

SCHEDULE AND ABSTRACTS FOR THE JOINT 2022 NARC/W-3008/STOP-THE-ROT MEETINGS

Registrants of the NARC/W-3008/StR meetings may also attend sessions of the Colorado Fruit and Vegetable Growers' Association on the lower levels in the conference center. Their schedule is available at <https://pheedloop.com/CFVGA22/site/schedule/>

Monday, February 28, 2022

Time	Activity
07:00 onward	Registration table will be located outside the Aspen Room . Posters may be hung in the Telluride and Aspen rooms after 7:00 AM. Please register before hanging your poster. Numbers assigned to posters (see list of poster abstracts below for numbers) will indicate where the poster should be hung.

Time	Chairs	Activity
08:00	Lindsey du Toit (Stop-the-Rot Team), Peter Rogers (W-3008), and Michael Havey (NARC)	Meeting opening in the Aspen Room and overview of schedule

Oral Session I: Diseases and Pests (Aspen Room)

Time	Authors	Affiliations	Title
08:10	<u>Douglas S Higgins</u> and Mary K Hausbeck	Michigan State University	EARLY FUNGICIDE APPLICATION TIMING CAN IMPROVE STEMPHYLIUM LEAF BLIGHT CONTROL IN ONION
08:30	<u>Gia Khuong Hoang Hua</u> , Robert Wilson, and Jeremiah Dung	Oregon State University	USE OF ALLIUMS AS TRAP CROPS TO REDUCE WHITE ROT INOCULUM IN INFESTED FIELD SOIL
08:50	<u>Rob Wilson</u> , Kevin Nicholson, and Brenna Aegerter	University of California	THE INFLUENCE OF IRRIGATION METHOD (SOLID-SET SPRINKLER IRRIGATION OR DRIP IRRIGATION) ON THE INCIDENCE AND SEVERITY OF BACTERIAL DISEASES IN ONIONS GROWN IN NORTHEAST CALIFORNIA
09:10	<u>Mei Zhao</u> , Shaun Stice, Gi Yoon Shin, Teresa Coutinho, Ron Gitaitis, Brian Kvitko, and Bhabesh Dutta	University of Georgia	A NOVEL BIOSYNTHETIC GENE CLUSTER ACROSS THE <i>PANTOEA</i> SPECIES COMPLEX IS IMPORTANT FOR PATHOGENICITY IN ONION
09:30	<u>Bhabesh Dutta</u> , Brian Kvitko, Mei Zhao, Brendon Myers, Gaurav Agarwal, Gi Yoon-Shin, Sujana Paudel, Santosh Koirala, Bamidele Sangoyomi, and Ronald Gitaitis	University of Georgia	AN OVERVIEW OF BASIC AND APPLIED RESEARCH ON DIVERSE BACTERIAL DISEASES ON ONION IN GEORGIA
09:50	<u>Gi Yoon Shin</u> , Bhabesh Dutta and Brian Kvitko	University of Georgia	GENETIC BASIS OF HIVIR, A PHYTOTOXIN GENE CLUSTER OF ONION PATHOGEN <i>PANTOEA ANANATIS</i>
10:10	Jo Ann Asselin, Mary Ann Karp, Paulina Rychlik, Margery Daughtrey, Melanie Filiatrault, and <u>Paul Stodghill</u>	Cornell University and USDA-ARS	GENOMIC COMPARISON OF <i>DICKEYA FANGZHONGDAI</i> STRAINS ISOLATED FROM DIFFERENT HOSTS AND PATHOGENICITY IN ONION (<i>ALLIUM CEPA</i>) BULBS

10:30 to 10:45 AM: Break with refreshments

Oral Session II: Production (Aspen Room)

Time	Authors	Affiliations	Title
10:45	<u>Mike Thornton</u> , Deron Beck, Kyler Beck, Ransey Portenier, and Oksana Morgan	University of Idaho	EVALUATION OF HERBICIDES FOR IMPACT ON THE INCIDENCE OF SINGLE CENTERS IN ONIONS
11:05	<u>Deron Beck</u> , Kyler Beck, Mike Thornton, Ransey Portenier, and Oksana Morgan	University of Idaho	STUNTING AND STAND LOSS IN DRIP-IRRIGATED ONIONS
11:25	<u>Brian A. Nault</u>	Cornell University	MANAGING INSECT PESTS OF <i>ALLIUM</i> CROPS IN THE EASTERN US
11:45	<u>Christy Hoepfing</u>	Cornell University	STUNNING WEED CONTROL IN MUCK-GROWN ONIONS WITH BICYCLOPYRONE

12:05 to 13:00 PM: Lunch Provided

Oral Session III: Breeding and Genetics (Aspen Room)

Time	Authors	Affiliations	Title
13:00	<u>Joseph B. Wood</u> , Christopher S. Cramer, Robert Steiner, Richard Heerema, Brian J. Schutte, and Ivette Guzman	New Mexico State University	ONIONS SELECTED FOR RESISTANCE TO <i>IRIS YELLOW SPOT VIRUS</i> HAVE HIGHER CARBON ASSIMILATION RATES
13:15	<u>Suman Sharma</u> and Christopher S Cramer	New Mexico State University	IMPROVING FUSARIUM BASAL ROT RESISTANCE OF SHORT-DAY ONION CULTIVARS THROUGH AN ARTIFICIAL INOCULATION MATURE BULB SCREENING
13:30	Elizabeth Straley, Jen Marzu, and <u>Michael J. Havey</u>	University of Wisconsin	GENETIC ANALYSIS OF RESISTANCE TO FUSARIUM BASAL ROT OF ONION
13:45	<u>Michael J. Havey</u>	University of Wisconsin	MODIFICATION OF EPICUTICULAR-WAX PROFILES TOWARDS THRIPS RESISTANCE IN ONION

Session IV: W-3008 Meeting (Aspen Room)

Time	Item
14:00	Welcome and introductions <ul style="list-style-type: none"> • State of W-3008 –Tracy Dougher • W-3008 Annual Report submission process and deadlines – Chair Peter Rogers • W-4008 Submission and next steps – Brian Nault
14:15	State reports with open forum discussions <ul style="list-style-type: none"> • <i>All states willing</i>: Colorado, New Mexico, Utah, Oregon, Idaho, Washington, California, Texas, Georgia, Pennsylvania, Michigan, New York, and others
15:15	Break
15:30	State reports continue with open discussions
16:30	Team business meeting for W-3008 <ul style="list-style-type: none"> • Communications and Alliumnet updates for W-3008 • Election of officers and lead into W-4008 • Next meeting – ideas and location?
16:45	Adjourn

Session V: Discussion regarding scheduling of next NARC (Aspen Room)

Time	Discussion leaders
16:45	Greg Yielding, National Onion Association, with the NARC/W-3008 groups

16.00-18.00: Reception with Colorado Fruit and Vegetable Growers Association (CFVGA) in the Ballroom. Snacks provided with a cash bar.

Tuesday, March 1, 2022

Research presentations on bacterial diseases by the “Stop-the-Rot” project team. Open to NARC/W-3008/CFVGA participants and members of the Stop-the-Rot Stakeholder Advisory Panel.

Oral Session I: Aspen Room

Time	Oral presentations	Presenter/lead
08:00	Welcome, introduction & overview of the project	du Toit
	Objective A: Characterization of onion bacterial diseases	
08.15	A1. Bacterial survey & NOBSC	du Toit
08.30	A2a. Onion bacterial pathogenomics	Kvitko
08.45	A2b. Microbiome studies	Coutinho
09.00	A3. Development of onion bacterial pathogen detection tools	Woodhall
09.15	A4. Development of onion phenotyping protocols for screening for resistance to bacterial pathogens	Dutta
09.30	<i>Break/Poster viewing</i>	
	Objective B: Management of onion bacterial diseases	
09.45	Overview of Objective B: How Stop the Rot work supports improved production, reduced losses, and better understanding of how to detect & manage bacterial diseases	du Toit
09.50	B1 & B2. Irrigation & fertility practices	LaHue
10.10	B4. Cultural practices	du Toit
10.25	B5. Post-harvest practices	Waters
10.40	<i>Break 20 mins for refreshments– include poster viewing during the break.</i>	
11.00	B6. Bacterial disease modeling	Rajagopalan
11.15	B8. Economics & surveys	Colson
11.30	B3. Bactericide/pesticide programs	Nischwitz
11.45	Closing, wrap up, questions. Session ends at 12.00 pm.	du Toit

Session II

(13:00 to 17:00): Closed meeting of the Stop-the-Rot project team (Aspen Room)

(13:00 to 17:00): Closed meeting of USDA-SCRI thrips/IYSV team (Telluride Room)

Wednesday, March 2, 2022

Session III

(08:00 to 12:00): Closed meeting of the Stop-the-Rot project team (Aspen Room)

ABSTRACTS OF ORAL PRESENTATIONS AT 2022 NARC/W-3008/STOP-THE-ROT MEETING

Monday, February 28, 2022

ORAL SESSION I: DISEASES AND PESTS

EARLY FUNGICIDE APPLICATION TIMING CAN IMPROVE STEMPHYLIUM LEAF BLIGHT CONTROL IN ONION

Douglas S Higgins¹ and Mary K Hausbeck (hausbec1@msu.edu)¹

¹Michigan State University, East Lansing, MI

Stemphylium leaf blight (SLB) has become Michigan's most common foliar disease of onion. A typical SLB fungicide program begins when symptoms first appear, and the most effective fungicides may be saved until symptoms become severe. However, preventative fungicide applications may improve disease control. In 2020 and 2021, replicated field experiments were established to evaluate (1) the initiation of fungicide applications according to five growth stages and (2) alternation of site-specific fungicides. In both years, fungicide applications initiated at the 3–4 and 5–7 leaf stages reduced disease severity over time by 35.9–67.4% and 14.4–36.4% compared to the nontreated control and 8–12 leaf stage initiation, respectively. Only the fungicide applications initiated at the 3–4 leaf stage increased total yield compared to the nontreated control in both years. In 2020, Miravis Prime (3 applications) alternated with Bravo Weather Stik (7 applications) improved disease control and increased yield when Miravis Prime was used earlier in the rotation. Overall, SLB fungicide programs should start early and not “save the good stuff” but consider using the most effective fungicides earlier in the season to ensure the best return.

USE OF ALLIUMS AS TRAP CROPS TO REDUCE WHITE ROT INOCULUM IN INFESTED FIELD SOIL

Gia Khuong Hoang Hua¹, Robert Wilson² and Jeremiah Dung (jeremiah.dung@oregonstate.edu)¹

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White rot, caused by *Sclerotium cepivorum*, is a serious disease that causes significant yield losses in Allium production. The pathogen persists in soil as sclerotia, which specifically germinate in response to Allium root exudates. The objective of this study was to investigate whether early-terminated Allium trap crops can reduce populations of white rot sclerotia in soil. Data obtained from growth chamber experiments with white-, red-, sweet-or bunching onion revealed that termination of all four Alliums at 3- and 7 weeks post-emergence (wpe) significantly ($P \leq 0.046$) reduced soil sclerotia populations by up to 55% and 59%, respectively. The reduction in sclerotia populations in soil due to early crop termination was also observed under greenhouse and field conditions. Sclerotia counts in soil collected 4 weeks after crop termination remained unchanged when seedlings were terminated at 3 wpe. Crop termination at 11 wpe resulted in the significant increase in sclerotia counts due to the pathogen reproduction on the trap crops. In general, greater reductions in sclerotia counts were observed when plants were terminated chemically compared to mechanically in both greenhouse and field trials. Together, our study demonstrates the potential for early termination of Allium trap crops to help reduce white rot inoculum in soil.

THE INFLUENCE OF IRRIGATION METHOD (SOLID-SET SPRINKLER OR DRIP) ON THE INCIDENCE AND SEVERITY OF BACTERIAL DISEASE IN ONIONS GROWN IN NORTHEAST CALIFORNIA

Rob Wilson¹, Kevin Nicholson¹, and Brenna Aegerter² (rgwilson@ucanr.edu)¹University of California ANR Intermountain Research and Extension Center and ²University of California Cooperative Extension San Joaquin County

Overhead sprinkler irrigation is often referenced as the primary means for bacterial disease spread in onions grown in arid climates. A study was conducted in 2021 in Tulelake, CA to compare the incidence and severity of bacterial disease under sprinkler and drip irrigation. The experiment was a split-plot randomized design with drip and solid-set sprinkler as the main plot treatments and inoculated and non-inoculated as the split-plot treatments. The entire study area was irrigated with solid-set sprinklers from planting until the 5-leaf stage to facilitate uniform onion germination and chemigation of herbicides for weed control. After the 5-leaf stage, irrigation treatments were imposed for the remainder of the growing season. Irrigation was scheduled using a combination of crop evapotranspiration and soil moisture monitoring. The total amount of irrigation water and irrigation frequency were similar for both irrigation methods. Bacterial leaf blight severity and the incidence of bulb rot were more than 10 times higher for solid-set sprinkler compared to drip. Total bulb yield and average bulb size were higher for drip compared to solid-set sprinkler.

A NOVEL BIOSYNTHETIC GENE CLUSTER ACROSS THE *PANTOEA* SPECIES COMPLEX IS IMPORTANT FOR PATHOGENICITY IN ONION

Mei Zhao¹, Shaun Stice², Gi Yoon Shin², Teresa Coutinho³, Ron Gitaitis¹, Brian Kvitko², and Bhabesh Dutta (bhabesh@uga.edu)¹

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Onion center rot is caused by *Pantoea* species complex. We found two distinct secondary metabolite biosynthetic clusters, one described in pathogenic *P. ananatis* (HiVir) and another a novel biosynthetic gene cluster (Halophos) in onion-pathogenic strains of *P. stewartii* subsp. *indologenes*. In contrast, only Halophos cluster was present in the onion-pathogenic *P. allii* strains evaluated. The contribution of HiVir- and Halophos-gene clusters in *Pantoea* spp. to pathogenesis was determined by deleting the phosphoenolpyruvate phosphomutase (*pepM*) gene from the gene clusters in *P. stewartii* subsp. *indologenes* strains (PNA03-3 (HiVir) and PNA14-12 (Halophos)) and in *P. allii* LMG24248^T (Halophos). The *pepM* deletion mutants of HiVir and Halophos clusters did not cause tissue necrosis on onion leaves or on red onion scales, and had significantly lower populations compared to the corresponding wildtype- and complemented-strains. Seven (*halB-halH*) out of eleven genes (*halA-halK*) in the Halophos cluster contributed to onion pathogenicity. Furthermore, culture filtrates of PNA14-12 expressing the Halophos cluster caused necrosis on onion leaves. Overall, this is the first report of onion pathogenicity determinants in *P. stewartii* subsp. *indologenes* and *P. allii*.

AN OVERVIEW OF BASIC AND APPLIED RESEARCH ON DIVERSE BACTERIAL DISEASES ON ONION IN GEORGIA

Bhabesh Dutta¹ (bhabesh@uga.edu), Brian Kvitko², Mei Zhao¹, Brendon Myers¹, Gaurav Agarwal¹, Gi Yoon-Shin², Sujan Paudel², Santosh Koirala¹, Bamidele Sangoyomi¹, Ronald Gitaitis¹

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Bacteria from a diverse number of genera and species can infect onion foliage and bulbs and can cause pre- and post-harvest economic losses. The common bacterial genera and species that are encountered regularly in Georgia are *Pantoea* (*P. ananatis*, *P. agglomerans*, *P. allii*, *P. stewartii* subsp. *indologenes*, *P. vagans*), *Pseudomonas* (*P. viridiflava*, *P. marginalis*), *Burkholderia* (*B. cepacia* complex, *B. cenocepacia*, *B. gladioli*), *Rahnella* spp., and *Enterobacter* spp. Recently, three new bacterial species (*Rouxiella badensis*,

Pseudomonas allivorans and *Pantoea eucalypti*) and one new pathovar of *P. stewartii* subsp. *indologenes* (pv. *cepacicola*) were associated with onion pathogenicity. Recently different pathogenicity and virulence factors in *Pantoea* spp., *Pseudomonas* spp. and *Rouxiiella* spp. were identified and validated. This indicates there is a range of genes/gene clusters that pathogenic bacteria utilize to infect onion. Multi-year assessments of bactericides, plant-growth regulators, thrips and weed management, cultural practices and varietal screens aided in identifying and optimizing integrated disease management practices that reduce losses due to bacterial diseases. Together, both basic and applied research on bacterial diseases of onion is continuously evolving and improving with the influx of novel information.

GENETIC BASIS OF HIVIR, A PHYTOXIN GENE CLUSTER OF ONION PATHOGEN *PANTOEA ANANATIS*

Gi Yoon Shin¹, Bhabesh Dutta² and *Brian Kvitko(bkvitko@uga.edu)¹

¹Department of Plant Pathology, University of Georgia, Athens GA USA, ²Department of Plant Pathology, University of Georgia, Tifton GA USA

Pantoea ananatis is a causative agent of Onion Center Rot that causes extensive water-soaked lesions and wilting of foliage and internal bulb necrosis of onion. In both pre-and post-harvest conditions, infection of *P. ananatis* in onion plants can result in substantial economic losses. Onion pathogenic strains of *P. ananatis* deploy the phytotoxin pantaphos whose production is associated with a positive red onion scale necrosis (RSN) phenotype. The pantaphos biosynthetic pathway is encoded by “the eleven hvr gene HiVir operon”. However, the requirements of most hvr genes for pantaphos production have not been determined. Furthermore, we have isolated aberrant *P. ananatis* strains that possessed the HiVir gene cluster yet were non-pathogenic. To determine the genetic requirements of individual hvr genes for the RSN phenotype, we constructed in-frame deletion mutants for the 11 hvr genes in the RSN positive *P. ananatis* strain PNA 97-1R. From the pathogenicity test, we found that majority of the hvr genes were necessary for the positive RSN. We have also identified single nucleotide polymorphisms (SNPs) present in *pepM/hvrA*, *hvrC*, and *hvrF* genes in aberrant strains and demonstrated genetically that these SNPs are sufficient for the loss of RSN thus rendering the strain non-pathogenic on onion.

GENOMIC COMPARISON OF *DICKEYA FANGZHONGDAI* STRAINS ISOLATED FROM DIFFERENT HOSTS AND PATHOGENICITY IN ONION (*ALLIUM CEPA*) BULBS

Jo Ann Asselin¹, Mary Ann Karp¹, Paulina Rychlik², Margery Daughtrey², Melanie Filiatrault^{1,2}, Paul Stodghill^{1,2} (paul.stodghill@usda.gov)

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Dickeya fangzhongdai is a bacterial plant pathogen that causes soft rot in Asian pears, potatoes, orchids, and other plants. Most *Dickeya* species have wide host ranges, but the extent of the host range of *D. fangzhongdai* is unknown. Previously, a *D. fangzhongdai* strain (AP6) was isolated from a diseased onion (*Allium cepa*), found to be pathogenic on this host, and its genome was partially sequenced. Recently, we isolated a *D. fangzhongdai* strain (643b) from *Aglaonema* (Chinese evergreen), showed that it causes soft rot in potatoes, and sequenced its genome. Preliminary analyses revealed that AP6 contains genes similar to the Allacin Tolerance (*alt*) cluster, which gives *Pantoea* tolerance to onion defense chemistry, and that strain 643b lacks this cluster. We inoculated onion bulbs with AP6 or 643b and incubated them for three days. Both strains caused maceration of onion tissue, though symptoms caused by strain AP6 appeared more severe. It is currently unclear whether *alt* plays a significant role in the virulence of AP6 in onion and whether other differences between the genomes of 643b and AP6 are important in this respect. We produced additional data to provide a complete genome sequence for strain AP6 to investigate further.

ORAL SESSION II: PRODUCTION

EVALUATION OF HERBICIDES FOR IMPACT ON THE INCIDENCE OF SINGLE CENTERS IN ONIONS

Mike Thornton (miket@uidaho.edu)¹, Deron Beck¹, Kyler Beck², Ransey Portenier¹, and Oksana Morgan¹
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A key characteristic of onion bulbs for processing is the proportion of single centers. Onion processors have reported a general decline in the proportion of single centers in the past few years. This decline in processing quality has been associated with an increase in the use of the herbicide ethofumesate. Research was conducted in replicated trials at the Parma Research and Extension Center from 2018 to 2020 to determine if rate and timing of ethofumesate application influences the proportion of single centered bulbs in a yellow sweet Spanish onion variety (SV6672NW). In all years of this trial there was a direct link between ethofumesate application and incidence of bulbs with multiple centers. The negative influence of herbicide application tends to be most pronounced at the higher rate (12 oz vs 8 oz/acre), and when applications are made relatively late in plant development (3 or 4 leaf stage). These results agree with previous studies that have identified the 3 to 4 leaf stage to be the most sensitive to stress in terms of developing multiple growing points.

STUNTING AND STAND LOSS IN DRIP-IRRIGATED ONIONS

Deron Beck (dbeck@uidaho.edu)¹, Kyler Beck², Mike Thornton¹, Ransey Portenier¹, and Oksana Morgan¹
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Early season stunting of onion seedlings is a common issue with growers in the Pacific Northwest. Reductions of plant size, stand and yield have been observed for the inside rows, closest to the drip tape for drip-irrigated onions. Growers have attributed this issue to several causes, including discrepancies in soil moisture, chemical exposure and soil compaction. Measurement of inner and outer row soil moisture and temperature at the 2-inch depth were conducted at the Parma Research and Extension Center from May 17 to May 30, 2021. Readings averaged during this period showed a 0.7% higher volumetric water content and a 0.4 °F lower soil temperature for the inner row. Reductions in plant stand, average bulb size and overall yield on the inside compared to outside row were also observed at harvest on September 15. Significant differences between the inside and outside row in pink root symptoms could be pointing to a key cause of these losses.

MANAGING INSECT PESTS OF ALLIUM CROPS IN THE EASTERN US

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Onion maggot (*Delia antiqua*), onion thrips (*Thrips tabaci*) and allium leafminer (*Phytomyza gymnostoma*) attack allium crops in the eastern USA. Management of all three pests have relied on the use of insecticides. However, current and likely changes in registration of certain insecticides, insecticide resistance and lack of new tools for managing these pests have made their control challenging. The purpose of this presentation is to provide an update on the performance of new management tools and tactics that appear promising for these three pests based on research conducted over the past several years in New York.

STUNNING WEED CONTROL IN MUCK-GROWN ONIONS WITH BICYCLOPYRONE

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Cornell Cooperative Extension – Cornell Vegetable Program

In New York, dry bulb onions are grown in muck soils where production is unique and intensive. Onions are poor competitors with weeds; control with herbicides is essential to ensure high quality and yield. Pendimethalin, dimethenamide-P and bromoxynil are used in various tank mix combinations for pre-emergence weed control. Bromoxynil, oxyfluorfen and flumioxazin are used for post-emergent weed

control. Unfortunately, common ragweed (*Ambrosia artemisiifolia*, AMBEL), perennial sowthistle (*Sonchus arvensis*, SONAR) and volunteer potatoes are not well controlled. Bicyclopyrone has a mode of action unlike any herbicides currently used in onion and its utility was investigated through on-farm small-plot field trials from 2016 to 2021. Highlights from these trials include: 1) Bicyclopyrone 50 + bromoxynil 133 g a.i./ha applied to 2-3 leaf onion resulted in almost 100% control of 1-2 inch escapes of AMBEL, marsh yellowcress (*Rorippa islandica*), Lady's Thumb (*Persicaria maculosa*) and Lamb's quarters (*Chenopodium album*); 2) Bicyclopyrone 50 + bromoxynil 133 g a.i./ha alternated with clopyralid 202 + flumioxazin 140 g a.i./ha applied to mid-rosette stage resulted in > 80% control of SONAR; 3) Bicyclopyrone 50 + flumioxazin 140 g a.i./ha controlled 2-inch volunteer potato when used with oxyfluorfen; 4) These bicyclopyrone tank mixes resulted in <10% crop injury.

ORAL SESSION III: BREEDING AND GENETICS

ONIONS SELECTED FOR RESISTANCE TO IRIS YELLOW SPOT VIRUS HAVE HIGHER CARBON ASSIMILATION RATES

Joseph B. Wood (joewood@nmsu.edu), Christopher S. Cramer, Robert Steiner, Richard Heerema, Brian J. Schutte, and Ivette Guzman

New Mexico State University, Las Cruces, NM USA

Bulb onion (*Allium cepa* L.) is an economically valuable vegetable crop in the United States. Onion production is threatened by onion thrips and the virus they vector, *Iris yellow spot virus* (IYSV). Previously selected accessions were shown to be less susceptible to IYSV, however, little is known about the effects of the virus on photosynthetic carbon assimilation rate. We hypothesized that four New Mexico State University (NMSU) previously bred less susceptible accessions, 12-243, 12-337, 12-236 and 12-238, would have higher carbon assimilation rates than susceptible cultivars 'Rumba' and 'Stockton Early Yellow'. To test this, a field study was conducted for three years at NMSU and carbon assimilation rate was measured five times throughout the season at two weeks intervals. At 10 and 12 weeks post transplanting, during bulb development and maturation, all NMSU accessions had higher rates of carbon assimilation when compared to the susceptible cultivar. For all accessions, carbon assimilation rate tended to decrease as bulbs approached maturation. Relatively high carbon assimilation rates for NMSU accessions at 10-12 weeks post transplanting coincided with higher bulb weights when compared to susceptible cultivars, resulting in larger, more marketable bulbs.

IMPROVING FUSARIUM BASAL ROT RESISTANCE OF SHORT-DAY ONION CULTIVARS THROUGH AN ARTIFICIAL INOCULATION MATURE BULB SCREENING

Suman Sharma¹ and Christopher S Cramer (csccramer@nmsu.edu)¹

¹New Mexico State University, Las Cruces, NM USA

Fusarium basal rot (FBR) in onion, caused by *Fusarium oxysporum* f. sp. *cepae* (FOC), causes significant bulb yield losses globally. FBR resistance is lacking in short-day onion cultivars. An artificial inoculation mature bulb screening (AIMBS) has been developed to improve FBR resistance in these cultivars. In 2021, first (FBR1) and advanced selected generations (FBR1-3 and FBR4) of seven, autumn-sown short-day cultivars along with two checks (susceptible and resistant), were evaluated for FBR disease development using the AIMBS. After harvesting, transversely-cut basal plates of 20 bulbs per plot were artificially inoculated with a virulent FOC isolate. At the 21st day after inoculation, basal plates were rated for disease severity using a scale of 1 (no disease) to 9 (70% or more disease) to calculate average disease severity. The AIMBS was successful in developing more disease in the susceptible check, 'NuMex Crimson', than the resistant check, 'Serrana'. All generations of the cultivar populations exhibited a less disease than 'NuMex Crimson'. The advanced generations for most of the cultivars exhibited a reduced disease severity when compared to the first generation. These results suggest that AIMBS is an effective method for improving FBR resistance in short-day onion cultivars.

FEBRUARY 21, 2022

GENETIC ANALYSIS OF RESISTANCE TO FUSARIUM BASAL ROT OF ONION

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Fusarium basal rot (FBR) is a serious disease of onion for which genetic resistance offers the best method of control. We identified sources of FBR resistance and studied its genetic control. Onion accessions were evaluated for FBR resistance and percent survival ranged from 0 to 78%. Survivors were intercrossed and progenies from one cycle of selection showed increased survival by 18 to 52%. Selections were crossed to male-sterile lines and hybrids showed specific combining ability for FBR resistance. Segregating families were produced and quantitative trait loci (QTL) were identified on chromosomes 2 and 4 conditioning FBR resistance. A second QTL on chromosome 4 was identified that decreased FBR resistance. Plants from families with different genotypes across the 1.5 logarithm of odds (LOD) regions on chromosomes 2 and 4 were self-pollinated and resulting families evaluated for FBR survival. Genomic regions on chromosomes 2 and 4 associated with resistance were validated at $P = 0.05$ and 0.10 , respectively. The region on chromosome 4 associated with increased susceptibility was validated at $P = 0.05$. These results are in agreement with previous studies reporting high heritability and specific combining ability for FBR resistance and should be useful for selection of FBR-resistant onions.

AN APPROACH TO MODIFY EPICUTICULAR-WAX PROFILES TOWARDS THRIPS RESISTANCE IN ONION

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Numerous studies have documented reduced feeding damage by thrips (*Thrips tabaci*) on onion foliage with unique profiles of epicuticular waxes. The blue-grey (“waxy”) foliage of wild-type onion suffers significant thrips damage and its waxes are composed primarily of fatty alcohols, alkanes, and the ketone H16. The foliage of “glossy” onion suffers significantly less thrips damage and accumulates less total wax relative to waxy onion; however glossy onions have not been widely grown due to poor field performance and susceptibility to leaf pathogens. The foliage of some onion accessions is visually intermediate between the glossy and waxy phenotypes and experiences less thrips damage compared with waxy plants. This intermediate phenotype is termed “semi-glossy” and can be due to less total wax or unique wax profiles. Specific semi-glossy selections were identified with the same total amount of leaf wax as waxy plants, but with a lower amount of H16 and higher amounts of fatty alcohols and/or alkanes. The fatty alcohols versus alkanes/H16 are synthesized by different branches of the biosynthetic pathway for leaf waxes; H16 is synthesized from alkane precursors in the same branch. Therefore a strategy for developing thrips resistant onions may be to select for reduced amounts of H16 and higher amounts of the fatty alcohols and/or alkanes to produce semi-glossy selections with adequate amounts of total leaf wax for commercial acceptance and suffering less thrips damage.

Tuesday, March 1, 2022

Stop-the-Rot Oral Session:

SURVEYS OF BACTERIAL PATHOGENS IN ONION PRODUCTION REGIONS OF THE U.S.: PRELIMINARY RESULTS FROM 2020 AND 2021

[Lindsey du Toit](mailto:dutoit@wsu.edu) (dutoit@wsu.edu),¹ Brian Kvitko², Heather MacKay¹ and the Stop the Rot project team members

¹Washington State University and ²University of Georgia

‘Stop the Rot’ is a USDA NIFA SCRI-funded project (2019-2023) investigating the host, pathogens, and environmental factors influencing bacterial diseases of onion. To characterize onion bacterial pathogens regionally, symptomatic onion foliage and bulbs were surveyed in 11 states in both 2020 and 2021. The

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most prevalent genera include *Pantoea*, *Pseudomonas*, *Burkholderia*, *Enterobacter* and *Erwinia*. However, the surveys revealed a very broad diversity of bacteria (≥ 84 genera identified to date) associated with symptomatic onions, although strains of very few caused symptoms in subsequent pathogenicity tests on onion. The distribution and pathogenicity of isolates of various genera differed among regions surveyed. Surveys in Georgia also showed a temporal progression in genera within seasons. Selected pathogenic and non-pathogenic bacterial strains are sent by regional labs for inclusion in the National Onion Bacterial Strain Collection (NOBSC) housed at the University of Georgia. The NOBSC will represent the diversity of onion-associated bacterial pathogens across the U.S. and will provide a repository of characterized onion bacterial strains that will be available publicly. Initially, we focused on strains of *Pantoea* species as onion pathogens of national concern, and strains of other genera are being added to the NOBSC as strains are characterized at the regional labs.

A PATHOGENOMICS APPROACH TO IDENTIFY VIRULENCE -ASSOCIATED GENES OF *PANTOEA* SPP. PATHOGENIC ON ONION

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Pantoea spp. are isolated routinely from diseased onions and can be aggressive onion pathogens. However, some strains of *Pantoea* spp., particularly *P. agglomerans*, often fail to cause symptoms when tested in onion pathogenicity assays. The isolation of non-pathogenic *Pantoea* spp. from symptomatic onion tissues can confound accurate diagnosis of onion diseases. Comparative genomics was used to identify genes unique to onion-pathogenic strains of *Pantoea* spp. Whole genome sequences were assembled for 88 *Pantoea* strains, including 40 *P. agglomerans*, 41 *P. ananatis*, 4 *P. allii*, 2 *P. eucalypti*, and 1 *P. vagans* isolated from WA, GA, TX, PA, NY, UT, OR, South Africa, Brazil and Uruguay. The *alt* thiosulfinate tolerance genes were identified in most of the onion-associated *Pantoea* strains, but this included non-pathogenic strains. Conversely, the HiVir phytotoxin biosynthesis genes were identified primarily from *Pantoea* strains that were pathogenic on onion with a red onion scale necrosis (RSN) assay. Universal HiVir PCR primers were designed based on alignment of HiVir sequences for 58 *Pantoea* strains, and used to screen 271 *Pantoea* strains, including 201 strains of *P. agglomerans*. There was 100% correlation between HiVir presence or absence and positive or negative RSN phenotypes of all 201 strains of *P. agglomerans*.

BACTERIAL BIOME OF STORAGE ONION BULBS SAMPLED IN GEORGIA AND WASHINGTON STATES

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A plant microbiome is defined as a microbial community living within the host plant. The aim of this study was to identify the bacterial biome in storage onion bulbs collected from two locations in the USA and attempt to decipher their role in these environments. Nine asymptomatic bulbs and nine bulbs with bacterial rot symptoms were collected from storage facilities in each of Georgia and Washington States in 2020. DNA was extracted from each bulb and amplicon sequencing of the hypervariable V₃-V₄ region of the 16S rRNA gene was performed. The total community metagenomes were also sequenced, from which metagenome-assembled sequences (MAGs) were analyzed. The 16S rRNA marker gene profiling revealed that rotten bulbs were characterized by polymicrobial infections dominated by *Pantoea* and *Burkholderia*. The extracted MAGs from diseased bulb metagenomes included *Pantoea ananatis*, *P. agglomerans*, *Burkholderia cepacia* and *B. gladioli*, known bulb rot pathogens. Functional metagenomics revealed that

these bacterial species possess genes linked to survival in this habitat. Few genes linked to virulence were identified. In conclusion, the onion bulbs were shown to host endophytic bacteria, including some that are potentially beneficial and some that are pathogenic to onion.

DEVELOPING NUCLEIC ACID AMPLIFICATION TESTS FOR ONION PATHOGENIC BACTERIA

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Rapid and accurate methods for the detection of the causal agents of diseases are often critical when implementing successful disease management strategies. Nucleic Acid Amplification Tests (NAAT) such as real-time PCR and loop-mediated amplification (LAMP) assays are often utilized in plant pathogen detection due to their sensitivity, speed, and accuracy compared to many other diagnostic methods. Real-time PCR assays are considered the gold standard diagnostic method while LAMP assays often are suitable for on-site testing. Bacterial diseases of onion are often difficult to diagnose as not all strains are pathogenic. For example, some strains of *Pantoea agglomerans* and *P. ananatis* do not appear to cause onion disease while other strains are highly aggressive. In this study, two real-time PCR assays were designed from the HiVir gene cluster present in pathogenic strains of both *P. agglomerans* and *P. ananatis*. These assays could differentiate between red scale assay positive and negative strains of each species. Field validation of the assays will take place in 2022 using plant material collected as part of the onion survey in year 3 of the Stop the Rot project. LAMP assays will be designed to the same targets, enabling in-field detection to be undertaken.

EVALUATION OF RESISTANCE SCREENING METHODS FOR BACTERIAL DISEASES OF ONION

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Four onion cultivars representing short- and long-day maturities of both sweet and dehydration types (Century, NUNSDST1.89, SV4053NU, and WSU1) were inoculated with *Pantoea ananatis* under greenhouse conditions for evaluation of two methods of screening for resistance: spray inoculation and cut leaf-tip inoculation (each at 10³ and 10⁶ CFU/leaf). For the cut tip method, all cultivars showed 100% disease incidence at both inoculum concentrations. For the spray-inoculation method, disease incidence ranged from 50-70% and was inconsistent among cultivars. Disease severity was inconsistent among cultivars for both methods. The hypothesis that sweet cultivars are more susceptible to *P. ananatis* than dehydration cultivars could not be validated with these methods. A field trial in Washington in 2020 screened 12 onion cultivars [3 in each of 4 maturity groups (MG)] for susceptibility to *Burkholderia gladioli* pv. *alliiicola* and *P. agglomerans*. Cultivars in each MG were inoculated at ~10% tops down and 2 weeks later. The longest maturity cultivars were most resistant, but results were confounded by the need to continue center pivot irrigation to finish later maturing cultivars. The trial was repeated in 2021 with sprinklers to enable termination of irrigation at the same growth stage for each MG for effective screening of cultivars.

REDUCING THE RISK OF ONION BACTERIAL DISEASES THROUGH IRRIGATION AND NITROGEN MANAGEMENT

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Bacterial diseases pose a major challenge for onion growers. Given the variable and limited efficacy of chemical control measures, and challenges at developing resistant cultivars, a holistic risk reduction strategy that focuses on sound agronomic management is necessary. The influence of irrigation and nitrogen (N) management on onion bacterial diseases was studied in field trials in California, Georgia, Oregon, Pennsylvania, New York, and Washington as part of the “Stop the Rot” project funded by USDA NIFA SCRI. Management practices studied included irrigation method (drip vs. sprinkler), irrigation frequency, timing of the final irrigation, N application rates, and N application timing. Results from the 2020 trials and preliminary results from 2021 trials highlight the importance of climate, soil properties, and regional production systems in determining how onion bacterial diseases develop in response to these management practices. Increased bacterial disease incidence was observed with sprinkler vs. drip irrigation (CA, GA), late termination of irrigation (WA), and late N applications (GA) under some conditions. The breadth of this project simultaneously allows for regional evaluation of relevant management practices and broad comparisons of results among regions to understand better how irrigation and N management can be modified to reduce losses to onion bacterial diseases.

EVALUATION OF BACTERICIDES AND PLANT DEFENSE INDUCERS TO MANAGE BACTERIAL ROTS OF ONION IN SEVEN STATES IN THE U.S.

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Bacterial rots of onion begin with infection in the field. Therefore, it is valuable to determine the efficacy of field applications of bactericides and plant defense inducers at reducing losses to bacterial rots. In 2021, field trials to evaluate products for management of bacterial rots of onion were established in California, Colorado, Georgia, New York, Oregon, Washington, and Utah. The trials spanned eight onion cultivars and either relied on natural bacterial infection (GA and NY) or were inoculated (CA, CO, OR, WA, and UT) with virulent strains of bacteria from each region, including *Burkholderia cepacia*, *B. gladioli*, *Pantoea agglomerans*, and *P. ananatis*. In total, the trials encompassed 24 treatments, including some mixtures (e.g., Kocide 3000 + Manzate Max), and a non-treated check. Efficacy of treatments varied among locations. In Georgia, internal bulb rot was reduced to various degrees by Agrititan, Champ, Forticept, Howler, Kocide 3000, LifeGard, ManKocide, MasterCop, Nordox, NUCop, and Oxidate 5.0, but not by Theia. In Utah and California, there was no significant reduction in bacterial bulb rot by any of the seven and nine treatments, respectively. Bulbs harvested from the trials in the other states will be evaluated for storage rots in the winter of 2021/22.

EVALUATION OF LATE-SEASON CULTURAL PRACTICES FOR MANAGING ONION BACTERIAL BULB ROTS

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Field trials in GA, NY, and WA in 2020 and 2021 evaluated late-season cultural practices for managing onion bacterial bulb rots. In 2020 and 2021 in GA, fewer bulbs harvested with a TopAir chain digger developed bacterial rot vs. bulbs harvested with a TopAir straight-blade undercutter. In both years, topping onion bulbs mechanically caused less bacterial bulb rot compared to topping bulbs manually. In 2021, manually cutting the tops of onion bulbs 5- or 3-inches long caused less bulb rot than cutting tops 1-inch long. In a 2020 NY trial, onion bulbs cured in the field developed more bacterial rot than bulbs cured indoors with forced air. In field trials in WA state in 2020, undercutting bulbs, rolling tops, and early vs. standard vs. late topping of bulbs did not affect marketable bulb yield or bacterial bulb rot at harvest or in storage. However, inoculation with *Burkholderia gladioli* and *Pantoea agglomerans* increased leaf blight and bulb rot, and reduced marketable yield. For repeat trials in WA in 2021, undercutting, topping, and rolling were initiated 10-14 days earlier to see if these practices impact less mature bulbs. Bulbs harvested from the trials will be evaluated in storage in February 2022.

EVALUATION OF POST-HARVEST TREATMENTS FOR CONTROL OF BACTERIAL ROTS IN ONIONS

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Bacterial rots of onions in storage result in millions of dollars of losses annually for U.S. onion producers. In an attempt to mitigate these potential losses, onion producers employ various treatments of onion bulbs post-harvest, including applications of disinfectant products to the bulbs. Onion bulbs were grown in field trials in Washington (2020 and 2021) and Colorado (2021) in plots inoculated with the bacterial pathogens *Burkholderia gladioli* and/or *Pantoea agglomerans*. Bulbs harvested from both inoculated and non-inoculated plots were placed in storage containers and treated with equipment used to treat bulbs in commercial storage facilities. Treatments included application of ozone using an ozone generator and various formulations of commercial products containing peroxyacetic acid + hydrogen peroxide. After treatment, the bulbs were placed in a commercial onion storage facility and rated for incidence and severity of internal bacterial rots after 130 days in storage in Washington and 60 days in storage in Colorado. In the 2020 WA trial, none of the products reduced the incidence or severity of bacterial rots in bulbs from inoculated or non-inoculated plots. Bulbs treated in 2021 are in storage and will be evaluated in December of 2021 (CO trial) and February of 2022 (WA trial).

PREDICTIVE MODELING OF BACTERIAL DISEASE RISK IN ONION BULB CROPS

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As part of the SCRI Stop the Rot project, our objective is to build a bacterial rot prediction model based on environmental and management drivers, in order to facilitate advance risk assessment and decision making. This is intended to help growers understand the risk of bulb rots so they can make informed decisions about late season field management practices and whether, and for how long, to store bulbs before shipping them to markets. The original plan of work was to mine producer-collected data on onion crop microclimates, and production/management practices (e.g., irrigation and fertilization records), and bacterial disease incidence data for onion crops and bulbs in storage. We have developed an analysis pipeline that can extract signals from data and help identify potential hypotheses. Given the challenges in gathering proprietary data from commercial fields, we have adapted the research plan to refocus on field data collected by research groups, including field trials within the Stop the Rot project. Updated plans and preliminary analysis on topological data will be shared.

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ECONOMIC ASSESSMENTS OF MANAGEMENT STRATEGIES FOR ONION BACTERIAL ROTS

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Bacterial diseases and marketable crop losses due to onion rots are significant economic issues for the onion industry. A survey of onion growers across the US as part of the ‘Stop the Rot’ USDA NIFA SCRI-funded project (2019-2023) indicates that bactericides and plant defense activators are common strategies used by growers to reduce losses from onion bacterial diseases. However, growers’ stated experience with the performance of these products ranges widely from not at all effective to very effective in mitigating bulb rots. To assess the economic returns to onion growers from bactericides and plant defense activators, field trial data from three states (GA, NY, and WA) on the efficacy of an array of commercial products are synthesized into a cost-benefit framework. Due to little or no significant reduction in bacterial bulb rot, evidence suggests a negative economic return from most of the analyzed products when used on onions. However, recognizing that for many growers, bactericides and plant defense activators are employed for risk reduction rather than profit maximization, we estimate the yield-variance reduction required from these products, conditional on grower-specific risk tolerance, for adoption in a risk-adjusted framework to potentially be optimal.

STOP THE ROT: OVERVIEW OF RESEARCH TO COMBAT ONION BACTERIAL DISEASES WITH PATHOGENOMIC TOOLS AND ENHANCED MANAGEMENT STRATEGIES

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‘Stop the Rot’ is a USDA NIFA SCRI-funded project (2019-2023) with 25 scientists from diverse disciplines in the U.S. and South Africa conducting research on the host, pathogens, and environmental factors influencing bacterial diseases of onion. The project aims to develop practical, economically-sound strategies for pathogen detection and management that improve profitability and sustainability of onion production. We conducted surveys in 7 onion production regions in the U.S. in 2020 and 2021 to assess the diversity of bacterial pathogens and compare the genomics of onion bacterial pathogens from different regions. The survey revealed broad diversity of bacteria (≥ 84 genera) associated with symptomatic onions, but few caused symptoms in onion pathogenicity tests. We are identifying virulence factors unique to pathogenic strains, to develop molecular diagnostic tools for rapid detection of specific bacterial pathogens. Initial efforts at developing diagnostic tests are targeting *Pantoea agglomerans*, a pathogen of national concern. We also are evaluating onion resistance screening methods. In 2020 and 2021, we investigated the impacts of irrigation, fertility, and cultural practices as well as pesticide programs and postharvest applications of disinfectants on development of onion bacterial diseases. Field trials focused on practices used by onion growers and reflect stakeholder concerns and priorities.

ABSTRACTS OF POSTER PRESENTATIONS AT THE 2022 NARC/W3008/STOP-THE-ROT MEETING

Posters will be on display throughout the meeting in the Aspen and Telluride Rooms

Poster Title, Authors, and Abstract

1 **IDENTIFICATION AND PATHOGENICITY OF *FUSARIUM PROLIFERATUM* CAUSING CLOVE ROT ON GARLIC**

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Clove rot, caused by *Fusarium proliferatum*, has been reported on garlic in different growing regions worldwide. The occurrence of this disease during drying, conditioning and storage can lead to severe economic losses. In this study, 116 *F. proliferatum* isolates collected from symptomatic garlic bulbs from New York, California, Washington and Oregon of the United States and mainland China were identified using EF-1 α sequencing. Phylogenetic analysis revealed that the isolates clustered into eight clades showing no association with their geographic origins. Pathogenicity tests were conducted twice with 25 isolates representing different phylogenetic clades. Symptoms recorded five weeks after inoculation showed that all tested *F. proliferatum* isolates, except isolate 20-181-F in the first pathogenicity trial, induced rot symptoms on garlic cloves. However, there were significant differences in their virulence. The most severe symptoms were observed on cloves inoculated with isolates 20-068-F, 20-081-F, 20-144-F, 20-024-F and 21-027-F in one or both pathogenicity tests. Isolates 20-179-F and 20-185-F were moderately pathogenic to garlic and the inoculation of these isolates resulted in 20% - 50% rotted cloves. These results confirm the pathogenic capacity of *F. proliferatum* and the infection of this pathogen is responsible for major losses of garlic postharvest.

2 **THE METAVIROME OF ONION BULBS IN THE USA**

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Viruses play a crucial, yet often overlooked role in shaping microbial communities across many ecosystems. In marine environments, bacteriophages drive nutrient cycling, population turnover, and exchange of genetic material. While the impact of viruses in land environments is not as well documented, recent studies have indicated that viral communities might also be an important fraction of the soil microbiome. To date, no studies have been performed on the viral fraction of the microbiome of onion bulbs, and their possible role in shaping the diseased onion microbiome. In the present study, we aimed to document the viral communities associated with diseased onion bulbs from two storage facilities in the U.S.A. Using a metagenomics-based approach, we extracted 89 unique viral genomes from two metagenomes of onion bulbs, 67 of which were successfully classified according to the viral RefSeq and IMG databases. These viral genomes were found to be differently represented across the two sampled locations, suggesting a degree of regional speciation. Screening of the viral population for possible auxiliary metabolic genes (AMGs) revealed several hits for genes involved in bacterial nutrient cycling and transport. This study represents the first report of viral populations in the endophytic microbiome of onions.

3 PAN-GENOMIC APPROACHES TO PREDICTING VIRULENCE FACTORS IN *PANTOEA ANANATIS* IN *ALLIUM PORRUM* AND *ALLIUM FISTULOSUM*

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Pantoea ananatis can cause foliar infection on diverse *Allium* crops including leek (*Allium porrum*) and bunching onion (*A. fistulosum*). This pathogen relies on a phosphonate biosynthetic gene-cluster (HiVir) and the thiosulfinate tolerance (*alt*) gene cluster for virulence in onion (*A. cepa*). However, virulence factors associated with *A. porrum* and *A. fistulosum* remain unknown. We used phenotype-dependent genome-wide association (GWAS) and phenotype-independent gene-pair coincidence (GPC) analyses for 106 *P. ananatis* strains, which were phenotyped for virulence on these *Allium* hosts. In general, strains of *P. ananatis* were highly aggressive on *A. fistulosum x cepa* hybrid as opposed to *A. porrum*. GWAS identified genes pathogenicity-associated genes for *A. fistulosum x cepa* hybrid (2701 genes) and *A. porrum* (232 genes) including genes in the HiVir and *alt* clusters. We confirmed the role of the intact HiVir cluster in *P. ananatis* pathogenicity on these hosts. Further, comparison of six *P. ananatis* strains that were pathogenic on either *A. porrum* or *A. fistulosum* identified an uncommon type III secretion system gene cluster. Overall, our results indicate that *P. ananatis* infection of *Allium* spp. involves some common virulence factors as well as and unique genes associated with different host species.

4 BREEDING FOR REDUCED IRIS YELLOW SPOT SYMPTOM EXPRESSION IN ONION

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Iris yellow spot (IYS) disease, causal organism *Iris yellow spot virus* (IYSV), is spread by onion thrips (*Thrips tabaci*) and can have detrimental effects on onion production during hot, dry weather. Breeding for onion germplasm that is less attractive to onion thrips and develops less severe IYS symptoms under these climatic conditions may help to reduce the impact of both issues. NMSU onion germplasm previously identified to possess fewer thrips per plant and less severe IYS was evaluated using cultural practices that encouraged thrips buildup and IYSV spread. Adult and juvenile thrips were counted on 10 plants per plot at 5 different times 2 weeks apart. At the last 3 observation dates, the same 10 plants were rated for IYS severity on a scale of 0 (no symptoms) to 4 (>50% dead leaf tissue). Plants of 'Stockton Early Yellow', an onion landrace possessing blue green, waxy foliage, possessed a great number of adult and juvenile thrips at the first two observation dates than plants of NMSU germplasm. This greater number of thrips may have contributed to a more severe IYS observed on plants of 'Stockton Early Yellow' at the early observation dates when compared to plants of NMSU germplasm.

5 MITOCHONDRIAL COI-BASED BARCODING OF *Thrips tabaci* POPULATIONS FROM ONION

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Thrips tabaci, an important pest of onion, is also a vector of Iris yellow spot virus. There are limited studies on the genetic variation within and among populations of *T. tabaci* on *Allium cepa* in the USA. In the present study, 84 *T. tabaci* specimens were collected from onion plants from 15 locations in four states of the USA. A total of 84 mitochondrial COI gene sequences were amplified from individual insects and sequenced. Combined with the nine COI gene sequences available in GenBank or Barcode of life data repository from Raleigh, NC, the genetic

diversity and population structure of *T. tabaci* were determined. Twelve haplotypes were found in 93 *T. tabaci* individuals from 16 different locations. The highest genetic variation was found in Raleigh and Elba with 7 haplotypes identified. The Mantel test showed a correlation between genetic distance and geographical distance. The results showed that haplotype 2 and 4 are more frequently prevailing haplotypes in pacific northwestern USA, while the populations on the US east coast consisted of more diverse groups of *T. tabaci* haplotypes, with haplotype 4 being the most common.

6 GLOBAL ANALYSIS OF THE POPULATION STRUCTURE, HAPLOTYPE DISTRIBUTION AND EVOLUTIONARY PATTERN OF *IRIS YELLOW SPOT VIRUS*

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Iris yellow spot, caused by *Iris yellow spot virus* (IYSV), is an important disease of Alliums. The complete N gene sequences of 142 IYSV isolates of curated sequence data from GenBank were used to determine the genetic diversity and evolutionary pattern. In silico restriction fragment length polymorphism (RFLP) analysis, codon-based maximum likelihood studies, genetic differentiation and gene flow within the populations of IYSV genotypes were investigated. Bayesian phylogenetic analysis was carried out to estimate the evolutionary rate. In silico RFLP analysis of N gene sequences categorized IYSV isolates into two major genotypes viz., IYSV Netherlands (IYSV-NL; 55.63%), IYSV Brazil (IYSV-BR; 38.73%) and the rest fell in neither group [IYSV other (IYSV-other; 5.63%)]. Phylogenetic tree largely corroborated the results of RFLP analysis and the IYSV genotypes clustered into IYSV-NL and IYSV-BR genotypes. Genetic diversity test revealed IYSV-other to be more diverse than IYSV-NL and IYSV-BR. IYSV-NL and IYSV-BR genotypes are under purifying selection and population expansion, whereas IYSV-other showed decreasing population size and hence appear to be under balancing selection. IYSV-BR is least differentiated from IYSV-other compared to IYSV-NL genotype based on nucleotide diversity. Three putative recombinant events were found in the N gene of IYSV isolates based on RDP analysis.

7 EVALUATION OF WEATHER-BASED MODELS FOR MANAGEMENT OF ONION DOWNY MILDEW IN CALIFORNIA

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Downy mildew has the potential to cause severe damage to onion seed or production crops in California. Models that describe how weather governs downy mildew development have been published, but they have not been evaluated for practical use in California. The objective of this research was to determine if weather-based models can be used to time fungicide applications targeting onion downy mildew. Field trials of dehydration onion were established in October from 2017 to 2020. Treatments consisted of fungicide applications triggered when conditions are favorable for growth or infection of the downy mildew pathogen, and were compared to a standard calendar control. In three of four study years, fungicide programs based on weather model output resulted in 1 to 3 fewer fungicide applications compared to the standard calendar, although downy mildew did not develop in these study areas. In one of four years, the same level of control was achieved by either two applications in a standard calendar schedule or one application on a weather-based model approach. Model output was automated in a cloud-based program. This research could lead to the availability of improved tools to more effectively manage downy mildew of onion while reducing overall fungicide use.

8 EVALUATION OF HERBICIDE COMBINATIONS FOR WEED CONTROL IN ONION IN NEW MEXICO

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Onion production in New Mexico is challenged by weeds that reduce yield and provide refuge for insect pests and crop disease. Weed control combinations of herbicides, tillage, and hand-hoeing to remove weeds from fields are significant production expenses. The objective of this research was to evaluate 11 herbicide programs that included registered and potential new herbicides to find cost-effective methods for weed control in autumn-sown onions. Seven different herbicides were applied preplant and at 1-4 leaf stages. Herbicide programs and a hand-hoed control were arranged in a randomized complete block design with four replications. To measure herbicide efficacy, the amount of time for one person to hoe weeds that escaped herbicide control was determined at two dates during crop growth. Once mature, onion bulbs were harvested and marketable yield calculated for each program. Bensulide and DCPA applied at seeding followed by bromoxynil at the 2-leaf stage provided the best control of weeds; however, this program was one of the most expensive. Based upon this research, a special local needs registration for the delayed pre-emergence application of pendimethalin is being sought as this herbicide use shows promise as a cost-effective means for controlling weeds in autumn-sown onions grown in New Mexico.

9 RESPONSE OF YELLOW SWEET SPANISH ONION VARIETIES TO ETHOFUMESATE HERBICIDE

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Efficiency of processing, especially for onion rings, is greatly reduced when onions have multiple centers. Our previous research showed that onions treated with ethofumesate had a significantly lower proportion of single-center bulbs compared to the non-treated control, but the magnitude of response may be variety specific. Research was conducted in replicated trials at the Parma Research and Extension Center in 2020 and 2021 to evaluate the response of eight yellow sweet Spanish varieties to three sequential applications of ethofumesate at 16 oz/acre starting at the two-leaf stage. Results clearly indicated that onion varieties are not equally susceptible to ethofumesate in terms of production of multiple-centered bulbs. Onion varieties that show little change in the proportion of large multiple centers show good processing stability with little effect from herbicide applications. Based on that criteria, Joaquin and Vaquero stood out in this trial, with Arcero being intermediate in reaction to herbicide application.

10 SELECTION PROGRESS FOR REDUCED IRIS YELLOW SPOT DISEASE SYMPTOMS AND ASSOCIATED TRAITS IN ONION

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Onion thrips (*Thrips tabaci*) and the associated virus that they transmit, *Iris yellow spot virus*, are substantial challenges to US onion production that cause an estimated economic loss of \$60-90 million annually. Selection for onion germplasm less attractive to thrips and/or less

likely to develop Iris yellow spot (IYS) symptoms under conducive environmental conditions may help to lessen the impact of both issues. Onion breeding line selections were evaluated for IYS symptoms, thrips numbers, plant size, and average bulb size in order to visualize progress when compared to previous generations and a thrips attractive landrace, 'Stockton Early Yellow' (SEY). A field layout was utilized that encouraged the spread of onion thrips and IYSV throughout the field. At several times throughout the 2021 season, the number of thrips/plant, plant size, and IYS severity were measured on 10 plants/plot. Plants of several selected breeding lines exhibited less severe IYS than plants of their previous generations and SEY. Plants of most selected lines exhibited fewer thrips/leaf than plants of SEY early in the season but not of plants from their previous generation. Plants of several selected lines exhibited a greater bulb size than plants of their previous generation and SEY.

11 SURVEYS OF BACTERIAL PATHOGENS IN ONION PRODUCTION REGIONS OF THE U.S.: PRELIMINARY RESULTS FROM 2020 AND 2021

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'Stop the Rot' is a USDA NIFA SCRI-funded project (2019-2023) investigating the host, pathogens, and environmental factors influencing bacterial diseases of onion. To characterize onion bacterial pathogens regionally, symptomatic onion foliage and bulbs were surveyed in 11 states in both 2020 and 2021. The most prevalent genera include *Pantoea*, *Pseudomonas*, *Burkholderia*, *Enterobacter* and *Erwinia*. However, the surveys revealed a very broad diversity of bacteria (≥ 84 genera identified to date) associated with symptomatic onions, although strains of very few caused symptoms in subsequent pathogenicity tests on onion. The distribution and pathogenicity of isolates of various genera differed among regions surveyed. Surveys in Georgia also showed a temporal progression in genera within seasons. Selected pathogenic and non-pathogenic bacterial strains are sent by regional labs for inclusion in the National Onion Bacterial Strain Collection (NOBSC) housed at the University of Georgia. The NOBSC will represent the diversity of onion-associated bacterial pathogens across the U.S. and will provide a repository of characterized onion bacterial strains that will be available publicly. Initially, we focused on strains of *Pantoea* species as onion pathogens of national concern, and strains of other genera are being added to the NOBSC as strains are characterized at the regional labs.

12 A STUDY ON THE BACTERIAL INHABITANTS OF STORAGE ONIONS: IDENTITY AND FUNCTION

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Within different parts of a plant, microbes establish a network of interactions that can influence plant health positively or negatively. Some bacterial species found in association with plants can switch lifestyle from non-pathogenic to pathogenic, causing diseases that have major impacts on crop production. The trigger for this switch is poorly understood. The aim of this study was to characterize bacterial communities present in storage onion bulbs collected from two locations in the USA, Georgia and Washington State, and attempt to decipher their role in onion health. DNA was extracted from healthy and diseased bulbs in each state, and used to profile the taxonomy and possible function of the bacteria in the bulbs. The taxonomic profiling

revealed that bacterial communities in diseased bulbs were less diverse and were dominated by a few bacterial genera with species known to cause bulb rots. Communities from diseased bulbs also had several DNA markers associated with pathogenicity and increased capacity for survival within onion bulbs. Together these results suggest that bulb rots may result from polymicrobial colonization of onion bulbs.

13 THE METAVIROME OF ONION BULBS SYMPTOMATIC AND ASYMPTOMATIC FOR BACTERIAL BULB ROTS SAMPLED FROM GEORGIA AND WASHINGTON STATES IN THE USA

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Viruses play a crucial, yet often overlooked role in shaping microbial communities across many ecosystems. In marine environments, bacteriophages drive nutrient cycling, population turnover, and exchange of genetic material. While the impact of viruses in land environments is not as well documented, recent studies indicate that viral communities might be important in the soil microbiome. To date, no studies have evaluated the viral fraction of the microbiome of onion bulbs, including their possible influence on the microbiome of diseased onions. In this study, we aimed to document viral communities associated with onion bulbs symptomatic and asymptomatic for bacterial rots sampled from storage facilities in Georgia and Washington States. Using a metagenomics-based approach, we extracted 89 unique viral genomes from metagenomes of onion bulbs, 67 of which were successfully classified according to the viral RefSeq and IMG databases. The viral genomes of symptomatic bulbs differed across the two locations, suggesting a degree of regional speciation, but few viral genomes were detected in the asymptomatic bulbs. Screening the viral populations for possible auxiliary metabolic genes (AMGs) revealed several genes involved in bacterial nutrient cycling and transport. This represents the first report of viral populations associated with endophytic bacterial microbiomes of onion bulbs.

14 INFLUENCE OF VARIETY ON BACTERIAL BULB ROT OF ONION IN NEW YORK STATE

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Effective control of bacterial diseases of onion involves an integrated approach that considers varietal resistance to bacterial pathogens. Five field trials conducted in New York in 2018-2020 evaluated 7-14 onion varieties per year for susceptibility to bulb rot and to identify traits associated with responses to bacterial bulb rot. Techniques for inoculation with virulent local strains of *Pantoea ananatis* and *P. agglomerans* varied by year, including a toothpick prick assay and spray inoculation as well as natural infection. Of the five varieties common to all trials, only one consistently ranked the same for susceptibility to bulb rot in all five trials. Montclair had levels of bulb rot that were not significantly different than the variety with the most bulb rot. Trailblazer had the least bulb rot in four trials, but some of the most rot in the fifth trial. Bulb rot levels for Catskill varied by region. Varietal responses to bulb rot varied between naturally-infected and inoculated plots. There were no consistent associations of bulb rot incidence with traits of leaf color, plant architecture, vigor and severity of thrips damage, except a general trend toward increased incidence of bulb rot as days to maturity and neck diameter increased.

15 INFLUENCE OF APPLIED NITROGEN ON BACTERIAL BULB ROT IN MUCK-GROWN ONION IN NEW YORK

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As control of bacterial bulb rot is unreliable with chemical measures and there are no fully resistant varieties, investigation into agronomic practices that may alleviate bulb rot is warranted. The influence of applied nitrogen fertilizer rates on bacterial bulb rot was studied across 5-12 varieties in onion crops on muck soils in each of Elba and Oswego in New York in 2018 and 2019. Nitrogen was pre-plant incorporated (PPI) at 37, 100, and 150 lb/A in 2018 and 10, 30, and 60 lb/A in 2019. Variety was the only factor that influenced bacterial bulb rot and bulb yield. Nitrogen rate did not have significant impacts on bacterial bulb rot in the four trials. Nitrogen rate also did not impact marketable yield except in the 2019 Oswego trial, where application of 60 lb N/A resulted in significantly greater yield than 10 and 30 lb N/A due to more jumbo-sized (3-4 inch) bulbs at the higher rate. Available nitrate-nitrogen in the soil at harvest ranged from 25 to 188 lb/A, often exceeding the amount of nitrogen applied. Our results suggest that applying 60 lb N/A is sufficient for muck-grown onions, but we are also studying the effects of 60-120 lb N/A.

16 EVALUATION OF BACTERICIDES AND PLANT DEFENSE INDUCERS TO MANAGE BACTERIAL ROTS OF ONION IN SEVEN STATES IN THE U.S.

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Bacterial rots of onion begin with infection in the field. Therefore, it is valuable to determine the efficacy of field applications of bactericides and plant defense inducers at reducing losses to bacterial rots. In 2021, field trials to evaluate products for management of bacterial rots of onion were established in California, Colorado, Georgia, New York, Oregon, Washington, and Utah. The trials spanned eight onion cultivars and either relied on natural bacterial infection (GA and NY) or were inoculated (CA, CO, OR, WA, and UT) with virulent strains of bacteria from each region, including *Burkholderia cepacia*, *B. gladioli*, *Pantoea agglomerans*, and *P. ananatis*. In total, the trials encompassed 24 treatments, including some mixtures (e.g., Kocide 3000 + Manzate Max), and a non-treated check. Efficacy of treatments varied among locations. In Georgia, internal bulb rot was reduced to various degrees by Agrititan, Champ, Forticept, Howler, Kocide 3000, LifeGard, ManKocide, MasterCop, Nordox, NUCop, and Oxidate 5.0, but not by Theia. In Utah and California, there was no significant reduction in bacterial bulb rot by any of the seven and nine treatments, respectively. Bulbs harvested from for the trials in the other states will be evaluated for storage rots in the winter of 2021/22.

17 ALLIUMNET.COM WEBSITE REDESIGN TO SUPPORT COLLABORATIVE ONION RESEARCH AND EXTENSION

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Alliumnet is a collaborative website focused on supporting the production of *Allium* species in the United States. The Alliumnet.com website has been maintained over the long term to provide a home for national onion research collaborations, including USDA projects,

information and proceedings associated with the National Allium Research Conference, links to onion industry association meetings and events, and reports, activities, and meeting details for the W-1008, W-2008, and W-3008 multistate onion projects. The website was recently redesigned and updated based on input from a User Reference group established within the USDA NIFA SCRI 'Stop the Rot' research project on onion bacterial diseases. The website is now live, and contains information on the latest research, resources for diagnosing diseases and other onion problems, links to regional extension resources, grower associations' notices about upcoming scientific meetings, and archives of prior research meetings. The website is now live. Comments and suggestions on the layout and content can be sent to the webmaster (webmaster@bugwood.org). The Alliumnet.com website is developed, maintained and hosted by the Southern IPM Center and the Center for Invasive Species and Ecosystem Health at the University of Georgia.

18 DOWNCAST IS AN EFFECTIVE MODEL FOR TIMING FUNGICIDE APPLICATIONS FOR ONION DOWNY MILDEW IN ONTARIO, CANADA

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Onion downy mildew, caused by the Oomycete *Peronospora destructor*, is a highly destructive foliar disease of onion, but does not occur every year in the Holland Marsh, Ontario, Canada. Symptoms develop 10 – 14 days after infection and fungicides must be applied prior to infection to be effective. Disease forecasting is used to indicate when no fungicides are needed and to properly time protective fungicide applications when there is disease risk. The integrated pest management program uses DOWNCASST to predict the risk of sporulation and infection based on temperature, relative humidity, leaf wetness duration and temperature in the days after infection. Fungicide sprays are also recommended if downy mildew is found in the region. Spore trapping was added to detect sporangia of *P. destructor* in the air. Rotorod spore traps are assessed three times a week. From 2012 to 2021, the forecasting program was accurate in eight of the 10 years, including two years where there was no disease risk, and no disease was found. Most years downy mildew developed 14 – 17 days after sporangia were found. While mostly effective, DOWNCASST can be improved with trapping of sporangia.

19 ONION MAGGOT AND ONION THRIPS OVER FORTY YEARS OF IPM

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An integrated pest management (IPM) program for onions began as a pilot project in the Holland Marsh, Ontario, Canada, in 1980. The IPM program has continued, with a few breaks, since then. The main onion insect pest in 1980 was onion maggot (*Delia antiqua*). Cutworms (Noctuidae) and onion thrips (*Thrips tabaci*) were considered occasional pests. Onion maggot flies were monitored with yellow sticky traps and damage was assessed in grower fields. Onion fly populations have decreased over time. Population highs were over 100 flies per trap per day in 1981, with most fields having over 20/trap/day. In 2020, the highest onion fly counts were 13/trap/day and most fields had less than 5/trap/day. Insecticide foliar sprays were applied to control onion flies in the 1980's. Currently, seed treatments clothianidin and imidacloprid are used for onion maggot. The reduction in onion fly populations is interesting, but the current levels can still cause over 50% damage in untreated onions. Onion thrips are scouted twice a week. The spray threshold for thrips was increased from one to three thrips per leaf, as the newer insecticides spirotetramat and spinetoram are very effective. In 2021 thrips counts were below the spray threshold throughout the season.