Grant Proposal to the Pacific Northwest Vegetable Association
2010

Title: Evaluation of conditions promoting bacterial rot in storage onions in Washington State.

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Summary
The overall objective of this proposal is to determine the conditions that promote development of bacterial storage rots caused by *Burkholdera cepacia*, *B. gladioli* and *Enterobacter cloacae*. More specifically, this project includes: 1) determining the impact of curing parameters on storage rots caused by *E. cloacae, B. cepacia and B. gladioli* in onion bulbs; 2) evaluating the reactions of commercially-available onion cultivars to *E. cloacae, B. cepacia and B. gladioli* using onions harvested from the WSU Onion Cultivar Trial; and 3) investigating factors that influence infection of onion plants by *E. cloacae*. The first two objectives are 2-year studies to generate information on the influence of curing and storage parameters on these storage rots. The third objective will enhance our understanding of how *E. cloacae* colonizes onion bulbs, to help identify effective management strategies. This proposal requests funding for continued support of research funded by the PNVA and WSCPR in 2009.

Statement of the problem:

Bacterial storage rots of onion bulbs were responsible for significant losses in onion packing sheds in the Columbia Basin (WA and OR) during the 2004-05 storage season. A bacterium isolated from symptomatic bulbs sampled from these storage sheds was identified as *Enterobacter cloacae* (H. Schwartz and L. du Toit, personal communication). The disease develops during storage, affecting physiologically mature bulbs, with the inner fleshy scales turning brown. Symptoms typically begin near the neck of the bulb and spread down individual fleshy scales. Infected scales typically are firmer than those infected with other bacterial pathogens such as *Dickeya* spp. or *Pectobacterium* spp. Interestingly, infected bulbs appear normal on the outside surface, making visual detection of the disease a challenge. In addition to *E. cloacae, Burkholderia cepacia* and *Burkholderia gladioli* have frequently (particularly the latter) been associated with storage bulb rots of onion bulbs in the Pacific Northwest (PNW). These pathogens cause sour skin and slippery skin, respectively. Symptoms produced by *B. cepacia* include the breakdown of inner fleshy scales resulting in a pale yellow to light brown discoloration which progresses across the scales. *B. gladioli* induces an inner fleshy scale rot resulting in water-soaked fleshy scales. The scales are discolored a light yellow to brown and, like Enterobacter bulb deay, the rot does not move between adjacent fleshy scales.

There is currently little knowledge on how curing parameters might impact progression of these bacterial storage rots of onion. Over the past two years, the investigators have completed bulb storage rot assays using *E. cloacae*. The results indicate that higher curing temperatures (35-40°C) significantly promote these storage rots compared to lower curing temperatures (25-30°C), and the longer the curing duration at the higher temperatures, the more severe the storage rot. This suggests that reduced curing temperatures for longer durations may result in less

Enterobacter bulb decay in storage than higher curing temperatures for shorter durations. Another question is how slippery skin and sour skin caused by *B. cepacia* and *B. gladioli*, respectively, develop under various curing parameters. *B. cepacia* and *B. gladioli* are more aggressive pathogens in storage than *E. cloacae*, based on the greater prevalence with which they have been found associated with storage rots in the PNW (particularly *B. gladioli*). These bacteria cause more severe onion bulb rots than *E. cloacae*, with greater potential for spread of the pathogen between bulbs within storage facilities. Determining the impact of curing parameters on these two diseases in comparison with Enterobacter bulb decay will provide valuable knowledge to stakeholders for management of onion bulbs in storage.

The onion cultivars grown in the Washington State University Onion Cultivar Trial in 2007 and 2008 were screened for resistance to *E. cloacae*. A range of reactions to *E. cloacae* were observed among the 69 cultivars screened (Schroeder et al., 2010, Plant Disease 94:236-243). Interestingly, the reactions of several cultivars, particularly some red cultivars, did not appear as severe as expected based on empirical stakeholder observations. Discussions with stakeholders indicates that, since *E. cloacae* is a relatively new pathogen of onion bulbs in the PNW, valuable information could be generated by completing similar resistance evaluations with *B. cepacia* and *B. gladioli* to compare cultivar responses to inoculation with *E. cloacae*.

Because our understanding of *E. cloacae* and Enterobacter bulb decay of onion is limited, it is difficult to recommend effective management practices to stakeholders. Therefore, continued studies on the colonization and movement of *E. cloacae* in onions will further our understanding of the epidemiology of this pathogen. Identification of factors that impact plant pathogen interactions is needed to develop effective management strategies for this disease.

We received funding for this project from the WSPCR in 2009. This proposal requests support in 2010 to continue this research on bacterial storage rots of onion bulbs. The overall objective is to identify conditions that promote development of bacterial storage rots caused by *B. cepacia*, *B. gladioli* and *E. cloacae*. More specifically, this project will entail: 1) determining the impact of curing parameters on progress of diseases caused by *E. cloacae*, *B. cepacia* and *B. gladioli* in onion bulbs in storage; 2) evaluating the reactions of commercially available onion cultivars to *E. cloacae, B. cepacia and B. gladioli* using onions harvested from the 2009 WSU Onion Cultivar Trial and placed in storage; and 3) investigating factors that influence infection of onion plants by *E. cloacae*. This proposal expands upon research initiated in 2007 which was funded by the Pacific Northwest Vegetable Association (2007, 2008, 2008) and the Washington State Commission on Pesticide Registration (2008, 2009, 2010).

Procedures:

Objective 1. Curing Parameters Experiments: Storage onion bulbs are typically heat-cured in onion packing sheds at temperatures ranging from 30 to 40°C for durations ranging from 1 to 14 days prior to long-term storage at cool temperatures (~2-5°C). In order to determine the impact of curing temperatures on bacterial storage rots caused by *B. cepacia*, *B. gladioli* and *E. cloacae*, a known pathogenic isolate of each will be injected into healthy onion bulbs at harvest (after topping the bulbs). Four replicates of 5 bulbs harvested from a grower-cooperator's field in the Columbia Basin will be cured at each of four temperatures (25, 30, 35 and 40°C) for each of two durations (2 days vs. 2 weeks) and then placed in storage at 5°C. Bulbs will be cut down the center and evaluated for severity of rot after 1, 2, and 3 months in storage. A water-inoculated control treatment and a non-inoculated control treatment will be included for comparison with each bacterial pathogen. Two cultivars, Redwing and Vaquero will be evaluated. Results of this

study will help identify whether curing temperature and duration can influence progress of bacterial storage rot caused by each of the three pathogens.

Objective 2. Evaluate the range in resistance reactions in commercially-available onion cultivars. We will screen the onion cultivars planted in the 2009-10 Washington State University Onion Cultivar Trial & Storage Demonstration Trial for resistance to *B. cepacia*, *B. gladioli* and *E. cloacae*. Fifty onion bulbs were harvested in September 2009 from each of three replicate plots of each cultivar in the trial. For each replicate of each cultivar, 10 bulbs were injected with a suspension of either *B. cepacia*, *B. gladioli* or *E. cloacae*; 10 bulbs were inoculated with sterile water; and 10 bulbs served as non-inoculated controls. The onions were placed in a commercial storage facility at Grigg & Sons, and will be evaluated for bacterial storage rot in mid-February 2010 by cutting each bulb lengthwise and rating the severity of bacterial storage rot symptoms. We will repeat this trial in 2010-11 to check consistency in reaction of diverse onion cultivars to the three pathogens under different curing parameters.

Objective 3. Movement of *E. cloacae* in onion plants and development of bulb rot. We have demonstrated that *E. cloacae* can colonize onion plants and move within leaves, but this foliar colonization is not associated with any symptoms on colonized leaves. We will assess whether *E. cloacae* can move from inoculated onion leaves into the onion bulb, leading to bulb rot in storage. In the greenhouse, 4 replicates of 5 onion plants will be inoculated using a wounding method to introduce a known amount of *E. cloacae* inoculum into the leaves at monthly intervals through the growing season. After inoculation, plants will be kept in the greenhouse until the bulbs mature. The tops will then be removed and the bulbs incubated at 30°C for two weeks before storage at 5°C. The bulbs will be monitored monthly for three months for development of Enterobacter bulb decay. The entire trial will be replicated. Published scientific literature suggest that a high temperature (>35°C) may promote development of Enterobacter bulb decay. Therefore, low, moderate and high temperatures of incubation and growth of the inoculated plants will be evaluated in a greenhouse. Control plants will be treated similarly with water.

Timeframe: 2010

Jan-Dec.	Greenhouse trials initiated in Jan. Seed planted, plants inoculated at intervals, bulbs harvested after they mature, and evaluated in storage at intervals.
Jan.	Evaluation of bulbs in the curing temperature storage trial.
Feb.	Evaluation of bulbs in the curing temperature storage trial and the WSU Cultivar Storage Trial.
Sep.	Harvest bulbs and set up storage trials.
Nov.	Results presented at the annual meeting of the PNVA in Tri-Cities, WA.
Dec.	Results submitted to Vol. 5 of *Plant Disease Management Reports* (APS Press).

Project Budget

Expenditure	WSCPR (Request)	Co-funding (CASH or IN-KIND)			TOTAL COST
		Source: Amount PNVA (CASH)	Source: Amount (IN-KIND)	Source: Amount (IN-KIND TIME)[5]	
Salaries[1]	$4,289	$1167		$1,000	$6,456
Employee Benefits	$423	$467			$890
Temporary or hourly workers	$900				$900
Travel[2]	$734	$166		$600	$1,500
Equipment					
Other: seed, onion bulbs[3]			$5,480		$5,480
Other: field & lab supplies[4]	$897	$200			$1,097
Total $7243.50 ~~$7,000~~		$2,000	$5,480	$1,600	$16,323

[1] Support for a graduate student in the summer + hourly support for cultivar storage assays and bulb ratings. Support for post-doctoral research associate for fall storage experiments. Graduate student salary ($4289) + 9.5% benefits ($407). Student receives stipend in spring and fall semesters from Dept. of Plant Pathology. Post-doctorate salary ($1167) + 40% benefits ($467). Time-slip wages ($900) + 2.4% benefits ($16).

[2] Travel between WSU-Pullman & Tri-Cities: ~300 miles round-trip for ~4 trips + presentation at PNVA annual meeting. Travel for Dr. du Toit and staff between WSU Mount Vernon NWREC & Tri-Cities: ~600 miles round-trip for 2 trips with overnight stays.

[3] Donations of onion bulbs for storage experiments (>8,000 bulbs) & cultivar trial (6,500 onions) and curing and storage facilities/resources; seed for greenhouse trials.

[4] Greenhouse and growth chamber fees ($600). Laboratory supplies, media, and consumables ($497).

[5] In-kind support from Nunhems, USA, Inc. in the form of assistance with bulb inoculations and storage rot evaluations.