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[http://www.scholars.pppmb.cals.cornell.edu/](http://www.scholars.pppmb.cals.cornell.edu/)
[1] Genetic characterization of *Spissistilus festinus* populations

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The three-cornered alfalfa hopper (TCAH, *Spissistilus festinus* [Say])—a member of the family Membracidae—was first described in 1831. It is the insect vector of grapevine red blotch virus (GRBV) of *Vitis* spp. TCAH is not considered a vineyard pest but can cause substantial yield loss in fabaceous crops such as alfalfa and soybean in the southeastern United States. TCAH has been observed in thirty-eight states, representing a spectrum of hosts and ecosystems across the United States. Information on intraspecies genetic diversity is lacking for TCAH. In this study, twenty *S. festinus* populations were collected in California, Alabama, Georgia, Virginia, North Carolina and Mississippi from grape, peanut, alfalfa, soybean, potato, weeds, and various clovers in 2015-2017. Populations were genotyped by PCR using total DNA extracted from individual specimens and appropriate primers followed by sequencing to characterize a 648-bp region from the 3’ end of the mitochondrial cytochrome C oxidase gene. Nucleotide sequences were aligned and compared using a sequence distance matrix. Specimens grouped into two distinct phylogenetic clades: one comprised of the California populations and the other comprised of populations from the southeastern US states. Within-clade genetic diversity was low (98-100% nucleotide sequence identity) and between-clade diversity was high (80-90% nucleotide sequence identity), suggesting that genetic variability among TCAH populations is dependent on location rather than host crop or collection date.

[2] Germination assays for RNAseq analysis of *Melampsora americana* on *Salix purpurea*

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The fungal plant pathogen *Melampsora americana* is the most damaging pathogen of the bioenergy crop *Salix purpurea*. It causes premature defoliation and can significantly reduce crop yield by up to 50%. To better understand the genes associated with initial stages of the plant-pathogen interaction, we will use comparative RNAseq of germinating rust uredospores on several media and time points to deduce what genes have increased transcription during appressoria formation. In order to conduct such an experiment, each medium chosen requires specific germination assays to ensure consistent germination and appressorium formation rates. Since *Melampsora americana* requires a hydrophobic surface with distinct ridges for appressorium production, as found on leaf surfaces, we inoculated polyethylene surfaces (germ tubes only), oil-collodion membranes (germ tubes and appressoria), and *Salix purpurea* leaf tissue with the pathogen. We then counted the number of spores, germ tubes, and appressoria in 20 microscope fields at 40X magnification at four, six, and 18 hour time points. Because appressoria do not form in the absence of guard cell-like ridges, RNAseq of the polyethylene treatment can be compared to those of collodion membranes and leaf tissue, where appressoria are expected to form. This information can be used to identify effectors related to the virulence of the willow rust pathogen in its initial interaction with the host leaf tissue.
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New York (NY) is the second largest producer of table beet in the United States and the majority of seed is sourced from the Pacific Northwest, including Washington (WA). *Phoma betae* is a destructive seedborne pathogen of table beet capable of causing damping-off, leaf spot, and root decay. The reproductive strategy of a plant pathogen can have substantial implications on population structure, inoculum sources, and disease management strategies. *P. betae* is a heterothallic ascomycete fungus implying that two individuals of opposite mating type (MAT1-1 and MAT1-2) are required for sexual reproduction. A 1:1 ratio of individuals with the opposite mating types may be indicative of random mating within a population. PCR-based assays for each of the *P. betae* mating type genes were developed and validated using isolates from NY and WA. Mating type markers were multiplexed and five *P. betae* populations (n = 214) were screened for each mating type. In one WA population and two NY populations, the ratio of mating types did not significantly deviate from a 1:1 ratio (χ² = 0.9 to 2.08; *P* > 0.05) and in the remaining WA populations did significantly deviate from a 1:1 ratio (χ² = 8.26 and 17.86; *P* < 0.004). Taken together with previous results of genotypic diversity and genetic differentiation studies, these results imply that both recombination and clonality may be occurring in *P. betae* populations.

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Genetic studies of traits important in breeding ornamental crabapples are lacking. It is also not known if the genetics of traits studied in the cultivated apple, *Malus domestica*, will apply. Most current crabapples are derived from open-pollination, so little is known of their pedigree or transmission of traits. In this study, we phenotyped three different interspecific crabapple progenies and studied the segregation of key traits of importance, including leaf color and shape, fruit size and flowering. Leaf color in crabapples follows a red dominant to green model, but variation of intensity of anthocyanin has been related to several transcription factors (MdMYB10, ERF, SPL, and WRKY) and promoter copy number. However, even in a small cross of two parents with leaf color which is fading red to green, we identified nine persistent purple leafed offspring. Fruit size was skewed towards the smallest size, which is desirable to prevent fruit litter. The segregation in a cross of two double flowered parents was a 2:1 ratio of double petals to single petals, indicating a lethal gene. Across all progenies, leaf lobing varied in expression depending on the parents chosen, with ‘Royal Raindrops™’ transmitting persistent lobing to many of its offspring. Even with small population sizes, we confirmed the inheritance of key traits and identified promising progeny for future breeding.
[5] Phenotypic analysis of *Phytophthora capsica* isolates and squash resistance trials

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The phenotypic analysis of several characteristics of a pathogen is the first approach toward finding a treatment and controlling its spread. *Phytophthora capsici* is an oomycete plant pathogen capable of infecting crops such as tomato, pepper, and squash, causing root and stem rot and subsequent death of the plant. Mating type and mefenoxam sensitivity were determined for 40 *P. capsici* samples that were collected in Ontario County. Furthermore, the resistance against this pathogen is being evaluated for 20 squash accessions having genetic similarities with the resistant accession PI 615089. A single zoospore was isolated from each sample to make every culture genetically homogenous. From these new isolates, the mating type (MT) of each sample was determined by growing them in presence of either an A1 or A2 reference isolate and checking for oospores after 5 days of growth. The fungicide assays consisted of transferring each sample to V8 media with increasing concentrations of mefenoxam and measuring relative growth after 3 days in the dark. We found a 1:1 distribution of mating types between all the samples and failed to identify fungicide insensitivity since no colony had relative growth greater than 40% on media amended with 5 µg/mL mefenoxam. Squash accessions will be inoculated in the field in the coming days. This information provides a starting point toward understanding plant pathogen characteristics that may lead to efficient treatment of the organism.


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*Erwinia amylovora* is the causal bacterial agent of fire blight, a major disease of rosaceous crops. Once fire blight becomes established, the plant discolors, and exudes bacterial ooze composed of bacterial sugars, plant sap, and bacteria. Insects, specifically dipterans, have been identified as potential vectors. They have been observed consuming ooze in the field, although whether bacteria persists in/on flies has not been determined. The goal of this study was to evaluate the persistence of *E. amylovora* within *Delia* flies, which are common in orchards. We observed *Delia* flies feeding on ooze in an infected Ida Red apple orchard, recorded total feeding time, captured them separately in tubes and returned them to the lab. Every day each fly was transferred to a new tube, and the old tube was washed, with an aliquot plated on selective media. CFU of *E. amylovora* was determined after incubating plates for 2 days. This process was continued until the fly died or no bacterium was observed. In a laboratory assay, field captured flies were starved and presented with oozing apple fruitlets before being treated as described above. *E. amylovora* persisted on/in the fly from 0-7 days (median =2.5 d) in sufficient titers to infect new hosts. Our results are consistent with the hypothesis that *Delia* flies have high potential to vector fire blight.
Why do powdery mildew epidemics stall early in the growing season?
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Podosphaera macularis, the causal agent of hop powdery mildew (HPM), is an obligate biotrophic pathogen. It is almost wholly external to the host, exposing it to a number of environmental stresses. An enigmatic feature of powdery mildew epidemics on many hosts is stalling of epidemic progress early in growing seasons, despite favorable average temperatures. Stalling of HPM epidemics was associated with acute overnight cold events (i.e., < 10°C) in a retrospective analysis of disease incidence in Pacific Northwest hop yards. Acute cold Stress Induced Disease Resistance (SIDR), a transient host resistance response noted in the grape powdery mildew pathosystem, may be a factor in this response. Cold SIDR makes ordinarily susceptible tissue more resistant to infection, and suppresses established mildew colonies. We subjected established colonies of P. macularis to cold events. Duration of latent period increased and sporulation decreased as cold events became longer and colder. Hyphal density and hyphal viability were reduced by both cold (4°C) events singly, and in sequence on multiple days. Acute overnight cold events occurred even in the warmest climates where hop is cultivated. Acute overnight cold may be responsible for inaccurate performance of predictive models which are based on average temperatures. Modelling of epidemic progress is likely to be improved by including the impact of acute overnight cold events upon host resistance and pathogen suppression.

Population survey and preventive treatments against the black stem borer in apples
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Black stem borer (Xylosandrus germanus [Blandford]), an ambrosia beetle native to eastern Asia, has recently been found in New York, causing damage to apple trees by boring into trunks, which causes tree decline and death. Like many insects, X. germanus is attracted to ethanol, which is given off by stressed plants. To further understand flight patterns and distribution of X. germanus, ethanol-baited traps were set at the edges and interior of apple orchards. Ethanol-soaked bolts of apple branches were also placed in forest habitats with X. germanus populations, with half of the bolts covered with insecticide-treated netting to test its efficacy as a repellent. Other repellent methods for X. germanus were also tested by trunk application of a wax-based paste containing verbenone, an anti-aggregation pheromone, and methyl salicylate, a plant defense chemical, onto potted apple trees stressed by flooding. X. germanus populations were highest during the second and fourth week of May and first week of July, and greater numbers occurring on the edges of orchards. Insecticide-treated netting reduced infestations significantly. The combination of verbenone and methyl salicylate prevented X. germanus attacks the most effectively. Future investigations will allow for further confirmation of X. germanus flight patterns and repellent potentials.
[9] In planta validation of a quantitative PCR assay for *Cercospora beticola*

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*Cercospora* leaf spot, caused by the fungus *Cercospora beticola*, is an important disease of table beet (*Beta vulgaris ssp. vulgaris*) in New York. The disease causes defoliation, reducing the ability of top-pulling machinery to harvest crops and increasing consumer rejection in the fresh market. A *C. beticola*-specific quantitative PCR assay would provide an improved tool for detecting and quantifying *C. beticola* in agricultural systems. Primers and probes specific for *C. beticola* were designed using unique sequences and were confirmed for specificity against DNA of multiple fungal species. Pre-existing primers and probes for *B. vulgaris* were confirmed to amplify DNA of 15 table beet varieties. *In planta* assessments were performed using table beet plants inoculated with a droplet of conidial suspension (10⁵ conidia/mL). Disease progression was monitored over 21 days and leaf discs were collected at 3-day intervals. Lesion presence and diameter was recorded, and DNA extracted from dried leaf tissue. Lesions were observed by day 6, and expanded to 16 mm by day 21. PCR quantification cycles, based on an increase in fluorescence relative to DNA quantity, were used to estimate *C. beticola* biomass in the leaf discs. *Cercospora beticola* was detected by day 3, with a 6000-fold biomass increase over the 21-day period. The ability to detect and quantify *C. beticola* DNA in beet leaf tissues will inform strategies for improved disease control.

[10] Gene mapping of a Cry1Ac resistance gene in *Trichoplusia ni*

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The Cabbage Looper, *Trichoplusia ni* is a major vegetable pest that has developed resistance to many pesticides, including the biopesticide *Bacillus thuringiensis* (Bt). This study aims to map a resistance gene of the Bt toxin Cry1Ac in *T. ni*, which will provide important knowledge about the mode of action of resistance and mechanism of resistance. A homozygous resistant *T. ni* strain was bred with a homozygous susceptible strain to create F1 progeny which were then backcrossed with the susceptible strain. Individuals from this backcross (BC1) were reared on Cry1Ac media to select for resistant individuals (BC1-Cry1AcR). After DNA extraction, PCR was performed on BC1-Cry1AcR using primer sets specific to *T. ni*’s 31 chromosomes. If the gene for the Cry1Ac resistance is not on the chromosome examined, PCR analysis of BC1 and BC1-Cry1AcR populations show roughly a 1:1 ratio of homozygous to heterozygous individuals. If the gene is on the chromosome examined, PCR analysis shows homozygous and heterozygous individuals in a 1:1 ratio in BC1; but all the individuals from BC1-Cry1AcR are heterozygous. Successful analysis of the linkage of the resistance gene with the 31 chromosomes determined that the putative Cry1Ac resistance gene is localized on chromosome 31. With this knowledge, further fine gene mapping will be conducted to locate the locus of the resistance gene and the gene mutation conferring Cry1Ac resistance in *T. ni*. 
[11] Rapid, precise and cost-effective apple fire blight pathogen detection in the orchard

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Fire blight, the most destructive bacterial disease of apples, is caused by Erwinia amylovora. E. amylovora has spread worldwide and costs approximately $100 million through crop loss and control in the U.S. E. amylovora enters a plant through wounds or natural openings, primarily nectarthodes in flowers. Infection through flowers leads to loss of the current year’s crop and in favorable conditions, infection may spread throughout the tree causing death. Early, precise, and cost-effective diagnosis is key for fire blight management strategies including pruning out infected tissues to limit its spread. Unfortunately, pruning out the disease is not always successful since the bacteria may linger in asymptomatic tissues ready to strike again. The objective of this research is to determine the most effective, both in cost and accuracy, method for early detection of fire blight in symptomatic and asymptomatic tissues. We have tested two nucleotide amplification based LAMP (Loop Mediated Isothermal Amplification) assays (BioRanger and in-house LAMP) and two lateral flow immunoassay kits, AgriStrip (Bioreba) and RPDT (Pocket Diagnostics). Serial dilutions were used to assess sensitivity of all assays. The minimum detection threshold of immunoassay strips is above 5 x 10^5 CFU and the minimum detection threshold of LAMP assay is 0.0025ng/µl. Though LAMP detection is more sensitive, it is more expensive, requires a higher skill level to perform, and is more easily contaminated than immunoassay strips.

[12] Understanding Passalora fulva: examination of tomato leaf mold in New York State high tunnels

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Passalora fulva is the pathogen responsible for tomato leaf mold. The disease is an economically important disease of tomatoes in high tunnels and greenhouses. The conditions favor the growth of tomatoes, but they also favor the spread of tomato leaf mold. It causes almost 50% yield loss per year. It has been a challenge to complete Koch's postulates and to carry out other in vitro studies due to the specific growth requirements of P. fulva and the close association it has with distantly related Cladosporium species. Three trials of whole plant assays and detached leaves assays were performed to standardize methodologies of inoculation and isolation of the fungus from plant tissue. Two susceptible tomato varieties, BHN589 and Moneymaker were used. The time in which the first symptoms were expected to develop varied between 7 and 11 days. In addition to this, an evaluation of the sporulation levels of P. fulva was compared at different time periods. The number of conidia was quantified every week, for four weeks with a hemocytometer. Finally, CTAB, SDS, and Qiagen DNEasy Plant Mini kit DNA extraction protocols were compared to determine the most appropriate DNA extraction protocol for this fungus based on yield and nucleic acid purity. Appropriate techniques developed in this research will help to better understand P. fulva populations in New York.
Quantitative trait loci analysis of leaf serration phenotype in *Brassica oleracea* using a double haploid mapping population

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Leafy greens such as kale, collards, cabbage, and Chinese kale are members of the highly morphologically diverse and economically important crop group, *Brassica oleracea*, which comprises ~$480 million/year in domestic farm-gate value. Current breeding efforts are targeting improved leaf morphology and textures favored by consumers. Here, we phenotyped a double haploid (DH) biparental population generated from a broccoli x Chinese kale cross \([N=183]\) segregating for leaf morphology traits (e.g. serrated leaf margin). Using genotype-by-sequencing, we generated 6891 high-quality single nucleotide polymorphisms (SNPs), saturating the entire genome (mean=14.9 SNPs/Mbp +/- 3.8). In two replicated trials, we detected a strong single quantitative trait locus (QTL) for serrated leaf margin that is 0.95 Mbp long, with logarithmic odds (LOD) of 15.0 in 2017 and 18.2 in 2018. This QTL was found on the third chromosome \((2017=S3_1034154, 2018=S3_638901)\) with ~200 potential candidate genes. The proportion of variation explained by this QTL was 31.2% in 2017 and 30.8% in 2018. This finding validates the suitability of using DH populations to locate critical morphology genes in *B oleracea*, and may assist in the development of molecular markers in further improvements to leaf morphology in *Brassica oleracea* crops.

Testing of a leaf disk assay method for screening powdery mildew (*Podosphaera aphanis*) resistance in fifteen strawberry varieties

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Strawberry powdery mildew (*Podosphaera aphanis*) is a concern to many strawberry growers with the increased use of low tunnels for production. Low tunnels create a humid climate where powdery mildew can thrive. Leaf disk assay protocols have been developed in other crops to screen for powdery mildew resistance. The goal of this study is to compare leaf disk screening of strawberry varieties for resistance to field screening. One centimeter diameter leaf disks were sampled from newly expanded leaves of fifteen strawberry varieties. Nine replicates, of ten disks each, were placed on agar plates on three dates and inoculated with spores. Disks were rated on percent severity after 5-7 days. The results of the disk screening were compared to the field screening, where five plants of each variety were inoculated under low tunnels and rated for disease severity on a scale of 1 to 5. The leaf disk screening produced a similar result in five varieties, but a different result in the other ten. Results of the field trial will continue to be monitored for the remainder of the year. The screening may be improved by staining the disks to rate them, which tends to be more accurate, but more labor intensive. The disk screening shows potential, however a modified protocol is needed to obtain results that more closely align to field screening.
Impact of low dose prohexadione calcium programs on blossom blight

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Fire blight (\textit{Erwinia amylovora}) is an economically destructive disease that infects apple and pear orchards in all major production regions of the United States. Antibiotics have been the industry standard for fire blight management since the 1950s but are controversial because of concerns of off target selection for antibiotic resistance. The purpose of this experiment was to evaluate a novel management strategy using the plant growth regulator prohexadione calcium (PhCa). Different rates and applications timings of PhCa were evaluated for efficacy against blossom and shoot blight and impacts on plant growth and development. Trees were treated with Apogee (prohexadione calcium) at different times of the season and inoculated with fire blight, strain Ea273 at 1x10\textsuperscript{6} CFU ml\textsuperscript{-1} at full bloom. While the non-treated controls had more than 55\% incidence of blossom blight, all the treatments had less than 15\% incidence of blossom blight. There were no statistically significant differences among the PhCa programs, the biological control standard, and the streptomycin standard. This work suggests that PhCa could be used as an alternative to antibiotics to control blossom blight in commercial orchards. Future work will evaluate other programs of prohexadione calcium and determine the most cost-effective program.

Can fungicide resistance in grape powdery mildew be predicted using genetics?

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Grape powdery mildew (\textit{Erysiphe necator}) is the most destructive disease of grapes worldwide. All varieties of the widely-planted wine grape species \textit{Vitis vinifera} are susceptible and require fungicides to achieve commercially-relevant disease suppression. Over time, fungicide applications can drive pathogen populations towards resistance. Bioassays are used to assess frequency and degree of resistance, but are laborious and expensive. Molecular methods have revealed genes associated with resistance, but cannot supplant bioassays unless they are sufficiently correlated with resistant phenotypes and the occurrence of field resistance. We assessed correlations of genotype and phenotype in seven isolates of \textit{E. necator}. DNA extracted via a DNeasy kit was subjected to PCR and Sanger sequencing to reveal key mutations within fungicide target regions. A leaf-disc bioassay was used to quantify resistance to four common fungicides: azoxystrobin, trifloxystrobin, difenoconazole, and flutriafol. Inexpensive and rapid molecular-based assessments may eventually replace bioassays in gauging the potential for fungicide resistance, allowing better implementation of disease management strategies, and more sustainable use of fungicides.
[17] Genetic survey of a candidate gene under the *WEEPING (W)* locus in *Malus*

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Weeping growth habit in *Malus* is of great interest for its unique canopy archetype. It is characterized by downward growing branches and is a genetically dominant trait. Knowing the genetic basis of weeping would improve our understanding of canopy development and plant growth in general. The purpose of this project was to characterize a weeping candidate gene called *Wp1*, identified in the *WEEPING (W)* region that was mapped previously on chromosome 13. It appears that there are putatively important mutations in the *Wp1* promoter and coding sequence when compared with the apple reference genome. To see if these mutations are conserved in *Malus*, regions in the genomic sequence of *Wp1* from these cultivars was obtained by Sanger sequencing. High resolution melting (HRM) markers were designed as well to target single nucleotide variants (SNVs) selected from the *W* region. The DNA sequence and HRM genotypic data revealed that several mutations are specific to weeping cultivars, including those putative mutations found in *Wp1*. These results support that *Wp1* is a strong candidate gene underlying *W* that largely controls the weeping phenotype. Further studies are underway to validate the function of *Wp1* through genetic complementation experiments.

[18] QTL mapping of flowering phenology in grapevine (*Vitis* spp.) populations *V. rupestris* × ‘Horizon’ and ‘Horizon’ × *V. cinerea*.

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By rapidly changing the growing conditions of vineyards around the world, global warming has made a genetic understanding of grapevine flowering phenology relevant to the development of cultivars suitable for the climates of the future. To identify QTL (Quantitative Trait Loci) controlling time of flowering, we have used the early and late flowering half-sib mapping populations *V. rupestris* × ‘Horizon’ and ‘Horizon’ × *V. cinerea*. We scored flowering using the modified Eichorn-Lorenz scale as vines progressed from first to last cap-fall. Applying CIM (Composite Interval Mapping) and HK (Haley-Knott) QTL mapping techniques to these data identified QTL associated with flowering time on chromosome 2 in the ‘Horizon’ × *V. cinerea* population and on chromosome 5 in the *V. rupestris* × ‘Horizon’ population. However, three different linear models—General Linear Model, Mixed Linear Model (PCA + K), and FarmCPU—showed significant associations only for the locus on chromosome 2 in the ‘Horizon’ × *V. cinerea* population. The tentative QTL on chromosome 5 thus requires confirmation, while the QTL on chromosome 2 deserves further study, including candidate gene identification to aid in the selection of early or late flowering phenotypes in future grapevine breeding efforts.
Effects of strawberry cultivar on two-spotted spider mite (Tetranychus urticae) oviposition and biocontrol efficacy by predatory mites N. fallacis and P. persimilis

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Two-spotted spider mites (Tetranychidae urticae) (“TSSM”), pests of strawberries, can be managed using biological control. Biological control agents Neoseiulus fallacis and Phytoseiulus persimilis express different behavior depending on host plant environment. Leaf pubescence is one such trait that differs between cultivars and can influence predatory mite behavior, namely feeding ecology. This study aims to understand how tri-trophic interactions, i.e. plant influences on herbivore and predator behavior, impacts biological control efficacy of TSSM. Among four cultivars, the two least and most pubescent cultivars were chosen. Leaf disc arenas were inoculated with four gravid TSSM females. Females remained on arenas for 48 hours and eggs were counted to determine oviposition rate. Predatory mite treatments were the following: two N. fallacis females, two P. persmilis females, one of each species, or a control, which were applied to arenas with TSSM eggs. Live eggs were recorded after 24 and 48 hours. Trichome quantification data confirmed the assumption that Cabrillo and Sweet Ann were the most and least glabrous, respectively, and thus were chosen for assays. Based on one-way ANOVA , 4 cultivars significantly differed for TSSM oviposition. Finally, releasing Neoseiulus fallacis and Phytoseiulus persimilis resulted in best and most consistent control across cultivars Understanding the influence of leaf pubescence on predatory mite efficacy would help tailor biological control by cultivar and rationalize unpredictable results biological control can yield.

Exploring Continued: Fine genetic mapping of the WEEPING (W) region in Malus

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Successful genetic improvement of plant architecture was the central element for Green Revolution, which drastically aided the yield increase of field crops, such as maize, rice and wheat. It is long considered that a better understanding of the genetic basis of apple tree growth habit would lead to optimized orchard management practices. The weeping growth habit in Malus is defined by downward growing branches, desirable not only for ornamental purposes, but also for certain apple orchard training systems. We previously mapped four loci of significant genetic effect on weeping growth habit, including the major locus Weeping (W) that has been delimited to 1.0-MB region on chromosome 13. To further characterize the W region, we studied four new weeping-segregating populations derived from cultivar ‘Cheal’s Weeping’. Genomic DNA was extracted from young leaves and genotyped with simple sequence repeat (SSR) and high-resolution melting (HRM) markers developed in the region. Marker-trait linkage analyses identified several informative recombination events. As a result, we further narrowed down the W region to 680 KB, reducing the number of predicted genes from 72 to 47, considerably facilitating the effort to identify strong candidate genes underpinning W.
[21] Seed-coat color and nutrient content in kidney and black beans (*Phaseolus vulgaris* L.)

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*Phaseolus vulgaris* L. (common bean) plays a vital role in global nutrition, serving as a significant source of key micronutrients, including iron. Enhanced pigment diversity and retention after cooking may impact the inherent nutritional content and create a more aesthetically appealing product that could promote bean consumption across world markets. The purpose of this research was to assess the nutritional content and color retention of black beans and pigmented kidney beans in relation to pigment intensity and seed size. We hypothesized that black beans with higher color retention post-processing would also have higher iron content. Further, we expected small-seeded beans to have relatively higher nutrition. Genotypes were visually selected on the basis of stability, pigment, and shape. After undergoing standard canning protocol, 18 genotypes were selected for density analysis and sent to the Cornell Nutrient Analysis Lab for nutrient analysis. Separately, black bean selections were subjected to UV-spectroscopy. Color intensity was determined by summing absorbance values measured at \(\lambda = 420\text{nm}, 520\text{nm},\) and \(620\text{nm}\). Results from a Spearman’s correlation test show no significant monotonic association between iron and pigment retention (\(r_s = 0.257\)) or seed size and nutrient content (\(r_s = 0.061\)) in our samples. The genetic base for seed-coat color retention may be more complex than mineral interaction and dependent on different phytochemicals.

[22] Tracking hop powdery mildew mating type distribution across commercial and wild plantings of hop

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Hop powdery mildew (*Podosphaera macularis*) is a major pathogen of hop in the northern hemisphere. It first arrived in the Pacific Northwest (PNW) in 1997, causing over $10 million in losses to the top US hop production region. *P. macularis* is heterothallic and is capable of producing viable chasmothecia in the presence of both mating types. Presence of chasmothecia poses additional management concerns through more efficient pathogen overwintering and increased pathogen genotypic diversity. Currently only the MAT1-1 mating type is thought to be present in the PNW, while both mating types have been confirmed in many Midwest and Eastern US states, including New York. Our ongoing study aims to create a high-density map of *P. macularis* mating type distribution. We designed a multiplexed RealTime qPCR protocol to survey the mating types of 213 *P. macularis* samples originating from 10 US states and 3 regions of Europe. The qPCR results further supported prior reports that only the MAT1-1 is currently present in the PNW. Results also indicate that only the MAT1-1 mating type was present in Midwest/ Eastern US commercial yards, while both mating types were found at 5 of 6 plantings of wild hop. This distribution of mating types suggests that pathogen introduction into commercial yards primarily occurs through the initial hop planting material, as opposed to arrival from wild mildew populations.
Traveling with *Clavibacter michiganensis* subsp. *michiganensis* through the vascular systems of wild tomatoes

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The Gram-positive bacterium *Clavibacter michiganensis* subsp. *michiganensis* (*Cmm*) is the causative agent of bacterial wilt and canker of tomato (*Solanum lycopersicum*). *Cmm* spreads through the xylem and forms biofilm-like aggregates that may create blockages leading to wilt of the plant. The tolerance to *Cmm* of wild tomato accessions *S. habrochaites* LA407, *S. habrochaites* LA2128, *S. peruvianum* LA2157, *S. peruvianum* LA2172 and the susceptible *S. lycopersicum* cv. Mt. Fresh were measured by inoculating via the cotyledon clip or needle stab method and rating plants for 21 days. *Cmm* systemic spread and colonization of the xylem was measured by dilution plating sections of plants at 14 and 21 days post inoculation. To determine if the xylem sap of the wild tomato species influenced aggregation, xylem sap of the tomato accessions was harvested and used to measure *in vitro* bacterial growth and to conduct an *in vitro* biofilm assay. *In vitro* growth and biofilm experiments in sap were repeated with two different NY *Cmm* isolates to test *Cmm* biological patterns between isolates. Three strains of *Cmm* (WT0317, WT0690 and an expansin knockout strain) were used to inoculate wild tomato accessions which were rated for 21 days to test the strains’ virulence. Understanding this movement of *Cmm* through wild tomatoes will allow for a deeper understanding of the host pathogen interaction, which may produce new targets for breeding tolerant tomatoes.

Preventing the development of SDHI fungicide resistance in *Venturia inaequalis*

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*Venturia inaequalis*, the causal agent of apple scab, damages fruit and foliage of apple trees leading to non-commercially viable fruits and defoliated trees, respectively. To manage apple scab, fungicides are applied multiple times seasonally, however repeated applications may select for the development of fungicide resistance. Succinate dehydrogenase inhibitors (SDHI), a class of fungicides that interfere with fungal aerobic respiration, are currently a great management option as they have both high field efficacy and no reported resistance. The goal of this study was to identify application methods of SDHIs that prevent development of resistance and promote sustainable fungicide use. Differential treatments of the SDHI fluxapyroxad were applied four times seasonally to Jonagold, Empire, and Jersey Mac from 2016-2018. Treatments included fluxapyroxad at a high dose, fluxapyroxad at a low dose, fluxapyroxad combined with a second single-site (pyraclostrobin), fluxapyroxad with a multi-site (mancozeb), and a control which received no fungicides. Following application, leaves with apple scab lesions were collected from each treatment and resulting isolates were subjected to a growth assay. The isolates were grown on varying concentrations of the SDHI fluxapyroxad and relative growth rates were determined to monitor for resistance or any reductions in sensitivity. These results will contribute to our understanding of how to best slow the development of resistance to this important class of fungicides.
Quantification of *Phytophthora capsici* biomass within *Cucurbita pepo* stem tissue using real-time PCR

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The causal organism for Phytophthora blight in many fabaceous, solanaceous and all cucurbit crops, *Phytophthora capsici* is of economic importance across the world. *Phytophthora capsici* is a soilborne oomycete that has a polycyclic life cycle, producing sexual and asexual spores, which contributes toward it being a highly virulent pathogen with multiple physiological races. The lack of highly resistant host varieties has increased the importance of investigating the relationship between pathogen infection and the development of disease symptoms. The goal of this project was to quantify *P. capsici* biomass in host tissues to further the understanding of plant susceptibility and provide a quantitative measurement of the degree of pathogen colonization of host tissue. Suspensions of 1 x 10⁴ zoospores/mL were used to inoculate 2 ½ week old Spineless Beauty and Dunja zucchini cultivars. Crown samples, 0.5-1cm in length, were collected each day throughout a four-day time series and used for DNA isolation. Quantitative PCR was then conducted using primers amplifying the ITS region. Amplification of pathogen DNA was observed with both varieties. A later experiment was conducted with 12 squash varieties to compare the correlation of visual disease ratings and qPCR estimates of pathogen abundance. Initial time series qPCR results suggested more tolerance within Spineless Beauty which showed less symptoms with higher pathogen DNA amounts whereas Dunja showed higher symptoms with less pathogen DNA present.

Comparing methods for high-throughput phenotyping of downy mildew disease on *Vitis* spp. leaves

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Downy mildew (*Plasmopara viticola*) remains one of the major diseases in viticulture worldwide. Quantitative trait loci (QTL) analyses to identify resistance loci require phenotyping large numbers of samples from segregating populations. The purpose of this project was to compare available methods for assessing downy mildew severity on grape leaf disks, to determine the most efficient, consistent, and accurate phenotyping method. Images from 2015 and 2016 leaf disk array experiments of F₁ *Vitis rupestris* B38 × ‘Horizon’ (RH) individuals were evaluated using manual rating, APS Assess 2.0, ImageJ, and published custom scripts. For each method, the total time for phenotyping was recorded and QTL analyses were run using phenotype data averaged from eight replications of 157 RH genotypes within two experiments. For total time phenotyping, the scripts were the highest-throughput method, requiring 72 minutes to assess 144 images containing multiple leaf disks. Manual ratings took an average (from two replications) of 229 minutes, and Assess 2.0 took 543 minutes. ImageJ was eliminated from consideration since it analyzed just one leaf disk at a time. QTL analyses showed that the phenotypes obtained from the custom scripts resulted in the most consistently and strongly identified locus on chromosome 7 with an LOD of 5.17. These results indicate that the custom scripts would be the preferred method over the other tested methods for phenotyping downy mildew severity in grapevine.
[27] **Optimization of grapevine fanleaf virus protein 1E\textsuperscript{Pol} detection in plants**

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The nepovirus grapevine fanleaf virus (GFLV) is one of the causal agents of fanleaf degeneration of grapevine. It is one of the most adverse viral diseases of grapevine worldwide. Infection with GFLV produces a rapid decline in overall health of the vine, causing poor yields and premature vine death, though the molecular mechanisms controlling these symptoms remain poorly understood. The GFLV strain GHu causes vein-clearing symptoms in the plant model species *Nicotiana benthamiana*, while the F13 strain is asymptomatic. The GFLV-GHu symptom determinant has recently been mapped to residue 802 of 1E\textsuperscript{Pol}. To further determine the mechanism of symptom development, the protein interactome of 1E\textsuperscript{Pol} and *N. benthamiana* will be investigated by co-immunoprecipitation and mass spectrometry. In this study, total proteins were extracted from GFLV-infected *N. benthamiana* using 15 different experimental buffers in order to identify a buffer suitable for 1E\textsuperscript{Pol} analysis in western blots. Furthermore, the GFLV titer was assessed in infected *N. benthamiana* in a time course study by RT-qPCR and DAS-ELISA to determine the optimal time for protein extraction. Together, this research lays the groundwork for future studies into the molecular mechanisms of symptom development by GFLV-GHu 1E\textsuperscript{Pol}.

[28] **Effect of Asian jumping worms on biological and chemical properties of turfgrass ecosystems**

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Earthworms play beneficial roles in soil as decomposers and ecosystem engineers, but they can also have detrimental effects in some ecosystems. Asian jumping worms (Clitellata: Megascolecidae) are rapidly expanding their range throughout the east coast with anecdotal reports of the worm damaging soil structure and consuming homeowner’s lawns. The purpose of this study was to evaluate how jumping worms impact turf grass and soil quality. In order to do this, we conducted a greenhouse pot study that mimicked a lawn-landscape ecosystem. Each pot contained a patch of turf grass on one side and one of three organic amendments (hardwood, compost, or no amendment) on the other. We then added three Asian jumping worms each to half of the pots. Over the course of 30 days, we tracked changes in turf quality, soil chemical, and biological traits to assess turf and soil responses to earthworms. Furthermore, we compared our results to soils collected from field locations in central, NY with and without existing jumping worm populations. Earthworms increased microbial peptidase activity and turf greenness by 34.8% (p= 0.0139) and 14.2% (p=0.002) respectively. These findings suggest jumping worms may benefit turf grass health in the short term, however, long-term assessment is needed to fully capture their impacts on turf. This study provides an important starting database for the Asian jumping worm as a backyard pest.
Characterizing strains of *Erwinia amylovora* across orchards in New York State using CRISPR elements

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Fire blight is a disease caused by the bacterium *Erwinia amylovora*, and it ranks among the most devastating diseases of Rosaceous tree fruit crops, causing physical and economic damage to the commercial apple industry on an annual basis. Recently, characterization of the patterns within CRISPR regions has been used to distinguish between different strains of *E. amylovora*, and over 700 patterns are currently described. In this study, our objective is to use these CRISPR patterns to track the spread of fire blight over space and time. Samples of infected tree shoots have been collected from orchards in Geneva in 2018 (29) and 2017 (20), the Hudson Valley in 2018 (11), and the Champlain Valley in 2018 (12), and we are now in the process of isolating and characterizing the pathogen using a dictionary of CRISPR patterns. The CRISPR 1 region from five representative Geneva samples has been genotyped, and it is likely that all five samples are of the same strain, but also of a different strain than Ea273- the experimental strain used to inoculate neighboring blocks. The CRISPR 1 region of the Hudson Valley and Champlain Valley samples are currently being sequenced, as well as CRISPR regions 2 and 3 for the five representative Geneva samples. This approach could be instrumental for the field of plant disease protection, as the ability to distinguish among different strains of a pathogen could allow for scientists to trace an incidence of disease back to a source inoculum and identify which strains are present in the three major growing regions of New York State.

Comparison of anthocyanin levels in the USDA tart cherry collection

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Tart cherries (*Prunus cerasus*) and sweet cherries (*Prunus avium*) have an abundance of anthocyanins, compounds with antioxidant properties and many health benefits. Industries that utilize tart cherries tend to only use one cultivar, 'Montmorency'. There are two major anthocyanins present in cherries, cyanidin 3-glucosylrutinoside and cyanidin 3-rutinoside. Cyanidin 3-glucosylrutinoside is found in higher levels in tart cherries while cyanidin 3-rutinoside is profuse in sweet cherries. Anthocyanins from tart cherries collected in 2011, 2013 and 2014, as well as sweet cherries from 2018 and commercial juice were extracted and quantified using reverse-phase HPLC. For two years ‘Almaz O.P’ had the highest anthocyanin levels, while ‘Montmorency’ and ‘M-209’ had the lowest levels for cyanidin 3-rutinoside and cyanidin 3-glucosylrutinoside, respectively. Overall, the concentration of cyanidin 3-glucosylrutinoside and cyanidin 3-rutinoside are dependent on the season, but cyanidin 3-rutinoside stays relatively low tart cherries. Concentrations of both anthocyanins decreased over time in the commercial juice. GBS data revealed greater genetic variation in *P. cerasus* than *P. avium*. This research demonstrates that cultivars other than 'Montmorency' can have the same if not more health benefits and marketable properties. Anyone with interests in the health benefits of cherries should expand the types of cultivars they use.
Revisiting hemp: The re-establishment of research and industrial uses of *Cannabis sativa* L. in New York State

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The Agricultural Act of 2014, or “Farm Bill,” raised the opportunity to grow non-psychoactive cultivars of *Cannabis sativa* L. (industrial hemp) for the first time in nearly a century. Industrial hemp is already of interest to growers and consumers for its potential use in food (e.g. hemp protein powder), fiber and health. The NYS Industrial Hemp Research Pilot Program was established in partnership with Cornell University to delve into the most economical methods of creating ideal conditions for the industrial production of hemp. This project has two objectives: 1) to gain a better understanding of the *C. sativa* microbiome and 2) to produce a new episode on Cornell’s Food + Science podcast platform. An initial microbiome study was performed in 2017, and one unique yet little-known microbe was potentially detected in the root microbiome. We developed a detection method for this microbe and found that the sequence amplified from all hemp tissues, therefore it is likely part of the hemp genome rather than a unique microbe. The bulk of the podcast is being constructed from 7 interviews conducted locally – of 2 Cornell researchers, an extension/outreach coordinator, grower, producer, and 2 customers at the downtown farmer’s market – to ultimately publicize an indirect dialogue between differing perspectives of hemp production in NYS.

Associations between onion growth characteristics and *Thrips tabaci* densities

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Onion thrips (*Thrips tabaci*) are a major onion crop pest that feeds on chlorophyll-rich mesophyll, reducing photosynthetic ability and indirectly repressing bulb growth, causing up to 60% yield loss. Although prior studies have demonstrated positive relationships between thrips densities and nitrogen levels in onion crops, relationships between thrips densities and onion growth metrics have not been established. We hypothesized that plants with more and longer leaves, wider necks, and more chlorophyll would yield higher thrips populations. An initial survey was conducted in a field of onions receiving varying rates of nitrogen to identify potential associations between onion growth characteristics and thrips densities. Plant neck width and leaf count, leaf length, chlorophyll content (SPAD), adult thrips and larval thrips were measured. Results showed that longer leaves and greater amounts of chlorophyll had a significant positive relationship with thrips larvae counts. No-choice assays were then set up in the greenhouse to evaluate chlorophyll and leaf length associations with larvae. We anticipate seeing similar results in the greenhouse assays as in the field. A better understanding of thrips behavior could help guide IPM strategies in the future by breeding cultivars with shorter leaves and lower chlorophyll that may be less appealing to thrips but still produce adequate yield.
Preliminary Evaluation of 50 Industrial Hemp Cultivars Across New York State

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Industrial hemp (Cannabis sativa) is emerging as a promising crop for New York State due to its multitude of uses and suitability to grow in the local climate. One barrier to rapid adoption is the lack of available yield and quality data about commercial cultivars as a result of the several-decade lapse in production. We aimed to characterize 50 industrial hemp cultivars for both immediate use by growers and potential value in a breeding program. Our trials were divided into four major groupings based on post-harvest use: grain production, fiber production, cannabidiol (CBD) production, and dual-purpose (grain & fiber) cultivars. To characterize the phenotypic diversity of these cultivars, we collected data throughout the lifetime of the plants – from seed germination to harvest – across several locations, soil types, and hardiness zones in New York. We collected initial data for this growing season, including: germination rates, stand counts, flowering status, branching patterns, and early-season heights. There were significant differences in all of these measurements both by site and cultivar that will be discussed in the context of cultivar suitability.

Exploring global apple diversity to identify fire blight resistant genotypes

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Genetic diversity of crop species is an important resource for mining alleles or genes to mitigate yield and quality losses. This study aimed to identify fire blight resistant accessions present in the USDA Malus germplasm, the world’s largest and most diverse apple germplasm. A total of 356 accessions from 26 species were evaluated in the greenhouse for variations in shoot growth rate, and for resistance to Erwinia amylovora, the causal agent of fire blight. The phenotypic data along with previously generated GBS (genotyping-by-sequencing) SNP data set were used to identify quantitative trait loci (QTL) linked to fire blight resistance through an association mapping approach. Principal component analysis suggested that a significant amount of diversity is present in shoot growth rate (GR) and percent lesion length (PLL) in the shoots and leaves amongst the 26 species of apples. The data showed there was no significant correlation between GR and any PLL, but a positive correlation of over 50% between the PLL of the shoots and leaves. Association mapping will identify potential candidate genomic regions linked with these traits for future genetic studies. Having a deeper knowledge of how each of these species varies in growth rates and disease progress will facilitate breeding efforts for increased resistance to fire blight.
[35] Genetic mapping of woolly apple aphid resistant genes in Malus x domestica

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Woolly Apple Aphids (*Eriosoma lanigerum*) infect apple trees (*Malus X domestica*) in high densities. As woolly apple aphids colonize they build a white, thin, cottony film and as the population increases aphids will move down to the roots. Woolly apple aphids survive by sucking the sap from their host and as they feed they release a toxic saliva. This toxic saliva can stunt the growth of apple trees as well as distort the fruit. The objective of this research is to discover new genes that may be responsible for resistance to aphids in apple trees derived from a cross between *Malus robusta* ‘Robusta 5’ and ‘Ottawa 3’ apple rootstock.

Greenhouse cultivation trials were managed to compare gene expression of hybrid resistant trees to trees that were not resistant. The six plants were organized by name, WAA10502, and by letters A-F. WAA10502 A-C appeared to be resistant to woolly apple aphids while WAA10502 D-F were not resistant. Raw RNA was extracted from apical leaves of all six WAA10502 samples and frozen in liquid nitrogen. These samples were then processed to build RNAseq libraries and sequenced using an Illumina Hi-Seq system. CLC Workbench and Geneious software were used to analyze and create a genetic mapping of raw reads to genes that may be responsible for resistance of woolly apple aphids. SNP markers in *Malus X domestica* genome (GDDH13 v.1 1) were mapped to markers in the WAA10502 samples and this revealed locations within chromosome 17 that may host differentially expressed genes, genes that contained mutations (truncations) or genes that had not yet been annotated responsible for resistance. We found 11 of new genes that were different between the resistant and susceptible genotypes. We will continue to characterize those candidate genes to see which ones are responsible for the trait.

[36] Identifying polymorphisms associated with sex-determination genes in Purple Osier Willow (*Salix purpurea*)

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Plant sex determination mechanisms in the Salicaceae have evolved differently in poplars and willows. Purple Osier willow (*Salix purpurea* L.) is an ideal dioecious plant for studying sex determination, because of its excellent genetic and genomic resources for studying the female heterogamety (ZW system). The purpose of this study is to find polymorphic markers in sex-related candidate genes within the *S. purpurea* sex determination region on chromosome 15. Regions of five genes with potential male- or female-specific restriction sites were PCR-amplified from 12 female, 12 male, and one hermaphrodite genotype. For each candidate gene, the PCR products were subjected to restriction enzyme digestion, and the banding patterns were screened on agarose gels for sex-specific polymorphisms. The results showed that all the selected genes did not display consistent patterns by sex. Thus, the polymorphisms in those genes do not appear to be specific to the Z and W haplotypes. Different genes in the sex-determination region that are a little farther away from this five gene area should be tested to see if they are associated with sex.
[37] Nitrogen status estimation in shrub willow fields with UAS aerial imaging

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In shrub willow (\textit{Salix spp.}), a dedicated woody biomass crop, nutrient status information informs breeding decisions, management practices, and may correspond with final crop yield. Leaf nitrogen status is highly correlated with leaf greenness and is typically measured with handheld SPAD 502 Plus Chlorophyll Meters. However, collecting these data manually is time consuming, especially for large fields, and the numbers can be variable depending on sensor placement. This project aims to enhance and accelerate nutrient status data collection by using unmanned aerial systems (UAS) for aerial imaging and analysis to generate vegetation indices that correlate well with SPAD readings. Aerial images of a second-year post-coppice field were captured with a Micasense RedEdge multispectral sensor mounted to a DJI Matrice 100. The field had four paired treatment blocks of fertilized and non-fertilized plots across ‘Preble’ and a polyculture mixture of shrub willow cultivars. Different techniques of excluding soil and weed pixels from vegetation indices were examined, and 22 index statistics were compared to ground-based SPAD measurements. Preliminary analyses indicate that fertilizer treatments significantly increased SPAD and vegetation indices, but there were no significant differences by cultivar treatment. We also found a number of significant correlations between SPAD readings and vegetation indices. This analysis demonstrates the usefulness of UAS imagery in generating vegetation indices and filtering masks for assessing shrub willow nutrient status.

[38] Effects of drought stress testing apple rootstocks in an enclosed aeroponics system

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Aeroponics, a relatively new method of farming, is the practice of growing crops with their roots exposed and unanchored with water and nutrients supplied via regular misting. As this field becomes more widespread and viable, research on how different species and breeds grow and develop is needed. Through experimental trials, more data can be obtained and used to further our knowledge and understanding of this technology to build upon it. With droughts becoming more widespread, conservation of water is more important than ever, and the ability of different apple trees to withstand such conditions can be invaluable. With this experiment trial of exposing 9 different apple rootstocks to increasingly dry conditions, we hope to lay some of the groundwork necessary to develop this practice and further research attempts. Using an enclosed aeroponics system and a timer, rootstocks were exposed to water stress conditions at 6, 9, and 12 minutes without water. Rootstock 969 survived and thrived under the conditions, G41 and M9 withstood them, while rootstock G890 suffered even before drought periods began. B9, G935, G210, G214, and G11 all died during the trials. This experiment highlights the potential for aeroponics in precision drought testing, as well as provides information for future experiments.