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Effects of postharvest onion curing parameters on bulb rot caused by *Pantoea agglomerans*, *Pantoea ananatis* and *Pantoea allii* in storage

C. Vahling-Armstrong[a], J. K. S. Dung[b], J. L. Humann[c] and B. K. Schroeder[c]*

[a]*USDA-ARS, Fort Pierce, FL 34945;* [b]*Oregon State University, Madras, OR 97741; and* [c]*Washington State University, Pullman, WA 99164, USA*

Crop loss of onion bulbs during storage carries an exceptionally high economic impact because a large portion of the production expenses has been expended before storage. Because of this, it is important to define practices that can reduce onion bulb losses caused by storage rots. This study investigates the impact of various curing parameters on disease development resulting from infection by *Pantoea agglomerans*, *P. ananatis* and *P. allii* on onion bulb cultivars Vaquero and Redwing, during storage. Overall, both the incidence and mean rot severity were similar amongst the bulbs under comparable conditions regardless of the species of *Pantoea* inoculated, although a significant difference was detected between the two onion bulb cultivars. In addition, a significant reduction of storage rot was observed when curing temperatures were ≤35°C. At temperatures >35°C, a shorter curing duration (2 days vs 14 days) decreased the severity of bulb rot due to *Pantoea*. This increased understanding of the inter-relationships between the parameters used for curing, and the incidence and severity of bulb rot caused by *Pantoea* helps provide guidance towards using the curing process as a means to reduce the level of damage resulting from post-harvest storage rot.

Keywords: bulb rot, onion curing, *Pantoea agglomerans*, *Pantoea allii*, *Pantoea ananatis*

Introduction

According to the United States Department of Agriculture, approximately 63 000 ha of land in the United States were used annually for onion (*Allium cepa*) production from 2010 to 2012 (National Agricultural Statistics Service, 2013). With 68% of the annual onion production intended for storage, bulb rots in storage have the potential to cause substantial economic losses for the onion industry. As production costs of storage onions accounted for $554 708 000 in 2012 alone, it is imperative that the nature of bulb rot diseases are understood in order to shield the crop from impending damages. There are 26 diverse pathogens currently known to cause storage bulb rot, of which 14 are fungal pathogens and 12 are bacterial pathogens (Schwartz & Mohan, 2008).

One group of bacterial storage rot pathogens belong to the genus *Pantoea*. As a member of the family Enterobacteriaceae, the genus *Pantoea* is composed of a collection of Gram-negative bacteria that have been isolated from water, soil, plants, animals and humans (Bergey & Holt, 2000). Depending upon the particular ecological niche they inhabit, *Pantoea* spp. can be regarded as epiphytes (Smith *et al.*, 2013), endophytes, plant pathogens or opportunistic human pathogens (Cruz *et al.*, 2007). Three different species of *Pantoea* have been reported to be pathogenic on onion: *Pantoea ananatis*, *Pantoea agglomerans*, and the recently described *Pantoea allii* (Brady *et al.*, 2011).

Pantoea infections usually begin as water-soaked lesions on the leaves that progress down the neck into the developing bulb causing a disease commonly known as centre rot (Conn *et al.*, 2012). *Pantoea ananatis* is the most well-studied of the *Pantoea* species on onion. Symptoms of *P. ananatis* bulb infection can be found on one or multiple scales with the infected scales being light brown to brown in colour (Carr *et al.*, 2010). In the absence of any secondary infections, the bulb tissue often does not show signs of maceration or emanate the foul odours that have been associated with other bacterial rots. The bacterial colonies appear yellow with dark centres when grown on the semiselective medium PA-20 (Goszczynska *et al.*, 2006). This bacterium can be transmitted via infected seed (Walcott *et al.*, 2002), tobacco thrips (*Frankiniella fusca*) (Gitaitis *et al.*, 2003) and onion thrips (*Thrips tabaci*) (Dutta *et al.*, 2014). Given the recent distinction between *P. allii* and *P. ananatis* (Brady *et al.*, 2011), true characterization of the novel *P. allii* species is in its infancy and previous literature on the bacterial onion pathogen designated as *P. ananatis* may actually be reporting on either *P. allii* or *P. ananatis* or possibly a combination the two. Previously used

*E-mail: bschroeder@uidaho.edu

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strains will have to be recharacterized in order to resolve this issue.

Bulb curing is a management strategy that is commonly used to minimize water loss as well as the occurrence of both fungal and bacterial rot in storage. Onions can be cured either in the field by lifting them to the surface and allowing them to dry via the sun and/or by artificially curing bulbs in a storage facility using forced air or heated forced air to dry down the neck and the outer layers of the onion (Opara, 2003). The curing process dries the neck resulting in a tight, dry wrapper around the onion, helping to protect the bulb during storage.

Depending on the environmental conditions present at the time of harvest, farmers may use a combination of the two curing methods mentioned above. Many parameters can be adjusted when using artificial means to cure bulbs, including the modification of curing temperatures, the rate of ventilation, the rate of temperature change, and the overall curing duration. Previous studies have shown that these factors can affect the severity of the bulb rot and that they are specific to the organism responsible for the rot. For example, the incidence of bulb rot caused by *Burkholderia cepacia*, *Burkholderia gladioli* pv. *allii* and *Enterobacter cloacae* increases significantly when bulbs are cured at higher temperatures (Schroeder & Du Toit, 2010; Schroeder *et al.*, 2012); however, the incidence of bulb rot decreases for *Botrytis* using higher temperatures (Maude *et al.*, 1984).

The purpose of this study was to delineate the progression of onion bulb rot caused by *P. ananatis*, *P. allii* and *P. agglomerans* in onion bulbs under different curing parameters. The effects of curing temperature, length of curing time, duration of storage, and cultivar were assessed. Defining the conditions that affect the progression of the rot caused by the different *Pantoea* spp. will provide a management tool to onion producers. Depending on the pathogens present, curing parameters can potentially be manipulated to reduce the severity or eliminate disease development in onion bulbs.

Materials and methods

Experimental design and source of onion bulbs

Onion cultivars Redwing and Vaquero were used for experiments, as representatives of common red and yellow storage cultivars grown in the Pacific Northwest region of the US. Individual trials were set up using a randomized complete block design with a factorial combination of the two onion cultivars, five inoculation treatments (*P. agglomerans*, *P. ananatis*, *P. allii*, water and non-inoculated bulbs), four curing temperatures (25, 30, 35 and 40°C), two curing durations (2 and 14 days), and three storage durations (1, 2 and 3 months). Four replicates containing five onion bulbs were used in each treatment combination. The first trial was carried out in the 2011/12 storage season and was repeated in the 2012/13 storage season on bulbs harvested in 2011 and 2012, respectively, from commercially produced onion crops. These crops had been grown using drip irrigation under the semi-arid conditions of the Columbia Basin in central Washington, using production practices typical for this region (Pelter & Sorensen, 2003). The bulbs ranged from 5·0 to 7·5 cm in diameter, and were removed from the field manually after each crop had been topped mechanically.

Onion bulb inoculations

Inoculum containing *P. agglomerans* strain C9-1, *P. ananatis* strain HortHill #31, and *P. allii* strain Blackshank #24 was prepared as previously described (Schroeder *et al.*, 2012). Briefly, a 5 mL overnight culture of each strain was used to seed 250 mL nutrient broth placed at 28°C with agitation. After an overnight incubation, cells were harvested by centrifugation, washed, and resuspended in sterile water to an optical density (OD_{600}) of 0·3 (approximately 10^8 CFU mL^{-1}). Each bacterial inoculum was then diluted, which resulted in a final concentration between $4{\cdot}0 \times 10^7$ and $5{\cdot}5 \times 10^7$ CFU mL^{-1}, and dispensed into a series of sterile test tubes. A 0·5 mL aliquot of inoculum or water was injected into the shoulder region of each bulb as described by Schroeder and du Toit (Schroeder *et al.*, 2010). In an effort to prevent cross-contamination, the inoculation site and syringe were swabbed with 70% ethanol for every injection and a separate tube of inoculum with a new, sterile syringe were used for each replicate of five onion bulbs. Control treatments consisted of bulbs injected with sterile water or non-inoculated bulbs, which allowed for the assessment of natural infections in the bulb lots used for the assay.

Bulb curing and storage

After inoculation, four replicate sets of five onion bulbs for each cultivar and inoculation treatment were placed in nylon mesh onion bags in incubators set at 25, 30, 35 or 40°C. The bulbs were cured for either 2 or 14 days in incubators that contained fans to aid air circulation. After curing, temperatures were reduced by 2·5°C daily until they reached 5°C. Bulbs were then stored at 5°C for 1, 2 or 3 months. At each time point, five bulbs were removed from each replicate bag in storage and evaluated for severity of bulb rot by slicing through the centre of the inoculation site on the bulb from the neck to the basal plate, and visually rating the fleshy scales for severity of bulb decay. The percentage of the cut surface area of the fleshy scales showing symptoms typical of bacterial rot was recorded for each bulb and photographs were taken.

Statistical analyses

Data were analysed essentially as described by Schroeder *et al.* (2012). Mean incidence and severity of bulb rot were calculated separately for Redwing and Vaquero onion cultivars. Bulb rot severity data were subjected to analysis of variance (ANOVA) using PROC MIXED in SAS v. 9.2 (SAS Institute). Main effects included cultivar, treatment, curing temperature, curing duration, and storage duration, with block and trial random effects. The full model, which included all two-, three-, four- and five-way interactions, was analysed using $\log_{10}$-transformed data to improve data normality and homogeneity of variances. Bulb rot severity in non-inoculated and water-inoculated treatments was low and data were not included in the analysis to reduce hetero-

geneity of variances. Separate ANOVAs were conducted for each combination of pathogen, curing duration and storage duration due to significant interactions in each trial. Analyses were performed using square root- or $\log_{10}$-transformed data when necessary to satisfy the assumptions of normality and homogeneity of variances required for ANOVA.

Results

Disease progression of *Pantoea*

Upon inoculation of both Redwing and Vaquero onion bulbs with *c.* $2{\cdot}5 \times 10^7$ CFU of *P. agglomerans*, *P. ananatis* or *P. allii*, symptoms were observed similar to those resulting from a natural infection of the bulb in field-derived material containing *Pantoea*. Symptoms resulting from infection by *P. agglomerans*, *P. ananatis* or *P. allii* were highly similar in nature and included a yellowing or browning of one or more of the internal fleshy scales and in some cases a drying/shrinking of those scales that resulted in a collapse of the tissue. Generally, extensive amounts of tissue damage were not seen on bulbs incubated below 40°C and symptoms developed mainly around the point of deepest penetration of the inoculation needle, where the inoculum was released (Fig. 1). Certain inoculated bulbs, especially bulbs incubated at 40°C, displayed severe symptoms including water-soaked lesions and tissue maceration in addition to an overall softening of the bulb. In comparison, most non-inoculated (indicative of natural infection) or water-inoculated bulbs were symptomless but those that did show disease symptoms presented severe symptoms similar to those described above (Fig. 2). However, because these bulbs were not inoculated with *Pantoea*, their symptoms may be the result of an infection with one of the other pathogens known to cause bulb rot in onion (Schwartz *et al.*, 2008).

Incidence and severity of bulb rot

All three *Pantoea* species caused rot in 100 ± 0·0% Redwing onion bulbs in the first trial. Inoculations with *P. agglomerans*, *P. allii* and *P. ananatis* resulted in 100 ± 0·0, 99 ± 10, and 100 ± 0·0% incidence of bulb rot, respectively, in the second trial and all three pathogens caused 100 ± 0·0% incidence of rot in Vaquero in both trials. Inoculations of Redwing bulbs using sterile water caused rot in 13 ± 33 and 8·3 ± 28% of bulbs in the first and second trial, respectively. Incidence of rot in Vaquero bulbs inoculated with sterile water was 18 ± 38% in the first trial and 30 ± 46% in the second trial. The incidence of natural infection for non-inoculated bulbs was found to be 7·3 ± 26% on Redwing in both trials and 6·3 ± 24 and 12 ± 32% on Vaquero in the first and second trial, respectively. This was measured by storing bulbs that were not inoculated or artificially wounded in any manner under the same conditions as those for the inoculated bulbs.

Severity of rot was similar among the three pathogens for Redwing and Vaquero cultivars but rot severity was significantly ($P < 0{\cdot}0001$) greater in Vaquero than Redwing following inoculation with any of the three *Pantoea* species when data were combined from all curing temperatures, curing durations and storage durations

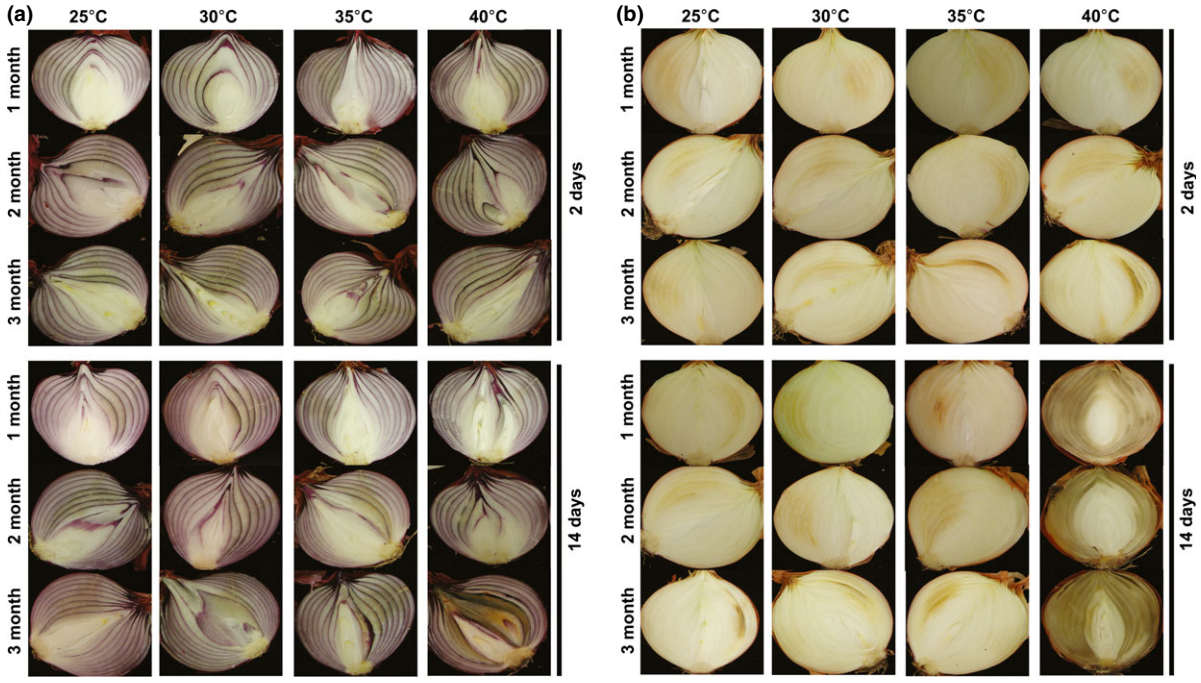

Figure 1 Disease progression of inoculated bulbs. Symptoms on (a) Redwing or (b) Vaquero onion bulbs after inoculation with *Pantoea allii*. Curing parameters of these bulbs included incubation for 2 or 14 days at four temperatures ranging from 25 to 40°C and storage at 4°C for 1–3 months.

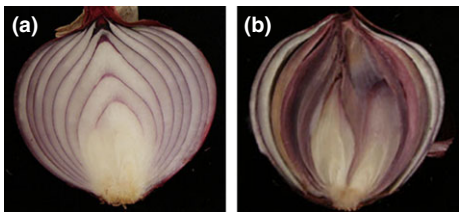

Figure 2 Natural infection of bulbs. Non-inoculated Redwing bulb showing (a) no symptoms or (b) severe symptoms after incubation at 25°C for 14 days and subsequent storage for 1 month.

(Table 1). Mean rot severity in Redwing onion bulbs inoculated with *P. agglomerans*, *P. allii* and *P. ananatis* was 20 ± 7·4, 21 ± 8·8 and 20 ± 6·9%, respectively, in the first trial and 19 ± 13, 19 ± 11 and 23 ± 15%, respectively, in the second trial. Mean severity of rot in Vaquero onion bulbs inoculated with *P. agglomerans*, *P. allii* and *P. ananatis* was 31 ± 6·9, 30 ± 9·3 and 32 ± 10%, respectively, in the first trial and 30 ± 13, 34 ± 17 and 35 ± 13%, respectively, in the second trial. Severity of rot in non-inoculated Redwing bulbs or Redwing bulbs inoculated with sterile water was low, with mean values ranging between 0·33 ± 1·6 and 1·0 ± 4·8 in both trials (Table 1). Mean rot severity in non-inoculated or sterile water-inoculated Vaquero bulbs was lower in the first trial (0·43 ± 2·3 and 1·3 ± 8·0%, respectively) compared to the second trial (5·2 ± 16 and 8·4 ± 20%, respectively).

Significant ($P < 0·0001$) effects of all five main effects (cultivar, treatment, curing temperature, curing duration and storage duration) were observed when ANOVA was conducted using the full model. Significant two-way interactions of cultivar × curing duration ($P = 0·013$), cultivar × storage duration ($P < 0·0001$), treatment × curing temperature ($P = 0·0042$), treatment × curing duration ($P = 0·0012$), curing temperature × curing duration ($P < 0·0001$), curing temperature × storage duration ($P = 0·034$) and curing duration × storage duration ($P < 0·0001$) were observed. Significant three-way interactions of cultivar × treatment × curing temperature ($P = 0·019$) and curing temperature × curing duration × storage duration ($P = 0·025$) were also observed. All other two- and three-way interactions were not significant ($P > 0·05$). Significant four-way or five-way interactions were not observed. Although the random effect of trial was not significant ($P = 0·059$) differences between trials were apparent (Tables 2, 3 & 4) and data for each trial were analysed separately. Separate ANOVAs were conducted for each trial and treatment combination based on the observed differences between trials and the significant interactions observed using ANOVA and the full model.

Inoculation with Pantoea agglomerans

Significant ($P < 0·0001$) effects of cultivar, curing duration and storage duration were observed in the first trial. Significant two-way interactions of cultivar × curing temperature ($P = 0·035$) and curing duration × storage duration ($P = 0·0008$) and a significant three-way sinteraction of curing temperature × curing duration × storage duration ($P = 0·023$) were observed in the first trial. In the second trial, the effect of cultivar, curing temperature, curing duration and storage duration was significant ($P < 0·0001$) and significant two-way interactions of cultivar × storage duration ($P = 0·020$) and curing temperature × curing duration ($P < 0·0001$) were observed. A significant three-way interaction of cultivar × curing temperature × curing duration ($P = 0·0003$) was observed in the second trial.

The effect of cultivar was significant ($P < 0·0001$) in both trials for onion bulbs cured for 2 days and for bulbs cured for 14 days ($P \leq 0·0009$) at all three storage durations (1, 2 and 3 months; Table 2; Fig. 3). The effect of curing temperature was significant ($P \leq 0·0073$) for bulbs cured for 14 days and stored for 1 month in both trials. The interaction of cultivar and curing temperature for bulbs cured for 2 days and stored for 3 months was significant in the second trial ($P = 0·0046$) but not in the first trial ($P = 0·056$).

Inoculation with Pantoea allii

Significant cultivar × curing duration ($P = 0·0003$), cultivar × storage duration ($P = 0·0007$), curing tem-

Table 1 Incidence and severity of rot in two onion cultivars (Redwing and Vaquero) following inoculation with *Pantoea agglomerans*, *P. allii*, *P. ananatis*, or sterile water

	Redwing		Vaquero	
Inoculation	Incidence (%)	Severity (%)	Incidence (%)	Severity (%)
Trial 1				
P. agglomerans	100 (±0·0)	20 (±7·4)	100 (±0·0)	31 (±6·9)
P. allii	100 (±0·0)	21 (±8·8)	100 (±0·0)	30 (±9·3)
P. ananatis	100 (±0·0)	20 (±6·9)	100 (±0·0)	32 (±10)
Water-inoculated	13 (±33)	0·67 (±2·4)	18 (±38)	1·3 (±8·0)
Non-inoculated	7·3 (±26)	0·33 (±1·6)	6·3 (±24)	0·43 (±2·3)
Trial 2				
P. agglomerans	100 (±0·0)	19 (±13)	100 (±0·0)	30 (±13)
P. allii	99 (±10)	19 (±11)	100 (±0·0)	34 (±17)
P. ananatis	100 (±0·0)	23 (±15)	100 (±0·0)	35 (±13)
Water-inoculated	8·3 (±28)	1·0 (±4·8)	30 (±46)	8·4 (±20)
Non-inoculated	7·3 (±26)	0·85 (±3·5)	12 (±32)	5·2 (±16)

Table 2 Effects of cultivar, curing temperature and cultivar × curing temperature interactions on mean bulb rot severity in cultivar Redwing and Vaquero onion bulbs inoculated with *Pantoea agglomerans*, cured for 2 or 14 days at 25, 30, 35 or 40°C, and stored for 1, 2 or 3 months[a]

	Trial 1			Trial 2		
	1 month	2 months	3 months	1 month	2 months	3 months
2 days						
Cultivar	<0·0001	<0·0001	<0·0001	<0·0001	<0·0001	<0·0001
Temperature	0·46	0·17	0·94	0·0026	0·033	0·23
Cultivar × temperature	0·12	0·68	0·056	0·45	0·42	0·0046
Transformation[b]	None	None	None	None	None	None
14 days						
Cultivar	0·0008	0·0009	0·0003	<0·0001	<0·0001	<0·0001
Temperature	0·0073	0·58	0·69	<0·0001	<0·0001	0·0001
Cultivar × temperature	0·19	0·60	0·52	0·84	0·54	0·088
Transformation[b]	None	None	None	None	None	Log_{10}

[a]Values are *P*-values from analysis of variance (ANOVA).
[b]Data were log_{10}-transformed to satisfy assumptions of normality and homogeneity of variances for ANOVA.

Table 3 Effects of cultivar, curing temperature and cultivar × curing temperature interactions on mean bulb rot severity in cultivar Redwing and Vaquero onion bulbs inoculated with *Pantoea allii*, cured for 2 or 14 days at 25, 30, 35 or 40°C, and stored for 1, 2 or 3 months[a]

	Trial 1			Trial 2		
	1 month	2 months	3 months	1 month	2 months	3 months
2 days						
Cultivar	<0·0001	<0·0001	<0·0001	<0·0001	<0·0001	<0·0001
Temperature	0·37	0·042	0·0077	0·056	0·0014	0·0058
Cultivar × temperature	0·11	0·14	0·93	0·58	0·88	0·44
Transformation[b]	None	None	None	None	None	None
14 days						
Cultivar	0·0017	0·0084	0·0034	<0·0001	<0·0001	<0·0001
Temperature	0·0024	<0·0001	0·22	<0·0001	<0·0001	<0·0001
Cultivar × temperature	0·95	0·40	0·25	0·60	0·78	<0·0001
Transformation[b]	Sqrt	Sqrt	None	Sqrt	Log_{10}	None

[a]Values are *P*-values from analysis of variance (ANOVA).
[b]Data were square-root (sqrt) or log_{10}-transformed to satisfy assumptions of normality and homogeneity of variances for ANOVA.

Table 4 Effects of cultivar, curing temperature and cultivar × curing temperature interactions on mean bulb rot severity in cultivar Redwing and Vaquero onion bulbs inoculated with *Pantoea ananatis*, cured for 2 or 14 days at 25, 30, 35 or 40°C, and stored for 1, 2 or 3 months[a]

	Trial 1			Trial 2		
	1 month	2 months	3 months	1 month	2 months	3 months
2 days						
Cultivar	<0·0001	< 0·0001	<0·0001	<0·0001	<0·0001	<0·0001
Temperature	0·0004	< 0·0001	0·049	0·0016	0·015	0·086
Cultivar × temperature	0·51	0·47	0·51	0·44	0·85	0·76
Transformation[b]	None	None	None	None	None	None
14 days						
Cultivar	<0·0001	0·0002	0·0005	<0·0001	0·0002	<0·0001
Temperature	0·0011	0·13	0·056	<0·0001	<0·0001	<0·0001
Cultivar × temperature	0·023	0·28	0·37	0·92	0·056	0·88
Transformation[b]	Sqrt	Log_{10}	None	None	Sqrt	Sqrt

[a]Values are *P*-values from analysis of variance (ANOVA).
[b]Data were square-root (sqrt) or log_{10}-transformed to satisfy assumptions of normality and homogeneity of variances for ANOVA.

perature × curing duration ($P = 0·0005$) and curing temperature × curing duration × storage duration ($P = 0·020$) interactions were observed in the first trial. Significant curing temperature × curing duration ($P < 0·0001$) and curing duration × storage duration ($P = 0·0082$) interactions were also observed in the second trial. Significant main effects were not observed in either trial.

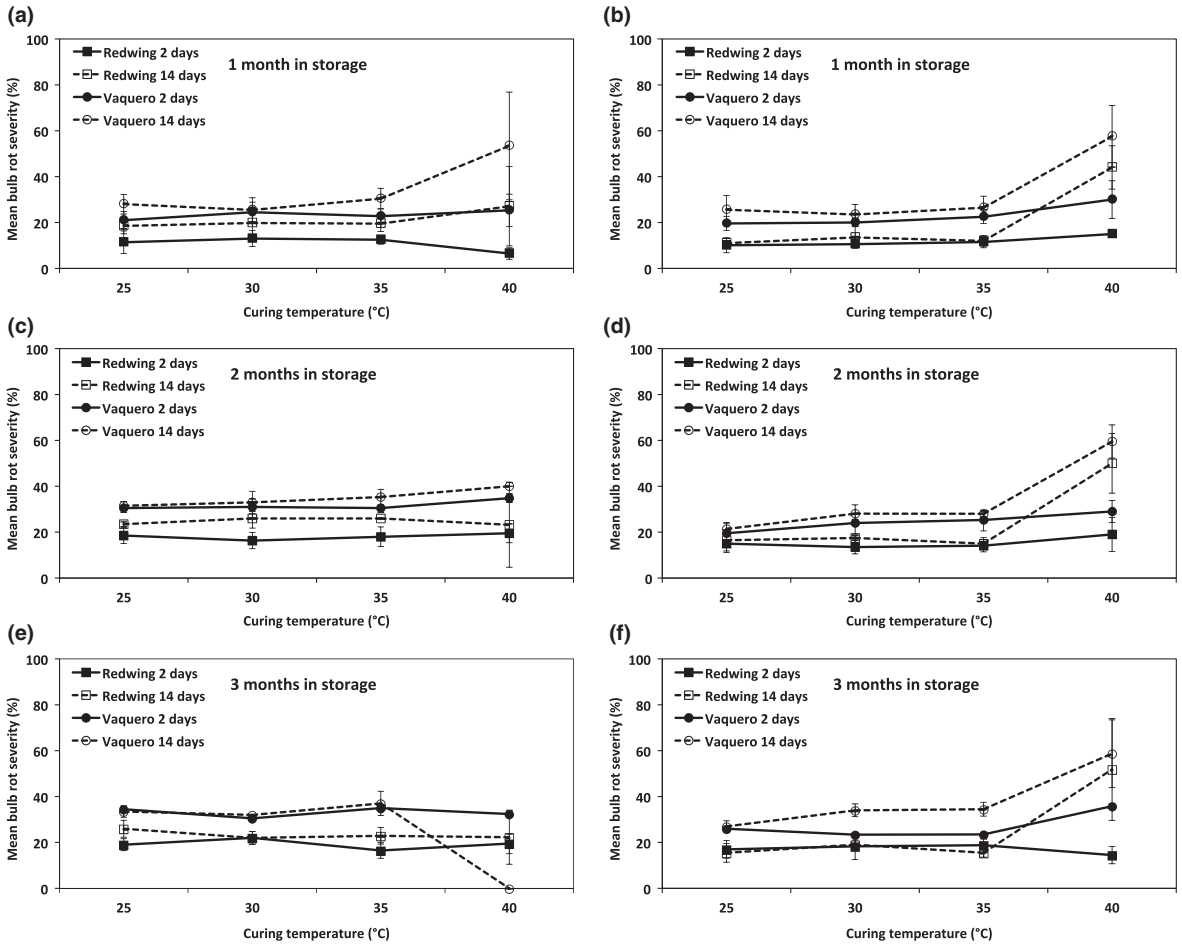

Figure 3 Mean severity of bulb rot (percentage of cut surface area of scales showing symptoms) in Redwing and Vaquero onion bulbs following inoculation with *Pantoea agglomerans*, cured for 2 or 14 days at four temperatures, and stored for either 1 month (a) Trial 1 and (b) Trial 2; 2 months (c) Trial 1 and (d) Trial 2; or 3 months (e) Trial 1 and (f) Trial 2. Error bars indicate standard deviations of mean values.

A significant effect of cultivar was observed in both trials for all three storage durations after bulbs were cured for 2 days ($P < 0{\cdot}0001$) or 14 days ($P \leq 0{\cdot}0084$; Table 3; Fig. 4). The effect of temperature was significant for bulbs cured for 2 days and stored for 2 months ($P \leq 0{\cdot}042$) or 3 months ($P \leq 0{\cdot}0058$) in both trials and for bulbs cured for 14 days and stored for 1 month ($P \leq 0{\cdot}0024$) or 2 months ($P < 0{\cdot}0001$) in both trials. A significant cultivar × curing temperature interaction was only observed for bulbs cured for 14 days and stored for 3 months in the second trial ($P < 0{\cdot}0001$).

Inoculation with Pantoea ananatis

Main effects of cultivar ($P < 0{\cdot}0001$), curing temperature ($P < 0{\cdot}0001$), curing duration ($P \leq 0{\cdot}014$) and storage duration ($P < 0{\cdot}0001$) were observed in both trials. Significant interactions of cultivar × curing temperature ($P \leq 0{\cdot}012$), cultivar × storage duration ($P \leq 0{\cdot}0006$), curing temperature × curing duration ($P \leq 0{\cdot}013$), curing temperature × storage duration ($P \leq 0{\cdot}014$) and curing duration × storage duration ($P \leq 0{\cdot}019$) were also observed in both trials. Significant three-way cultivar × curing temperature × curing duration ($P = 0{\cdot}011$) and curing temperature × curing duration × storage duration ($P = 0{\cdot}0093$) interactions were observed in the first trial.

A significant effect of cultivar was observed in both trials for onion bulbs cured for 2 ($P < 0{\cdot}0001$) and 14 days ($P \leq 0{\cdot}0005$) at all three storage durations (Table 4; Fig. 5). The main effect of curing temperature was significant for onion bulbs cured for 2 days and stored for 1 month ($P \leq 0{\cdot}0016$) or 2 months ($P \leq 0{\cdot}015$) in both trials and bulbs stored for 3 months in the first trial ($P = 0{\cdot}049$) but not the second trial ($P = 0{\cdot}086$). The effect of temperature was significant for bulbs cured for 14 days and stored for 1 month in the first trial ($P = 0{\cdot}0011$) and all three storage durations in the second trial ($P < 0{\cdot}0001$). A significant cultivar × temperature interaction was observed in the first trial for onion bulbs cured for 14 days and stored for 1 month.

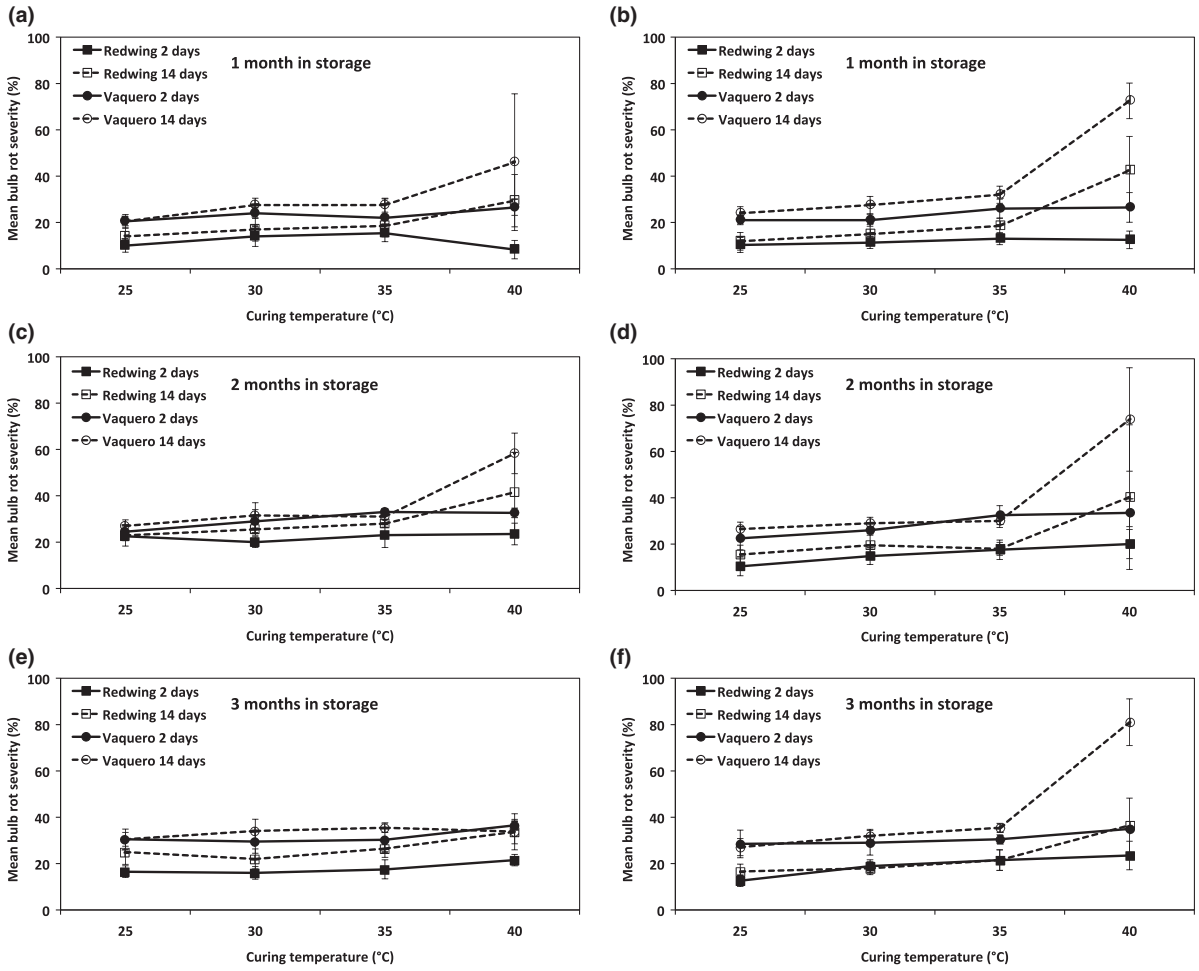

Figure 4 Mean severity of bulb rot (percentage of cut surface area of scales showing symptoms) in Redwing and Vaquero onion bulbs following inoculation with *Pantoea allii*, cured for 2 or 14 days at four temperatures, and stored for either 1 month (a) Trial 1 and (b) Trial 2; 2 months (c) Trial 1 and (d) Trial 2; or 3 months (e) Trial 1 and (f) Trial 2. Error bars indicate standard deviations of mean values.

No other significant interactions were observed ($P \geq 0{\cdot}056$).

Discussion

The curing process for onion bulbs dries down the neck and outer layers. This reduces the potential for pathogens to travel from the dry leaf tissue into the more moist bulbs and cause bulb rot. The tight outer wrapper of dry scales also helps to prevent moisture loss and, subsequently, shrinkage during storage. Because a single standard protocol detailing the curing process for all onions does not exist, individual growers will often follow a procedure that has worked well for them in previous years. However, as pathogen response to the various curing parameters may differ, the most effective way to prevent storage rot of onion may be to alter the curing process depending upon the pathogens present at the time of harvest. The process of curing involves a variety of parameters including the environmental conditions during harvest, the temperatures at which the bulbs are cured, and the length of time that the bulbs are cured. Because pathogen growth and disease progression can also be affected by these same parameters, determining the inter-relationship amongst them is critical to controlling the level of damage incurred in any given growing season. The present study examined the effects of two of these curing parameters, both temperature and duration, on the severity of rot resulting from infection by *P. agglomerans*, *P. ananatis* and *P. allii* in combination with the duration of storage on two different onion cultivars.

Overall, the effects of curing time and temperature on the development of disease caused by *Pantoea* spp. were significant in both cultivars tested. The severity of storage rot in both Vaquero and Redwing onion bulbs was lower when bulbs were cured at temperatures ≤35°C compared to bulbs cured at temperatures above this threshold. Additional factors, such as curing duration, also have an effect, although it was only significant when

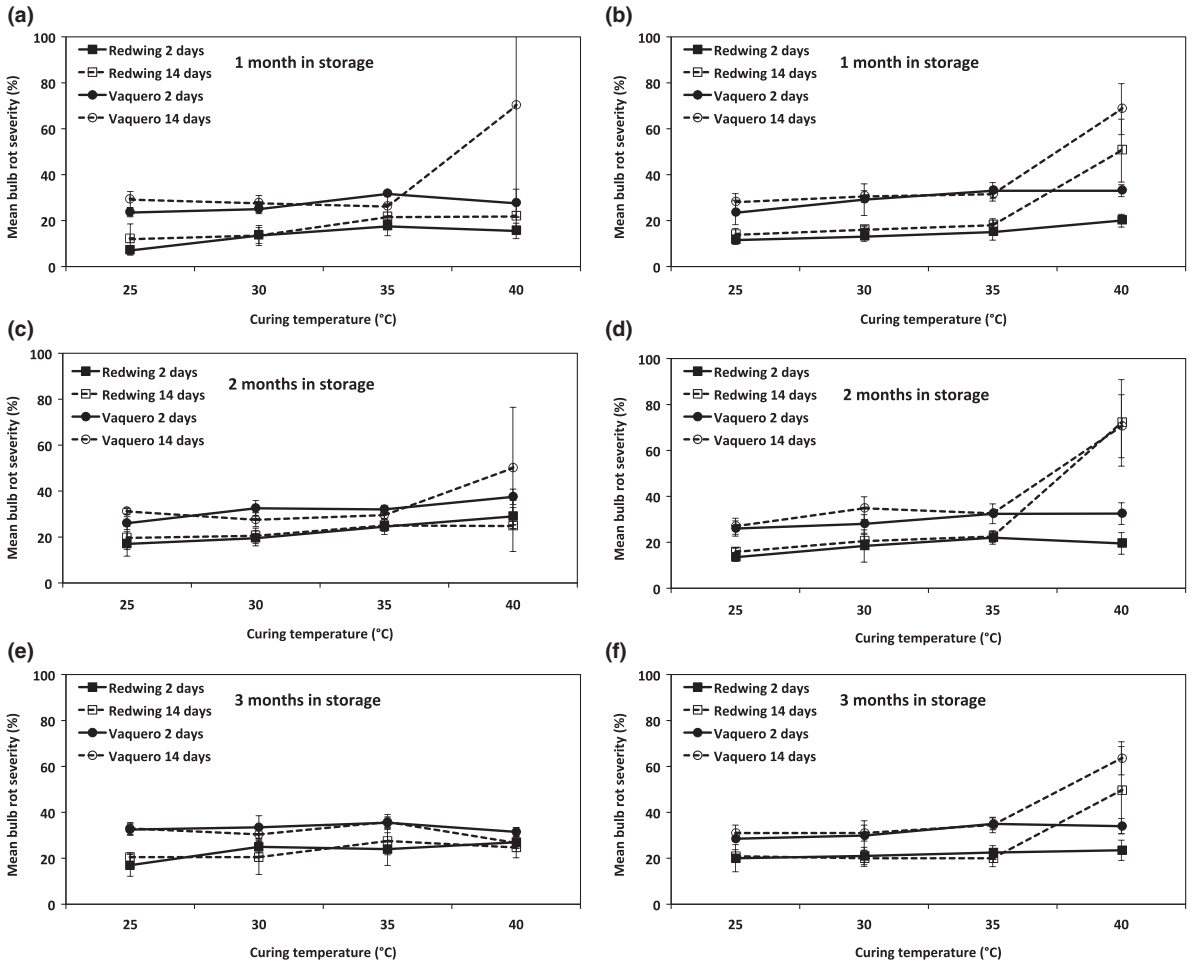

Figure 5 Mean severity of bulb rot (percentage of cut surface area of scales showing symptoms) in Redwing and Vaquero onion bulbs following inoculation with *Pantoea ananatis*, cured for 2 or 14 days at four temperatures, and stored for either 1 month (a) Trial 1 and (b) Trial 2; 2 months (c) Trial 1 and (d) Trial 2; or 3 months (e) Trial 1 and (f) Trial 2. Error bars indicate standard deviations of mean values.

bulbs were cured at 40°C. Under these conditions, shorter curing durations (2 vs 14 days) reduced the severity of rot seen in the bulbs. However, some caution must be used in interpreting the data involving the curing temperature of 40°C because of the greater variability observed in bulbs cured at this temperature compared to the other temperatures. For example, in the first trial a 40°C curing temperature for 14 days resulted in an increase in bulb rot in bulbs inoculated with *P. agglomerans* and stored for 1 month and bulbs inoculated with *P. allii* and stored for 1 or 2 months but not at all for *P. ananatis*. Taken together, these data suggest that conditions during curing are important factors in disease progression and that manipulating the conditions by reducing the temperature and shortening the duration used for curing can be a valid approach to controlling bulb rots caused by *Pantoea* species.

In addition to curing temperature and duration, the effects of storage duration on disease progression were also investigated. Interestingly, the severity of the disease did not increase significantly with increased time in storage for any of the *Pantoea* pathogens. There are several explanations for this, such as the possibility that the damage to the bulb is generated early in the disease process or that a critical mass of the pathogen was reached at an early stage in the infection process, thus the extent of the damage did not continue to progress past the initial 1 month time point. As with all diseases, symptoms eventually cease to advance, although the timing of this coincides more closely with bacterial diseases of onion caused by *B. cepacia* compared to *B. gladioli* (Schroeder *et al.*, 2012). Additional experiments beyond the scope of this study would be necessary to clarify the reasons behind these observations.

In contrast to investigations comparing different species of other bulb rot pathogens (Schroeder *et al.*, 2012), all three known storage rot pathogens of onion from the genus *Pantoea* appear to be equally pathogenic, based on the incidence and severity data in this study (Table 1). This implies that the factors tested here affect these

pathogens in a similar fashion and that parameters used for control of one member of the genus may also be effective for the other members. This is advantageous, considering that it may make identification of the *Pantoea* pathogens to a species level unnecessary for proper treatment to be implemented in a post-harvest situation.

Although not impervious to infection, the cultivar known as Redwing (a late maturing, firm, red globe onion) appears to have some resistance to bacterial pathogens. In previous studies with *Enterobacter* (Schroeder *et al.*, 2010), *Burkholderia* (Schroeder *et al.*, 2012) and *Xanthomonas* (Schwartz & Gent, 2011), this cultivar has also shown a lower degree of bulb rot severity, which indicates a superior resistance to known bacterial bulb rot pathogens. The present study on bulb rot caused by *Pantoea* was consistent with these previous findings because the mean bulb rot was found to be significantly reduced in Redwing compared to Vaquero for all of the *Pantoea* inoculation treatments in both trials. In addition, Redwing bulbs may have contained less natural infection than Vaquero bulbs, because the water-inoculated bulbs exhibited a reduced mean rot incidence and severity in both trials. Although many differences exist between Redwing and Vaquero, ascertaining the precise mechanisms behind this resistance may lead to more resilient onion cultivars in the future.

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