

## SHORT COMMUNICATION

SURVIVAL OF *RALSTONIA SOLANACEARUM* ON WOOD, HIGH DENSITY POLYETHYLENE AND ON JUTE FABRIC IN COLD STORAGED. Pasqua di Bisceglie<sup>1</sup>, A. Saccardi<sup>1</sup>, S. Giosue<sup>2</sup>, F. Traversa<sup>3</sup> and U. Mazzucchi<sup>3</sup><sup>1</sup> Servizio Fitosanitario Regionale, Regione del Veneto, Viale dell'Agricoltura 1/a, 37060 Buttapietra, Verona, Italy<sup>2</sup> Istituto di Entomologia e Patologia Vegetale, Università Cattolica del Sacro Cuore, Via Emilia Parmense 84, 29100 Piacenza, Italy<sup>3</sup> Dipartimento di Scienze e Tecnologie Agroambientali, Università degli Studi di Bologna, Viale Fanin 40, 40127 Bologna, Italy

## SUMMARY

Containers used for transport and warehouse storage of potatoes may be contaminated by *Ralstonia solanacearum* Yabuucki *et al.* and become sources of inoculum and the means of spreading infection over short, medium and long distances. The materials of which the containers are made could be an important factor influencing the survival of contaminating bacteria. This paper describes the study of survival of *R. solanacearum* in cold storage at 4°C on poplar and oak wood, on high-density polyethylene and on jute sack fabric. Survival was quantitatively assessed on concentrates obtained by washing samples of contaminated materials and centrifuging the washing liquids. The plate count was performed on two semi-selective media, Kelman's and SMSA. Contamination survived on oak for approximately 4 days and on poplar for 17 days. On high density polyethylene survival was zero after 2 days. On jute fabric, the number of surviving bacteria had dropped considerably after 24 hours, but subsequent decrease was moderate and the population was only zero after 78 days.

*Key words:* brown rot, potato, oak wood, poplar wood.

Hygiene is an important factor for preventing the introduction of *Ralstonia solanacearum* Yabuucki *et al.* on potato growing farms (Janse and Wenneker, 2002). Indeed, it is essential for those growers who produce basic planting material. *R. solanacearum* cells contaminating machinery, tools, storage and packaging materials can be spread over short, medium and long distances during agronomic operations and trade, thus becoming sources of inoculum.

*R. solanacearum* is a harmful organism and its introduction and spread is prohibited in the European Union (Directive 2000/29/EC; Annex I, Sect. II, comma b, 2). All EU member states must adopt measures to prevent the introduction and spreading of *R. solanacearum*

(Directive 98/57/EC). In those cases where foci are detected in the open field or infected tubers in a warehouse, all objects which have come into contact with these tubers, including packaging, must be considered as effective vehicles of infection and thus cleaned and, if necessary, disinfected (Directive 98/57/EC, Art 5; Annex VI, commas 3, 4C, 4.2, aa).

The efficacy of cleaning and disinfection of surfaces contaminated with *R. solanacearum* cells depends on the number of bacteria, their type of aggregation, on or inside the material, as well as the disinfectant and application method used (Gudmestad and Secor, 1993; Secor and Gudmestad, 1993; Roggi *et al.*, 2000). The survival of *R. solanacearum* in potato storage warehouses and the type of materials contaminated may therefore influence the results achieved.

This study describes the survival of *R. solanacearum* on poplar and oak wood, on high-density polyethylene (HDPE) and on jute sack fabric in a cold store. Parallelepiped samples of oak and poplar wood and HDPE with similar surface areas (Ceroni *et al.*, 2004) and fabric squares (2x2 cm) from new jute sacks were contaminated by immersion in a water suspension  $0.2 A_{660}$  (approx.  $5.10^8$  CFU ml<sup>-1</sup>) of the virulent strain *R. solanacearum* PD 2762, grown for 48 h on TZ agar (TZ) (Kelman, 1954) at 27°C. After air-drying at room temperature, the materials were stored in open trays in a cold store at 4°C and 80-90% RH, alongside the crates containing ware potatoes (Cooperative S. Pietro, Roveredo di Guà, Verona, Italy).

Survival in the cold store was assessed in terms of CFU cm<sup>-2</sup> on 18 parallelepiped samples of oak, poplar and HDPE and as CFU g<sup>-1</sup> on 20 square weighed samples of jute fabric. Samples were collected the day after contamination, after air drying (time 0), and after 1, 4, 10, 17, 38, 78, 108 and 161 days of storage. For the jute fabric, the slow air-drying only made it possible to collect the 0 time sample 24 hours after contamination. Each sample was washed in sterile distilled water in a suitable rotating glass cylinder (Ceroni *et al.*, 2004). The washing liquid was centrifuged (10,000 g, 15 min, 8-10°C). The pellet was suspended in 1.5 ml (final extract) and tenfold dilutions were plated for counting (Mazzucchi and Comelli, 1977) on TZ and on SMSA

**Table 1.** Survival of *R. solanacearum* on poplar and oak wood, high-density polyethylene (HDPE) and jute sacks. The data are expressed as CFU cm<sup>-2</sup> (wood and HDPE) or CFU g<sup>-1</sup> (jute fabric). The materials were contaminated on day 0 and survival was assessed in a cold store at 4°C and 80-90% RH. Parallel reisolations were made for each material on TZ and SMSA.

Days	Poplar		Oak		HDPE		Jute	
	TZ	SMSA	TZ	SMSA	TZ	SMSA	TZ	SMSA
0	1.22·10 <sup>5</sup>	1.27·10 <sup>3</sup>	1.56·10 <sup>3</sup>	1.27·10 <sup>3</sup>	5.39·10 <sup>2</sup>	2.45·10 <sup>3</sup>	1.38·10 <sup>8</sup>	1.26·10 <sup>8</sup>
1	5·10 <sup>1</sup>	1.2·10 <sup>1</sup>	2.4·10 <sup>1</sup>	2.1·10 <sup>1</sup>	< 1	< 1	2.43·10 <sup>4</sup>	1.94·10 <sup>4</sup>
2	1.2·10 <sup>1</sup>	2.4·10 <sup>1</sup>	0	0	< 1	0	1.16·10 <sup>4</sup>	1.0·10 <sup>4</sup>
4	8	1	3	3	0	0	2.42·10 <sup>3</sup>	2.5·10 <sup>3</sup>
10	4	2	< 1	< 1	0	0	7.52·10 <sup>2</sup>	4.88·10 <sup>2</sup>
17	3.19	1.47	0.13	0.10	0	0	1.89·10 <sup>2</sup>	1.51·10 <sup>2</sup>
38	< 1	< 1	0	0			1.9·10 <sup>1</sup>	1.8·10 <sup>1</sup>
78	< 1	< 1	0	0			< 1	< 1
108							0	0
161							0	0

**Table 2.** Parameters and statistics of the regression model  $y = a \cdot e^{(-x/b)}$  fitting the relationship between the survival percentage of *R. solanacearum* on different materials and on two re-isolation media (TZ and SMSA)<sup>a</sup>. Standard errors of parameters are indicated in brackets.

Medium	Poplar			Oak			HDPE			Jute		
	a	b	R2	a	b	R2	a	b	R2	a	b	R2
TZ	100 (0.0052)	0.128 (0.0021)	0.99	100 (0.077)	0.239 (0.003)	0.99	100 (0.003)	0.137 (0.001)	0.99	100 (0.003)	0.116 (0.002)	0.99
SMSA	100 (0.787)	0.219 (0.036)	0.99	100 (0.083)	0.244 (0.003)	0.99	100 (8.7·10 <sup>-9</sup> )	0.089 (5.2·10 <sup>-8</sup> )	0.99	100 (0.003)	0.114 (0.002)	0.99

<sup>a</sup> a and b are the parameters of the exponential equation.

(Elphinstone *et al.*, 1996).

The colonies were morphologically similar to strain *R. solanacearum* PD2762, producing a characteristic amplicon of 288 bp with PCR using the primers OLI-1 and Y-2 (Seal *et al.*, 1993). Survival was expressed as CFU cm<sup>-2</sup> on wood and HDPE, and as CFU g<sup>-1</sup> on the jute fabric and as percentages for all the materials with reference to the CFU cm<sup>-2</sup> and CFU g<sup>-1</sup> at time 0 (100%).

On oak and poplar wood the number of CFU cm<sup>-2</sup> rapidly decreased within 24 and 48 h with survival percentages were 0.001% and 0.01%, respectively. On poplar, a very low number of CFU cm<sup>-2</sup> was found up to day 17, but on oak only up to day 4 (Table 1). Clearly poplar wood offered better survival for the first two weeks after contamination. Indeed, *Erwinia amylovora* also survives better for the first 15-20 days of cold storage on poplar than on oak (Ceroni *et al.*, 2004). As expected, the porosity of poplar wood caused greater absorption of the contaminating suspension and also offered protection to the bacteria against dehydration and removal with washing water. The survival of *R. solanacearum* on oak for just a few days is in line with the results of Wenneker *et al.* (1998) on heat-treated wood, stored in the dark at room temperature.

On HDPE there was already a marked decrease in the number of CFU cm<sup>-2</sup> after 24 h, and after 2 days this

was zero on both re-isolation media. The percentage of surviving bacteria decreased drastically to approximately 0.06% and 0.001% after just 24 hours on TZ and SMSA, respectively.

On jute there was a marked decrease in the number of CFU g<sup>-1</sup> within the first 24 h, which became slow and progressive in the following weeks reaching almost zero after 78 days on both re-isolation media. The pattern was similar for both media. The percentage of surviving bacteria on jute dropped to approximately 0.018% and 0.015% 24 h after contamination on TZ and SMSA, respectively.

A non-linear exponential model was applied to the experimental data, using SPSS (version 11.0, SPSS Inc., 2002), using the equation:

$$y = a \cdot e^{\frac{-x}{b}}$$

where: y = survival percentage; x = storage period (days); a, b = equation parameters.

The goodness of fit was evaluated using a regression analysis of variance, the standard error of parameters, the coefficient of determination (R<sup>2</sup>) and distribution of residuals against the independent variable. The regression curves were compared using the parallelism test, which compares the b regression coefficients, using the t test (Fowler and Cohen, 1990) after linearization of the experimental model.

The exponential model showed a satisfactory fit to experimental data. In all cases the regression analysis of variance was highly significant ( $P < 0.01$ ) and parameter standard errors always lower than parameter values (Table 2). Moreover the residues were always randomly distributed (not shown).

When the materials were compared using TZ medium, the test showed a significant difference ( $P < 0.05$ ) between the survival dynamics observed on poplar and on jute and a highly significant difference ( $P < 0.01$ ) between the dynamics observed on HDPE and jute, these being the materials on which the lowest and highest survival levels were observed. When SMSA was used, the results of the parallelism test were similar to those obtained for TZ medium, but comparisons between the regression coefficients obtained for poplar and jute were not significant.

These results indicate that survival of *R. solanacearum* is markedly reduced on HDPE as compared to wood and that the risk of spreading *R. solanacearum* with the movement of bins is zero after just a few days. Clearly, the HDPE bins are more effective than wood bins in reducing phytosanitary risk. Moreover, it is easier to clean HDPE bins than wooden ones.

The number of CFU  $g^{-1}$  on jute fabric showed a sharp drop in the first 24 hours and the survival percentage was approximately 0.015 %, similar to that on poplar wood. Subsequently, however, the decrease was moderate and survival was only zero after 78-108 days. Jute fabric was therefore the material, which allowed the best survival of *R. solanacearum*.

Survival of on jute was however much shorter than that reported for *Clavibacter michiganensis* subsp. *sepedonicus* (*Cms*) (Slack 1987; Gudmestad and Secor, 1993). In fact *Cms* can survive for at least 24 months at 5°C and 12% RH, but this period is reduced to <14 months if the RH increases to 94%. Jute sacks contaminated with rotting or exuding infected tubers are therefore a probable source of inoculum and means of spreading infection and must therefore be thoroughly cleaned before being reused.

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