RESEARCH YIELDS GREATER UNDERSTANDING OF BACTERIAL DISEASES OF ONION IN NEW YORK

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Introduction:

Our program became involved with bacterial diseases for two reasons. Prof. Jim Lorbeer requested our assistance early in 2007 in characterizing what seemed to be a new bacterial disease that was affecting New York onions following storage. The problem turned out to be center rot, caused by the bacterium *Pantoea ananatis*. Later, we found symptoms of center rot in fields of onions growing in several New York muck-land areas. Center rot had been described earlier in Colorado and Georgia. Secondly, onion growers and other industry officials in New York reported that bacterial problems had become more severe in recent years, and no effective means were available to consistently reduce losses. Thus, more definitive information was needed for development of effective control strategies. During 2011, we found several bacteria infecting onions that were not previously recognized in New York. Further, we found that muck-soil may be the key important source of overwintering inoculum for several of the bacterial diseases. Also, we made progress in developing techniques useful for evaluation of potential controls. In addition, the results of pilot studies indicated that further study and evaluation of some new control strategies might be justified. If further studies prove promising, these strategies may be worthy of adoption by New York onion growers.

Bacteria Associated with Cull Onions:

New York growers have reported losing 10% to 40% of their stored onions to storage decay. Although sour skin, caused by *Burkholderia cepacia,* and center rot, caused by *Pantoea ananatis*, are familiar, these two pathogens were not responsible for all the unmarketable bulbs that growers were culling during grading following storage. The critical question becomes, "What is responsible for the tremendous losses that growers sustain after storage of onions in New York?"

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To answer the question, we must identify the bacterial pathogens responsible for the losses. Why? Because, each bacterial pathogen likely behaves differently from others. It's source of inoculum is likely to be different, it initiates disease differently and the conditions favoring or not favoring disease development are likely to differ for those for other bacterial pathogens. The key to control or management of plant disease is to interfere with the disease cycle, so details of the cycle should be known. To put the situation in military terms: to defeat the enemy, we have to know all about it, its nature, habits and weaknesses!

Thus, with the cooperation of several onion growers in Central and Western New York, we analyzed hundreds of unmarketable onions that grower-packers had sorted out because they suspected bacterial decay. We characterized symptoms of each bisected bulb, and then attempted to isolate bacteria from each cull onion. Once purified, colonies of the isolated bacteria were identified using traditional microbiological and biochemical tests. The suspected pathogens *Burkholderia cepacia* and *Pantoea ananatis* were tentatively identified often using these tests. However, we isolated many other bacteria and tried to identify them and determine their capability to cause rot in onions.

Techniques for Identification of Microbes from Onions:

To identify the microbes, we developed molecular-based techniques that were based on a combination of PCR (Polymerase Chain Reactions), digestion of the PCR-amplified DNA fragment with a particular enzyme and sequencing of the fragment. The latter technique was effective because the entire genome sequences of many organisms have been determined and are available for comparison with sequences that we, and other researchers, can determine with available techniques. We used PCR to generate a major portion of the *gyrB* gene. That DNA fragment was further fragmented enzymatically to produce an array of fragments, which were compared to the arrays from authentic strains of bacteria. Comparison of the DNA sequences with those of sequenced *gyrB* genes in Genbank led to a definitive identification of the bacterium in question. Using these techniques, we identified many bacteria including *Pantoea ananatis*, *P. agglomerans, P. vagans, Enterobacter cloacae, Pseudomonas fluorescens and other Pseudomonas spp.* Also, rather surprisingly, we isolated and identified *Rahnella species* from about 40% of the culls. *Rahnella spp.* had not been reported as a pathogen of onion anywhere to our knowledge; it occurs rather commonly in water.

Several of the microbes isolated from cull onions did not have morphological features characteristic of bacteria, and they failed to amplify with PCR primers that recognize bacterial genes. However, when we used primers for amplification of genes of yeasts and other eukaryotic microbes, products were obtained. Further studies are needed to determine whether the microbes we isolated from the culls are known pathogens of onions.

The following table summarizes the data on the bacteria isolated from the eight lots of cull onions assayed in 2011. Note, that generally many different bacteria were isolated from each lot of culls. However, some bacteria, like *Burkholderia sp*. and *Enterobacter sp*., were not isolated from some lots, whereas those same bacteria were commonly isolated from other lots. One thing is quite clear: the bacteria isolated from the culls were those present in the culls at the

time they were analyzed. That does not mean that the isolated bacteria were, in fact, responsible for the decayed conditions of the bulbs. Strains of *Burkholderia sp*., *Enterobacter sp.,* and *Pantoea sp.* clearly can cause decay symptoms in inoculated onions. Strains of *Rahnella sp.* also are capable of causing decay in onion bulbs and sets to different degrees depending on the strain and the environmental conditions following inoculation. Although strains of *Pseudomonas sp.* frequently were isolated from cull onions, generally these strains failed to cause any disease symptoms following inoculation into mature onion leaves, bulbs or sets.

Table 1. The percent of cull onions from which specific bacteria were isolated from eight lots of cull onions grown in 2010 and graded in 2011 in Central and Western New York.

	Lot A	Lot B	Lot C	Lot E	Lot F	Lot G	Lot H	Lot D
Burkholderia sp.	14	16	0	4	10	0	2	18
Enterobacter sp.	14	33	12	12	4	0	4	7
Pantoea sp.	10	13	4	8	2	4	2	6
Pseudomonas sp.	27	10	38	65	71	14	18	16
Rahnella sp.	22	3	75	33	47	70	44	9
Total[1]	87	75	129	122	134	88	70	56

Pathogenic Ability of Microbes Isolated from Onions:

To determine the relevance of these bacteria to decay, we initiated studies to determine whether the isolated strains could cause decay following inoculation into putatively healthy onion tissues. We used mature bulbs and onion sets in the laboratory and sprouted onion bulbs and young transplants growing in the lab, greenhouse and controlled environment chambers. Strains of *Pantoea ananatis, Pantoea agglomerans, Burkholderia cepacia* and *Enterobacter cloacae* caused symptoms in inoculated whole onion bulbs or sets. Incubation temperature following inoculation affected the results in a differential manner with respect to the bacteria introduced. Only strains of *Burkholderia cepacia* exhibited characteristic symptoms within a few days of inoculating slices of large bulbs incubated in Petri dishes. Only some strains of *P. ananatis, P. agglomerans and P. vagans* caused lesions in inoculated leaf tissues. (Note, recent taxonomic revisions have led to further distinction of strains of *Pantoea spp*. that once were all considered strains of *Erwinia herbicola*.) Similarly, only some strains of *P. ananatis* and *P. agglomerans* caused a hypersensitive-like response in tobacco leaves in response to infiltration of leaf panels. The yeast-like microbes isolated from the culls have not yet been sufficiently tested to determine their pathogenic capability to onions, but pathogenic yeasts are known.

Sources of Bacterial Pathogens of Onions:

In 2010, we conducted limited tests of materials destined for planting in New York onion soils. We had attempted to isolate bacterial pathogens from several lots of onion seed and transplants and from several lots of muck-land soil. Very few of the samples tested yielded known pathogens of onions, a perplexing result. Because our testing in 2010 was rather limited, we expanded testing in 2011. We also increased our attention to muck-land soils by assessing a greater number of samples and modifying our assay procedures. Soils were collected within a few weeks of onion planting generally before we might expect bacterial multiplication to occur in the plants in the spring. The seed, transplant and soil samples assessed were derived from the same onion plots that were included in the Northeast IPM program study aimed at identifying management factors related (positively or negatively) to the incidence of bacterial decay.

As in 2010, very few of the samples of seed and transplants yielded bacteria pathogenic to onion. However, many strains of *Pantoea agglomerans* were isolated from several of the transplant samples, but with the exception of two tested samples they all failed to infect onions in our tests. The results with the muck-land soil differed radically from the seed and transplant assessments.

More than 78 samples of soil were assessed and several hundred bacterial strains were isolated and characterized from them. The strains isolated included ALL the bacterial pathogens mentioned above, including *Rahnella spp*. Thus, muck-land soils collected following winter and close to the time of onion planting yielded the several pathogens that we had previously isolated from cull onions. This result suggests that soil may be THE important source of inoculum for the several pathogens that are responsible for the extensive losses sustained by growers. Furthermore, since the pathogens appear to be present in soil, perhaps efforts to reduce the populations of the pathogens in soil may reduce the extent of losses caused by these organisms. We intend to address this relationship in our future research.

Thrips have been found to vector the center rot pathogen in Georgia where tobacco thrips, *Frankliniella fusca,* are serious pests of onions grown there. In New York onion thrips, *Thrips tabaci*, plague onions, and it is not known whether these thrips vector *P. ananatis* or any other pathogenic bacterium of onions. As an initial step to address this question, we collected thrips from onion foliage and attempted to isolate bacteria from the surfaces of sub-samples by rinsing the insects vigorously with buffer and then plating the rinse solution on medium that supports the growth of bacteria that we have found are commonly associated with onion tissues. Other sub-samples of thrips were surface-disinfested by treating with dilute bleach, rinsed with sterile water and then macerated. Dilutions of the macerate were plated in an attempt to recover bacteria from the internal thrips tissues. *P. ananatis*, *P. agglomerans, E. cloacae*, and *B. cepacia* were recovered from the external surfaces. *P. ananatis, P. agglomerans, P. vagans* and *E. cloacae* were recovered from the internal tissues. Samples of the recovered bacteria were tested for pathogenicity to onions. Generally, the strains induced the same sort of symptoms as the same bacteria that had been recovered from cull onions earlier. Thus, onion thrips collected from onion foliage rather late in the 2011 growing season harbored pathogenic bacteria both on

their surfaces and in their internal tissues. Whether the bacteria recovered were incidental or capable of being transmitted to bacteria-free plants requires further investigation.

Effects of Cultural Practices on Bacterial Decay of Onions:

Earlier studies were conducted in plots of sweet onion transplants grown on mineral soils in Seneca County, New York and Lancaster County, Pennsylvania. The results indicated that onions grown at wide, vs. narrow, spacing sustained significantly greater losses from bacterial decay. Also, in a trial aimed at assessing the effect of different levels of nitrogen fertilizer on damage from onion thrips, bacterial decay was significantly greater in plots that had been treated with high levels of nitrogen in comparison to plots that received less nitrogen fertilizer. To determine if similar relationships occur in direct-seeded onions grown on a large scale in muck-land soils, a substantial and factorial field trial was arranged with a collaborating grower located in Oswego County. At harvest, onions in each replicated plot were graded for size, counted and weighed. The numbers of obviously decayed onions were determined by manual assessment. The yield from each replicated plot was bagged separately and stored in the conventional manner by the grower. Following storage for several months, losses due to bacterial decay will be determined as well as organisms associated with samples of the decayed onions.

Effects of Sprays of Resistance Inducers on Bacterial Decay:

As an alternative to treating onions with bactericidal chemicals, we decided to investigate the possibility that certain chemicals that have been found to induce pathogen resistance in plants following application, might induce resistance to pathogens of onion that cause bulb decay. Resistance inducers stimulate certain metabolic pathways in the plant that result in enhanced resistance to a broad range of pathogens, and in some cases pests. As the enhanced resistance depends on plant metabolism to develop, the materials must be applied several days in advance of anticipated disease initiation.

To increase our chances of obtaining results from the application of the putative resistance inducers, we designed experiments that included procedures to initiate disease in the onions to be sprayed with the resistance inducing materials. We used methods for inoculating leaf tissues that we had developed earlier in our lab and controlled environment studies. For initiating center rot, we pierced leaves with toothpicks freshly dipped in suspensions of the bacteria. This technique was simple, rapid and reproducible. For initiating sour skin, we devised a technique that involved depositing a suspension of *Burkholderia cepacia* into the lumen of an onion leaf, the top of which had been clipped off to facilitate inoculation.

Projects Planned for 2012

The results of our studies in 2011 clearly have shaped our plans for research in 2012. The cull onion survey revealed the organisms that are associated with unmarketable bulbs following storage. That survey pointed out that Enterobacter bulb decay, which was not known in New York, previously was rather common in Central and Western New York onion growing areas in the 2010 crop. In addition, finding the bacterial pathogen responsible in symptomatic growing onions is good evidence that the disease can be initiated in the field. Furthermore, the presence of *Enterobacter cloacae* in muck-land soil near to onion-planting time suggests that this

pathogen is soil-borne, and perhaps strategies to reduce its population in soil may be effective to reduce losses from Enterobacter bulb decay. Perhaps, similarly, finding *Rahnella* spp. in 40% of the cull onions analyzed requires further investigation of that bacterium. We intend to determine the conditions under which it can be problematic to growers, where it resides when not associated with onions and how it behaves in stored onions.

Tests to induce disease in the field were very promising. We intend to further refine our inoculation techniques for both center rot and sour skin and to develop techniques to initiate Enterobacter bulb decay. The purpose of developing techniques to initiate disease is to have reliable means of inducing disease so that materials and strategies that potentially could reduce disease could be tested effectively and efficiently.

Disease reduction strategies that were tested in 2011 with promising results will be evaluated again in 2012. These include alteration in cultural practices including reducing the amount of space allocated to each onion, both between rows and within rows, and reducing the amount of pre-plant nitrogenous fertilizer applied. In addition, our tests of resistance-inducing materials applied to onions by spraying will be repeated and expanded. Results in 2011 were especially interesting for reducing center rot. We hope to further refine our testing protocols and to determine whether resistance inducers are likely to be effective in non-inoculated grower trials.

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