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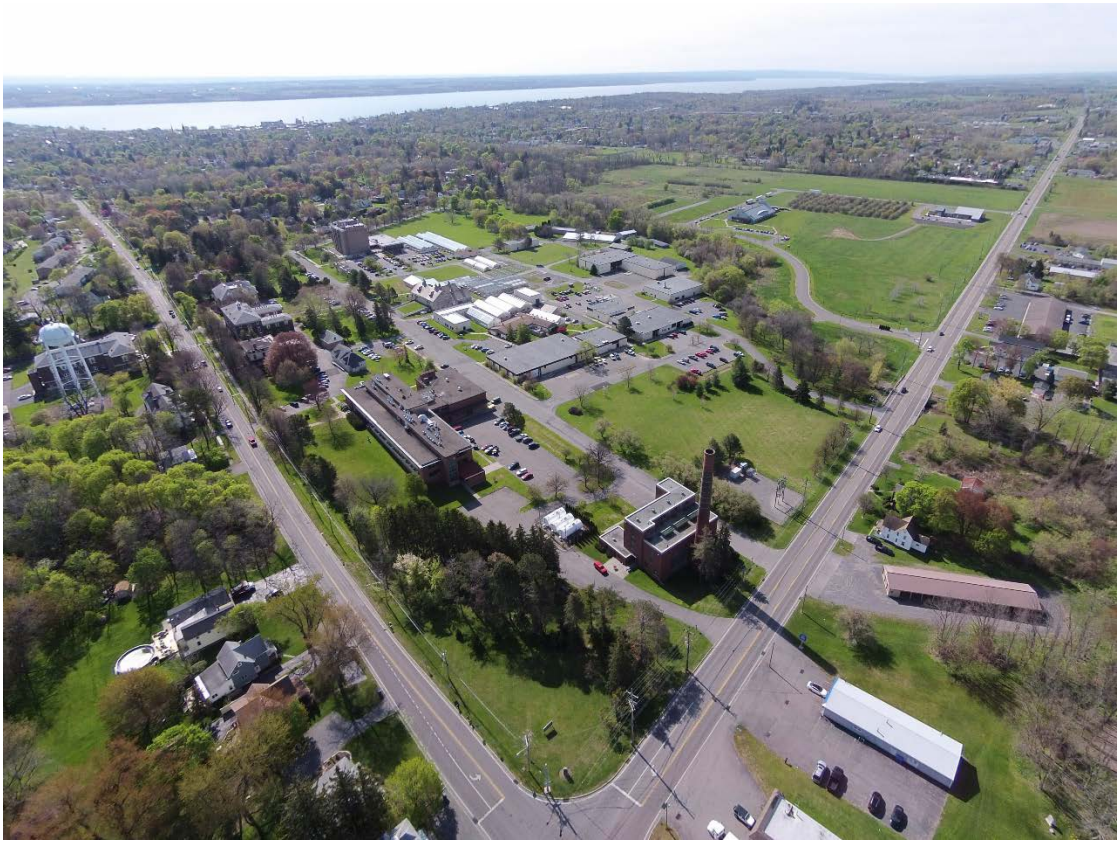

Cornell University
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[1] The effects of light, fertilizer, and temperature stress on the accumulation of anthocyanins in Redbor kale (*Brassica oleracea* var. *acephala*)

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Increasing consumer concern over the use of artificial food colors has prompted research on the identification of natural alternatives. Anthocyanins are water-soluble pigments derived from flavonoids and are responsible for a diverse range of colors, including blue, purple, violet, and bright red. Benefits such as reduced risk of cardiovascular disease, degenerative diseases and cancer have also been observed with the dietary intake of anthocyanins. Previous research showed that temperature, fertilization, and light stress created variations of anthocyanin concentrations in Brassica plants. The objective of this project was to understand how the combination of these stresses influenced anthocyanin accumulation. Eight different growing conditions tested these stresses on 'Redbor' kale (Brassica oleracea var. acephala) during a 10-day period. Plants were grown hydroponically in growth chambers at 10°C and 21°C with either fluorescent or incandescent light bulbs. In each of the conditions, plants were treated with either deionized water or a full fertilizer solution. Results showed strong differences within each of the treatments. Plants produced the highest concentration of anthocyanins in 10°C conditions, with fluorescent bulbs, in deionized water. There was little to no accumulation of pigments observed in the 21°C, incandescent, and fully fertilized water conditions. These results will be used to optimize juvenile kale growth conditions for controlled environment agriculture in order to derive a cost efficient source of natural colors for large-scale supply and consumption.

[2] Phytochemical analysis of strawberries harvested in the early morning versus mid-afternoon

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Strawberry (*Fragaria x ananassa*) growers often harvest their crop early in the morning when the temperature is cool in order to obtain a higher quality berry. The purpose of this project was to determine if an early morning harvest yields higher quality strawberries. Strawberries were harvested in the early morning and mid-afternoon on three dates for each of six cultivars (NY 02-56, 'Dream', 'Archer', 'Amandine', 'Deluxe', and 'Walker') to compare fruit quality attributes. Each sample was analyzed for fruit firmness, total soluble solid content, titratable acidity, total phenolics, and total anthocyanins. Overall, there were no significant differences in quality based on these parameters. However, there were significant differences at some harvest dates. At the first harvest date, the anthocyanin content of the morning harvest was 60% higher than the afternoon. This date had the greatest temperature difference between morning and afternoon. Our findings suggest that growers do not have to harvest in the early morning to maximize the quality of berries except possibly on exceptionally hot days.

[3] Searching for alternative hosts of grapevine red blotch virus

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Red blotch is an emerging viral disease of *Vitis* species that threatens North American vineyards by negatively impacting fruit quality and ripening. Identified in 2011, grapevine red blotch virus (GRBV) of the family *Geminiviridae* has a genome comprised of single-stranded, circular DNA. The only known vector of GRBV, the three-cornered alfalfa treehopper (*Spissistilus festinus*) is not generally considered a vineyard pest, but can cause yield loss in fabaceous crops, which are commonly sown as cover crops between vineyard rows. The main objective of this project was to evaluate common vineyard cover crop species for their potential to host GRBV. Individual plants were inoculated with *Agrobacterium tumefaciens* carrying an infectious cDNA clone of GRBV and tested for the accumulation of GRBV at local and systemic sites. Virus replication was verified by reverse transcription polymerase chain reaction (RT-PCR) at 7 days post-inoculation (dpi) in locally infected leaves. Systemic movement of the virus was detected by multiplex PCR targeting two regions of the GRBV genomic DNA at 14 dpi in apical tissue. Cover crop species that test positive for GRBV will be evaluated for virus transmissibility by *S. festinus*. An improved understanding of the ecology and epidemiology of GRBV is important for the development of strategies to mitigate disease spread.

[4] Monitoring aphid activity and Cucumber mosaic virus incidence in snap bean fields in New York State

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Cucumber mosaic virus (CMV) caused catastrophic losses for the snap bean industry in the Great Lakes region of the United States in some years from 2001 through 2010. CMV is spread by aphids, especially the soybean aphid (*Aphis glycines).* Soybean aphid populations have decreased dramatically in recent years, which could be attributed to the widespread adoption of neonicotinoid-treated soybean seeds. The decline in soybean aphid populations may be responsible for the decrease in CMV incidence in snap bean fields. The purpose of this project was to monitor aphid activity and CMV incidence in snap bean fields and relate findings to years when epidemics occurred. Soybean aphid activity in commercial snap bean fields was monitored weekly using water pan traps. CMV incidence was estimated from 18 snap bean fields with a total of 500 plants per field (9,000 plants total) using a double antibody sandwich enzyme-linked immunosorbent assay (DAS-ELISA). Results indicated that few aphids were active in snap bean fields as only an average of 0.2 aphids (range 0 to 2.8) were captured per trap per day across all fields sampled. Of the 18 fields sampled, none were infected with CMV. Implications of these results suggest that snap bean growers may expand their cultivar choices beyond the predominate CMV-tolerant snap bean variety 'Huntington' towards varieties with other desirable traits.

[5] Examining squash tolerance and susceptibility to *Phytophthora capsici* using confocal laser-scanning microscopy

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Phytophthora capsici causes crown and root rot of many solanaceous and cucurbit crops. Tolerance and susceptibility to *P. capsici* is variable amongst varieties of squash, but there is no variety that is resistant to the pathogen and mechanisms of tolerance are not well understood. Confocal laser-scanning microscopy was used to examine infection of root tissue by an isolate of *P. capsici* expressing green fluorescent protein (GFP) in susceptible commercial zucchini variety Dunja and tolerant Cornell breeding line 17-G-009. Two-week-old seedlings were each inoculated at their crown with a suspension of 1 x 10^4 zoospores/mL, and latitudinal cross-sections of the tap root were observed at 3 and 6 days post-inoculation. Pathogen hyphae and colonized vascular bundles were more prevalent in cross-sections taken from Dunja than those taken from 17-G-009. In a separate greenhouse experiment, Dunja and 17-G-009 were evaluated for tolerance against the GFP *P. capsici* isolate and 3 additional isolates over a 3-week period. No difference in virulence of the isolates was observed. The difference in taproot colonization of susceptible and tolerant squash indicates that there is a mechanism in 17-G-009 to inhibit colonization by *P. capsici.* Further work may be done to determine what this mechanism is and how it may be utilized to prevent disease.

[6] Role of a single amino acid of grapevine fanleaf virus in symptom expression

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Grapevine fanleaf virus (GFLV) has a bipartite RNA genome and causes fanleaf degeneration, the oldest known viral disease of grape. The disease is characterized by a variety of symptoms, including malformed leaves and clearing of veins, the molecular mechanisms behind which are not understood. Our goal was to create GFLV mutants and determine how the mutations change the virus' ability to cause symptoms in a model host plant, *Nicotiana benthamiana*. Previous work identified a segment of the RNA1-encoded RNA-dependent RNA polymerase ($1E^{Pol}$) as the key symptom determinant. By characterizing chimeras of two GFLV strains, wild type GHu, which causes vein-clearing symptoms in *N. benthamiana*, and wild type F13, which does not cause symptoms, this region was further narrowed to a couple of amino acids, including the residue in position 802 of $1E^{Pol}$. We used site-directed mutagenesis to create GHu mutants where K^{802} (lysine) was replaced by asparagine, glutamic acid, glutamine, or proline, and used these mutants to agroinoculate *N. benthamiana* seedlings. Plants were observed for symptoms for 3 weeks post-inoculation and tested for local and systemic GFLV infection. Replacing K^{802} with other amino acids altered symptoms and rates of systemic infection. This suggested that K^{802} of $1E^{Pol}$ not only plays a key role in triggering vein clearing symptoms of GFLV, but may also be involved in other processes that enable the virus to spread within the host.

[7] Evaluating pollen tube growth and ovule fertilization in atypical fruit morphologies of tomato (*Solanum lycopersicum*)

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Cornell's vegetable breeding program has developed small-fruited tomato (*S. lycopersicum*), breeding lines that exhibit atypical morphologies, including shapes similar to that of a chili pepper. These atypical fruit shapes develop from similar anomalies in ovary shape, and exhibit decreased seed production in mature fruit. We believe that the morphology is interfering with either pollen germination or pollen tube elongation, contributing to issues with seed production. To test this hypothesis, three morphological types of small-fruited tomatoes were evaluated: a green, round cherry tomato (HP 113), a pink heart-shaped tomato (HP 14), and a small chili pepper shape (HP 32-1). Within each fruit type, pollen tube growth was studied in flowers over the first four days of pollination, which begins 24 hours after flower opening. Three plants of each morphological type were included as replicates, and two flowers from each plant were collected at each of the four time points. (day 0, 1, 2, and 3). Flowers were stored in formaldehyde-acetic acid alcohol (FAA) until processing for study by fluorescence microscope. During microscopy, pollen germination and pollen tube growth were scored within each sample using a seven-point categorical scale. Pollen germination and pollen tube growth to the ovary and ovule of the flower was considered an indicator of fertilization. Our null hypothesis was rejected when fertilization was visualized. Statistical methods, final results, and conclusions will be discussed in greater detail on the poster.

[8] An inside job: Investigating intracellular localization of *Clavibacter michiganensis michiganensis*

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Clavibacter michiganensis subspecies *michiganensis (Cmm)* is a Gram-positive bacterial pathogen which causes bacterial canker in tomato plants. Though *Cmm* has previously been thought to inhabit vascular tissue and intercellular spaces exclusively, previous data from the Smart lab indicates that *Cmm* may spread intracellularly and non-lytically between plant cells. To investigate this possibility, leaves from *Nicotiana benthamiana,* another susceptible host, were infiltrated with suspensions of three different strains of eGFP expressing *Cmm* at OD_{600}=0.8. Protoplasts were extracted from these leaves at 24 and 48 hours post inoculation (hpi) and imaged under a confocal microscope to determine whether intact protoplasts contained intracellular *Cmm*. Additionally, epidermal peels of infiltrated leaves were imaged at 24 and 48 hpi. None of the protoplasts observed using fluorescence confocal microscopy contained intracellular *Cmm*. However, some aggregates of Cmm were observed in areas in and around cells that were collected from epidermal peels of infiltrated leaves. This particular finding which suggests non-lytic intracellular invasion of plant cells by *Cmm* warrants further study of *Cmm* localization during pathogenesis.

[9] Exploration of diversity in *Melampsora* willow rust populations in Northeastern America

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Melampsora rust is one of the predominant pathogens of the biofuel crop shrub willow (*Salix spp*.) in the Northeastern United States. The two major willow rust pathogen species in New York State are *M. americana*, which alternates on balsam fir as a host, and *M. paradoxa*, with larch as an alternate host. To manage willow rust in the field, knowledge of the diversity of the rust population in New York State is required for breeding resistant cultivars. To achieve this, we extracted DNA from willow rust isolates collected in the summers of 2015 and 2016 and then compared internal transcribed spacer (ITS) region sequences for these isolates. A phylogenetic tree was generated using sequences from New York isolates and three type species ITS sequences for *M. americana*, *M. paradoxa*, and *M. ribesii-purpurea* as an out group. Of 103 isolates sequenced, we found that 90.7 percent were of the species clustered with *M. americana*, and 9.3 percent were of the species clustered with *M. paradoxa*. This work suggests that shrub willow cultivars with resistance to *M. americana* should be more heavily emphasized in shrub willow breeding programs in the Northeastern United States.

[10] DNA sequence analysis of microbiomes on the surface of sour rot-infected grapes

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Sour rot is a grape disease about which research into its cause and management is limited. The disease negatively impacts fruit quality as ethanol oxidizes into acetic acid within the infected grape berries. The goal of this project was to determine which bacteria and fungi are more prevalent in the surface microbiomes of sour rot-infected versus asymptomatic grapes. Microbiome samples of 22 asymptomatic clusters and 29 sour rot-infected clusters were collected in a vineyard of Vitis interspecific hybrid cv. Vignoles in Geneva, NY in 2015. The total DNA was extracted from rinsate of 3-berry samples, and the 16s rRNA and ITS regions were amplified and sequenced. Qiime (Quantitative Insights Into Microbial Ecology) software was used to quality filter the sequences and identify the OTUs (operational taxonomic units) present in each sample. The taxonomies of these OTUs were assigned using the Greengenes and Unite databases. Statistical analysis was done in STAMP (Statistical Analysis of Metagenomic (and other) Profiles). Among 31 sequenced bacterial orders, the order *Rhodospirillales* had a significantly increased incidence in sour rot-infected grapes ($p < 0.001$) while *Burkholderiales* had a decreased incidence ($p = 0.024$). Among the 21 sequenced fungal species, *Talaromyces marneffei* was the only species with an increased rate of detection in the sour rot-infected group ($p = 0.004$). An understanding of which microbes are present on the surface of symptomatic and asymptomatic grapes may lead to a better understanding of the sour rot disease complex and aid in developing targeted treatments.

[11] Analysis of mutations and expression of ABCA1 and ABCA2 genes in cabbage loopers resistant to Bt toxin Cry2Ab

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The cabbage looper, *Trichoplusia ni* (Hübner), is a pest of crucifers and other crops that has developed resistance to the microbial pesticide, *Bacillus thuringiensis* (Bt). The resistance to Bt toxin Cry2Ab in *T. ni* has been genetically mapped to the ABCA1 and ABCA2 gene locus. This project aims to identify mutations and analyze the expression of these two genes in the midgut of Cry2Ab-resistant *T. ni*. The cDNAs of the ABCA1 and ABCA2 genes were obtained by RT-PCR of midgut mRNA from the susceptible and Cry2Ab-resistant *T. ni* larvae and were subsequently sequenced to identify mutations in Cry2Ab-resistant *T. ni*. Real-time RT-PCR analysis was used to quantify the expression levels of the two genes in the larval midgut and carcass from both the susceptible and resistant strains of *T. ni*. Results indicated that ABCA2 expression was abundant in the larval midgut compared to that in the carcass. In contrast, ACBA1 was expressed mostly in the carcass while expression in the midgut was rare. Missense and deletion mutations were found in the ABCA2 gene in the resistant strain. These findings provide information for further studies on the association of mutations in the ABCA2 gene with Cry2Ab resistance, which is crucial for management of Bt resistance in insects.

[12] Insect vectors of grapevine red blotch virus in a Napa County, CA vineyard

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Grapevine red blotch virus (GRBV), the causal agent of red blotch disease, is an emerging virus of grapevine. In 2015 and 2016, insect vector candidates of GRBV were identified in a *Vitis vinifera* Cabernet franc vineyard in Napa County, CA where secondary spread of GRBV was documented. Among the vector candidates, the three-cornered alfalfa treehopper (*Spissistilus festinus* [Say]) was recognized as a vector of epidemiological significance. The objective of this study was to investigate the community of insects in a nearby Cabernet Sauvignon vineyard where GRBV is present, but is not spreading. Sticky card traps were placed in the aforementioned Cabernet Sauvignon vineyard from April to June, replaced weekly, and shipped to us for analysis. Individual insect specimens were identified and removed from the sticky card traps for isolation of DNA. Diagnostic multiplex polymerase chain reaction targeting two regions of the GRBV genomic DNA was performed to determine if insects had ingested GRBV. Previously identified vector candidates comprised less than 0.02% of the total specimens on the traps. Insects that consistently test positive for GRBV will be evaluated for their ability to transmit GRBV from infected to healthy vines. Identifying potential insect vectors of GRBV and understanding their seasonal population dynamics in vineyards is critical for optimal disease management.

[13] The role of acute cold events in inhibiting *Podosphaera macularis* on hop

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Powdery Mildew, caused by *Podosphaera macularis*, was a major factor in the decline of the New York hop industry in the early 20th century. With the recent return of hop production to New York, powdery mildew once again threatens sustainable production. The early growing season (mid-May – June) provides generally favorable temperatures for epidemics. However, epidemics often inexplicably stall during this period. Our investigation set out to determine if the acute overnight low temperatures reached in the early growing season are capable of slowing progression of powdery mildew during otherwise favorable conditions. We assessed the initial colony establishment, viability, latency period, and sporulation density of powdery mildew colonies subjected to acute cold events of the following temperatures: 4, 8, 12, and 19℃ for the following durations: 30min and 3-4 h. Our results indicate that cold events lasting 3h at 4 and 8℃ significantly increased the length of the latent period of *P. macularis* and significantly decreased sporulation density and that treatments for 4hrs at 4℃ significantly reduced colony viability. This is relevant to the epidemiology and disease progression models for *P. macularis*, as naturally occurring cold shock may slow the progression of the pathogen in New York hop yards and result in a reduced need for fungicide application early in the growing season.

[14] Soil cultural practices alter efficacy of entomopathogenic nematodes (*Steinernema feltiae* and *Heterorhabditis bacteriophora*) as biocontrol agents in low maintenance turf

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Entomopathogenic nematodes infect and kill soil-dwelling insects and are an increasingly important tool for pest management in low-maintenance turfgrass. Despite their promise as an alternative to chemical insecticides, their inconsistent performance under field conditions has hindered their adoption into pest management programs. Abiotic factors such as soil compaction and soil texture may be partially responsible for the variable efficacy of EPNs. The aim of this project was to assess the effect of soil cultural practices on the efficacy of two species of entomopathogenic nematode (*Heterorhabditis bacteriophora* and *Steinernema feltiae*) as pest control agents in low maintenance lawn-type turf. Experimental plots were core aerified, aerified followed by sand amendment (top-dressing), or left unaltered. Subplots received an application of one of two nematode species (S.*feltiae* or H.*bacteriophora*) or remained nematode free. Wax moth larvae (*Galleria mellonella*) were confined to the soil in mesh sleeves under the soil surface in all plots to assess EPN infection rates among different soil and nematode treatments. After seven days, larvae were removed from the soil and monitored for signs of nematode infection. Infection by *S. feltiae* was greater than infection by *H. bacteriophora* in all soil treatments. Overall infection was also greater in aerified plots as compared to aerified and topdressed plots. This suggests that aerification, but not topdressing, of turf may be a valuable option to help improve pest control by entomopathogenic nematodes.

[15] Evaluating fungicide management practices to promote sustainable SDHI fungicide use for apple scab

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Apple scab, caused by *Venturia inaequalis*, is one of the most economically devastating diseases of fresh-market apples grown in temperate climates. In such climates, fungicides are needed to control apple scab. Unfortunately, there is a history of resistance development to new fungicide chemistries. In this regard, experiments were conducted to understand the selection of fungicide resistance following application of the newly labeled succinate dehydrogenase inhibitor (SDHI) fungicides. Field trials were conducted in two mature apple orchards at the New York State Agricultural Experiment Station in Geneva, NY. Replicate plots in each orchard were treated with applications of commercially formulated products of SDHI fluxapyroxad in different rates and combinations with other single and multi-site fungicides four times throughout the growing season. Single-lesion isolates were obtained from leaf lesions and subjected to microscopy-aided relative germination assays on medium amended with fluxapyroxad. Our results indicate that plots receiving low rates fluxapyroxad have populations with reduced sensitivity to fluxapyroxad compared to plots receiving high application rates. Applications of fluxapyroxad alone have had populations with reduced sensitivity to fluxapyroxad compared to plots receiving fluxapyroxad and another single or multi-site fungicide. It may be important that growers apply SDHIs (at high or low rates or with single or multi-fungicides) to improve disease management, and ensure sustainable agricultural production.

[16] Optimization of DNA isolation protocols and fungicide resistance gene primers across *Erysiphe* and *Podosphaera* species

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Powdery mildew is a fungal plant pathogen that costs grape growers millions of dollars in fungicides every year in the United States. Widespread use of these chemicals has caused fungicide resistance to develop in some powdery mildews. Amplicon Sequencing (AmpSeq) is a high-throughput technology that is used to study these fungicide resistance genes through the development of molecular markers for each target trait. The purpose of this study was to improve AmpSeq protocols by identifying the best DNA isolation protocols and designing efficient primer sets for different powdery mildew taxa. NanoDrop spectrophotometry results indicated that a CTAB DNA isolation protocol yielded the most consistent DNA product. Primers for fungicide resistance genes *CYP51*, *CYTb*, and *SDHb* were designed using polymorphisms found in previously generated AmpSeq sequences, as well as from sequences found in the National Center for Biotechnology Information (NCBI) database. The newly designed primers were tested for functionality across grape, hop, and strawberry powdery mildew species using quantitative PCR (qPCR). Primers designed using *Podosphaera* sequences showed increased amplification among *Podosphaera* samples compared to the original primers designed for *Erysiphe necator*, and vice versa. Our results will influence experimental and primer design in future runs of AmpSeq, allowing for greater success in identification of fungicide resistant alleles in *Erysiphe* and *Podosphaera*.

[17] Assessment of the potential for using loop mediated isothermal amplification for detection of *Meloidogyne hapla*

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Northern root knot nematode (RKN; *Meloidogyne hapla*) is a major pest of vegetables in temperate production regions and the predominant *Meloidogyne* species in New York. RKN causes crop losses through reduced yields or quality of produce such as blistering and galling of root and tubers, making fresh market products unmarketable. The pesticides used to control RKN populations are expensive and dangerous for farmers. Moreover, they are often used without knowledge of populations relative to a damage threshold. Traditional methods for enumerating nematode populations such as pie pan extractions and counting under high magnification can take weeks to provide results to farmers and requires tedious morphological identification, making delivery of robust, time sensitive management information difficult. An experiment was designed to test a protocol for a *Meloidogyne*-specific loop mediated isothermal amplification (LAMP) assay that may reduce the time to produce information on the presence of RKN. Specificity and sensitivity of the LAMP assay was quantified. The LAMP assay was able to detect *M. hapla*, as well as two other RKN species, *M. incognita*, and *M. arenaria*. The LAMP assay was able to detect the presence of *M. hapla* target DNA down to 12 pg/uL. LAMP technology is simple to use and economical which may aid in its potential to be used in routine nematode diagnostics in the future. Potential pitfalls of the technique are discussed.

[18] Genetics of sex determination in the purple osier willow (*Salix purpurea* L.)

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The genetics of sex determination in dioecious plants have become an area of increasing interest to better understand the evolution of sex chromosomes. Previous research in the family Salicaceae, including *Populus trichocarpa*, has demonstrated that this species uses an XY system with the sex determination regions (SDR) on Chr.19. However, recent research has shown that *Salix purpurea* utilizes a ZW system with an SDR on Chr. 15. The purpose of this study was to survey male, female, and hermaphrodite *S. purpurea* parent and progeny genotypes for polymorphisms in candidate sex determination genes. Primers were designed for candidate genes to amplify fragments from genomic DNA via PCR. Products were then Sanger sequenced and aligned to the reference genome for variant discovery. Our results confirm that these SDR genes are heterozygous in females and homozygous in males. Hermaphrodites also tended to be heterozygous for some gene fragments, but homozygous for others, suggesting a cross-over between the Z and W versions of Chr. 15, which may contribute to hermaphrodism. Genetic mapping has suggested that genes on Chr. 19 may also be involved in sex determination, and polymorphisms were identified in those target genes; however, it was not clear that polymorphisms on Chr. 19 correlated with sex. Further mapping and characterization of candidate genes in additional willow species will further improve knowledge of sex determination in the Salicaceae family.

[19] Effect of single amino acid mutations in the RNA-dependent RNA polymerase of grapevine fanleaf virus on pathogenicity in *Nicotiana benthamiana*

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Grapevine fanleaf virus (GFLV) causes fanleaf degeneration, a major disease of grapevine worldwide. GFLV belongs to the genus *Nepovirus* in the family *Secoviridae*. The genome of GFLV is composed of bipartite single-stranded RNA, and it requires both RNA1 and RNA2 to induce systemic viral infection in plants. Two naturally occurring strains of GFLV, GHu and F13, can establish systemic infection in *Nicotiana benthamiana*, but only strain GHu causes symptoms, i.e. chlorosis and vein clearing, while F13 elicits an asymptomatic infection. Previous studies have shown that the 3' end of the RNA1-encoded RNA-dependent RNA polymerase ($1E^{Pol}$) of GFLV is responsible for symptom development in *N. benthamiana*. The purpose of this study was to examine the effect of a single amino acid mutation in the symptom determinant region of GFLV strain GHu by mutating a lysine (K) residue at position 802 of $1E^{Pol}$ using site-directed mutagenesis. GFLV-GHu RNA1 mutants were characterized *in planta* for their ability to establish local and systemic infection, and elicit symptoms following agroinoculation in *N. benthamiana*. The fidelity of virus progeny was verified by sequencing of RT-PCR products. Some mutations abolished symptom development while others prevented even the establishment of local infection. These results provided insight into the pivotal role of residue 802 of GFLV-GHu $1E^{Pol}$ in both infectivity and symptom development in *N. benthamiana*.

[20] Looking at Extremes: Towards Genetic and Phenotypic Characterization of Columnar and Tip-Bearing Trait in Apple (*Malus domestica*.)

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Apple tree architecture influences orchard management practice and yield related traits. Two extreme architecture types, columnar and tip-bearing, are investigated to characterize their genetic control. The columnar trait of 'Wijick', a bud mutation from 'Macintosh', is mainly caused by a retrotransposon insertion in an intergenic region on chromosome 10. An ongoing study in a *COco* × *COco* population has identified another locus (*CO2*) that acts as a genetic modifier in the presence of the *CO* insertion. To fine-map *CO2*, two additional *COco* × *coco* populations, of 588 progeny in total, were examined. Genotyping with two *CO*-linked markers identified 334 progeny carrying the *CO* insertion, of which 35 individuals exhibit standard phenotype, providing useful information for chartering the *CO2* locus. Tip-bearing trees are associated with the desirable non-biennial fruit bearing trait. To quantify this trait for genome-wide association studies, tip-bearing percentage and spur density from two-year old branches were evaluated for 18 accessions in the USDA *Malus* germplasm collection. Four distinct fruit bearing types were observed. Accessions could be considered tip-bearing when tip-bearing percentages are above 75%. However, spur density does not strongly correlate with tip-bearing. These findings offer a reliable descriptor for phenotyping the fruit tip-bearing trait.

[21] Phenotypic and genetic characterization of pest resistance in interspecific F_1 shrub willow (*Salix* spp.) families

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As the demand for bioenergy grows, there is need to develop feedstock cultivars with improved yield and sustainability. A breeding goal for yield improvement in shrub willow (*Salix* spp.) is increased resistance to insect pests. Potato leafhopper (PLH, *Empoasca fabae*) and imported willow leaf beetle (*Plagiodera versicolora*) pose serious threats to shrub willow productivity, and greater knowledge of the genetic basis for resistance could speed breeding and selection of new cultivars. In order to characterize phenotypic and genetic resistance to these pests, a suite of mapping populations were generated, including an F_2 *S. purpurea* family as well as eight interspecific F_1 families with *S. purpurea* as a common parent. The family progeny and parents were genotyped by genotyping-by-sequencing (GBS) to identify loci associated with pest severity traits in the field. Surveys of these mapping populations demonstrated varying levels of susceptibility to PLH and willow leaf beetle. Expression of candidate genes involved in PLH resistance response identified from previous greenhouse no-choice feeding studies will be confirmed using qRT-PCR of field-grown leaves collected shortly after initial feeding by PLH. Identification of these genes involved in insect pest resistance will be used to develop tools for marker assisted selection and introgression of resistance into improved cultivars.

[22] Confirmation of two genetic loci associated with weeping phenotype in *Malus*

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Apple (*Malus*) is a woody species of diverse tree architecture forms. One form, which is characterized by downward growing branches, is called weeping. The weeping growth habit is often observed in crabapple cultivars and desirable for ornamental purposes. Early studies showed that the weeping phenotype is a dominant trait. In an ongoing effort to better understand the genetic control of the weeping trait, millions of DNA variants from 38 progeny of a 'Weeping × Standard' cross were obtained and analyzed, resulting in mapping four loci, including *W* and *W2* of large genetic effect. To confirm the findings, a population of 178 progeny derived from open-pollinated seeds of 'Cheals Weeping' were evaluated, scoring 74 of standard growth habit, 22 standard-like, 29 weeping-like, and 53 weeping. Genotyping the population with six simple sequence repeat (SSR) markers from the *W* and *W2* regions demonstrated that the markers are linked to the weeping trait. However, *W* is more tightly linked to the phenotype than *W2*, suggesting that *W* is a major genetic locus for weeping while *W2* is a minor. The data and results obtained by this project provide important information not only for fine mapping of *W* and *W2*, but also for identification of candidate causal genes for the weeping trait.

[23] Occurrence and distribution of the invasive *X. germanus* in apple orchards

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The ambrosia beetle *Xylosandrus germanus*, also known as the black stem borer, is an invasive species known to damage commercial apple trees with their nesting galleries, causing tree wilting and death. In previous years, preventive pesticide applications did not noticeably provide control for this pest. Applications of the repellent Verbenone to trees may lead to fewer economically significant infestations. Beetle populations were monitored in the interior and wooded edges of orchards using ethanol baited traps in order to monitor populations. Verbenone, in different commercially available and experimental formulations, was topically applied to intentionally stressed apple trees containing additional ethanol lures. Trees were then examined and dissected for borer damage. During a preliminary evaluation, only two trees contained beetle damage, one treated tree and one untreated tree. It was found that the mean trap catches were approximately three times higher in outside traps as compared to inside traps, and that this difference was statistically significant. Implications of our findings suggest that there are higher numbers of *X. germanus* occupying the wooded areas bordering orchards in Wayne Co. than in the interior. Additionally, there may be a trend of BSB peak capture and second flight onset occurring after fewer degree days each year. These data may be able to be used to monitor population movements of this pest in apple systems.

[24] High-throughput resistance phenotyping for fire blight infection of apple blossom via image analysis

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Fire blight bacteria, *Erwinia amylovora*, can enter through natural openings in apple plants, making flowers a vulnerable area for infection. Blossom blight response may be different than shoot blight response requiring further investigation. Quantifying severity of infection in apple flowers by *E.* amylovora is difficult and time consuming. Image analysis is a powerful and objective method to observe the presence of cultivar-specific resistance to fire blight. Flowers collected from the field for 3 commercially valuable cultivars, Empire, Gala, and Golden Delicious, were inoculated with three strains of *E. amylovora* (Ea273, E2002a, E4001a) and combinations of these strains. Pictures of dissected flowers were taken 2, 4, 6, 8, days after inoculation. Image segmentation in ImageJ selected diseased regions of interest on 288 images. The bacteria on the flowers was plated and images of corresponding plates were analyzed for the number of bacterial colonies present using Wolfram Mathematica software. This method successfully quantified the amount of disease on flowers in a high-throughput manner. We aim to explain the relation between disease area, the visual rating scale, and the number of bacterial colonies present. Accuracy can be increased with more uniform imaging procedures and improved global thresholds.

[25] Acquisition of *Erwinia amylovora* by *Drosophila melanogaster*

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Fire blight (*Erwinia amylovora*) is a common affliction of rosaceous trees such as apple and pear. This bacteria can spread in as few as thirty-five cells and leads to the eventual death of the tree, making it a significant pest for growers. One symptom of the disease is ooze, a bacteria-laden sugary substance that leaks from diseased tissue and is anecdotally attractive to insects, especially flies (Diptera). We hypothesize that insects feeding on ooze can acquire and transmit *E. amylovora*, however there is not specific research to date. Using *Drosophila melanogaster* as our model fly species, our goal was to show that flies interacted with and spread E. amylovora, specifically evaluating whether the flies were capable of acquiring the bacteria. To investigate acquisition we exposed individual *D. melanogaster* adults for different lengths of time (0.5, 1.5, 3, & 15 hours) to oozing immature apple fruit artificially inoculated with lab-grown *E. amylovora*. Flies left with infected fruits feed and walk on the ooze, picking up the fire blight. Initial trials showed that about 6% of flies tested positive for *E. amylovora* after being exposed to an infected fruit, with the highest levels of positive samples found with the longest time of exposure (15 hours). Overall, our data shows that flies can acquire fire blight, but additional study is necessary to further characterize these interactions.

[26] Precise and cost-effective detection of *E. amylovora*, the causative agent of fire blight in the orchard

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Erwinia amylovora, the causative agent of fire blight, is an economically important pathogen of apple species worldwide. In the United States, yield losses and control measures due to fire blight in apples cost over $100 million annually. Pruning infected plant tissue and vigilant antibiotic spray programs are the primary control methods in the field. Thus, early pathogen detection and identification of alternative sources of inoculum can be critical for effective implementation of control measures. We modified a Loop Mediated Isothermal Amplification (LAMP) assay to detect small quantities of *E. amylovora* DNA in less than one hour. This assay utilizes *E. amylovora*-specific primers and a colorimetric indicator to confirm the presence of the pathogen, showing potential for rapid diagnosis. Apple cultivars 'Gala' and 'McIntosh' inoculated with *E. amylovora* strain Ea_273 showed varying degrees of susceptibility to infection and rates of infection progress over time. Asymptomatic progress of fire blight infection can vary according to genetic resistance of the host, virulence of pathogen, and their interaction, therefore LAMP-based detection of the pathogen would be valuable for measuring genetic resistance-specific disease progression and to optimize pruning of asymptomatic tissues quickly and cost-effectively, directly in the orchard. We compiled a list of quantitative trait loci for fire blight resistance, to be used for further investigation of genetic resistance and pathogen virulence-specific bacterial movement in the tissue to optimize pruning.

[27] Genetic analysis of architecture and leaf traits in interspecific apple progeny (*Malus prunifolia* × *Malus* × *domestica*)

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Malus prunifolia is a species of crabapple useful in breeding for its resistance to fireblight, apple scab and drought, and columnars have been studied for their novel architecture and breeding potential. Progeny of *Malus prunifolia* (PI 19651) and a columnar *Malus domestica* (152) selection were phenotyped in June 2017 (n=254) for architecture type, presence and length of basal branches, number of laterals, and branch angles as well as leaf pubescence, shininess, lobing, aspect ratio (length/width), and area. Quantitative trait loci (QTL) analysis was performed using genotyping by sequencing (GBS) data to identify QTL's associated with the traits studied. Significant associations were not found between architecture type and either leaf width, lobing, or branch angle. Columnar types had significantly fewer and shorter basal branches, more pubescence, greater shininess, and bigger, longer leaves with a larger leaf aspect ratio than standard types. Average leaf length, average leaf area, average leaf aspect ratio, number of laterals, and architecture were all linked to a major QTL on linkage group (LG) 10, the known location of the *Co* gene and a minor QTL on LG11.The QTL on LG11 has not been reported in any of the many studies on columnar apple trees.

[28] Assessing and minimizing threat of fire blight following mechanical thinning and mechanical hedging

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The use of mechanical bloom thinners and hedgers in the apple industry has many beneficial effects, but has the potential to predispose trees to developing fire blight, a devastating apple disease. To determine the risk of blossom blight and shoot blight infection and spread after mechanical thinning and hedging, the first tree in each row of an apple orchard was inoculated with fire blight at bloom. After inoculation, a Darwin 250 mechanical thinner was used at king bloom in accordance with standard practice. In late July, a vertical mechanical apple hedger was run through active shoot blight at the beginning of each row. After the mechanized production practices, trees were sprayed with either streptomycin or Serenade Optimum (OMRI approved biological control) and the incidence of fire blight was measured 21 and 31 days later. The incidence of blossom blight (BB) and shoot blight (SB) in rows that only received mechanical thinning (BB 9.6% SB 21.0%) was higher than the non-mechanized, non-treated, but inoculated rows (BB 5.3% SB 15%). Little to no fire blight (< 1.0%) developed due to hedging. Hence, mechanical thinning and hedging were not found to spread fire blight beyond natural distribution if streptomycin or biologicals were applied.

[29] Continued investigation of low dose prohexadione-calcium programs & natural systemic acquired resistance (SAR) inducers for fire blight management in apples

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Fire blight, caused by the bacterium *Erwinia amylovora*, can severely compromise the productivity and longevity of an apple planting. Antibiotics and biologicals are used to manage blossom blight, while prohexadione-calcium, a growth regulator, is the best option for shoot blight management. Unfortunately, the overuse of antibiotics can lead to resistance development in the pathogen and can retard the vigor and productivity of the orchard, especially in establishment years. A trial was conducted in a 16-year-old 'Gala' orchard to investigate the use of bloom time applications of prohexadione-calcium and biological systemic acquired resistance (SAR) inducers as a means of managing blossom blight and subsequent development of shoot blight in the absence of antibiotics. Treatments were applied either early on from pink to full bloom or at lower rates through the season until terminal elongation, and trees were inoculated with *E. amylovora* at bloom. The incidence of blossom blight and shoot blight was evaluated 21 days after inoculation, and tree productivity was evaluated every 14 days. Antibiotics had the most control of blossom and shoot blight, although all treatments provided some level of control. Applications at pink of prohexadione-calcium were not significantly different in managing blossom blight than bloom and trickle programs. Pink and full bloom applications had similar impacts on shoot growth and tree productivity, but the full season programs of prohexadione-calcium were more variable.

[30] Implementing Oxford Nanopore MinION technology to obtain long DNA sequencing reads of *Vitis spp.*

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Short-read Illumina DNA sequencing of 50 bp to 300 bp has become widely popular, but computational analysis of short-read data can result in imperfect or wrong conclusions about the genome, particularly in duplicated regions. Here, Oxford Nanopore Technology's (ONT's) new long-read sequencing technology (MinION) was used to sequence seven genes, including two tandemly duplicated genes, from 96 diverse wild *Vitis* accessions. To test sequence quality, an 8.0 kb lambda DNA control was sequenced with a raw average read length of 7.2kb, 97.8% nucleotide identity, but only 83.9% raw data accuracy. The seven genes, which ranged in size from 1.3 kb to 7.8 kb, were amplified by long-range, multiplex PCR and barcoded for multiplexed sequencing, followed by computational analysis using a combination of ONT (MinKNOW, Albacore) and custom software, including CLC Genomics Workbench. The first sequencing generated 128,400 reads, with 91% of the data being short reads that did not align to the grape reference. The shortest gene produced 82.9% of the remaining data, indicating size bias within the PCR multiplexing. The aligned reads ranged from 0.9kb to 7.8kb, and the sequence identities neared 100% for conserved gene regions, which attest to high sequence accuracy. The MinION provides long reads of more than 1 kb, but more work is needed to optimize multiplexing strategies for sequencing of long amplicons.

[31] Characterization of fungi associated with tomato leaf mold in New York high tunnels

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Tomato leaf mold, caused by the ascomycete *Passalora fulva (P. fulva)*, is a worldwide concern given its potential to significantly reduce the production of tomatoes grown in greenhouses and high tunnels. Symptoms include chlorosis, heavy sporulation on the underside of leaflets, and in the long term, defoliation. In order to provide appropriate management strategies for growers, isolation of the pathogen and a better understanding of population dynamics are needed. Sequences of the ITS and actin region of a representative sample of isolates from 2016 and 2017 were analyzed and matched to reference genes. It was found that most of the isolates from the year 2016, belonged to the *Cladosporium* genus, which has not been reported pathogenic to tomatoes. A phylogenetic tree was constructed and shows genetic relationships between the isolates. Successful isolation of *Passalora fulva* from symptomatic leaves of samples found from a variety of locations in 2017 was achieved. A microscopy profile was done in order to differentiate *Passalora fulva* from *Cladosporium sp* in cultured leaf discs. Experimental high tunnels were inoculated with *P. fulva* and symptoms appeared within a week. Future work will look at possible association of fungi with the *Cladosporium* genus. Diversity studies of isolates from New York will also be conducted in order to develop better management strategies.