, specifically, the novel antifungal protein YvgO. Future studies involving YvgO may lead to the development of antifungals for application to fruits, vegetables, juices, dairy, and other perishable produce.

[27] Geneva Blight Genetics: Genotyping-by-Sequencing a Bi-parental Population of *Phytophthora capsici*

Michael R. Fulcher^{1,2} Maryn O. Carlson² and Christine D. Smart²

¹Virginia Polytechnic Institute and State University, Blacksburg, VA 2003. ²Cornell University, New York State Agricultural Experiment Station, Geneva, NY 14456

Phytophthora capsici causes Phytophthora Blight, a devastating disease of solanaceous, fabaceous, and cucurbit crops. This oomycete spreads in surface and irrigation water, soil, and infected plant material. Approximately 30% of New York's vegetable acreage is susceptible to Phytophthora Blight, and recent floods have introduced *P. capsici* into previously uninfested fields. Asexual zoospores allow for rapid proliferation of the pathogen within a growing season, while sexual recombination between two mating types, A1 and A2, produces oospores that are persistent in the soil for many years, making disinfesting a field near impossible. Also complicating control is an emerging resistance to the widely used fungicide mefenoxam. Prior studies using microsatellite loci support a theoretical mitotic loss of heterozygosity, a potential means for rapid fixation of new advantageous alleles. To better understand the population genetics of this pathogen, bi-parental populations were founded in both the field and lab in 2008. Our study characterizes these populations with genotyping-by-sequencing (GBS), a method that can be used to generate genotypes based on single nucleotide polymorphisms (SNPs) drawn from a reduced representation genome. GBS targets non-repetitive sites of high gene density with a methylation sensitive restriction enzyme digest. This study provides a direct comparison of *in vivo* and *in vitro* *P. capsici* populations and insight into population structure, genotypic variation, changes in allele frequency, and loss of heterozygosity.

[28] Black rot resistance in North American *Vitis* Species

Alexander D. Isby, Elizabeth M. Takacs, and Bruce I. Reisch

Department of Horticulture, NYSAES Cornell University, Geneva, NY

Black rot, caused by the pathogen *Guignardia bidwellii*, has become an increasingly important disease in viticultural regions. Black rot will target any new growth of the grapevine and is nearly impossible to control via organic means (Weigle, T. and Carroll, J. 2014). Symptoms on shoot tips and leaves appear as tannish lesions wherein pycnidia develop. On developing berries, symptoms appear as an engulfing rot that eventually leads to mummification. Depending on the environmental conditions and cultivar resistance, black rot can cause miniscule damage to almost complete crop loss. Resistance to the disease varies among grapevine species. The European grape, *Vitis vinifera*, is highly susceptible to black rot, whereas North American *Vitis* species are more resistant (Barrett, H. 1953). Overall, the main goal of the present project was to determine if germination frequency correlates with the variation in black rot resistance of North American *Vitis* species. In order to assess the natural resistance to the pathogen, 20 accessions representing eight grapevine species were cut into leaf discs and inoculated. The onset of infection was quantified by observing germination frequency with a microscope.

References:

- Barrett, H. (1953) A survey of black rot resistance on the foliage of wild grapes. *Proceedings of the American Society for Horticultural Sciences*, 62, 319-322.
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[29] *Grapevine fanleaf virus* symptom development *in planta*: caused by a viral protein or by viral nucleotides?

Kaitley Wozer^{1,2}, Larissa Osterbaan², Marc Fuchs²

¹Hobart and William Smith Colleges, Geneva, NY 14456; ²Cornell University, Dept. of Plant Pathology and Plant-Microbe Biology, Geneva, NY 14456

Grapevine fanleaf virus (GFLV) is present in most regions where grapevines are grown. Infected vines experience progressive decline, shortened lifespan, and yield reductions up to 80%. The GFLV genome consists of two RNAs, called RNA1 and RNA2. While systemic infection requires both RNAs, a small 408 bp region at the 3' end of the RNA1-encoded RNA-dependent RNA polymerase (1E^{Pol}) coding region of strain GHu is responsible for symptom development in *Nicotiana benthamiana*. Another strain, F13, induces an asymptomatic infection on this host. Whether symptom development is dependent on the nucleotide or amino acid sequence of the 1E^{Pol} segment remains unclear. Five gene constructs were designed in the F13 1E^{Pol} region to be analogous to previously-constructed GHu constructs for comparative analysis of symptom development in *N. benthamiana*. Some of these constructs were engineered so that no peptide can be produced. The five constructs were individually cloned into the cDNA of GFLV-GHu RNA2, called pROX, after a double enzyme digest. The recombinant plasmids were then transformed into *Escherichia coli* strain DH5 α , sequenced to confirm integrity, and electroporated into *Agrobacterium tumefaciens* strain GV3101. Such constructs, as well as other previously-designed ones, were used for agroinfiltration of *N. benthamiana* to assess symptom development.