MODELS TO PREDICT THE COMBINED EFFECTS OF TEMPERATURE AND RELATIVE HUMIDITY ON *PECTOBACTERIUM ATROSEPTICUM* AND *PECTOBACTERIUM CAROTOVORUM* subsp. *CAROTOVORUM* POPULATION DENSITY AND SOFT ROT DISEASE DEVELOPMENT AT THE SURFACE OF WOUNDED POTATO TUBERS

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SUMMARY

The main objectives of this study were to evaluate and model the influence of temperature (10, 15 and 20°C), relative humidity (86, 96 and 100%) and initial concentration of bacterial inoculum (10^5, 10^7 et 10^9 CFU ml^{-1}) on the population density of *Pectobacterium atrosepticum* (*Pba*) and *Pectobacterium carotovorum* subsp. *carotovorum* (*Pcc*) which are important potato pathogens in temperate climates, and on the development of soft rot symptoms caused by these bacteria at the surface of wounded potatoes tubers under controlled conditions. Experiments were carried out according to a Box-Behnken experimental design, simplifying prediction of the combined effects of three controlled factors. With both bacterial species, statistical analysis showed a significant effect of temperature, relative humidity and initially applied bacterial concentration on population dynamics and soft rot development at the surface of wounded potato tubers. Multiple regression analyses and the contour plots showed that the temperature is the most important factor, followed by the initially applied bacteria concentration and relative humidity. More than 64% of the variability of the soft rot symptoms observed could be explained by the presence of *Pba* and *Pcc* at the level of wounded potato tubers under the combined effect of tested factors. The quadratic polynomial models developed in our research should integrate the heterogeneity of tested bacteria belonging to the same species (which was not evaluated in this preliminary investigation) in further research.

Key words: *Pectobacterium* spp., temperature, relative humidity, potato tubers, predictive microbiology.

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INTRODUCTION

Pectobacterium atrosepticum (Dye 1969; Gardan *et al.*, 2003) (former name *Erwinia carotovora* subsp. *atroseptica*) (*Pba*), *Pectobacterium carotovorum* subsp. *carotovorum* (Dye 1969; Gardan *et al.*, 2003) (former name *Erwinia carotovora* subsp. *carotovora*) (*Pcc*) and *Dickeya* spp. (Burkholder 1953; Samson *et al.*, 2005) (former name *Erwinia chrysanthemi*) are pathogenic to potato crops (Pérombelon and Kelman, 1980). They cause blackleg in the field and soft rot of tubers in storage (Pérombelon and Kelman, 1987; Pérombelon, 2002; de Haan *et al.*, 2008). These bacteria are Gram-negative, non-spore-forming, facultative anaerobes, produce a large variety of extracellular pectic enzymes, including pectate lyase (PEL) and pectin methyl esterase (PME), that are major virulence factors in soft rot development (Smadja *et al.*, 2004).

Soft rot is a serious problem for potato production and can cause significant economic losses during plant growth, harvest, transport and storage (Latour *et al.*, 2008). The three bacteria are found on plant surfaces and in the soil where they can penetrate potato tubers through natural openings (lenticels) and wounds (Toth *et al.*, 2003). Once inside the plant, they may reside in the vascular tissue and intercellular spaces of suberized or parenchymatous tissues where they remain (latent stage) until the environmental conditions, including temperature and humidity, become suitable for disease development (Pérombelon and Salmond, 1995). Macerated tissues are wet, cream to tan in colour, with a soft, slightly granular consistency. In spite of much research, no effective chemical treatment (Diallo *et al.*, 2009; Evans *et al.*, 2010) nor resistant cultivars are available for controlling potato soft rot (Rasche *et al.*, 2006). Potato shippers (producers and traders) need information that would enable them to predict the conditions favourable for bacterial and soft rot symptoms development.

Kendrick *et al.* (1959) used a temperature/relative humidity index to predict the incidence of bacterial soft rot on naturally infected potato tubers, but the species and the quantity of bacteria involved in disease development were not specified. Kushalappa and Zulfiqar (2001) tried to model soft rot of potato tubers with re-

spect to temperature, relative humidity, and duration of storage, but their predictive model was developed for *Pcc* only. Moreover, this model did not take into account the population level of *Pcc* present at the infection site. Several studies were dedicated to ecology, epidemiology and detection of *Pectobacterium* spp. infecting potato tubers (Pérombelon and Kelman, 1980; Pérombelon, 1992; Toth *et al*., 1999; Diallo *et al*., 2009). To our knowledge, however, no mathematical model has been developed to describe *Pectobacterium* spp. and *Dickeya* spp. population dynamics and symptom development under the combined effect of the most important factors of storage environment. Although an outbreak of *Dickeya* spp., known as a potato pathogen in warm regions (Pérombelon, 2002), has been reported during recent decades from western and northern Europe (Laurila *et al*., 2008; Toth *et al*., 2011), this study will focus on *Pba* and *Pcc,* because they are associated to potato mainly in temperate countries (Czajkowski *et al*., 2011).

The main aims of this work were, on one hand, the use of a tool for the quantification of *Pectobacterium* spp. populations (in order to follow bacterial population densities under various ecological conditions at the level of wounded potato tuber) and, on the other hand, to connect the observed bacterial population densities with a risk factor for the development of potato tubers soft rot.

MATERIALS AND METHODS

Biological materials. Strains used in this study (*Pba* 03034/1 and *Pcc* 030033) were isolated and characterised by the Walloon Agricultural Research Centre (CRA-W) of Libramont (Belgium). Strains were stored frozen at -80°C in glycerol 25% (v/v) for long-term storage and were always replicated at least twice on nutrient agar (NA) (Difco, Belgium) before use in the experiments. A pathogenicity test was first performed by inoculating a suspension of 10^7 CFU ml^{-1} of a 2-day-old NA culture on sterilised potato tuber slices in Petri dishes with both bacteria (*Pba* 03034/1 and *Pcc* 030033).

Healthy tubers of the highly soft rot-susceptible cv. Bintje, purchased from local retail stores, were washed to remove excess soil, were surface-sterilized in 10% sodium hypochlorite for 20 min, rinsed three times in distilled water for 10 min and air-dried for *ca.* 20 min in a laminar flow hood. Cylinders (without epidermis) with a diameter of 12 mm and 20 mm in length, were extracted with a cork borer from the tubers.

Culture media for plating. Four semi-selective media, viz. CVPB (Bdliya and Langerfeld, 2005), CVPM (Ahmed, 2001), CVP-S1 and CVP-S2 (Hyman *et al.*, 2001), based on crystal violet pectate, were selected from the literature. The double layer CVP-S2 (Hyman *et al*., 2001) was retained for assays because this medium allows a better enumeration of *Pba* 03034/1 and *Pcc* 030033 as compared to the three other media.

Controlling chamber humidity. Desiccators (miniature growth chambers) with a maximal capacity of 1 litre of water were used. The relative humidity (RH) inside desiccators was controlled using two saturated salt solutions, KCl (86%) and KNO_3 (96%) (Winston and Bates, 1960; Lahlali *et al*., 2008) and distilled water (100%). Pure salts were dissoved in 1 litre of distilled water and stirred until a saturated solution was obtained. The resulting RH varied slightly and gradually with the temperature (Winston and Bates, 1960). For each temperature-humidity combination, there was one desiccator, containing 250 ml of an appropriate saturated salt solution with excess of the solid phase of the salt to maintain the desired RH. Desiccators with differents humidities were incubated for 48 h at the experimental temperature (10, 15 and 20°C) before introducing inoculated potato tubers. RH was monitored by means of a thermohygrometer in each desiccator.

Experimental design. The Box and Behnken (1960) experimental design (BBD) was used to check, on one hand, the isolated and combined effect of temperature (T), RH and initially applied bacterial concentration (Con) on the final bacteria density at the surface of the wounds of potato tubers, expressed as Log(CFU cm^{-2}) and, on the other hand, the effect on the development of soft rot symptoms induced at the surface of the wounded potato tubers, expressed as percentage of rotted tissue. BBD allows to studying the effects of three factors in a single block of 15 sets of test conditions and three central points. The order of the experiments was fully randomized. Three levels were attributed to each factor, coded as –1, 0, +1, so that each experiment could be located by its three coded values (Table 1 and 2). Multiple regression analysis was carried out by using the software package Minitab version 15 to establish a quadratic polynomial model which would allow to predict the answer. This model had the following form:

$$Y = \beta_0 + \beta_1 X_T + \beta_2 X_{RH} + \beta_3 X_{Con} + \beta_{11}(X_T)^2 + \beta_{22}(X_{RH})^2 + \beta_{33}(X_{Con})^2 + \beta_{12} X_T X_{RH} + \beta_{13} X_T X_{Con} + \beta_{23} X_{RH} X_{Con}$$

where Y is the response expressed as the logarithm of the final bacteria density at the surface of the wounds of potato tubers, Log(CFU cm^{-2}) or as percentage of quantity of macerated tissue at the levels of these wounds, β_0 is a constant coefficient and the regression coefficients (β_1, β_2 and β_3), (β_{11}, β_{22}, and β_{33}) and (β_{12}, β_{13}, and β_{23}) represent, respectively, the linear, quadratic, and interaction effects of the model, estimated by multiple regression analysis. X is the coded value (between –1 and +1) of the factor indicated by the attached subscript (T, RH and Con). Interpretation of the data was based on

Table 1. Experimental and predicted values of population densities of *Pba*, expressed in $\log_{10}$(CFU cm^{-2}), at the surface of potato tuber wounds and the development of soft rot at the wound surface, expressed as percentage (%) of rotted tissue.

Experiments	Temperature (°C)	Relative humidity (%)	Applied concentration (CFU ml^{-1})	*Pba* population density Log (CFU cm^{-2})		Symptoms (% of rotted tissue)	
				Observed values	Predicted values	Observed values	Predicted values
E_1	10	96	10^9	7.90 ± 0.02	7.93	13.46 ± 0.83	14.73
E_2	15	96	10^7	7.45 ± 0.25	7.44	12.15 ± 0.66	11.81
E_3	20	86	10^7	7.60 ± 0.09	7.52	19.70 ± 0.29	22.01
E_4	10	86	10^7	5.84 ± 0.01	5.83	6.05 ± 0.40	5.12
E_5	10	96	10^5	3.89 ± 0.05	3.80	3.89 ± 0.31	5.87
E_6	20	100	10^7	7.71 ± 0.09	7.72	26.08 ± 1.25	27.01
E_7	15	96	10^7	7.42 ± 0.27	7.44	12.12 ± 1.83	11.81
E_8	20	96	10^9	9.49 ± 0.17	9.58	52.06 ± 1.46	50.08
E_9	10	100	10^7	5.90 ± 0.01	5.98	7.33 ± 0.50	5.02
E_{10}	15	100	10^5	5.35 ± 0.04	5.37	8.84 ± 0.43	9.17
E_{11}	20	96	10^5	5.59 ± 0.23	5.57	10.67 ± 0.78	9.40
E_{12}	15	100	10^9	9.70 ± 0.01	9.59	33.18 ± 2.86	34.22
E_{13}	15	86	10^9	9.28 ± 0.01	9.26	31.83 ± 2.47	31.50
E_{14}	15	86	10^5	5.24 ± 0.01	5.34	8.05 ± 0.06	7.00
E_{15}	15	96	10^7	7.43 ± 0.16	7.44	11.17 ± 1.28	11.81

the signs (positive or negative effect on the response) and statistical significance of coefficients ($P<0.05$). Interaction between two factors could appear as an antagonistic effect (negative coefficient) or a synergic effect (positive coefficient). The quality of fit of the polynomial model equation was expressed by the coefficient of determination R^2.

Preparation of the inoculum. Bacteria were plated twice on NA before incubation overnight in 50 ml of liquid Luria Bertani medium (LB) at 27°C with an agitation of 120 trs/min. Cells were harvested by centrifugation at 2,500 rpm for 10 min and the pellet was resuspended in sterile saline (0.85% NaCl). To determine bacterial concentration, the optical density (OD) was measured as the absorption at 600 nm with a Prim light spectrophotometer 230 V (Secomam, France) and the bacterial density of *Pba* and *Pcc* was determined by a calibration curve according to the formula:

$$CFU\ ml^{-1} = 2\times10^{8}\times OD_{600} - 10^{7}$$

Effect of temperature, relative humidity and initially applied inoculum on *Pba* 03034/1 and *Pcc* 030033 population densities. On each cylinder of potato tuber tissue two millipore filters (sterile support for cells applied) with a diameter of 12 mm and 0.2 µm pore size were deposited. The bacterial suspension (20 µl) was placed on the first filter which was not in direct contact with potato tuber wounds for avoiding possible contaminations that could affect the quantification of bacteria in Petri dishes. Negative control tubers were inoculated with sterile saline (0.85% NaCl). Four potato tuber cylinders, one of which was the negative control, were used for each treatment, three potato tuber cylinders representing a triplicate treatment. Groups of four cylinders were taken from three different potato tubers.

Effect of temperature, relative humidity and initially applied inoculum concentration on the development of soft rot. A similar experiment as described above was carried out with the difference that bacterial suspensions were inoculated directly on potato tuber cylinders. To account for the loss of weight in water by cylinders due to differents RHs inside desiccators, three non inoculated potato tuber cylinders were used for each combination tested. Desiccators containing the three types of treatments described above were incubated at the set temperatures (10, 15 and 20°C) for 3 days.

Counting of bacteria. At the third day of incubation, the first filter with bacteria was removed aseptically and suspended for *ca.* 10 min in Falcon tubes containing 5 ml of sterile saline. Decimal dilution-plating was done on CVP-S2. Based on the indicatations of preliminary trials, 100 µl suspensions were plated, which allowed counting the bacteria after 48 or 72 h of incubation at 27°C on plates that showed 30 to 300 colonies.

Quantification of macerated tissues. Three days after incubation, two measurements were made. The quantity of macerated tissue (Qmt) was expressed as percentage by the formula:

$$Qmt\ (\%) = P1 - P2/P1$$

where P1 is the weight (g) of potato tuber cylinders inoculated before incubation, P2 is the weight (g) of cylinders inoculated after removal of the macerated tissue with a sterile scalpel.

The quantity of the water lost (Qwl) by the cylinders was also expressed as a percentage by the formula:

$$Qwl\ (\%) = P'1 - P'2/P'1$$

where P'1 is the weight (g) of non inoculated cylinders before incubation, P'2 is the weight (g) of non inoculated cylinders after three days incubation.

Finally, Qmt and Qwl were used to estimate the net quantity of removed macerated tissue (Qn) by the formula:

$$Qn\ (\%) = Qmt - Qwl$$

Correlation between the population density of *Pba* 03034/1 and *Pcc* 030033 and the development of soft rot symptoms. An analysis of the correlation between the population density of *Pba* and *Pcc* and the development of soft rot symptoms was made with the software package minitab version 15 (Minitab Inc. 2006, USA).

RESULTS AND DISCUSSION

Effect of temperature, relative humidity and initially applied inoculum concentration on the population density of *Pba* 03034/1 and *Pcc* 030033 at the surface of the wounds of potato tubers. The quadratic models describing simultaneously the effect of T, RH and Con on the population density of both bacteria at the surface of the wounds of potato tubers were as follows:

(1) $Y_1 = 7.438 + 0.856X_T + 0.088X_{RH} + 2.036X_{Con} - 0.675(X_T)^2 - 0.001(X_{RH})^2 - 0.045\ (X_{Con})^2 + 0.012X_TX_{RH} + 0.076X_TX_{Con} + 0.07X_{RH}X_{Con}$

(2) $Y_2 = 6.555 + 0.379X_T - 0.063X_{RH} + 1.989X_{Con} - 0.465(X_T)^2 + 0.019(X_{RH})^2 + 0.166(X_{Con})^2 + 0.030X_TX_{RH} + 0.033X_TX_{Con} + 0.019X_{RH}X_{Con}$

where Y_1 and Y_2, are, respectively, the predicted population density of *Pba* and *Pcc* (Log CFU cm^{-2}), at the surface of the wounds. X_T (temperature), X_{RH} (relative humidity), and X_{Con} (initial concentration of bacteria) are coded variables ranging from –1 to +1. The results of observed and predicted average values of population densities of *Pba* and *Pcc* under various ecological condi-

Table 2. Experimental and predicted values of population densities of *Pcc*, expressed in $\log_{10}$(CFU cm^{-2}), at the surface of potato tuber wounds and the development of soft rot at the wound surface, expressed in percentage (%) of rotted tissue.

Experiments	Temperature (°C)	Relative humidity (%)	Applied concentration (CFU ml^{-1})	*Pcc* population density Log (CFU cm^{-2})		Symptoms (% of rotted tissue)	
				Observed values	Predicted values	Observed values	Predicted values
E_1	10	96	10^9	7.78 ± 0.03	7.83	10.30 ± 1.06	12.01
E_2	15	96	10^7	6.59 ± 0.13	6.55	9.31 ± 1.01	9.27
E_3	20	86	10^7	6.35 ± 0.09	6.39	16.93 ± 0.73	18.69
E_4	10	86	10^7	5.75 ± 0.03	5.70	4.14 ± 0.17	2.68
E_5	10	96	10^5	3.88 ± 0.02	3.92	3.01 ± 0.57	4.53
E_6	20	100	10^7	6.53 ± 0.12	6.58	19.63 ± 1.27	21.09
E_7	15	96	10^7	6.45 ± 0.28	6.55	9.27 ± 0.74	9.27
E_8	20	96	10^9	8.70 ± 0.12	8.66	43.49 ± 2.04	41.97
E_9	10	100	10^7	5.81 ± 0.03	5.76	6.43 ± 0.20	4.67
E_{10}	15	100	10^5	4.79 ± 0.05	4.79	7.01 ± 0.07	7.26
E_{11}	20	96	10^5	4.67 ± 0.13	4.61	8,72 ± 0.34	7.01
E_{12}	15	100	10^9	8.82 ± 0.07	8.81	29.78 ± 1.08	29.85
E_{13}	15	86	10^9	8.65 ± 0.02	8.65	26.53 ± 1.16	26.28
E_{14}	15	86	10^5	4.70 ± 0.04	4.71	6.50 ± 0.59	6.44
E_{15}	15	96	10^7	6.63 ± 0.14	6.55	9.24 ± 0.54	9.27

tions tested are recorded respectively in Tables 1 and 2 (columns 5 and 6.) With both bacterial species, differences were slight (near zero) between the predicted and observed values for all tested combinations.

The results of the multiple regression analysis, which provided the estimates of the model coefficients applicable to *Pba* and *Pcc* are listed, respectively, in Tables 3 and 4 (column 3). The greater the absolute value of a linear coefficient (β_1, β_2, or β_3), the more important was the influence of the corresponding factor (Box and Draper, 1987) on predicted bacterial densities. With both bacteria, the coefficients β_1 (T) and β_3 (Con) were highly significant, and the coefficient β_2 (RH) was significant. These results demonstrate the importance of tested storage factors for the control of *Pba* and *Pcc* growth, also found by Pringle *et al.* (1991) and Pérombelon and Salmond (1995). For the *Pba* model, the coefficients β_{11}, β_{22}, and β_{33}, describing the quadratic effect of T, RH and Con, have a highly significant effect for β_{11}, but not for β_{22}, and β_{33}. All quadratic coefficients of the *Pcc* model appeared to be higly significant, except for coefficient β_{22} (quadratic effect of RH) which was not significant. All interactions coefficients, T x RH (β_{12}), T x Con (β_{13}), RH x Con (β_{23}) were not significant in both models. These results suggested that the interaction effect of the tested factors does not seem

Table 3. Model coefficients and their significant effects on population densities of *Pba* and the development of soft rot symptoms at the surface of the wounds of potato tubers.

Parameters	Coefficients	*Pba* density Log(CFU cm^{-2})	Symptoms (% of rotted tissue)
R^2		99.06	97.63
Response mean	β_0	7.438***	11.814***
T	β_1	0.856***	9.721***
RH	β_2	0.088*	1.225*
Con	β_3	2.036***	12.386***
T^2	β_{11}	-0.675***	1.261^{ns}
RH^2	β_{22}	-0.001^{ns}	1.715*
Con^2	β_{33}	-0.045^{ns}	6.944***
T x RH	β_{12}	0.012^{ns}	1.276^{ns}
T x Con	β_{13}	0.076^{ns}	7.955***
RH x Con	β_{23}	0.07^{ns}	0.137^{ns}

^{ns} = not significant; * = significant ($P < 0.05$); ** = highly significant ($P < 0.01$), *** = highly significant ($P < 0.001$).

Table 4. Model coefficients and their significant effects on population densities of *Pcc* and the development of soft rot symptoms at the surface of the wounds of potato tubers.

Parameters	Coefficients	*Pcc* density Log(CFU cm^{-2})	Symptoms (% of rotted tissue)
R^2		99.40	97.00
Response mean	β_0	6.555***	9.272***
T	β_1	0.379***	8.109***
RH	β_2	0.063*	1.095*
Con	β_3	1.989***	10.609***
T^2	β_{11}	-0.465***	0.717^{ns}
RH^2	β_{22}	0.019^{ns}	1.794*
Con^2	β_{33}	0.166***	1.35***
T x RH	β_{12}	0.030^{ns}	0.102^{ns}
T x Con	β_{13}	0.033^{ns}	6.869***
RH x Con	β_{23}	0.019^{ns}	0.686^{ns}

^{ns} = not significant; * = significant ($P < 0.05$); ** = highly significant ($P < 0.01$); *** = highly significant ($P < 0.001$)

to influence the growth model of the two bacteria within the range of the experimental domain.

Statistical analysis of the experimental responses for *Pba* and *Pcc* models showed that the three factors tested, strongly affect cells multiplication of *Pba* and *Pcc* at the surface of wounded potato tubers. The sensitivity of *Pba* and *Pcc* populations to environmental conditions offers good prospects for integrated control. Indeed, the knowledge of the range of environmental factors within which the pathogens can proliferate is an important step for selecting biocontrol agents. It is known that *Pectobacterium* spp. require free moisture at the site of growth (Pringle *et al.*, 1991) and, therefore, it could be interesting to incorporate to the model the moisture level observed directly at the site of bacterial growth, instead of the moisture detected in the storage atmosphere. But, in this case, this adapted method would need an instrument to check the moisture at the site of bacterial growth.

The coefficients of determination (R^2) for the two models constructed were calculated. When the value of R^2 is close to 1.00, the model is said statistically able to predict the answer [$\log_{10}$(CFU cm^{-2})] at the surface of the wounded potato tubers. In terms of percentage, the coefficient R^2 is interpreted as being the part of variation of the answer explained by variables involved in model conception (Box and Draper, 1987). R^2 values were 99.06 for *Pba* 03034/1 (Table 3) and 99.40 for *Pcc* 030033 (Table 4). These results mean that the observed 99.06% variation of the population density of *Pba* and 99.40% variation of that of *Pcc*, are explainable by the models.

The contour plots describing the combined effect of temperature and RH on the logarithm of population densities were drawn for each inoculum concentration from the equations for *Pba* (Fig. 1a, b and c) and *Pcc* (Fig. 2a, b and c). These graphs show that, the logarithm of the *Pba* and *Pcc* population density increases with the incubation temperature within the range of the experimental data. These results indicate that the growth of both bacteria is temperature-dependent. A similar temperature-dependent increase in the number of bacteria was also reported for *Pba* on wounded potato two days post inoculation by van Vuurde and de Vries (1994). The increasing populations of *Pba* and *Pcc* with temperature suggest also an increase of the risk of soft rot initiation. As observed by several authors, when a critical number of *Pectobacterium* spp. (10^7-10^8 cells) is reached at the infection site, the bacteria produce and secrete several extracellular enzymes including pecti-

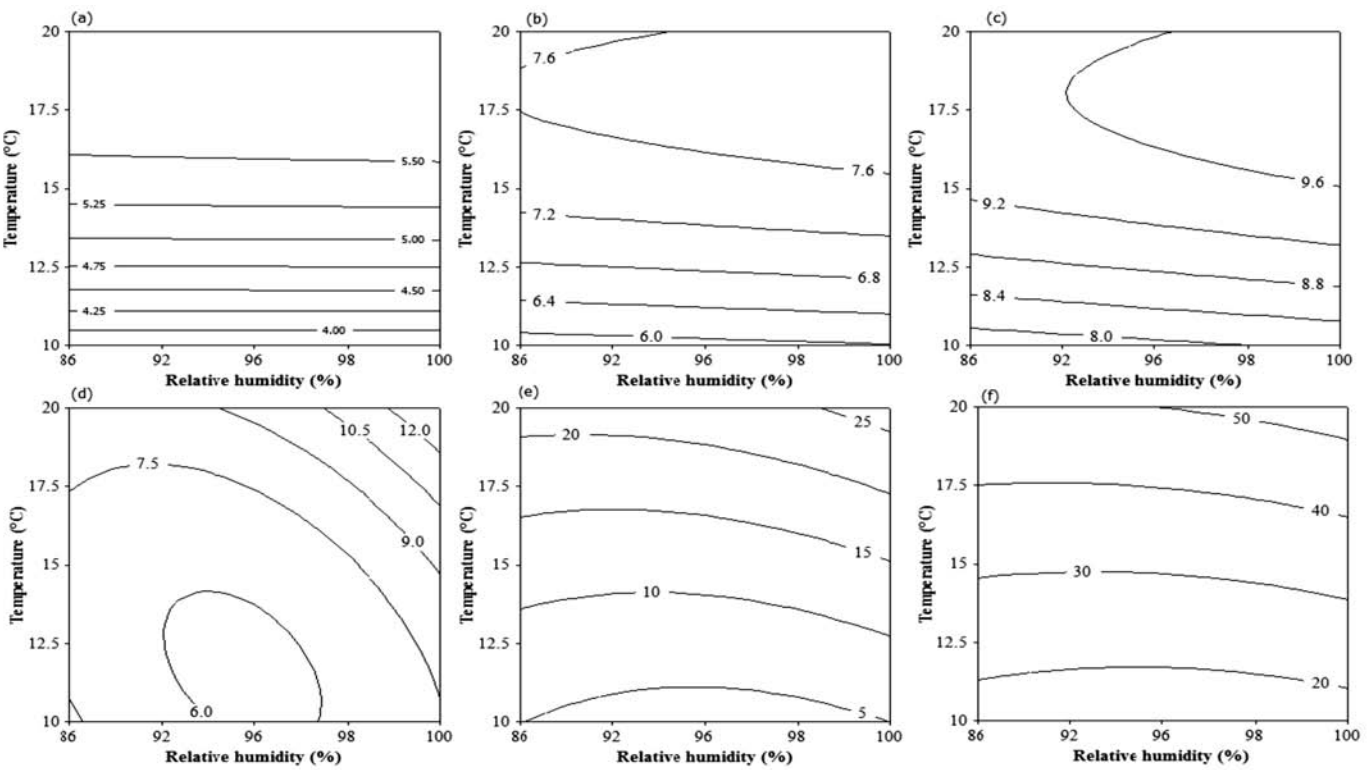

Fig. 1. Contour plots showing the predicted effect of temperature and relative humidity, on the population density of *Pba*, expressed in number of $\log_{10}$(CFU cm^{-2}), at the surface of the wounds of potato tubers inoculated with 10^5 (a), 10^7 (b) and 10^9 (c) CFU ml^{-1} of *Pba*; and on the development of soft rot symptoms at the surface of the wounds, expressed as percentage (%) of rotted tissue, inoculated with 10^5 (d), 10^7 (e) and 10^9 (f) CFU ml^{-1} *Pba*.

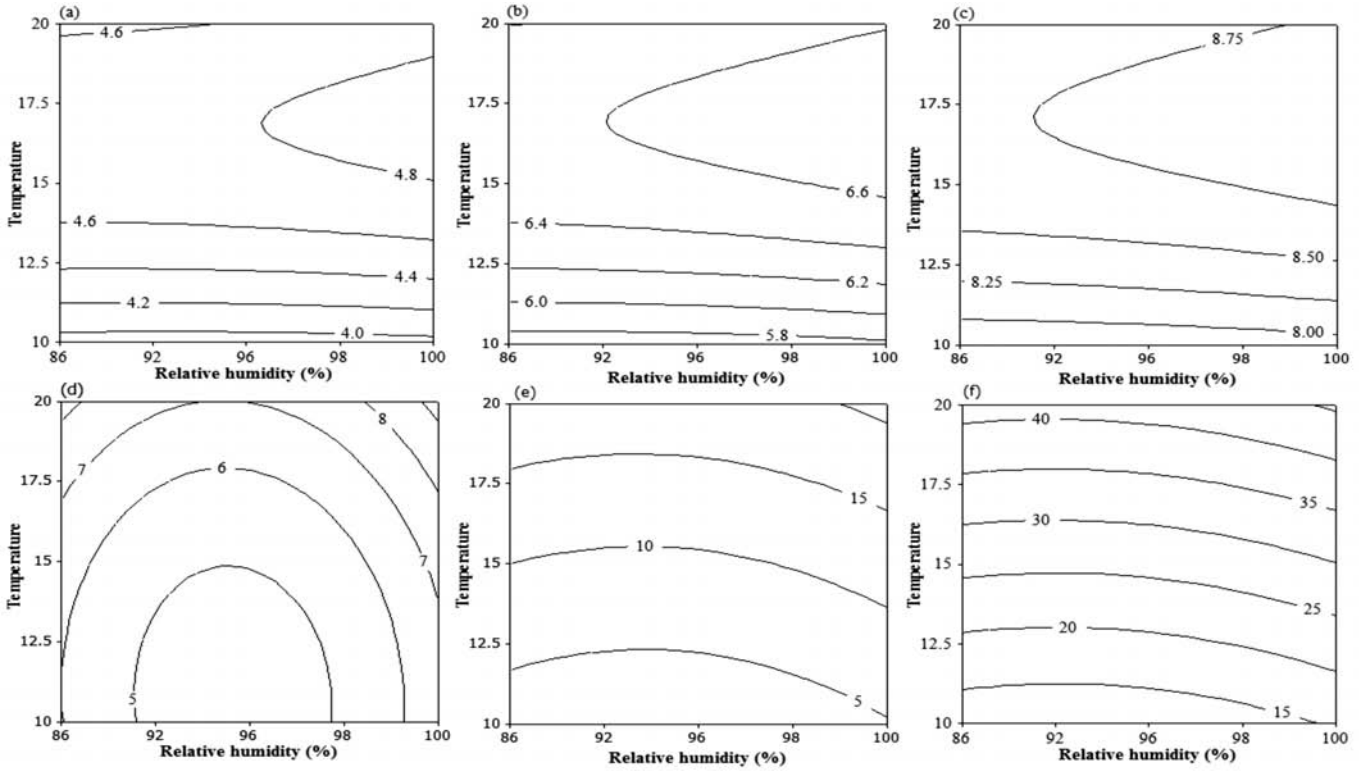

Fig. 2. Contour plots showing the predicted effect of temperature and relative humidity on the population density of *Pcc*, expressed in number of $\log_{10}$(CFU cm^{-2}), at the surface of the wounds of potato tubers inoculated with 10^5 (a), 10^7 (b) and 10^9 (c) CFU ml^{-1} of *Pcc*; and on the development of soft rot symptoms at the surface of the wounds, expressed as percentage (%) of rotted tissue inoculated with 10^5 (d), 10^7 (e) and 10^9 (f) CFU ml^{-1} *Pcc*.

nolytic enzymes essential for virulence (Barras *et al*., 1994; Maë *et al*., 2001; Pérombelon 2002; Latour *et al*., 2008). This production is controlled by a quorum-sensing process that relies upon the production of *N*-acylhomoserine lactones (HSL) (Toth *et al*., 2004; Cirou *et al*., 2010). An increase of the risk of soft rot initiation at the surface of wounded potato tubers could also result in an increase of the risk of further disease development. Indeed, when the infection was initiated any further disease development was dependent on the water potential of potato tubers (Pérombelon and Salmond, 1995; Pérombelon, 2002). In our experiments, the growth of *Pba* and *Pcc* was found to be more dependent on T than on RH regardless of Con. Furthermore, the minimum and maximum growth rate of both bacteria were found to be 10°C and 20°C, respectively, irrespective of the initial *Pectobacterium* concentration applied. We also observed that maximum growth of *Pba* for the three initial concentrations were about tenfold higher than those of *Pcc*. These results suggest that *Pba* grows more quickly than *Pcc* at the surface of potato tuber wounds.

In this work, population dynamics of *Pectobacterium* were followed at the level of potato tuber wounds. It would be interesting to follow *Pectobacterium* population dynamics in the natural openings on the tuber surface (lenticels) and population development over longer periods of storage. But this would require an adequate inoculation method via lenticels such as vacuum infiltration and an appropriate experimental design.

Effect of temperature, relative humidity and inoculum concentration on the development of potato tuber soft rot symptoms. The percentage of the quantity of macerated tissue of potato tubers at the wound level due to bacteria and combined effect of T, RH and Con was predicted by the following quadratic polynomial equations:

(3) $Y_3 = 11.814 + 9.721X_T + 1.225X_{HR} + 12.386X_{Con} + 1.261(X_T)^2 + 1.715(X_{HR})^2 + 6.944(X_{Con})^2 + 1.276X_TX_{HR} + 7.955X_TX_{Con} + 0.137X_{HR}X_{Con}$

(4) $Y_4 = 9.272 + 8.109X_T + 1.095X_{HR} + 10.609X_{Con} + 0.717(X_T)^2 + 1.794(X_{HR})^2 + 1.35(X_{Con})^2 + 0.102X_TX_{HR} + 6.869X_TX_{Con} + 0.686X_{HR}X_{Con}$

where Y_3 and Y_4 represent, respectively, the predicted percentages of the quantity of rotted of potato tuber tissues by *Pba* and *Pcc*; X, is the coded values (between -1 and +1) of the factor indicated by the attached subscript (T, RH and Con). The results of measuring and predicting percentage of rotted tissue at the wound level are presented in Tables 1 and 2 (columns 7 and 8). There is no substantial difference between the observed

and predicted percentage of rotted tissue at the surface of wounded potato tubers caused by each tested bacterium (*Pba* and *Pcc*).

Tables 3 and 4 (columns 4 and 4, respectively) summarize the estimated regression coefficients given by the multiple regression analysis. All linear coefficients of both models, β_1, β_2, and β_3 which represent, respectively, the linear effects of T, RH and Con, are highly significant for β_1 and β_3 but only significant for β_2. These results are in accordance with the data reported by Togbé (2007) on soft rot development in potato tubers slices inoculated with *Pba*.

Several authors have pointed out the importance of each factor separately on disease development by *Pba* and *Pcc* (Pérombelon and Kelman, 1980; Pérombelon, 1992; Kushalappa and Zulfiqar, 2001). The coefficients β_{11}, β_{22}, and β_{33} which describe the quadratic effect of T, RH and Con for the *Pba* and *Pcc* model were, respectively, not significant, significant and highly significant. The interactions T x RH and RH x Con were not significant for the two models. Quadratic or interaction effect of those factors that were not significant, appear to suggest that they did not influence the percentage of rotted tissue predicted by each model in our conditions, contrary to those that were significant. Latent infection of potato tubers by *Pectobacterium* spp. is widespread (Pérombelon, 2002; Toth *et al*., 2003) and if high RH (85 to 95%) is applied to storage rooms (Martin and Gravoueille, 2001) so that the interaction RH x Con is significant, massive cases of sot rot development in storage rooms can be expected at the surface of wounded potato tubers. The combined effect of T x Con was positive and very highly significant in both models. These results suggest a synergistic effect of this combination on the maceration of potato tubers. R^2 values show that T, RH and Con account for 97.63% (Table 3) and 97.00% (Table 4) of the percentage of rotted tissue variation observed in the *Pba* and *Pcc* model, respectively.

The contour plots showing the combined effect of T and RH on the percentage of potato tubers diseased at the wound level were also drawn for each bacterium concentration from the model for *Pba* (Fig. 1d, e and f) and *Pcc* (Fig. 2d, e and f). The choice of each bacterium concentration for contour plots construction was based on the fact that the quantity of initial inoculum present on potato tubers at the time of storage was fixed, whereas the two other ecological factors (T and RH) varied. With reference to the *Pba* and *Pcc* growth observed in this study, the percentage of diseased potato tubers seemed to depend more on T than RH, regardless of Con. Togbé (2007) obtained similars results with *Pba* on potatoes tubers slices. Moreover, the highest percentage of potato tuber tissue diseased by *Pba* and *Pcc* was observed when T and RH were highest (20°C and 100%) with a more important value for *Pcc,* independently of the the initial bacterial concentration used. These results suggest that *Pcc* produced more soft rot than *Pba* in our experimental conditions. The differential effect of temperature on the pathogenic behaviour of *Pba* and *Pcc* may be attributed to its effect on pectate lyase production (Smadja *et al*., 2004).

All polynomial models described in this investigation with multi-factorial analysis are valid only within the experimental domain of the tested key environmental factors, for the *Pectobacterium* strains employed and the potato cv. Bintje (Kushalappa and Zulfiqar, 2001; Lui and Kushalappa, 2003).

The modelling approach chosen for this study was the "response surface methodology" (RSM) applied to the Box and Behnken (1960) experimental design. RSM is a mathematical approach most often used to describe the simultaneous effect of several factors on microbial behaviour (Delignette-Muller, 2009; Moh *et al*., 2011). BBD, an experimental design of RSM, was preferred because relatively few experimental combinations of the variables are adequate to estimate complex response functions. BBD has been widely used to predict the growth of food-borne pathogens (Sautour *et al*., 2003; Lahlali *et al*., 2008) or plant pathogens (Togbé *et al*., 2007) in relation to at least three environmental factors.

Correlation between the population density of *Pba* 03034/1 and *Pcc* 030033 and the development of soft rot symptoms. Analysis of the correlation between the population densities of Pectobacteria and the development of potato tuber soft rot shows a linear coefficient of correlation (r) equal to 0.83 (R^2 = 69%) and 0.80 (R^2 = 64%) for *Pba* and *Pcc*, respectively. These results indicate that more than 64% of the variability of soft rot symptoms observed could be explained by the presence of *Pba* and *Pcc* at the level of wounded potato tubers with the combined effect of T, RH and Con. The combined effect of these factors, however, may not be the only incitant of soft rot development at the surface of wounded potato tubers. Other factors that were not controlled in the experiment, such as the O_2 status in the dessicator, the physiological resistance of the cultivar used, the harvesting, or the storage conditions of the tuber could also interfere with disease development (Kushalappa and Zulfiqar, 2001; Lui and Kushalappa, 2003). Moreover, the growth behaviour of the tested bacteria on a non natural growth support (millipore) would not be the same if they were grown directly on potato tubers tissue.

In conclusion, this work constitutes a preliminary study addressing the evaluation and modelling the influence of T, RH and Con on the population dynamics of *Pba* and *Pcc*, and on the development of soft rot symptoms caused by these bacteria at the surface of wounded potatoes tubers. The four models developed in this study had a good capacity of response prediction (R^2 value is close to 1.00 for each model). However, for using these

models as warning systems to make management decisions, some adaptations to practical conditions involving different strains of bacteria, potato cultivars, physiology of potato tubers, harvesting and storage conditions are required. All polynomial models developed in this investigation did not include the heterogeneity of the tested bacteria belonging to the same species. This is why further research should take into consideration the large genetic variation within the genus *Pectobacterium* (*Pba* and *Pcc*) and *Dickeya* spp. that were isolated from potatoes in western and northern Europe during recent years. In this investigation the risk factor of disease development was qualitative i.e. a low or high *Pectobacterium* population corresponded respectively to a low or high risk of disease development. It could be interesting to quantify this risk factor i.e. associate qualitative risk (low or high) of soft rot development with the number of potato tubers diseased (disease incidence). To better approximate naturals conditions of soft rot development further study should also be conducted on entire potato tubers inoculated at the lenticel level.

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