XANTHOMONAS CAMPESTRIS pv. *VITICOLA* ON GRAPEVINE CUTTING TOOLS AND WATER: SURVIVAL AND DISINFECTION

C.R. Naue[1], V.S.O. Costa[2], M.A.G. Barbosa[2], D.C. Batista[2], E.B. Souza[3] and R.L.R. Mariano[1]

[1] Universidade Federal Rural de Pernambuco, Departamento de Agronomia, 52171-900 Recife, Pernambuco, Brasil
[2] Embrapa Semiárido, 56302-970, Petrolina-Pernambuco, Brasil
[3] Universidade Federal Rural de Pernambuco, Departamento de Biologia, 52171-900 Recife, Pernambuco, Brasil

SUMMARY

Bacterial canker (*Xanthomonas campestris* pv. *viticola*) (*Xcv*) is the most important disease of grapevine in northeast of Brazil. The survival of *Xcv*2^{Rif} was evaluated from 0 to 42 h following the dipping of the harvest shears in a pathogen suspension. An *in vitro* test of *Xcv* sensitivity to sanitizing agents and initial tests on shears were performed with dodecyl dimethyl ammonium chloride (1,140 ppm), benzalkonium chloride (245 ppm), sodium dichloroisocyanurate (16.2 ppm), sodium hypochlorite (20,000 ppm), calcium hypochlorite (130 ppm), calcium oxychloride (97,500 ppm) and chlorine dioxide (41 ppm). The ability of dodecyl dimethyl ammonium chloride (1,140 ppm) and sodium hypochlorite (20,000 ppm) to disinfect shears was tested with 50 sequential cuts on vine leaves. The maintenance of the efficacy of the sanitizing agents was tested between 0 and 8 h following solution preparation. The ability of five sanitizing agents to disinfect *Xcv*-contaminated water was also tested. *Xcv* survived for 24 h on harvest shears. Sodium hypochlorite and dodecyl dimethyl ammonium chloride presented the largest growth inhibition zones to four *Xcv* isolates and were 100% effective in disinfecting contaminated harvest shears. These two sanitizing agents maintained their efficacy for 8 h. *Xcv* was spread by contaminated harvest shears until the 24th cut, in average. Water disinfection was successfully achieved with dodecyl dimethyl ammonium chloride (570 ppm), sodium hypochlorite (5,000 ppm) and benzalkonium chloride (122.5 ppm).

Key words: *Vitis* sp., grapevine bacterial canker, spreading, disease management, sanitizing agents

Corresponding author: R.L.R Mariano
Fax: +55.81.33206205
E-mail: rrmbac@gmail.com

INTRODUCTION

The São Francisco Valley (Vale do Submédio São Francisco) in Brazil has become increasingly recognized for production of high quality grapes. Pruning, water management, and the use of pesticides, fertilizers and physiology-regulating chemical agents guarantee the high productivity of the crop and allow up to 2.5 harvests per year. The Valley is responsible for 99% of the annual Brazilian export of fine table grapes (Valexport, 2009).

The intensification of grapevine cultivation, the planting of susceptible varieties, the favorable climate conditions of the Valley and the exchange of germplasm among farmers have promoted the occurrence of phytosanitary problems, such as the grapevine bacterial canker caused by *Xanthomonas campestris* pv. *viticola* (Nayudu) Dye (*Xcv*) (Tavares and Menezes, 1991; Tavares, 1995). This bacterium is a quarantine pest (A2) present in the states of Bahia, Pernambuco and Ceará. It was detected in and eradicated from the states of Piauí, Roraima, São Paulo, Goiás and Paraná (Freire and Oliveira, 2001; Halfeld-Vieira and Nechet, 2006; Junqueira *et al*., 2006; Rodrigues Neto *et al*., 2011; Tomaz *et al*., 2011). Grapevine bacterial canker threatens the productivity of Valley crops, limits the sale of grapes in both internal and external markets and prevents the transfer of grapevine propagating material from states where the bacterium has been detected.

Symptoms of grapevine bacterial canker in the leaves begin with sparse, small, necrotic and angular lesions that coalesce, causing blight and desiccation of large foliar areas. Elongated necrotic spots, forming cankers, can appear in the leaf veins and petioles. In the stems, external darkening of extensive areas occurs, which is generally accompanied by necrosis, canker formation and systemic tissue colonization, causing discoloration of the vascular region. Symptoms can also be observed on the inflorescences as necrotic spots, cankers on the rachis, and dark rounded lesions 1 to 3 mm in diameter on the berries (Nayudu, 1972; Malavolta Jr. *et al*., 1999). The severity of symptoms varies with the climatic conditions and the level of disease resistance of grapevine cultivars (Lima, 2000). In the São Francisco Valley, the disease occurs with greatest severity in the rainy season and under conditions of high relative humidity and temperature.

Between production periods, *Xcv* can survive in infected plants, in symptomless plants as an epiphyte (Araujo, 2001), in crop debris (Silva *et al*., 2012) and in alternative hosts (Peixoto *et al*., 2007). The pathogen is introduced in vineyards mainly through infected planting and propagative materials (Nascimento and Mariano, 2004), and its dissemination can take place via rain or irrigation splash water, by adherence to vehicles, clothes, containers, gloves, and by cutting tools that have not been disinfected. Because *Xcv* is present both at the surface and within the tissues and veins of infected plants (Araujo *et al*., 2002), one of the main routes of dissemination within or between vineyards is through the use of contaminated cutting tools. In the São Francisco Valley, the water where cuttings are soaked to facilitate rooting prior to plantation was suspected to be an additional source of *Xcv*, as infected and non-infected cuttings are kept in the same container. This was confirmed in 2012 with the detection of the pathogen in this water by Bio-PCR, technique which consists in cultivate bacteria on agar media prior to PCR (M.A.V. Ferreira, personal communication).

Because there is no single most efficient method for the control of grapevine bacterial canker, a set of preventive control and eradication measures is recommended. The use of sanitizing agents for disinfection of cutting tools is one such measure of preventing disease spreading. Several sanitizing agents, including chlorinated compounds that possess a broad spectrum of biocidal activity against bacteria, fungi and viruses, are commercially available. Because of its low cost, sodium hypochlorite is the inorganic source of chlorine most widely used for the sterilization of surfaces, food and water. Recently, organic sources of chlorine such as sodium dichloroisocyanurate have also been used because of their higher stability and efficacy and less intense corrosive properties (Ribeiro *et al*., 2008).

The use of sanitizing agents for the disinfection of pathogen-contaminated tools is recommended as an efficient preventive measure in several crops and has been found to be of high practical value (Vida *et al*., 2004; Yakabe *et al*., 2012**)**.

This technique is effective in controlling diseases transmitted by mechanical contact, and has become an important measure in plant disease control programs (Ventura and Costa, 2002; Zambolim *et al*., 2002). The presence of *Xcv* in the water used for soaking cuttings and the lack of recommended products for the disinfection of contaminated water put forward the need to investigate water disinfection procedures in the production of grapevine-rooted grafts. Because it is active against several pathogens, chlorine has long been used on a broad scale in the disinfection of irrigation water (Hong *et al*., 2003; Lacy *et al*., 1981; Raudales *et al*., 2011; Rosner *et al*., 2006; Thompson, 1965).

Despite the importance of the grapevine in the São Francisco Valley, no studies have been conducted to assess the survival of *Xcv* in cutting tools, to determine if they represent an effective means of pathogen dissemination or to recommend products that are effective for the disinfection of *Xcv*-contaminated tools and water. Likewise, the sanitizing efficiency of different product concentrations, the persistence of product efficacy in the field and other aspects of product action have not been investigated. The aims of this work were to confirm the survival of *Xcv* on harvest shears and to select efficient sanitizing agents for the disinfection of cutting tools and water used in the production of grapevine-rooted grafts.

MATERIALS AND METHODS

Isolates and preparation of the mutant and the bacterial suspension. The *Xcv*2^{Rif} isolate, a spontaneous and stable mutant resistant to 100 ppm rifampicin was used to study the survival of *Xcv*, the disinfection of harvest shears and water and the maintenance of the efficacy of the sanitizing agents. This mutant has similar growth rates in NYD liquid growth medium (dextrose 10 g, meat extract 3 g, yeast extract 5 g, and peptone 5 g) and similar pathogenicity to grapevine as the *Xcv*2 isolate (Silva *et al*., 2012). The molecular identification of the wild-type isolate *Xcv*2 and of the mutant was done by PCR using the specific primers *Xcv*1F/*Xcv*3R that amplify a *hrp*B gene fragment of 243 bp (Trindade *et al*., 2005, 2007).

All of the *Xcv* isolates (*Xcv*5, *Xcv*83, *Xcv*113 and *Xcv*2) used in the present study came from the Bacteria Collection of the Phytopathology Laboratory of the Brazilian Enterprise for Agricultural Research (Laboratório de Fitopatologia da Embrapa Semiárido). The isolates were kept in sterilized distilled water (SDW) and reactivated in NYDA growth medium (NYD plus agar 18 g l^{-1}), with the exception of the *Xcv*2^{Rif} isolate which was reactivated in NYDAM^{Rif} growth medium (NYDA plus 100 ppm ampicillin and 100 ppm rifampicin). For preparation of the suspensions, the isolates were grown in NYDA or NYDAM^{Rif} media at 28°C for 48 h. The concentration of the bacterial suspension in SDW was adjusted to $A_{570} = 0.4$ (10^8 CFU ml^{-1}) using a photocolorimeter (Analyser 500M, Brazil).

***Xanthomonas campestris* pv. *viticola* survival on harvest shears.** A survey of the type of cutting tools used in the São Francisco Valley vineyards showed that those of stainless steel were the most commonly used. To make the results obtained comparable and because of their higher handling practicability, harvest shears of the brand LIMMAT (model 610, 15 cm long) with stainless steel blades were used throughout. A 2-cm portion of the blade tips of all shears was dipped in the *Xcv*2^{Rif} suspension for 30 sec. Each scissor was then hanged from a holder and placed in an incubator at 28°C. Sampling was 0, 6, 12, 18, 24, 30, 36 and 42 h after dipping. The survival of bacteria on the shears was evaluated at each sampling time by steeping the tips of each shear in a glass container with 20 ml SDW and gently stirring the liquid with circular

motions for 20 sec. A series of dilutions up to 10^{-4} were then made in test tubes containing 4.5 ml SDW and 100-µl aliquots of each dilution were plated on NYDAM^{Rif} in three replicates. The plates were incubated for 72 h at 28°C, and the number of bacterial colonies was counted.

The experiment consisted of eight treatments (corresponding to the sampling times 0, 6, 12, 18, 24, 30, 36 and 42 h after infection), after which the survival of the bacteria was evaluated. There were three repetitions per treatment, each represented by one shear. The experimental design was completely randomized and the experiment was repeated twice. The data obtained from the three experiments was log (x + 1) transformed [where x is CFU $(cm^2)^{-1}$] and tested for homogeneity of variance (Levene test). Average values were calculated and a non-linear regression was performed. A negative exponential model was fitted to the data. Statistical analyses of the data were done using the SAS System version 9.0 software (SAS Institute, USA).

***In vitro* sensitivity of *Xanthomonas campestris* pv. *viticola* to sanitizing agents.** Seven chlorinated sanitizing agents were tested: dodecyl dimethyl ammonium chloride (1,140 ppm), benzalkonium chloride (245 ppm), sodium dichloroisocyanurate (16.2 ppm), sodium hypochlorite (20,000 ppm), calcium hypochlorite (130 ppm), calcium oxychloride (97,500 ppm) and chlorine dioxide (41 ppm) at the concentrations recommended by the manufacturers. All solutions were made in SDW.

The antibiogram methodology used in the present study was a modified version of that described by Romeiro (2005a). A layer of approximately 1 mm of water-agar was spread on disposable Petri-dishes, followed by a layer of semi-solid NYDA medium to which a 10^8 CFU ml^{-1} suspension of the isolates *Xcv*5, *Xcv*83, *Xcv*113 and *Xcv*2 had been added (200 µl 10 ml^{-1} NYDA). Following the hardening of the medium, paper discs were soaked in the sanitizing agent solutions to be tested and placed on top of the medium. Four paper discs of the same concentration were placed equidistantly onto each plate and a control disc soaked in SDW was placed in the center of the plate. The plates were incubated at 28°C for 48 h. The diameter of the growth inhibition zone was measured in two opposite directions using a ruler, and the average diameter was calculated. The experimental design was completely randomized with five repetitions per treatment. Statistical analyses of the data were done using the SAS System version 9.0 software. Averages were compared using the Tukey test ($P \leq 0.01$).

Selection of sanitizing agents for the disinfection of harvest shears contaminated with *Xanthomonas campestris* pv. *viticola*. To this aim, a 2-cm portion of the blade tips was sequentially dipped in the *Xcv*2^{Rif} suspension, then in the sanitizing agent solutions and finally in the 20 ml of SDW, where the shears tips were gently stirred with circular motions for 20 sec. A series of dilutions up to 10^{-4} of the original suspension was made in SDW and aliquots (100 µl) of each dilution were plated on NYDAM^{Rif} medium in three replicates for each dilution. The plates were incubated for 48 h at 28°C, and the number of *Xcv*2^{Rif} colonies was counted. The experimental design was completely randomized, with eight treatments (seven sanitizing agents plus the control) and four replicates per treatment (individual shears). The experiment was repeated four times. The data were tested for homogeneity of variance (Levene test) and analyzed together when homogeneous. The analysis of variance (ANOVA) was performed; when significant, the means of the treatments were compared using the Tukey test ($P \leq 0.05$). CFU $(cm^2)^{-1}$ data were log10 transformed prior to analysis. Statistical analyses of the data were performed using the SAS System version 9.0 software.

Validation of the efficiency of sanitizing agents used for disinfecting harvest shears. A 2-cm portion of the blade tips was sequentially dipped in a suspension of the mutant *Xcv*2^{Rif} (10^8 CFU ml^{-1}), and immediately in dodecyl dimethyl ammonium chloride (1,140 ppm) or sodium hypochlorite (20,000 ppm), the two sanitizing agents found to be most efficient for tool disinfection in a previous experiment. Immediately following dipping in the inoculum and disinfectant, 50 sequential cuts were made in the leaves of cv. Red Globe vines with each shear. Ten cuts were made per plant and three per leaf (one in the main vein and two in secondary veins). The 10th cut was made in a fourth leaf (main vein). This procedure allowed the monitoring of the infection capacity of *Xcv* from the moment of inoculation. All cuts were numbered. Shears dipped only in a *Xcv*2^{Rif} suspension were used as control. The plants were maintained in a nursery and observed for 30 days. The presence of *Xcv* in the cut tissue was confirmed via isolation in NYDAM^{Rif} medium. The experimental design was completely randomized, with three treatments (two sanitizing agents plus control) and four repetitions per treatment (individual shears).

Persistence of the efficacy of the sanitizing agent solutions for the disinfection of harvest shears contaminated with *Xanthomonas campestris* pv. *viticola*. This experiment was carried out on a working bench at the Plantlets Nursery of Embrapa Semiárido which is covered with 70% Sombrite® screen. This coverage ensured that field conditions of solar incidence (500 µmol), temperature (27°C to 35°C) and relative humidity (66% to 39%) were similar to the ones found in local vineyards. To simulate sanitizing agent solutions containing plant fragments resulting from pruning, grapevine canes were cut with pruning shears (Tramontina; stainless steel blade) every four minutes (average pruning time of one plant in the field). After each cut, shear tips were dipped in the sanitizing agent solutions totalizing 17 dips in one hour.

Table 1. Effect of sanitizing agents on the *in vitro* growth of the *Xanthomonas campestris* pv. *viticola* isolates *Xcv*5, *Xcv*81, *Xcv*113 and *Xcv2* in agar-agar + NYDA semi-solid growth medium.

Treatment	Growth inhibition zones (mm)			
	*Xcv*5	*Xcv*81	*Xcv*113	*Xcv*2
Sodium hypochlorite (20,000 ppm)	13.4[1] aA	13.9 aA	14.5 aA	8.8 bB
Dodecyl dimethyl ammonium chloride (1,140 ppm)	11.3 bA	10.0 bB	11.1 bB	12.0 aA
Benzalkonium chloride (245 ppm)	8.4 cA	0 cB	8.6 cA	8.8 bA
Sodium dichloroisocyanurate (16.2 ppm)	0 dA	0 cA	0 dA	0 cA
Calcium oxychloride (97,500 ppm)	0 dA	0 cA	0 dA	0 cA
Chlorine dioxide (41 ppm)	0 dA	0 cA	0 dA	0 cA
Calcium hypochlorite (130 ppm)	0 dA	0 cA	0 dA	0 cA
Control (water)	0 dA	0 cA	0 dA	0 cA

[1]Average of five repetitions. Averages followed by the same lower or uppercase letter are not significantly different according to the Tukey test ($P \leq 0.05$).

Table 2. Disinfection of harvest shears infected with *Xanthomonas campestris* pv. *viticola* isolate *Xcv*2^{Rif} by different sanitizing agents.

Treatment	Log CFU $(cm^2)^{-1}$	
Control	13.0[1]	a
Calcium hypochlorite (130 ppm)	12.6	a
Chlorine dioxide (41 ppm)	11.7	ab
Sodium dichloroisocyanurate (16.2 ppm)	10.4	b
Calcium oxychloride (97,500 ppm)	7.9	c
Benzalkonium chloride (245 ppm)	6.9	c
Dodecyl dimethyl ammonium chloride (1,140 ppm)	0	d
Sodium hypochlorite (20,000 ppm)	0	d

[1]Average of sixteen repetitions. Averages followed by the same lower case letters are not significantly different according to the Tukey test ($P \leq 0.05$). Data were log (x + 1) transformed.

No pruning shears were dipped in the solutions sampled at 08:00 h (beginning of the experiment). Pruning shears were dipped 34 times in the solutions sampled at 10:00 h (2 h after the beginning of the experiment), 68 times at 12:00 h (4 h after the beginning of the experiment), 136 times at 14:00 (6 h after the beginning of the experiment) and 272 times at 16:00 h (8 h after the beginning of the experiment). The amount of organic matter in the sanitizing agent solutions was quantified at 08:00 and 16:00 h using the Oxygen Chemical Demand method (OCD) according to The Standard Methods for the Examination of Water and Wastewater (Rice *et al*., 2012). The index of changes in organic matter (ICOM) was calculated for the three treatments by the following equation ICOM = [(IOM – FOM)/IOM] × 100, where IOM = initial organic matter, and FOM = final organic matter. The experiment started at 8:00 h and finished at 16:00 h. The sanitizing agent's dodecyl dimethyl ammonium chloride (1,140 ppm) and sodium hypochlorite (20,000 ppm) were tested.

A 2-cm portion of the blade tips of the harvest shears to be tested was flamed and dipped in the suspension of *Xcv*2^{Rif} to simulate the acquisition of inoculum from an infected plant. After 0, 2, 4, 6 and 8 h, the harvest shear tips were sequentially dipped in the sanitizing agent solutions and in the 20 ml of SDW. The isolation and counting of *Xcv*2^{Rif} colonies were performed as previously described. For the control treatment, *Xcv*2^{Rif} contaminated harvest shears were dipped in SDW and maintained under the same conditions as the sanitizing agent solutions.

The experimental design was completely randomized, with three treatments (sodium hypochlorite, dodecyl dimethyl ammonium chloride, and control) and four repetitions per treatment (individual harvest shears). The experiment was repeated three times.

Selection of sanitizing agents for the disinfection of water used in the production of rooted grafts. Five sanitizing agents were tested at five different concentrations: oxytetracycline + copper sulfate (150+2,000, 165+2,200, 180+2,400, 195+2,600 and 210+2,800 ppm), oxytetracycline (600, 700, 800, 900 and 1,000 ppm), dodecyl dimethyl ammonium chloride (570, 1,140, 1,710, 2,280 and 2,850 ppm), sodium hypochlorite (5,000, 10,000, 20,000, 30,000 and 40,000 ppm) and benzalkonium chloride (122.5, 163.6, 245, 327.3 and 490 ppm). Mutant bacterial suspension (1 ml) was placed in test tubes with 9 ml of the tested solutions to a final volume of 10 ml and a final bacterial concentration of 10^7 CFU ml^{-1}. Following incubation at 28°C for 30 min, the tubes were vortexed, and a series of dilutions up to 10^{-4} were made with three repetitions. Aliquots of 0.1 ml of the suspension were plated on NYDAM-^{Rif} medium. The plates were incubated at 28°C for 72 h, the bacterial population was determined as CFU ml^{-1}, and the population decrease was calculated. The experiment was made twice. The experimental design was completely randomized. Five concentrations were tested for each of the products with four repetitions (each one consisting of an individual test tube). The data were tested for homogeneity of variance (Levene test), submitted to ANOVA and, when significant, the comparison of the means was performed by the Tukey test ($P \leq 0.05$). Statistical analyses of the data were performed using the SAS System version 9.0 software.

RESULTS

***Xanthomonas campestris* pv. *viticola* survival on harvest shears.** The *Xcv*2^{Rif} mutant survived for 24 h on the shears (Fig. 1). In samplings made immediately following dipping of the shears in the bacterial suspension, the population was approximately 10^7 CFU $(cm^2)^{-1}$ per shear.

After 6 h, the population had decreased to less than half of the initial amount then it stabilized between 12 and 24 h. *Xcv*2^{Rif} was no longer detected after 30 h and remained undetectable until the end of the experiment, at 42 h.

***In vitro* sensitivity of *Xanthomonas campestris* pv. *viticola* to sanitizing agents.** The widest inhibition zones of *Xcv* growth were obtained with sodium hypochlorite and dodecyl dimethyl ammonium chloride (Table 1). Benzalkonium chloride came third, except for isolate *Xcv*2. Significant interaction ($P \leq 0.01$) was observed between *Xcv* isolates and sanitizing agents. Isolate *Xcv*81 was found to be resistant to benzalkonium chloride. All tested isolates proved to be resistant to sodium dichloroisocyanurate, calcium oxychloride, chlorine dioxide and calcium hypochlorite (Table 1).

Selection of sanitizing agents for the disinfection of harvest shears contaminated with *Xanthomonas campestris* pv. *viticola*. Of the seven tested sanitizing agents, five significantly reduced the population of *Xcv*2^{Rif} in harvest shears (Table 2). However, only the use of sodium hypochlorite (20,000 ppm) and dodecyl dimethyl ammonium chloride (1,140 ppm) resulted in the total disinfection of the treated shears (Table 2).

Validation of the efficiency of sanitizing agents used for disinfecting harvest shears. Although shears dipped in the *Xcv*2^{Rif} suspension were able to inoculate the pathogen when used for cutting up to 42 healthy grapevine leaves in a row, pathogen spreading occurred in average until 24 cuts. Nevertheless, this happened in a discontinuous fashion, i.e., the bacteria were sometimes not found on certain cut but were present in the following one. Treating the shears with a single dip in dodecyl dimethyl ammonium chloride (1,140 ppm) and sodium hypochlorite (20,000 ppm) resulted in successful disinfection and prevented pathogen spreading.

Persistence of the efficacy of the sanitizing agent solutions for the disinfection of harvest shears contaminated with *Xanthomonas campestris* pv. *viticola*. Dodecyl dimethyl ammonium chloride (1,140 ppm) and sodium hypochlorite (20,000 ppm) solutions successfully disinfected *Xcv*-contaminated shears from time 0 (no solar exposure and absence of organic matter fragments) until the end of the experiment (eight hours of solar exposure and presence of organic matter fragments) (Fig. 2). The disinfection was confirmed by the absence of bacterial growth in the culture medium, showing that the efficacy of the solutions was maintained for eight hours, regardless of solar exposure, temperature and relative humidity. At time 0, water control and sodium hypochlorite did not show organic matter while dodecyl dimethyl ammonium chloride showed 257.3±21.3 mg l^{-1}. After eight hours, the index of change in organic matter in the water control reached 209.0±30.4 mg l^{-1}, and in sodium hypochlorite reached 87.7±5.0 mg l^{-1}. In dodecyl dimethyl ammonium chloride, this index decreased from 257.3±21.3 mg l^{-1} (time 0) to 154.2±40.0 mg l^{-1} (time 8).

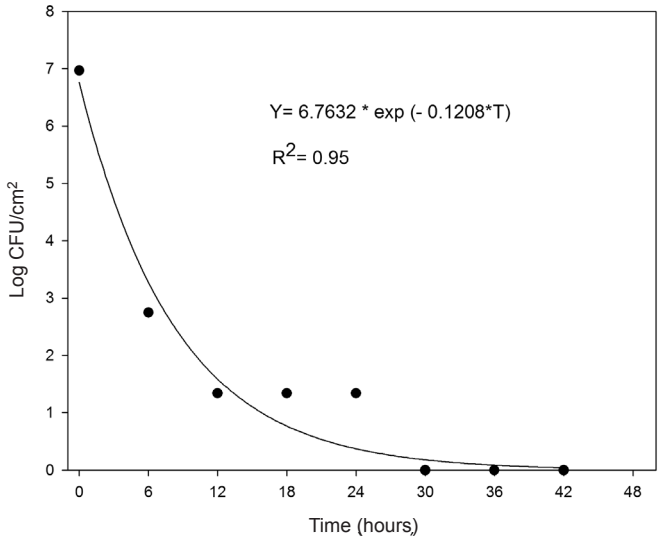

Fig. 1. *Xanthomonas campestris* pv. *viticola* survival in grapevine harvest shears following dipping in bacterial suspensions and incubation at 28°C.

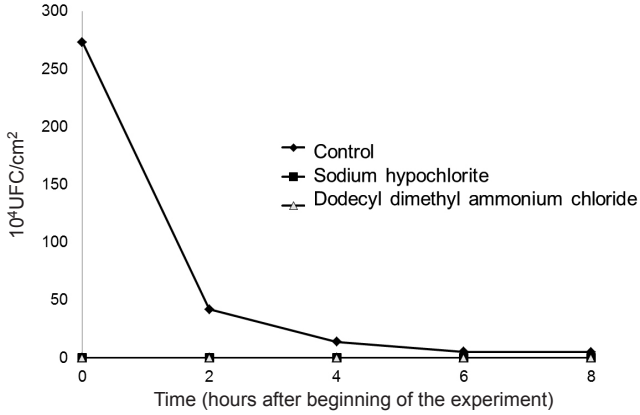

Fig. 2. Persistence of the efficacy of sanitizing agents sodium hypochlorite (20,000 ppm) and dodecyl dimethyl ammonium chloride (1,140 ppm) in disinfecting harvest shears contaminated with *Xanthomonas campestris* pv. *viticola*, evaluated by the remaining bacterial population on shears after treatment in each sampling time (mean of three experiments).

Selection of sanitizing agents for the disinfection of water used in the production of rooted grafts. The *Xcv*2^{Rif} population in the water was significantly reduced ($P \leq 0.05$) by all the sanitizing agents at all concentrations except for oxytetracycline at 800 ppm (99.8%). However, only dodecyl dimethyl ammonium chloride, sodium hypochlorite, and benzalkonium chloride at all concentrations, oxytetracycline at 900 and 1,000 ppm and oxytetracycline + copper sulfate at 195+2,600 and 210+2,800 ppm achieved complete (100%) *Xcv*2^{Rif} eradication.

DISCUSSION

Phytopathogenic bacteria survive adverse conditions such as radiation, heat stress, desiccation, microbial antagonism, oxygen deficiency and high/low pH, regardless of whether they are associated with a host or not. These bacteria possess several survival mechanisms, including encapsulation, endospore formation, latency, antagonism, biochemical and physiological versatility, sheltering in ecological niches, and alternative hosts (Romeiro, 2005b); the presence of these defense mechanisms depend on the bacteria-habitat-mechanism interaction. *Xcv* can survive in healthy or infected grapevines, in the epiphytic (Araujo, 2001) or endophytic stages, in alternative hosts (Peixoto *et al*., 2007) and crop debris (Silva *et al*., 2012). However, no studies have been performed examining their survival on inert objects such as cutting tools. In the present study, *Xcv* survived for at least 24 h on harvest shears without organic residues (Fig. 1); this short survival period may have been due to the lack of available nutrients on the tested material. Because the blades of the shears selected for this study were made of stainless steel *Xcv* is likely to survive on other cutting tools, (e.g. budding knives and pruning shears) made with the same material. The survival mechanisms of this pathogen in environments with low nutrient levels are unknown. The bacteria possess an extrapolysaccharide layer that protects the cells against desiccation (Romeiro, 2005b) and may aid in nutrition (Sutherland, 1988). The survival of *Xcv* on harvest shears indicates that such tools are important sources of inoculum for grapevine bacterial canker, especially when producers use the same non-disinfected tools in crops of different areas within 24 h. This was likely one of the causes of the rapid spreading of the disease in the vineyards of the São Francisco Valley following its introduction in 1998. Tool disinfection is therefore of great importance for the control of this disease.

In the test of the *in vitro* sensitivity of four *Xcv* isolates to seven sanitizing agents, sodium hypochlorite at 20,000 ppm and dodecyl dimethyl ammonium chloride at 1,140 ppm produced the greatest growth inhibition areas (Table 1) and were the only tested products that successfully disinfected *Xcv*-contaminated shears (Table 2). When a suspension of *Rhizobium radiobacter* (Ophel and Kerr) Young et al. (sin. *Agrobacterium tumefaciens*) was exposed to nine different products at 100, 1,000 and 10,000 ppm for 30 min, benzalkonium chloride and cetyltrimethylammonium bromide were the only sanitizing agents that completely eliminated the pathogen population at the three tested concentrations. Furthermore, in another study, 30 sec of exposure to these two sanitizing agents were able to extinguish the pathogen at 70, 100 and 500 ppm (Yakabe *et al*., 2012).

In the present study, *Xcv* was inoculated in grapevine leaf tissue by non-disinfected shears, and the inoculum progressively decreased with successive cuts, likely since it was progressively diluted until disappearance. Similar results were obtained with *Datura stramonium* and walnut trees cv. Paradox when 10 and six sequential cuts were performed, respectively, with scalpels contaminated with *R. radiobacter*; in this study, the bacteria were detected until the eighth and sixth cuts, respectively (Yakabe *et al*., 2012). The disinfection of harvest shears with dodecyl dimethyl ammonium chloride and sodium hypochlorite prevented the spread of *Xcv* in grapevine leaf tissue. The disinfection efficacy of these products has also been observed in tools used in other crops. In *D. stramonium*, the percentage of cuttings with *R. radiobacter* galls was significantly decreased when the blades used for grafting were disinfected with benzalkonium chloride, and no gall formation occurred when a 5,000 ppm concentration was used (Yakabe *et al*., 2012). In melons, the disinfection of pruning shears with 2% sodium hypochlorite usually decreased the spreading of *Didymella bryoniae* by 72.5% (Vida *et al*., 2004).

Based on the results obtained on the maintenance of efficacy of the studied solutions, sodium hypochlorite and dodecyl dimethyl ammonium chloride can be recommended for the disinfection of *Xcv*-contaminated tools, as the efficacy of these products was maintained for eight hours, under conditions similar to the ones found in local vineyards and in the presence of plant fragments (Fig. 2). Indeed, the presence of organic matter is one of the factors that can interfere with the action of chlorinated compounds because it uses part of the disinfecting agent, thus decreasing the initial concentration (Ribeiro *et al*., 2008). The efficacy of sodium hypochlorite (0.02 ppm), benzalkonium chloride (2.5 ppm), cetyltrimethylammonium bromide (2 ppm) and n-alkyl dimethyl benzyl ammonium chloride/n-alkyl ethyl benzyl ammonium chloride (0.80 ppm) against *R. radiobacter* was reduced in the presence of 0.7, 1.5, 2 and 3 g/ml of total solids. Sodium hypochlorite efficiency was the most affected with a reduction in efficacy of 64% in the presence of the lowest concentration of total solids and of 91% in the presence of the highest concentration of total solids. For the remaining products, the highest concentration of total solids tested resulted in reduced efficacies of 21, 28 and 31%, respectively (Yakabe *et al*., 2012). In this work, even in presence of organic matter in their solutions, the efficacy of sodium hypochlorite and dodecyl dimethyl ammonium chloride was 100%, which means complete eradication of *Xcv* from shears. Solutions of dodecyl dimethyl ammonium chloride showed organic matter in the beginning of the experiment since this is an organic compound. The reduction of organic matter observed in this sanitizing agent was probably due to the removal of the product by the shears during the 8 h of consecutive dipping and washing, since the same solution, without dipping the shears, remained with the same amount of organic matter as in the beginning of the experiment.

The use of fungicides to disinfect irrigation water can result in the development of resistant isolates and has a negative environmental impact. The importance of sanitizing agents as an alternative to fungicides has previously been reported (Cayanan *et al*., 2009). Of the five sanitizing agents tested for the disinfection of *Xcv*-contaminated water, only dodecyl dimethyl ammonium chloride, sodium hypochlorite, and benzalkonium chloride were 100% effective at all concentrations tested. The concentrations of sanitizing agents used for the disinfection of water may vary with the pathogen genus and species (Hong *et al*., 2003). *Erwinia carotovora* subsp. *zeae* (sin. *Dickeya zeae*) was successfully eliminated from water with 1 mg l^{-1} of active chlorine (Thompson, 1965), but *Erwinia chrysanthemi* (sin. *Dickeya chrysanthemi*) and *E. carotovora* subsp. *carotovora* (sin. *Pectobacterium carotovorum* subsp. *carotovorum*) were less sensitive and survived up to 10 mg l^{-1} of active chlorine (Lacy *et al*., 1981). The ideal concentration for the elimination of *R. radiobacter* in recycled water used on rosebush crops was found to be 4 mg l^{-1} of active chlorine (Poncet *et al*., 2001). Instead, no dosage tested of dodecyl dimethyl ammonium chloride eradicated *Ralstonia solanacearum* from water (Bagnall, 2007).

Benzalkonium chloride, sodium hypochlorite and dodecyl dimethyl ammonium chloride were also found to inhibit the *in vitro* growth of *Xcv* isolates (Table 1), except for benzalkonium chloride, which did not inhibit *Xcv*81; but they were unsuccessful at eliminating the pathogen from thinning shears (Table 2). To eliminate the pathogen, benzalkonium chloride would need an immediate disinfecting action on shears but this was not verified. However, this sanitizing agent was effective in eliminating *Xcv* from infected water (Table 3), most likely because the treatment lasted 30 min. Because sodium hypochlorite, dodecyl dimethyl ammonium chloride and benzalkonium chloride were effective at all tested concentrations, any of these products can be used for the disinfection of *Xcv*-contaminated water at the lower tested concentrations of 5,000, 570 and 122.5 ppm, respectively. Further investigation is needed to evaluate the effect of organic matter (acquired during the 48 h of immersion of the cuttings) on the efficacy of the products. The efficacy of shorter exposure times and other product concentrations in eliminating *Xcv* from water should also be investigated.

The present study has shown that *Xcv* survives for at least 24 h on harvest shears, indicating that cutting tools are sources of inoculum and can introduce and spread grapevine bacterial canker. Disinfecting the tools with sodium hypochlorite (20,000 ppm) and dodecyl dimethyl ammonium chloride (1,140 ppm) is therefore recommended. This preventive control measure should be performed during grapevine cutting procedures, between plants and at the end of the working day. The solutions of these sanitizing agents remain effective when maintained under the environmental conditions of the vineyards located at the São Francisco Valley and when solutions contain plant fragments for at least eight hours. For the disinfection of *Xcv*-contaminated water, dodecyl dimethyl ammonium chloride (570 ppm), sodium hypochlorite (5,000 ppm) and benzalkonium chloride (122.5 ppm) can be used.

ACKNOWLEDGEMENTS

The authors thank FACEPE for the doctorate scholarship and graduate mobility aid awarded to Carine Rosa Naue and CNPq for the scholarship in Research Productivity granted to Elineide B. Souza and Rosa L.R. Mariano, and also for the financial support (CNPq Proc. 472.555/2009-0).

REFERENCES

Araujo J.S.P., 2001. Perfil epidemiológico e subsídios para o controle de *Xanthomonas campestris* pv. *viticola* (Nayudu) Dye, agente do cancro bacteriano da videira (*Vitis vinifera* L.) no Brasil. PhD Thesis. Universidade Federal Rural do Rio de Janeiro, Rio de Janeiro, Brasil.

Araujo J.S.P., Olivares F.L., Reis Junior F.B., Robbs C.F., 2002. Movimento sistêmico de *Xanthomonas campestris* pv. *viticola* em videira. *Fitopatologia Brasileira* **27**: 53-54.

Bagnall R., 2007. Control of *Pythium* wilt and root rot of hydroponically grown lettuce by means of chemical treatment of the nutrient solution. M.Sc. Thesis, University of Pretoria, Pretoria, South Africa.

Cayanan D.F., Zhang P., Liu W., Dixon M., Zheng Y., 2009. Efficacy of chlorine in controlling five common plant pathogens. *HortScience* **44**: 157-163.

Freire F.C.O., Oliveira A.D.S., 2001. Ocorrência do cancro-bacteriano da videira no Estado do Ceará. Comunicado Técnico **62**. Embrapa Agroindústria Tropical, Fortaleza, CE, Brazil.

Halfeld-Vieira B.A., Nechet K.L., 2006. Bacterial canker of grapevine in Roraima, Brazil. *Fitopatologia Brasileira* **31**: 6098.

Hong C.X., Richardson R.A., Kong P., Bush E.A., 2003. Efficacy of chlorine on multiple species of *Phytophthora* in recycled nursery irrigation water. *Plant Disease* **87**: 1183-1189.

Junqueira N.T.V., Junqueira K.P., Braga M.F., Vaz C.F., Santos E.C., Junqueira L.P., Souza L.S., Lima C.A., 2006. Ocorrência do Cancro Bacteriano da Videira na Região do Entorno do Distrito Federal. *Anais do XIX Congresso Brasileiro de Fruticultura*, *Cabo Frio 2006*: 323.

Lacy G.H., Lambe R.C., Berg C.M., 1981. Iris soft rot caused by *Erwinia chrysanthemi* associated with overhead irrigation and its control by chlorination. *Proceedings International Plant Propagators Society* **31**: 626-634.

Lima M.F., 2000. Cancro bacteriano da videira causado por *Xanthomonas campestris* pv. *viticola*: Epidemiologia e Manejo. Circular Técnica **54**. Embrapa semiárido, Petrolina, PE, Brazil.

Malavolta Jr. V.A., Almeida I.M.G., Sugimori M.H., Ribeiro I.J.A, Rodrigues Neto J., Pires E.J.P., Nogueira E.M.C., 1999.

Ocorrência de *Xanthomonas campestris* pv. *viticola* em videira no Brasil. *Summa Phytopathologica* **25:** 262-264.

Nascimento A.R.P., Mariano R.L.R., 2004. Cancro-bacteriano da videira: etiologia, epidemiologia e medidas de controle. *Ciência Rural* **34:** 301-307.

Nayudu M.V., 1972. *Pseudomonas viticola* sp. nov., incitant of a new bacterial disease of grapevine. *Phytopathologische Zeitschrift* **73**: 183-186.

Peixoto A.R.P., Mariano R.L.R., Moreira J.O.T., Viana I.O., 2007. Hospedeiros alternativos de *Xanthomonas campestris* pv. *viticola. Fitopatologia Brasileira* **32**: 161-164.

Poncet C., Offroy M., Bonnet G., Brun R., 2001. Disinfection of recycling water in rose cultures. *Acta Horticulturae* **547**: 121-127.

Raudales E.R., Fisher P.R., Harmon C.L., Mackay B.R., 2011. Review of efficacy tests for chlorination of irrigation systems. *Proceedings Florida State Horticultural Society* **124**: 285-288.

Ribeiro J.M., Canuto K.M., Veschi J.L.A., 2008. Compostos clorados: aspectos gerais e sua utilização como agente sanitizante na agricultura, micropropagação e pecuária. Documentos **207**. Embrapa Semi-Árido, Petrolina, PE, Brazil.

Rice E.W., Baird R.B., Eaton A.D., Clesceri L.S., 2012. Standard Methods for the Examination of Water and Wastewater, 22nd ed. American Public Health Association, Washinton, DC, USA.

Rodrigues Neto J., Destéfano S.A.L., Rodrigues L.M.R., Pelosso D.S., Oliveira Junior L.C., 2011. Grapevine bacterial canker in the State of São Paulo, Brazil: detection and eradication. *Tropical Plant Pathology*. **36:** 42-44.

Romeiro R.S., 2005a. Antibióticos e seu emprego em pesquisa com bactérias fitopatogênicas. In: Romeiro R.S. (ed). Métodos em Bacteriologia de Plantas, pp. 127-162. Editora UFV, Viçosa, Brazil.

Romeiro R.S., 2005b. Bactérias Fitopatogênicas, 2nd ed. Editora UFV, Viçosa, MG, Brazil.

Rosner A., Lachman O., Pearlsman M., Feigelson L., Maslenin L., Antignus Y., 2006. Characterization of *Cucumber leaf spot virus* isolated from recycled irrigation water of soil-less cucumber cultures. *Annals of Applied Biology* **149**: 313-316.

Silva A.M.F., Menezes E.F., Souza E.B., Melo N.F., Mariano R.L.M., 2012. Sobrevivência de *Xanthomonas campestris* pv. *viticola* em tecido infectado de videira. *Revista Brasileira de Fruticultura* **34:** 757-765.

Sutherland I.W., 1988. Bacterial surface polysaccharides: structure and function. *International Review of Cytology* **113**: 187-231.

Tavares S.C.C.H., 1995. Principais Doenças das culturas de: manga, uva, acerola e banana. Apostila do Curso de Atualização Técnica para Engenheiros Agrônomos do Banco do Brasil. Embrapa semiárido, Petrolina, PE, Brazil.

Tavares S.C.C.H., Menezes M., 1991. Processo de infecção de *Botryodiplodia theobromae* em plantas de mangueira e videira no Trópico Semi-Árido brasileiro. *Fitopatologia Brasileira* **16**: 55.

Thompson D.L., 1965. Control of bacterial stalk rot of corn by chlorination of water in sprinkler irrigation. *Crop Science* **5**: 369-370.

Tomaz R., Scremin R.M., Ferreira M.A.S.V., Barbosa M.A.G., 2011. Detecção e erradicação de videiras com sintomas do cancro bacteriano no Estado do Paraná. *Tropical Plant Pathology* **36**: 896.

Trindade L.C., Lima M.F., Ferreira M.A.S.V., 2005. Molecular characterization of brazilian strains of *Xanthomonas campestris* pv. *viticola* by rep-PCR fingerpriting. *Fitopatologia Brasileira* **30:** 46-54.

Trindade L.C., Marques E., Lopes D.B., Ferreira M.A.S.V., 2007. Development of molecular method for detection and identification of *Xanthomonas campestris* pv. *viticola*. *Summa Phytopathologica* **33:** 16-23.

Valexport, 2009. Valexport há 21 Anos Unindo Forças para o Desenvolvimento do Vale do São Francisco e da Fruticultura Brasileira. Valexport, Petrolina, PE, Brazil.

Ventura J.A., Costa H., 2002. Manejo integrado das doenças de fruteiras tropicais: abacaxi, banana e mamão. In: Zambolim L. (ed**).** Manejo Integrado, Fruteiras Tropicais: Doenças e Pragas, pp. 279-350. Editora UFV, Viçosa, Brazil.

Vida J.B., Tessmann D.J., Zambolim L., Verzignassi J.R., Brandão Filho J.U.T., 2004. Controle da podridão gomosa em melão rendilhado em cultivo protegido por sanitização de ferramenta de poda. *Fitopatologia Brasileira* **29:** 626-630.

Yakabe L.E., Parker S.R., Kluepfel D.A., 2012. Cationic surfactants: Potential surface disinfectants to manage *Agrobacterium tumefaciens* biovar 1 contamination of grafting tools**.** *Plant Disease* **96:** 409-415.

Zambolim L., Costa H., Ventura J.A., Vale F.X.R., 2002. Controle de doenças em pós-colheita de fruteiras tropicais, pp. 443-511. In: Zambolim L. (ed.) Manejo Integrado, Fruteiras Tropicais: Doenças e Pragas**.** Editora UFV, Viçosa, Brazil.

Received April 25, 2013
Accepted May 28, 2014