2009 Pennsylvania Vegetable Marketing and Research Program
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Elucidation and management of bacterial diseases occurring on onion in Pennsylvania

Principle Investigator:

Beth K. Gugino, Dept. of Plant Pathology, The Pennsylvania State University, University Park, PA 16802; (814) 865-7328; bkgugino@psu.edu.

Collaborators:

Michele Mansfield, Dept. of Plant Pathology, The Pennsylvania State University, University Park, PA 16802; (814) 863-0565; man203@psu.edu.

Timothy Elkner, Penn State Cooperative Extension, Lancaster County, 1383 Arcadia Road, Room 140, Lancaster, PA 17601; 717-394-6851; tee2@psu.edu.

Tim McNellis, Dept. of Plant Pathology, The Pennsylvania State University, University Park, PA 16802; (814) 863-7646; mcnellis@psu.edu.

Jeff Stoltzfus, Eastern Lancaster School District, 669 East Main Street, New Holland, PA 17557; (717) 354-1500; jeff_stoltzfus@elanco.k12.pa.us.

Lee Young, Penn State Cooperative Extension, Washington County, 100 West Beau Street, Suite 601, Washington, PA 15301; (724) 228-6881; ljs32@psu.edu.

Introduction:

Onions are plagued by a number of bacterial diseases that cause both bulb and leaf decay. Yield losses as a result of reduced bulb size and decay can be considerable and have been attributed to diseases such as sour skin, slippery skin, bacterial soft rot and Xanthomonas leaf blight to name a few. It was estimated in 2008 that 30% of the sweet onion crop in Lancaster County was culled in the packing shed as a result of bacterial diseases. These losses occurred despite the effort to reduce losses by harvesting the crop early. In late June 2008 just prior to the early harvest several onion samples were collected from heavily symptomatic fields. The onion leaves typically had elongated bleached-white streaks on the center one or two leaves. The streaks on more severely infected plants progressed down the entire leaf which then became soft and wilted. Cross-sectioning the bulb often revealed one or two discolored bulb scales. The recently recognized bacterial pathogen *Pantoea ananatis*, which causes center rot, was isolated from several of the symptomatic onions. It was first identified in Georgia in 1997 and has since been reported only in Colorado, Michigan and New York in the United States. This is the first report of this pathogen on sweet onion in PA. Disease development is favored by moderate to high temperatures (82 - 95°F) that coincide with rainfall during or after bulb initiation. Symptoms begin as bleached white area with a water soaked margin that run the length of the leaf and eventually downward into the bulb causing a soft rot. Initially, only one or two of the center leaves are infected and become wilted but on severely infected plants all the leaves can become bleached and wilted.

Management is difficult especially when the environmental conditions favor the pathogen, therefore preventing the initiation of epidemics is critical. The strategies tend to center around planting pathogen-free seed, eliminating weeds, volunteer onions and cull piles to reduce initial inoculum and then creating a less favorable environment for the pathogen by avoiding overhead

irrigation and promoting good air circulation through row orientation and increased row spacing. Varieties also differ in their susceptibility. Unfortunately cv. 'Candy', one of the more popular varieties in the PA Simply Sweet Onion program, is one of the most susceptible cultivars to center rot.

One management strategy may be to alter the microclimate temperature within the onion canopy during bulb initiation by using different types of mulches other than black plastic. Trials using different mulch types with Vidalia onions in Georgia found center rot to be most severe on onions grown on black plastic and least severe on onions grown on straw mulch. The researchers attributed the increased disease severity to the higher soil temperatures associated with black plastic which triggered the earlier onset of the disease. However, the onion grown on straw mulch had delayed maturity and reduced yields which may negate the benefits of the straw mulch. In addition it has been demonstrated that thrips, specifically tobacco thrips, are able to transmit pathogenic strains of the center rot pathogen to onion. However, the role of onion thrips, the most common species of thrips in Pennsylvania onion fields, is not currently known.

Here, we report our efforts during 2009 to address the following objectives.

Objectives:

The purpose of this project is to:

1. Survey onion fields across the state to identify and characterize the bacteria that are causing and/or associated with the symptoms observed in the field, placing an emphasis on the center to pathogen, *Pantoea ananatis*.
2. Evaluate the effect of bare soil and different types/colors of plastic mulches compared to the standard black plastic mulch on crop canopy temperature and bacterial disease incidence and severity at the SE Research and Extension Center in a plot where bacterial diseases have previously been observed.
3. Incorporate results into grower newsletters and presentations at summer twilight and winter meetings.

Methods and Results:

Objective 1: *Survey onion fields across the state to identify and characterize the bacteria that are causing and/or associated with the symptoms observed in the field, placing an emphasis on the center rot pathogen, Pantoea ananatis.*

In collaboration with Jeff Stoltzfus and Lee Young, symptomatic sweet onions were collected from 15 growers' fields in five counties including Lancaster (8), Butler (3), Beaver (2), Fayette (1) and Somerset (1) in mid-June. Bacteria were isolated from the leaf tissue by cutting 0.5 g of material from the margin between healthy and diseased tissue and macerating in a buffer. 100 µL of this material was directly plated onto PA20, a medium selective for *P. ananatis*. A serial dilution of 10^6 was also plated on King's B medium and Nutrient Agar, which are traditional media for culturing and identifying plant pathogenic bacteria. Each sample was plated in duplicate on all three media, grown for 3-5 days and the resulting colonies isolated to generate pure cultures. Pure cultures were plated back onto the three media for identification and evaluated for pathogenicity by surface sterilizing a white pearl onion, slicing it in half and inoculating one half with a 10^6 dilution of bacterial cells of the isolate of interest. Plates and

onion slices were incubated for 7 days and evaluated for identification and pathogenicity, respectively. Known cultures of the major bacterial onion pathogens were obtained from Dr. Ron Gitaitis from the University of Georgia to use as reference isolates to which we could compare both our culture plates and pathogenicity tests.

To augment the identification process, bacterial isolates found to be pathogenic to onion bulbs and those cultured from PA20 also had a portion of their 16S ribosomal gene sequenced which is a standard gene region used for bacterial identification. Sequences from our isolates are then compared to other known isolates in various web databases and enables a more accurate identification of our bacterial isolates.

Results: From the commercial field samples, the major bacterial pathogens isolated included: two soft rot pathogens (*Pseudomonas marginalis* and *Pectobacterium caratovora*), two center rot pathogens (*Pantoea agglomerans* and *P. ananatis*), Xanthomonas leaf blight (*Xanthomonas axonopodis)* and Pseudomonas leaf streak (*Pseudomonas viridiflava*) (Table 1). The latter two are primarily foliar pathogens that cause foliar streaking and blighting symptoms.

Table 1. Bacterial pathogens isolated and identified from symptomatic onions collected from 15 sweet onion fields in 5 counties in Pennsylvania in June 2009.

	Sample Location (County, Field)														
	Lancaster								**Butler**			**Beaver**		**Fayette**	**Somerset**
Bacterial Pathogen	**JKa**	**MNa**	**MNb**	**SZa**	**SZb**	**MZa**	**MZb**	**ALa**	**HTa**	**SLa**	**SNa**	**JCa**	**JCb**	**DFa**	**KSa**
Pseudomonas marginalis	X			X	X	X			X	X			X	X	X
Pectobacterium caratovora	X	X	X	X						X		X			X
Pantoea agglomerans	X		X			X	X					X	X		
Pantoea ananatis					X							X	X		
Xanthomonas axonopodis							X		X						
Pseudomonas viridiflava										X					

Objective 2: *Evaluate the effect of bare soil and different types/colors of plastic mulches compared to the standard black plastic mulch on crop canopy temperature and bacterial disease incidence and severity at the SE Research and Extension Center in a plot where bacterial diseases have previously been observed.*

To evaluate the effect of mulch types on the development of bacterial diseases of onion, in collaboration with Tim Elkner, a trial was established at the PSU Southeast Research and Education Center in Landisville, PA in a field that had experienced an outbreak of onion bacterial diseases in 2008. The different mulch treatments, arranged in a randomized complete block design with 4 replications, were as follows: 1) bare soil; 2) straw mulch; 3) clear plastic; 4)

black biodegradable; 5) standard black with thrips control; 6) standard black with no thrips control; 7) black with kaolin clay; 8) white plastic with thrips control; 9) white plastic with no thrips control and 10) metalized silver. The each plot was planted with 4-rows of onion transplants cv. 'Candy' purchased from Dixondale Farms in Texas and irrigated using drip irrigation. In-field Onset HOBO data loggers were used to monitor soil and onion canopy temperatures reps 1 and 3. Onion pests and fungal diseases were managed using a standard spray program as recommended in the Commercial Vegetable Production Guide except in the thrips comparison treatments which did not receive any thrips control. The trial was scouted regularly during the season and bacterial disease incidence and severity assessed. Data was collected from the center two rows of each plot. Thrips populations were counted on 15 June and 29 June. Also on 29 June select symptomatic onions were collected for culturing and bacterial identification. At harvest on 22 July, 5ft of bulbs were collected from the center two rows and evaluated for both quantity and quality. From each plot an additional 6ft of the center two rows was harvested, placed in burlap onion sacks, dried on greenhouse benches and then stored at 40°F in a storage room at the Horticulture Farm at Rock Springs and evaluated every 3 to 4 weeks for post-harvest quality over three months. During the last evaluation, bulbs showing symptoms of bacterial rot were set aside for culturing and bacterial identification.

Results: From the 11 symptomatic plants collected mid-season from the research trial, the major bacterial pathogens identified in order of frequency were *P. marginalis* (6 samples), *P. caratovora* (6), *P. agglomerans* (6), *P. viridiflava* (3) and *P. ananatis* (1). Interestingly, *P. viridiflava* was only recovered from samples grown on white plastic during the mid-season evaluation otherwise in 2009, there was no noticeable correlation between the incidence of bacterial symptoms and particular mulch types. This may in part be due to the cool wet weather that persisted during the season. In fact, analysis of the temperature data collected using from the onion canopy and 2 in. soil depth found no significant differences in average daily max. and min. temperatures between the different mulch treatments (data not shown). Not surprisingly, thrips counts were numerically higher on onions grown on white plastic (3.6 and 5.4 thrips per leaf) compared to black plastic (1.4 and 2.8 thrips per leaf) on 15 June and 29 June, respectively. Correlating these counts with bacterial disease incidence was difficult due to the low incidence of disease in the trial this season.

The bacterial pathogens isolated and identified from 16 symptomatic plants collected at harvest were *P. marginalis* (11 samples), *P. caratovora* (3), *P. agglomerans* (9) and *P. ananatis* (2). No bacterial pathogens were recovered from 3 of the samples. The straw mulch treatment had the lowest marketable yield and smallest bulb weights while the black plastic treatments, metalized silver and bare soil treatments had slightly higher marketable yields (Table 2). In general the percent of onions showing visible symptoms of bacterial diseases at harvest was relatively low. A similar mulch trial was conducted on a commercial farm in collaboration with Jeff Stoltzfus and the onions grown on bare soil, although smaller as a result of cooler soil temperatures early in the season, had only 2.4% of the onions harvested with bacterial symptoms compared to 6.2 to 8.8% for the other mulch treatments (Table 3).

Two major pathogens were isolated from the symptomatic onion bulbs collected at the end of the onion storage evaluation. The soft rot pathogen, *Pectobacterium caratovora*, was isolated from two samples collected from the bare soil treatment as well as from single samples collected from

the white plastic (no thrips control) and metalized silver treatments. The center rot pathogen, *Pantoea agglomerans*, was isolated from an onion grown in the straw mulch treatment as well as from two samples collected from the black biodegradable and white plastic (no thrips control) treatments. Both pathogens were isolated from the standard black plastic and black plastic with kaolin clay treatments. In general, the storability of onions (fewest onions culled due to softness and bacterial disease symptoms) grown on bare soil was the highest while those grown on white plastic was the lowest.

Table 2. Effect of different mulch types on the yield and incidence of bacterial diseases in sweet onion cv. 'Candy' in replicated research trial at the SEAREC, Lancaster Co., 2009.

Mulch treatment	Mrkt yield (lb) per 5ft row	Ave. weight (oz) per bulb	% onions w/bacterial symptoms at harvest
Bare soil	30.9 abc	12.0 bc	1.2
Straw mulch	27.5 c	10.9 c	0.7
Clear plastic	33.9 a	14.8 a	3.7
Black biodegradable	32.8 ab	13.4 ab	2.0
Black w/ kaolin clay	33.5 ab	14.4 a	2.0
Standard black	35.0 a	13.7 a	1.9
White plastic	28.7 bc	12.0 bc	2.7
Metalized silver	31.5 abc	13.8 ab	0
Fisher's LSD	$P = 0.0493$	$P = 0.0034$	

Table 3. Effect of different mulch types on the yield and incidence of bacterial diseases in sweet onion cv. 'Candy' in an on-farm grower trial, Lancaster Co., 2009.

	Yield data per 5 ft of row			
Mulch treatment	No. bulbs	Total bulb weight (lb)	Weight (lb) > 3in. diameter	% onions w/bacterial symptoms at harvest
Bare soil	34.0	13.9 c	7.5 c	2.4
Metalized silver	31.0	18.7 b	15.9 ab	8.8
Silver on black	34.5	23.6 a	21.6 a	7.3
Black biodegradable	37.2	19.6 b	15.2 b	6.2
Standard black	33.8	21.8 ab	18.3 b	8.4
Fisher's LSD	NS	$P = 0.0116$	$P = 0.0191$	

Objective 3: *Incorporate results into grower newsletters and presentations at summer twilight and winter meetings.*

Results from research as well information about the biology and epidemiology of onion bacterial diseases was presented at the onion twilight meetings held at Harvest Valley Farms in Butler Co. on 23 June with Lee Young and at John King and Amos Lapp's farms in Lancaster Co. on 30 June with Jeff Stoltzfus. In addition, presentations that included this information were made at

the Northeast Division of the American Phytopathological Society meeting in Quebec City, Canada in October and at the Vegetable and Small Fruit Roundtable and Mid-Atlantic Vegetable Workers meeting in November 2009. These research results will also be the subject of a presentation during the onion session of the 2010 Mid-Atlantic Fruit and Vegetable Convention and will be summarized for one of the upcoming winter editions of the Vegetable and Small Fruit Gazette.

Table 3. Effect of different mulch types on the onion quality as measured by bacterial disease incidence after being harvested 22 Jul, dried and placed in storage on 2 Sep at the Horticulture Farm, Rock Springs, 2009.

	Average percent of soft bulbs per treatment (43 to 51 bulbs stored per plot)		
Mulch treatment	**9 Sep**	**28 Sep**	**26 Oct**
Bare soil	2.8	4.7	4.4
Straw mulch	2.6	4.0	9.2
Clear plastic	13.3	0.7	3.5
Black biodegradable	5.2	10.0	10.4
Black w/ kaolin clay	1.6	5.9	9.0
Standard black	13.6	3.4	6.3
Std. black w/o thrips control*	19.4	7.0	4.9
White plastic	11.9	12.1	20.3
White w/o thrips control*	4.4	3.2	6.0
Metalized silver	4.6	8.1	14.3

* The trial was sprayed regularly for thrips control except for these two treatments.

The data collected from these trials will continue to be mined for more information that can help us further understand which bacterial pathogens are causing significant yield losses for sweet onion growers in Pennsylvania. We will also continue to work towards identifying alternative mulching practices as well as other cultural practices that will provide growers with additional integrated pest management tools and will lead to more sustainable sweet onion production in PA. For next season, we would like to fine-tune and repeat the onion survey and mulch trial well as investigate the role of imported transplants, thrips and weeds as reservoirs of the identified major bacterial pathogens. Using the molecular sequence data generated from identification of the bacterial pathogens, we are in the process of developing a multi-plex PCR method that will enable us to screen for multiple bacterial pathogens simultaneously from a sample conserving both materials and labor as opposed to traditional culturing techniques.

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