Review

Effect of storage conditions on bacterial loading of seed potato tubers

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Review Authors: [1]Elphinstone J.G., [2]Cahill G., [2]Davey T., [3]Harper G., [4]Humphris S., [2]Saddler G.S., [4]Toth I.K. and [5]Wale S.

[1]Food and Environment Research Agency (Fera), Sand Hutton, York, YO41 1LZ
[2]Science and Advice for Scottish Agriculture (SASA), Roddinglaw Road, Edinburgh, EH12 9FJ
[3]AHDB Potatoes, Sutton Bridge Crop Storage Research, Sutton Bridge, Spalding, PE12 9YD
[4]James Hutton Institute, Invergowrie, Dundee, DD2 5DA
[5]SAC Aberdeen Campus, Craibstone Estate, Aberdeen AB21 9YA

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INTRODUCTION

It has long been recognised that the risks of development of potato blackleg disease in the field and tuber soft rot during storage and transport are heavily influenced by the loading of pectolytic bacteria that inhabit vascular tissues, lenticels and wounds of the planted seed tubers or the harvested progeny tubers going into store (Perombelon and Kelman, 1980; Czajkowski *et al*., 2011). Control strategies have therefore been based on minimising bacterial loading on seed potato tubers. Current strategies start with pathogen-free pre-basic material and attempts to minimise contamination in the field through reliance on certification schemes during cycles of seed multiplication. However, the degree of control achieved is erratic and heavily dependent on the prevailing weather during growth of the seed crops. Depending on weather conditions, heavily contaminated seed can give rise to little or no disease, and the converse is also true. Higher reliance can be placed on the control of storage conditions with the aim to prevent bacterial multiplication and eventually reduce pathogen loading and the risk of soft rot during the storage period. Currently, reduction of bacterial contamination in stored seed potatoes involves a difficult balancing act of drying tubers into store, encouraging effective wound healing and skin setting to reduce infection points and then gradually reducing temperatures to minimise bacterial growth whilst maintaining appropriate ventilation to keep the tubers dry and uniformly aerated, whilst maintaining their quality and vitality.

The volume of research reported over the last 50 years, describing the various interacting factors which contribute to variation in bacterial loading on potato tubers, reflects the continued importance of the pectolytic bacteria as a constraint to seed potato production. It is the intention of this review to summarise and compare some of the key observations and conclusions of this research so that it can be interpreted and used to underpin, focus and optimise further combined efforts by researchers, agronomists and growers to refine best practice measures which, when properly integrated, are most likely to minimise accumulation of pectolytic bacteria during seed multiplication. The review considers:

1. The relationship between inoculum loading and risk of disease.

2. The likelihood of increasing inoculum loading during handling of harvested potatoes.
3. The environmental effects of temperature, humidity/water availability and oxygen/carbon dioxide levels on inoculum loading.
4. The importance of wound healing and curing in reducing potential infection sites.
5. The development of models that aim to predict the risk of disease from measurements of tuber inoculum loading.
6. The limited success of physical, chemical and biological control measures that have been previously investigated.
7. Potential novel control methods that may reduce inoculum loading in future.

Due to its ability to multiply at lower prevailing temperatures, *Pectobacterium atrosepticum* currently presents the highest risk to seed production under UK growing and storage conditions. However, as the taxonomy of the blackleg and soft rot-causing bacteria has become clarified, several distinct organisms within the genera *Pectobacterium* (Czajkowski *et al*., 2016a; van der Wolf *et al.,* 2017) and *Dickeya* (Toth *et al*., 2011; van der Wolf *et al*., 2014) are now known to also be able to contribute to disease. Interpretation of historical reports can be complicated where former nomenclature has been used. For example, bacteria formerly referred to as *Ewinia carotovora* can now be recognised as *Pectobacterium atrosepticum*, *P. carotovorum* subsp. *carotovorum*, *P. carotovorum* subsp. *oderiferum*, *P. carotovorum* subsp. *brasiliense*, or *P. wasabiae*. Similarly, bacteria formerly referred to as *Erwinia chrysanthemi* are now known to comprise at least six species of the new genus *Dickeya*, including *D. dianthicola* and *D. solani* which have been found causing disease on European seed potatoes. Bacteria in all these taxa can cause potato soft rot and blackleg disease and/or stem soft rot in the field, although the influence of environmental conditions on their growth and pathogenicity can vary. In particular, *P. wasabiae* has clearly been historically misidentified as *P. carotovorum* in some reports. Furthermore, the optimum conditions for pathogenicity of the more recently discovered *P. carotovorum* subsp. *brasilense*, which is emerging as an aggressive pathogen in some European countries, are not yet fully understood. During the preparation of this review, the currently accepted names (where known) have been substituted for those given in the original reports. However, some degree of caution

has been necessary when interpreting the detail of some of those reports where the true identity of the pathogens involved remains ambiguous.

Effect of inoculum concentration on soft rot and blackleg development

Bacterial soft rot potential of potato tubers is related to the inoculum loading (Bartz and Kelman, 1984). Similarly, blackleg incidence is known to be related to seed contamination level, the minimum threshold level for disease development has been suggested at around 10^3 viable cells of *P. atrosepticum* per tuber (Pérombelon, 2000), although the accuracy of measurement of inoculum loading can vary with the method used. Low inoculum levels can still lead to high disease incidence under persistent disease-conducive conditions (van der Wolf *et al*., 2017). In general, the higher the bacterial density on seed potatoes, the more likely the pathogen will predominate at the infection site and the sooner rotting is initiated (Bain *et al*., 1990).

Spread of bacteria during handling

Monitoring tuber contamination during seed multiplication in Scotland showed that farms which regularly applied hygienic measures consistently produced healthier seed than the others (Pérombelon *et al*., 1980). Similar results were recently obtained in Scotland under AHDB project 114R475. Washing and disinfection of machines used when planting, spraying, haulm flailing, harvesting and grading in store are therefore recommended to reduce risks of introducing soft rot bacteria in a pathogen-free crop (Pérombelon & Kelman, 1980; Elphinstone and Pérombelon, 1986; Pérombelon, 2002). Removal of rotten tubers during harvesting and grading is recommended to reduce spreading and smearing of the bacteria in a seed lot, although it is very difficult to achieve in practice since smearing is likely already to have occurred during the process. Avoidance of wounding by correct machinery adjustment during harvesting and grading is important to reduce the risks of wounding, as bacteria can multiply at wound sites and survive after wound healing (Pérombelon, 1992; van Vuurde & de Vries, 1994). Use of mature tubers with a well-developed periderm also reduces risks of wounding.

Cardinal temperatures and pathogenicity

The occurrence of disease and the scale of the damage are both temperature dependent (Smadja *et al*., 2004). Disease development starts with multiplication of the bacteria in the infection area (e.g. lenticels, wounds or vascular tissues) followed by production of numerous extracellular lytic (pectolytic) enzymes that degrade plant cells, causing tissue rotting. Enzyme secretion only initiates when the bacteria reach critical populations (10^7–10^8 cells/g of tissue), under a quorum sensing control mechanism that involves build-up of signal molecules (homoserine lactones) (Kotoujansky, 1987; Pérombelon, 2002; Pirhonen *et al*. 1993; Barras *et al.,* 1994; Pérombelon and Salmond, 1995; Salmond *et al*., 1995; Byers *et al*. 2002). Smadja *et al.* (2004) showed that the effects of temperature on both growth in minimal media and *in vitro* pectate lyase activities differed for *P. atrosepticum* and *P. carotovorum* subsp. *carotovorum*. The optimal temperature for pathogenicity was found to be a compromise between the optimal growth temperature and the temperature at which maximum expression of pectate lyase occurred. The optimal temperature for pathogenicity is estimated to be between 15-20°C for *P. atrosepticum* and around 25°C for *P. carotovorum* (Pérombelon and Salmond, 1995; Priou and Jouan 1996). At low temperatures, growth is much slower and only apparent when measured over several weeks; isolates of *P. atrosepticum* were still able to grow (and therefore survive) at temperatures as low as 1°C, whereas *P. carotovorum* isolates did not grow below 8°C (Smadja *et al*., 2004). Bacterial soft rot has been observed to occur very slowly in inoculated potato tubers at storage temperatures of less than 10°C (Kushalappa and Zulfiqar, 2001; Wicks *et al*., 2017).

Du Raan *et al*. (2016) recently compared cardinal (minimum, optimum and maximum) growth temperatures for a selection of isolates of *P. atrosepticum*, *P. carotovorum* subsp. *carotovorum*, *P. carotovorum* subsp. *brasiliense*, *P. wasabiae*, *D. dadantii* and *D. solani*. *In vitro* growth was recorded in nutrient broth over a period of only 24 hours, or 48 hours for the slower growing *P. atrosepticum* (Table 1).

Table 1: Cardinal temperatures determined by monitoring *in vitro* growth in nutrient broth over 24-48 hours (Du Raan *et al*., 2016).

Bacterium	**Minimum temperatures (°C)[1]**	**Optimum temperatures (°C)[2]**	**Maximum temperatures (°C)[1]**
Pectobacterium atrosepticum	18.1-20.0	25.7-26.7	30.8-31.2
P. carotovorum subsp. *carotovorum*	20.7-21.6	30.5-33.6	35.7-39.9
P. carotovorum subsp. *brasiliense*	20.2-20.9	30.8-32.1	37.8-38.6
P. wasabiae[3]	20.6	30.2	33.8
Dickeya dadantii	20.1-23.8	30.7-34.4	36.5-38.8
D. solani[3]	23.8	35.3	42.0

[1]No growth after 24 hours, or after 48 hours for *P. atrosepticum.*

[2]Maximum growth after 24 hours, or after 48 hours for *P. atrosepticum.*

[3]Only one isolate tested.

In the case of mixed infections with different soft rot bacteria, the temperature also determines which pathogen predominates at the infection site (Pérombelon, 2002). In rotting tubers, *P. atrosepticum* predominates at temperatures <22°C, whereas at higher temperatures, *P. carotovorum* predominates. Only in a narrow temperature range close to 22°C do both bacteria subspecies develop at equal rates (Pérombelon *et al*., 1979; Pérombelon and Kelman, 1980). In storage conditions, *P. atrosepticum* will therefore predominate in rots developing in tubers with mixed infections. In the field in Scotland, where soil temperatures averaged 17-20°C, *P. atrosepticum* and *P. carotovorum* were detected equally in rotting mother tubers (Perombelon *et al*., 1987). When the same seed stocks were grown under Israeli field conditions with soil temperatures >20°C, *P. carotovorum* predominated.

D. solani can grow at temperatures as high as 39°C and is more aggressive than *D. dianthicola* at higher temperatures, as determined by greenhouse studies and experiments in high-temperature regions in Israel (Laurila *et al*., 2008; Sławiak *et al*., 2009b; Tsror *et al*., 2009). Both *D. solani* and *P. atrosepticum* were more aggressive than isolates of *D. dianthicola* on tubers and stems at 22–23°C (Laurila et al., 2008). In Israel, *D. solani* was highly aggressive in potato stem assays at day-time temperatures of 28–30°C, whilst *P. atrosepticum* failed to cause disease under these

conditions (Tsror *et al*., 2009). Similarly, in Spain, *D. solani* was more aggressive in *in vitro* stem tests than *D. dianthicola* at 28°C (Palacio-Bielsa *et al.*, 2006). In recent studies conducted under AHDB project R475, *D. dianthicola* strains showed variable aggressiveness with optimal temperatures between 21 and 27°C. The least aggressive of these were similar in aggressiveness to isolates of *P. atrosepticum* on tubers, and had similar optimal temperatures of around 21°C. However, aggressiveness on stems at 21- 27°C, appeared to be greater for *D. dianthicola* isolates than for *P. atrosepticum*. At 27°C, both *D. dianthicola* and *D. solani* isolates were more aggressive than *P. atrosepticum*.

The traditional method to conserve seed tubers in good health has been storage in ventilated stores at low temperatures (c. 3-5°C). Historically, these conditions have been achieved in stores by regulating temperatures by ventilating with cold outside air over the winter. However, recent warmer and wetter winter conditions have presented difficulties in maintaining the consistent levels of low temperature and appropriate humidity. Thus increasingly, the use of effective systems for refrigeration have been installed. Sometimes the refrigeration system has been linked to positive ventilation. Although most storage facilities are equipped with systems to control average temperature, RH and ventilation, dead air pockets in the pile or boxes can still cause local increases in temperature, induce condensation on tubers (Pringle, 1996) and result in unfavourable changes in oxygen concentration or other factors related to proliferation of soft rot (De Boer & Kelman, 1978).

Effect of humidity, oxygen level and drying on bacterial loading

It is known that the presence of a water film on the tuber surface induces development of anaerobic conditions in potato tubers, thereby favouring bacterial multiplication and initiation of rotting (Pérombelon *et al*., 1989b). If tubers remain wet long enough, tuber decay can ensue, resulting in further spread of the bacteria when tubers are handled, and sometimes massive tuber decay resulting in wastage of whole stocks (Pérombelon, 2000). Tubers covered by a thin but persistent film (only 30 µm average depth) of water become anaerobic after 6 hours at 10°C or 2.5 hours at 21°C (Burton and Wiggington, 1970). Under such conditions, pectolytic bacteria (present in suberised lenticels of most potato stocks) actively multiply and can induce soft rot.

Such water films can result from a number of causes (Pringle, 1996; Pringle and Robinson, 1996):

- Inadequate drying on entry of wet tubers into store or after washing.
- Insufficient ventilation leading to condensation (e.g. in plastic bags).
- Ineffective ventilation leading to differential temperatures and condensation in stored potatoes.
- Storing in an environment which is warmer than the tubers.

The bacteria are facultative anaerobes and therefore have a competitive advantage at low oxygen concentration over obligate aerobic microorganisms which may be present at infection sites. Furthermore, anaerobiosis affects oxygen-dependent host resistance, allowing unhindered bacterial multiplication and production of pectolytic cell wall-degrading enzymes, resulting in a rotting lesion (Fuqua *et al*., 2001). The water film also facilitates mobility of the bacteria, aiding colonisation of infection sites.

Growing of potatoes in poorly drained or waterlogged soils therefore encourages rotting of the seed tubers and development of blackleg. It is also critical to avoid harvesting in wet conditions or exposing harvested tubers to rain or high volume chemical sprays. Washing of ware potatoes also significantly increases the risk of multiplication and spread of soft rot bacteria. Bartz and Kelman (1985) found that disease severity decreased when inoculated tubers were air-dried immediately. It is therefore essential, prior to reducing temperatures to the holding temperature of seed, to dry harvested tubers rapidly by ventilation. In practice, this means the early storage period will often involve maintaining tuber temperature close to that on arrival in store to facilitate a period of curing or wound healing, after which temperatures are reduced. Both drying and cooling avoids increasing the tuber inoculum load and reduces the risk of tuber decay and blackleg in the subsequent crop (Wale & Robinson, 1986; Wale *et al*., 1986). Seed growers have adopted systems of passive and positive ventilation to dry tubers on entry into store and limit bacterial multiplication (Clayton & Cunnington, 1996). Effective ventilation during storage, besides ensuring that the crop temperature is relatively even, and condensation is prevented, also ensures that pockets of CO_2 build-up are prevented. Reduced oxygen concentration can increase susceptibility to bacterial soft rot (Boer & Kelman, 1978).

Effect of curing conditions on bacterial loading and soft rot development

Given the importance of wounds as infection sites, and temperature and humidity as key factors affecting bacterial loading and soft rot development, the regime used to cure potatoes entering stores needs to ensure a compromise between achieving rapid wound healing and limiting multiplication of soft rot bacteria. For example, experimental studies by Knowles *et al*. (1982) concluded that wound-healing at approximately 9°C for 25 days was the best compromise for keeping rot progress and weight loss at a minimum and minimising aging and sugar accumulation, while allowing the process of healing and skin maturation to occur. Webber (1988) showed that tuber susceptibility to soft rot caused by *P. atrosepticum* decreased after a 24-hour wound healing period. Wound healing was inhibited at temperatures below 10°C, reduced oxygen levels below 5% and increased CO_2 levels above 20%. Moisture loss in stored tubers also reduced their susceptibility to soft rot compared with freshly harvested tubers. Soft rot initiation was best prevented by removal of water, storage at 15°C, maintaining oxygen levels above 10% and avoiding CO_2 accumulation above 20%. Workman *et al.* (1976) showed that storage at 0°C rather than 5°C and increasing concentration of CO_2 (up to 8%) increased sucrose content and membrane permeability of potato tubers, which was highly correlated with an increased rate of decay caused by *P. atrosepticum*.

Modelling the effects of storage conditions on bacterial loading and soft rot development

Kushalappa and Zulfiqar (2001) modelled the effects of time of tuber wetness and temperature on tuber soft rot initiation, and the effects of storage time and temperature on the severity of soft rot which developed following wound inoculation of the cv Russet Burbank with *P. carotovorum*. They concluded that soft rot did not develop at temperatures between 10-25°C during the first 6 hours of tuber wetness. Soft rot started to develop when inoculated tubers remained wet for more than 6 hours and infection rates increased with temperature and time of wetness, reaching a maximum at 48 hrs wetness at temperatures above 15°C. Following infection, lesion development (disease severity) increased over time at a constant 95% RH depending on the temperature. Increasing storage temperature levels >12 °C increased disease progress over storage time, with a particularly dramatic increase in disease severity

observed after 60 days at 16 °C. Cubic regression models were developed which accounted for 95 and 96% of the variations in disease initiation and subsequent severity, respectively (Fig. 1). These studies were conducted with a single isolate of *P. carotovorum* and did not consider the effect of initial inoculum concentration.

Equation 1: Cubic regression model to predict infection potential (**X**) in potatoes wound-inoculated with *P. carotovorum* as a function of wet incubation time (**W** hr) and incubation temperature (**T** °C) where **X** is estimated in terms of the % of the maximum observed disease severity under optimum conditions:

$$\mathbf{X} = 0.013497 + (0.024845\mathbf{W^2}) - (0.005072\mathbf{TW^2}) + (0.000321\mathbf{T^2W^2}) - (0.000449\mathbf{W^3}) + (0.000091544\mathbf{TW^3}) - (0.000005773\mathbf{T^2W^3}) + (0.000004289\mathbf{T^3W}) - (0.000006375\mathbf{T^3W^2}) + (0.000000113\mathbf{T^3W^3})$$

Equation 2: Cubic regression model to predict disease severity potential (**Y**) in potatoes wound-inoculated with *P. carotovorum* as a function of storage temperature (**T** °C) and storage time (**S** days) where **Y** is estimated in terms of the % of the maximum observed disease severity under optimum conditions:

$$\mathbf{Y} = 0.121659 + (0.000199\mathbf{TS}) - (0.000000871\mathbf{S^3}) + (0.000000394\mathbf{TS^3}) - (0.00000005172628\mathbf{T^2S^3}) + (0.0000000021073839\mathbf{T^3S^3})$$

Figure 1 (above): Regression models developed by Kushalappa and Zulfiqar (2001) to predict the effects of temperature and tuber wetting period on initial soft rot development (Equation 1) and the effects of temperature and storage period on soft rot severity following disease initiation (Equation 2).

Moh *et al*. (2012) developed quadratic polynomial models to predict the combined effects of temperature and relative humidity on *P. atrosepticum* and *P. carotovorum* population densities and soft rot disease development at the surface of wounded tubers (Fig. 2). Experiments were conducted at three temperatures (10, 15 and 20 °C), three RH levels (86, 96 and 100%) and three initial inoculum concentrations (10^5, 10^7 and 10^9 CFU per ml). For both bacterial species, all three variables contributed independently to the population densities and soft rot disease that developed at the surface of wounded tubers in the 72 hrs after inoculation, with temperature having the greatest influence. The highest percentage of potato tuber tissue diseased by *P. atrosepticum* and *P. carotovorum* was observed at the highest temperature (20°C) and relative humidity (100%), independent of the initial bacterial inoculum concentration. The models indicated that 99.06% of the observed variation in *P. atrosepticum* population density and 99.40% of the observed variation in *P. carotovorum* populations could be accounted for by the three variables. In terms of severity of soft

rot development, the three variables accounted for 97.63 and 97.00% of the observed variation for *P. atrosepticum* and *P. carotovorum* respectively.

Equation 1: Quadratic regression model to predict the population density ($\mathbf{X_1}$ Log CFU cm^{-2}) of *P. atrosepticum* at the surface of wounded potato tubers as a function of temperature (**T**), relative humidity (**H**) and the initial inoculum concentration (**C**), each as coded variables of –1, 0 or +1 (for low, medium and high values of each respectively).

$$\mathbf{X_1} = 7.438 + (0.856\mathbf{T}) + (0.088\mathbf{H}) + (2.036\mathbf{C}) – (0.675\mathbf{T^2}) – (0.001\mathbf{H^2}) – (0.045\mathbf{C^2}) + (0.012\mathbf{TH}) + (0.076\mathbf{TC}) + (0.07\mathbf{HC})$$

Equation 2: Quadratic regression model to predict the population density ($\mathbf{X_2}$ Log CFU cm^{-2}) of *P. carotovorum* at the surface of wounded potato tubers as a function of temperature (**T**), relative humidity (**H**) and the initial inoculum concentration (**C**), each as coded variables of –1, 0 or +1 (for low, medium and high values of each respectively).

$$\mathbf{X_2} = 6.555 + (0.379\mathbf{T}) – (0.063\mathbf{H}) + (1.989\mathbf{C}) – (0.465\mathbf{T^2}) + (0.019\mathbf{H^2}) + (0.166\mathbf{C^2}) + (0.030\mathbf{TH}) + (0.033\mathbf{TC}) + (0.019\mathbf{HC})$$

Equation 3: Quadratic regression model to predict tuber soft rot development ($\mathbf{Y_1}$) by *P. atrosepticum* at the surface of wounded potato tubers as a function of temperature (**T**), relative humidity (**H**) and the initial inoculum concentration (**C**), each as coded variables of –1, 0 or +1 (for low, medium and high values of each respectively).

$$\mathbf{Y_1} = 11.814 + (9.721\mathbf{T}) + (1.225\mathbf{H}) + (12.386\mathbf{C}) + (1.261\mathbf{T^2}) + (1.715\mathbf{H^2}) + (6.944\mathbf{C^2}) + (1.276\mathbf{TH}) + (7.955\mathbf{TC}) + (0.137\mathbf{HC})$$

Equation 4: Quadratic regression model to predict tuber soft rot development (Y1) by *P. carotovorum* at the surface of wounded potato tubers as a function of temperature (T), relative humidity (H) and the initial inoculum concentration (C), each as coded variables of –1, 0 or +1 (for low, medium and high values of each respectively).

$$\mathbf{Y_2} = 9.272 + (8.109\mathbf{T}) + (1.095\mathbf{H}) + (10.609\mathbf{C}) + (0.717\mathbf{T^2}) + (1.794\mathbf{H^2}) + (1.35\mathbf{C^2}) + (0.102\mathbf{TH}) + (6.869\mathbf{TC}) + (0.686\mathbf{HC})$$

Figure 2 (above): Regression models developed by Moh *et al*. (2012) to predict the effects of temperature, relative humidity and the initial inoculum loading on population density of *P. atrosepticum* and *P. carotovorum* and tuber soft rot development at the surface of wound-inoculated tubers

Moh *et al*. (2011) also previously modelled the combined effect of temperature and water activity on the *in vitro* growth of *P. atrosepticum*, *P. carotovorum* and *Dickeya* sp. They showed that the maximum specific growth rate of isolates of all three soft rot bacteria were positively influenced by both temperature (over the range 10, 15 or 20 °C) and water activity (at 0.960, 0.980 or 0.997).

***In vivo* evaluations of the effect of storage on bacterial contamination of tubers**

Monitoring of contamination by *Pectobacterium* sp. in commercial seed stocks in a range of stores over different seasons has generally concluded that under good storage conditions contamination will fall over a typical storage period of six months. Markos (1988) monitored a heavily contaminated stock of cv. Desiree stored in either a commercial ambient store or experimental ambient store (with or without positive ventilation) over a six-month period. Despite a short period of condensation recorded during one period over winter, the contamination fell from an average of 10^5 to around $10^{3.9}$ *Pectobacterium* sp./tuber in each store. When samples were subsequently placed in small trays for chitting for nearly 7 weeks the contamination fell further to around 10^2 *Pectobacterium* sp./tuber.

Pringle *et al*. (1991) stored a heavily contaminated stock of cv. Desiree in four different ambient farm stores and monitored tuber contamination over a four-month storage period. In general, contamination fell rapidly immediately after harvest, although the extent of reduction varied between stores. The greatest early reduction was from $10^{5.5}$ to $10^{2.1}$ *Pectobacterium* sp./tuber after one-month storage. In all stores contamination rose later in storage, in one store almost back to the original level of contamination. There was no clear relationship between store temperature, RH and changes in contamination. However, there was some evidence that reduction in contamination was related to duration of ventilation and rise in contamination to onset of sprouting.

Monitoring of tuber contamination was carried out by Pringle & Robinson (1996) in three stocks of cv. Record in a commercial box store where different ventilation treatments were applied. *P. atrosepticum* and *P. carotovorum* contamination declined in all stocks and ventilation treatments but the fastest decline was with forced air (positive) ventilation. This ventilation treatment also resulted in the fewest condensation events. This paper summarises comparative rates of *P. carotovorum* decline during storage from a range of experiments. These range from $10^{0.98}$ to $10^{1.96}$ per 100 days storage.

Efficacy of control measures aimed at reducing bacterial loading

Control measures for *Pectobacterium* and *Dickeya* sp. were reviewed by Czajkowsky *et al.* (2011). Physical, chemical and biological factors that can affect bacterial loading on potato tubers were described:

Physical seed tuber treatments

The first trials on hot water treatment of potato tubers to control soft rot bacteria contamination (Mackay & Shipton, 1983) showed that viable *P. atrospeticum* and *P. carotovorum* were no longer detected in tuber peel after dipping naturally infected potato tubers for 10 min in water at 55°C. No blackleg was observed in plants grown from the treated tubers. Similar results were obtained by Wale & Robinson (1986), Robinson & Foster (1987) and Shirsat et al. (1991), where incubation in water at 44·5°C for 30 min or at 56°C for 5 min significantly reduced periderm and lenticel contamination of seed potatoes and consequently blackleg incidence in the field. Steam was also tested as an alternative to hot water treatment to remove fungi and bacteria, especially *P. carotovorum* and *P. atrosepticum* present superficially in the tuber periderm. The use of steam treatment reduced infestation of tuber periderm from 26–59% to 1–3% (Afek & Orenstein, 2002).

Thorough drying of tubers after hot water treatments is critical to avoid anaerobic conditions and post-treatment multiplication of residual bacteria. Pérombelon *et al*., (1989a) followed hot water treatment in which 50 kg batches were continuously treated for 5 min at 55°C with drying under forced ventilation with air knives. Blackleg control was observed in field experiments after hot water treatment of both vacuum-infiltrated and naturally contaminated seed tubers. The treatment also reduced several fungal pathogens causing gangrene, skin spot, silver scurf and black scurf (Dashwood et al., 1991). Bartz & Kelman (1985) reported elimination of external but not internal populations of *Pectobacterium* spp. from washed tubers by application of hot dry air at 50°C.

Continuous UV radiation was demonstrated to eliminate *P. carotovorum* when vacuum infiltrated tubers were treated with a low dose (15 kJ m^{-2}) 6 hours later (Ranganna *et al*., 1997). Rocha *et al*. (2015) applied UV-C light (254 nm) to *in vitro* cultures of *P. carotovorum* subsp. *carotovorum* and *in vivo* to *P. carotovorum* subsp. *carotovorum* inoculated potato tubers of cvs. Agata and Monalisa. In an additional treatment, tubers

were also exposed with and without fluorescent light. A dose of 34.5 kJ m^{-2} radiation completely inhibited *in vitro* bacterial development. UV-C and fluorescent light or fluorescent light alone were more effective at controlling disease development than the control. UV-C and dark treatment was intermediate, demonstrating some effect for this treatment alone. The authors attributed the best protective effect to an increase in glycoalkaloid concentration induced by exposure to fluorescent light. Pulsed treatment can deliver short durations of very high intensity UV light and this method is currently being investigated for efficacy.

Physical control has potential advantages over biological and chemical methods as it does not require registration and may be effective against a broad range of pathogens. However, physical procedures affect not only superficially located pathogens but also beneficial microorganisms and may negatively influence tuber emergence and quality. Heat treatments are expensive and difficult to standardise and so have not been adopted commercially. The temperature/time combination is critical and may vary from variety to variety and with initial inoculum loading. Overheating can cause delayed sprouting of seed tubers or even tuber death (Robinson & Foster, 1987; Pérombelon *et al*., 1989a). Hot air is beneficial because it dries the tubers and stimulates wound healing but heat transmission by air is less effective than by water, requiring longer treatment times, which could adversely affect tuber physiology.

Chemical seed treatments

No control measures are effective once rotting has initiated. A wide range of chemical compounds has been evaluated over the years with the aim to reduce surface contamination or prevent multiplication of internal latent infections by *Pectobacterium* spp. and *Dickeya* spp. Early attempts using antibiotics, such as streptomycin and oxytetracycline hypochloride or streptomycin and mercury (Bonde and de Souza, 1954), kasugamycin or virginiamycin (Wyatt & Lund, 1981; Bartz, 1999) provided some control against blackleg and soft rot but are not approved because of the risks of antibiotic resistance development. Some organic compounds, including hydroxyquinoline (Harris, 1979), 5-nitro-8-hydroxyquinoline bromopol (2-bromo-2-nitropropane-1,3-diol) and 7-chloro-1-methyl-6-fluoro-1,4-dihydro-4-oxo-3-quinolinic carboxylic acid (Bartz & Kelman, 1986), reduced development of soft rot in wounded potato tubers. Immersion of potato tubers in citric, acetic, ascorbic or malonic acids

also reduced rotting by *P. carotovorum* in freshly vacuum-infiltrated potato tubers without affecting sprouting. (Bartz & Kelman, 1986). Mills et al. (2006) showed that a range of inorganic and organic salts, including aluminium acetate, sodium metabisulphate, propyl paraben, sodium benzoate, alum (hydrated potassium aluminium sulphate), potassium sorbate, calcium propionate, sodium hypochloride, sodium bicarbonate, aluminium chloride and copper sulphate, inhibit *in vitro* growth of *P. carotovorum* and *P. atrosepticum* due to their effects on pH or cell membrane protein function. Promising protection against tuber tissue rotting has also been demonstrated using synthetic antimicrobial peptides (Kamysz *et al*., 2005). The disinfectant peracetic acid was tested as a seed tuber dip and low volume seed tuber treatment over a number of years for the reduction of blackleg in the subsequent crop. No consistent reduction was recorded (Wale, S., personal communication).

Despite repeated experimental activity in this area, there has been no approval of chemical bactericides for specific reduction of bacterial loading on potato tubers. Apart from the high registration costs for a limited market and the risks of phytotoxicity, a key factor limiting development of chemical controls is the difficulty of targeting bacteria that are well protected in suberized lenticels and wounds or in the tuber vascular tissues.

Biological control

Several potential biological control agents have been shown to suppress growth of *Pectobacterium* and/or *Dickeya in vitro* or on tuber tissue during laboratory experiments. These include *Pseudomonas* spp. (Kloepper, 1983; Colyer & Mount, 1984; Cronin *et al*., 1997; Kastelein *et al*., 1999), lactic acid bacteria (Trias *et al.,* 2008), biosurfactant-producing strains of *Bacillus thuringiensis*, *B. cereus*, *B. subtilis*, *B. licheniformis, B. megaterium* and *B. pumilus* (Sharga & Lyon, 1998; Cladera-Olivera *et al*., 2006; Issazadeh et al., 2012), *Methylobacterium* sp. (Ardanov *et al.,* 2012), antibiotic-producing *Streptomyces* sp. (Baz *et al*., 2012; Park *et al*., 2012; Balaraju *et al*., 2016), and an antibiotic- and surfactant-producing strain of *Serratia plymuthica* which inhibits blackleg and colonised potato tissue, even at low temperature and in aerobic or anaerobic conditions (Czajkowski *et al*., 2012). Several specific bacteria have been identified that quench quorum sensing signal molecules (N-acylhomoserine lactones), preventing production of pectinolytic enzymes by *Pectobacterium* and

Dickeya spp. in potato tissues. These include species of *Agrobacterium*, *Arthrobacter*, *Bacillus*, *Comamonas*, *Delftia*, *Lysinibacillus*, *Mesorhizobium*, *Ochrobactrum*, *Pseudomonas*, *Rhodococcus*, *Streptomyces* and *Variovorax* (Uroz, 2003; Jafra *et al.,* 2006a and 2006b; Mahmoudi *et al.,* 2011; Crepin *et al.,* 2012a and 2012b; Chankhamhaengdecha *et al.,* 2013; Garge & Nerurkar, 2016).

A further focus of recent biocontrol research has been on the identification and use of lytic bacteriophage, naturally-occurring viruses that specifically infect and lyse cells of *Pectobacterium* and *Dickeya* spp. (Czajkowski, 2016; des Essarts *et al*., 2016). Successful phage therapy has been demonstrated experimentally with lytic bacteriophages for prevention of potato tuber decay against *P. carotovorum* subsp. *carotovorum* (Eayre *et al*., 1995), *P. atrosepticum* (Balogh *et al*., 2010) and *D. solani* (Czajkowski, 2016), and control of *P. carotovorum* subsp. *carotovorum* infections in calla lily (Ravensdale *et al*., 2007). Although they are widely dispersed in the environment, only a small number of phage with activities against *Pectobacterium* and *Dickeya* spp. have been fully characterised (Adriaenssens *et al*., 2012a and 2012b; Korol and Tovkach, 2012; Lee *et al*., 2012a and 2012b; Lim *et al*., 2013, 2014 and 2015; Czajkowski *et al*., 2014; Czajkowski *et al*., 2015; Kalischuk *et al*., 2015; Hirata *et al*., 2016; Blower *et al*., 2017). Because of the high strain specificity of individual phages and the speed at which their target bacteria can acquire resistance, a cocktail of different phage isolates is usually required, and the degree of control can be variable depending on the genetic variation and phage resistance in the bacterial populations present in specific environments. A phage treatment, Biolyse® (APS Biocontrol Ltd., Dundee), is currently commercially available for use as a processing aid for potatoes and other fresh produce. The phage suspension is applied as a mist post-washing to reduce the risk of soft rots in packed produce and the related cost of rejections of consignments during distribution and retail. The effect of applying similar phage cocktails to seed potatoes prior to planting as a possible control for blackleg disease is currently being investigated.

CONCLUSIONS

1. In general, the higher the bacterial density on seed potatoes, the more likely the pathogen will predominate at infection sites and the sooner disease is initiated. Pathogen populations need to multiply at an infection site to reach a threshold

population before pectolytic enzymes and other pathogenicity factors are produced and disease symptoms can develop.

2. Infection sites on potato tubers include lenticels, vascular tissues and wounds. Handling of potato tubers creates wounds and efficiently spreads large numbers of bacteria from a low number of rotting tubers to fresh wounds on a high number of healthy tubers.
3. Multiplication and motility of the bacteria at the infection sites requires free moisture and so efficient drying of tubers after handling and on entry to the store is fundamental to preventing inoculum build-up.
4. Efficient curing of tubers allows rapid wound healing and skin setting and helps to reduce the number of potential infection sites. However, care is needed so that curing conditions do not also favour rapid bacterial multiplication. Curing conditions of 9°C for 25 days were proposed in one study for minimising bacterial multiplication, tuber weight loss, sugar accumulation and ageing while still allowing wound healing and skin maturation. However, optimum conditions are likely to vary with the variety of potato, the predominant pectolytic bacteria present and the type of store/ventilation used.
5. The rate of pathogen multiplication at infection sites is affected by temperature, humidity and oxygen potential. Careful control of all three parameters during the whole storage period should result in a decrease in tuber inoculum loading.
6. Different taxa of pectolytic bacteria have different minimum, optimum and maximum growth temperatures. The optimum temperature for pathogenicity is a compromise between the optimum growth temperature and the temperature at which the maximum expression of pectolytic enzymes occurs. For *P. atrosepticum* this is estimated between 15-20°C and for *P. carotovorum* around 25°C. Recently calculated minimal cardinal growth temperatures for the various pectolytic taxa suggest that no detectable growth occurs for any of the taxa over 48 hours at temperatures below 18°C. However, over longer periods, *P. atrosepticum* has been shown to multiply (and therefore survive) at temperatures above 1°C and *P. carotovorum* at temperatures above 8°C. For other taxa of pectolytic bacteria the optimal temperatures for pathogenicity and minimal growth temperatures are less fully understood.
7. A film of water on the tuber surface induces anaerobic conditions after 6 hours at 10°C or 2.5 hours at 21°C. Anaerobic conditions favour multiplication of the

pectolytic bacteria and inhibit oxygen-dependent resistance mechanisms in the potato tissues. Tuber respiration in poorly ventilated pockets of a store can also result in low oxygen/high carbon dioxide conditions and favour pathogen multiplication.

8. Different statistical models have been developed to confirm that temperature, relative humidity time of tuber wetness and initial inoculum loading contribute independently to observed variation in pathogen multiplication at infection sites and soft rot development and severity over the storage period.
9. The efficacy of physical control measures, including heat and ultra-violet (UV)/fluorescent light treatments, has been evaluated. Heating tubers in hot water (44.5°C for 5 min. to 55°C for 30 min.), steam or hot air (50°C) effectively reduced inoculum loading in several trials but was expensive to apply, difficult to standardise across varieties and had a high risk of negatively affecting tuber physiology and quality. Some success in trials with UV radiation and fluorescent light treatments, attributed to an increase in glycoalkaloid levels and associated increased resistance to the bacteria, justifies further evaluation.
10. A wide variety of chemical treatments has been evaluated, including organic and inorganic acids and salts, synthetic antimicrobial peptides and common disinfectants, with the aim to reduce tuber inoculum loading. No chemical control was found effective once rotting initiated and none have been approved for reduction of bacterial loading on potato tubers. A main difficulty is targeting bacteria that are protected in suberized lenticels and wounds or in the vascular tissues.
11. Several potential biological control agents have been identified that either suppress growth of *Pectobacterium* and/or *Dickeya* spp. in laboratory experiments or that quench quorum sensing signals and prevent production of pectolytic enzymes in infected potato tissues. As yet, none of these potential biocontrol agents has been developed commercially. A more promising approach involves the use of lytic bacteriophage, naturally occurring viruses that specifically infect and kill *Pectobacterium* and *Dickeya* spp. A commercial product Biolyse® (APS Biocontrol Ltd., Dundee) is currently under evaluation as a seed and ware potato tuber treatment.

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