2010 Pennsylvania Vegetable Marketing and Research Program
Pennsylvania Vegetable Growers Association Report
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Title: Further elucidation and management of bacterial diseases occurring on onion in Pennsylvania

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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 in the field can be considerable and further exacerbated by bulb decay in storage. Previous survey research and pathogenicity testing have identified a number of bacterial pathogens associated with symptomatic onion in PA including the soft rot bacteria *Pseudomonas marginalis* and *Pectobacterum caratovora*; the center rot pathogens *P. ananatis* and *P. agglomerans*; the Xanothomonas leaf blight pathogen *X. axonopodis*, and Pseudomonas leaf streak and bulb rot pathogen *P. viridiflava* and most recently *Enterobacter cloacae*, a significant storage pathogen in the Pacific Northwest. In many cases, multiple pathogens have been isolated and identified from a single sample. These bacterial isolates as well as known type cultures from Dr. Ron Gitaitis from the University of Georgia were used to develop primers based on the single copy gyrase B gene so now we can more accurately and efficiently identify both bacterial cultures as well as determine associated bacterial pathogens directly from symptomatic tissue.

So, now that several bacterial pathogens have been identified in association with symptomatic onions, one of the next questions is what is the source(s) of pathogen inoculum? One hypothesis is infested onion transplants. Transplants are a source for a number of other bacterial pathogens (e.g. bacterial canker on tomato) and although transplants are increasingly being produced locally, the majority of transplants are still being imported from out of state. If the transplants are found to be a source of bacterial pathogens then management strategies can be developed to focus on both the production and treatment of imported as well as local onion transplants.

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 transplants (as previously described), eliminating weeds, volunteer onions and cull piles to reduce initial inoculum and creating a less favorable environment for the pathogen by avoiding overhead irrigation and promoting good air circulation through row orientation and increased row spacing. 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. Although there was no noticeable correlation between the incidence of bacterial symptoms and particular mulch types in 2009, in part due to the cool weather, placing the trial in the same location as last year will increase potential disease pressure. In a similar mulch trial conducted on a commercial farm in collaboration with Jeff Stoltzfus and a grower in Lancaster County, 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 crop showing signs of bacterial symptoms compared to 6.2 to 8.8% for the other mulch treatments.

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 2010 to address the following objectives.

1. Screen imported transplants as well as locally grown transplants for bacterial pathogens using cultural and molecular multi-plex PCR techniques.
2. Conduct a second survey of onion fields across the state to further identify and characterize the bacteria that are causing and/or associated with the symptoms observed in the field.
3. Further evaluate the effect of bare soil, and different colored plastic mulches by comparing them to the standard black plastic mulch on crop canopy temperature and bacterial disease incidence and severity at the SE Research and Extension Center in the same location as the 2009 trial.
4. Incorporate results into grower newsletters and presentations at summer twilight and winter meetings.

Methods and Results:

Objective 1: *Screen imported transplants as well as locally grown transplants for bacterial pathogens using cultural and molecular multiplex PCR techniques.*

In collaboration with Lee Young and Jeff Stoltzfus, transplants were collected from 15 sources representing locally grown transplants (4) as well as those imported from Arizona (4) and Texas (7) in April 2010. A subset of approximately 10 to 100 transplants were selected from each group, roots removed and epiphytic populations recovered and concentrated in a phosphate buffer wash and dilution plated in duplicate onto appropriate microbiological media. Bacterial isolates were further identified using a combination of culturing and molecular techniques (described previously). The same transplants were then surface-disinfested and homogenized in a blender and dilution plated the same as for the surface washes. Bacterial strains isolated were pathogenicity tested by inoculating onion sets. Onion sets were surface-disinfested, cut in half and one half inoculated near the shoulder with a bacterial suspension using a hypodermic needle and syringe. The two halves were incubated in a sterile petri-plate for 7-days and then observed for the development of symptoms.

Results:

One or more bacterial pathogens were isolated from the surface of all 15 onion transplants samples that were collected from either locally grown or imported from Arizona or Texas. The soft rot pathogens, *Pectobacterium carotovora* and *Pseuodomonas marginalis* as well as the center rot pathogen *Pantoea agglomerans* were isolated most frequently (Table 1). These bacterial species were also most frequently isolated from the internal tissues of the transplants. Surprisingly, more diverse bacterial species were isolated from the surface of locally grown transplants (6 species) compared to those imported from Texas (5 species) and Arizona (3 species). Pathogenicity testing indicated that most of the bacterial strains isolated were to some degree pathogenic to onion sets. Further research is needed to understand between season variability in the bacterial populations associated with transplants and their potential role in disease development later in the season in order to determine the most effective way of achieving pathogen-free transplants.

Objective 2: *Conduct a second survey of onion fields across the state to further identify and characterize the bacteria that are causing and/or associated with the symptoms observed in the field.*

In late June, representative symptomatic onions representing the typical symptoms observed were collected from fields corresponding to the transplants collected as part of Objective 1. Individual onions were photographed and symptomatic tissue marked to indicate where the isolation would be made. Bacteria were isolated and identified using a combination of culturing and molecular techniques.

Results:

A total of 63 symptomatic onions cv. 'Candy' were collected from commercial production fields that were planted with transplants either grown locally or imported from Texas or Arizona. To-date bacterial strains have been isolated and identified from 57 of the 63 plants collected. The bacterial species most frequently isolated and identified are consistent 2009 data and include: *Pectobacterium carotovora*, *Pantoea agglomerans* and *Pseudomonas marginalis* (Table 2). Based on this small data set, it appears that the bacteria most frequently isolated from the transplants are consistent with those being isolated from symptomatic tissue in the field and that the pathogens isolated did not vary between the transplant source. Due to time constraints, bacterial disease incidence was not quantified in these fields. Additional research and a larger sample set is needed to confirm these results.

Objective 3: *Further evaluate the effect of bare soil, and different colored plastic mulches by comparing them to the standard black plastic mulch on crop canopy temperature and bacterial disease incidence and severity at the SE Research and Extension Center in the same location as the 2009 trial.*

To further evaluate the effect of mulch types on the development of bacterial diseases of onion, a replicated research trial was established at the PSU Southeast Research and Education Center in Landisville, PA in the field that had been planted to onion the past two years. 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 plastic; 5) standard black plastic; 6) black plastic with no insecticides applied; 7) black plastic with kaolin clay applied at bulbing; 8) white plastic; 9) white plastic with no insecticides applied and 10) metalized silver plastic. 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 2 and 4. 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 (6 and 9) which did not receive any insecticide applications. 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 counts were made on 15 and 29 June. At harvest on 26 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 for 3-weeks and then stored in the cold storage room at the Plant Pathology Farm at Rock Springs. Post-harvest quality was evaluated on 19 Aug, 14 Sep and 14 Oct. During the last evaluation, bulbs showing symptoms of bacterial rot were set aside for culturing and bacterial identification.

Results:

Mulch treatments: Total marketable yield was highest in the standard black plastic mulch treatment although, it was only significantly different from the clear plastic and metalized silver mulch treatments (Table 3). Severe weed competition underneath the clear plastic mulch increased the number the small bulbs (< 2.5 in. diameter) and significantly reduced the number of larger bulbs (> 3 in. diameter). In general, marketable yields were comparable to those observed in this trial last year. At harvest, total unmarketable yield ranged between 13.5% (bare soil) and 41% (metalized silver plastic) with between 8.5

and 23.7% of those losses as a result of bacterial bulb rots culled during harvest (Table 3). The bacterial pathogens most frequently isolated were the soft rot pathogens, *Pectobacterium caratovora* and *Pseudomonas marginalis* and the center rot pathogen, *Pantoea agglomerans*. Storage evaluations were conducted on an additional 6 ft of row harvested on 26 July. Due to significant bulb rot, on Aug 19, the onions were rated for disease incidence after drying but before going into cold storage which resulted in between 15 and 31% of the harvested onions being culled. By 14 Oct total losses ranged between 31 and 47%. Although these losses are considerable, there were no significant differences between mulch treatments (data not shown). Frequently, the onions felt firm and the rot was not realized until the onion was cut open (Fig. 1). Work is currently on-going to identify the bacteria associated with the storage bulb rots.

Fig. 1. Bacterial bulb rot symptoms 80-d post-harvest on onion cv. 'Candy'. Note that the shoulder remain firm and rot is not apparent until the onions are cut open.

Insecticide treatments: By mid-June, thrips counts in the trial were above economic threshold levels in both the black and white plastic mulch treatments and weekly insecticide applications were initiated. Within two weeks, the average thrips per leaf in non-insecticide treated plots were over five times that found in the comparable sprayed plots (Table 4). Although the increased thrips damage in the unsprayed plots did not translate into significantly reduced marketable yields compared to the sprayed plots, there was a higher percent of bacterial bulb rot in the unsprayed plots at harvest in late July (Table 4). In the second set of onion harvested for storage evaluations, significant differences in loss due to bacterial rots in storage was observed between the sprayed (34%) and unsprayed (57.5%) black plastic treatments 80 days post-harvest.

Based on these results at this time, thrips damage appears to be more closely associated with increased losses from bacterial bulb rots than the type of mulch used in the production system. However, in on-farm research trial conducted in collaboration with Cornell and with funding from the NE-IPM Partnership Grant program in 2010, the black plastic mulch treatment still resulted in significantly reduced yields and increased bacterial onion rots compared to the reflective silver and black biodegradable mulches.

Objective 4: *Incorporate results into grower newsletters and presentations at summer twilight and winter meetings.*

These research results will be the subject of a presentation during the onion session of the 2011 Mid-Atlantic Fruit and Vegetable Convention and will also be the subject of at least one winter meeting in Lancaster County in March 2011. In addition, a poster titled 'Multiplex PCR for simultaneous detection of eight major onion bacterial pathogens' was presented at the 2010 Annual American Phytopathological Society meeting in Charlotte, NC in August 2010.

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 other cultural practices that will provide growers with additional integrated pest management tools and will lead to more sustainable sweet onion production in PA.

The project investigators would like to acknowledge the help of John Stepanchak and Jim Bollinger in the establishment, maintenance and harvest evaluation of the Landisville trial as well as all the grower cooperators who willing provided transplant samples and allowing us to conduct our mid-season sampling.

Table 1. Bacterial species isolated from the surface or from macerated tissue of transplants grown locally in Pennsylvania or imported from Arizona or Texas in April 2010. Table includes Texas transplant samples collected from the research trial in Landisville as part of Obj 3.

	Transplant source							
	Arizona (4 samples)		**Texas** (7 samples)		**Locally grown** (4 samples)		**Overall (%)**	
Bacterial species isolated	**Surface**	**Tissue**	**Surface**	**Tissue**	**Surface**	**Tissue**	**Surface**	**Tissue**
Pectobacterium carotovora	0/4	0/4	3/7	2/7	4/4	3/4	47%	33%
Pantoea ananatis	1/4	2/4	0/7	0/7	2/4	0/4	19%	12%
Pantoea agglomerans	3/4	3/4	3/7	3/7	2/4	0/4	53%	40%
Pseudomonas marginalis	3/4	4/4	1/7	1/7	2/4	3/4	37%	50%
Pseudomonas viridiflava	0/4	0/4	2/7	0/7	0/4	1/4	12%	6%
Xanthomonas axonopodis	0/4	0/4	0/7	0/7	2/4	0/4	12%	0%
Burkholderia cepacia	0/4	0/4	0/7	0/7	1/4	1/4	6%	6%
Burkholderia gladioli	0/4	0/4	2/7	1/7	0/4	0/4	12%	6%

Table 2. Bacterial species isolated and identified from symptomatic onion plants cv. 'Candy' collected in June 2010 from commercial fields planted with transplants corresponding with those collected in April 2010.

	Transplant source		
Bacterial species isolated	**Arizona** (31 plants)	**Texas** (4 plants)	**Locally grown** (22 plants)
Pectobacterium carotovora	14/31	3/4	13/22
Pantoea ananatis	3/31	1/4	1/22
Pantoea agglomerans	14/31	3/4	10/22
Pseudomonas marginalis	10/31	2/4	10/22
Pseudomonas viridiflava	3/31	0/4	1/22
Xanthomonas axonopodis	0/31	0/4	3/22
Burkholderia cepacia	2/31	1/4	1/22
Burkholderia gladioli	0/31	0/4	0/22

Table 3. Effect of different mulch types on the yield of sweet onion cv. 'Candy' (per 5 ft of row) at harvest on 26 July 2010.

Mulch treatment	Total weight harvested (lb)		Marketable Yield: Total weight (lb)		Size category (bulb diameter): < 2.5 in.		2.5 to 3.0 in.		> 3.0 in.		Total unmarketable weight (lb)		% onion bulbs with bacterial symptoms
Bare soil……………………………………...	22.9	ab[z]	19.8	ab	0.7	b	4.6	ab	14.5	a	3.1	c	10.5
Straw mulch…………………………………….	23.6	ab	20.1	ab	0.7	b	6.4	ab	13.0	a	3.3	c	8.5
Clear plastic…………………………………….	16.1	c	12.8	c	2.9	a	6.8	ab	3.3	b	3.1	c	11.8
Black biodegradable plastic……………………	23.1	ab	18.1	ab	0.4	b	5.3	ab	12.3	a	5.1	bc	16.7
Black plastic……………………………………	26.0	a	20.9	a	0.9	b	8.2	a	11.9	a	5.1	bc	14.0
Black plastic sprayed w/ kaolin clay at bulbing..	21.6	b	15.9	bc	0.4	b	3.3	bc	11.9	a	5.7	bc	19.7
White plastic……………………………………	24.2	ab	16.5	ab	0.4	b	4.0	bc	12.3	a	7.7	ab	22.1
Metalized silver plastic…………………………	26.9	a	15.9	bc	0.4	b	1.8	c	13.7	a	11.0	a	23.7
P-value	0.0017		0.0347		0.0002		0.0229		0.0218		0.0047		

[z] Values within each column followed by the same letter are not significantly different (*P*=0.05) according to Fisher's Least Significant Difference test.

Table 4. Effect of mulch type and insecticide applications on thrips populations and post-harvest bacterial disease incidence in sweet onion cv. 'Candy'.

Mulch type/ Insecticide applications	Ave. thrips/ leaf: 15 June		29 June		% onion bulbs with bacterial symptoms at harvest on 26 July	Post-harvest storage evaluation (cumulative % symptomatic onion bulbs): 19 Aug (24 days)		14 Sep (50 days)		14 Oct (80 days)	
Black plastic Insecticides applied	2.4	a	0.6	a	14.0	19.0	a	25.7	a	34.0	a
Not applied	3.0	a	3.1	b	17.6	49.3	b	53.8	b	57.5	b
P-value	ns		0.0035			0.0020		0.0016		0.0050	
White plastic Insecticides applied	4.3	a	1.1	a	22.1	25.9	a	36.7	a	47.5	a
Not applied	3.8	a	6.2	b	27.6	44.0	a	50.3	a	60.6	a
P-value	ns		<0.0001			ns		ns		ns	

[z] Values within each column followed by the same letter are not significantly different (*P*=0.05) according to a t-test.

Budget for the 2010 onion project:

Hourly wages (+ fringe benefits 8.5%) for research technician and summer help to assist in conducting proposed field and lab work for 12 wks at 12hrs/wk at $16 and $8/hr, respectively…………………………………………………………....	$ 3750
Supplies to conduct field survey, cultural and molecular bacterial culture identification and greenhouse pathogenicity tests………………………………………	$ 1500
Supplies to establish, maintain, harvest and evaluate field trial…………………………	$ 1000
Travel (towards car rental expenses to conduct surveys & establish, maintain and harvest field trial)…………………………………………………………….….	$ 900
Total………	**$ 7150**