Plant Pathology (2012) **61**, 665–676 Doi: 10.1111/j.1365-3059.2011.02559.x

Environmentally friendly treatment alternatives to Bordeaux mixture for controlling bacterial apical necrosis (BAN) of mango

J. A. Gutiérrez-Barranquero[a], E. Arrebola[b], N. Bonilla[a], D. Sarmiento[c], F. M. Cazorla[a] and A. de Vicente[a]*

[a]*Instituto de Hortofruticultura Subtropical y Mediterránea "La Mayora" (IHSM-UMA-CSIC), Departamento de Microbiología, Facultad de Ciencias, Universidad de Málaga, Unidad Asociada al CSIC, Campus de Teatinos s/n, E-29071 Málaga;* [b]*Instituto de Hortofruticultura Subtropical y Mediterránea "La Mayora" (IHSM-UMA-CSIC), Estación Experimental "La Mayora", A/garrobo costa, E-29750, Málaga; and* [c]*SAT 2803 TROPS, Vélez-Málaga, Málaga, E-29700, Spain*

Bacterial apical necrosis (BAN), caused by the bacterium *Pseudomonas syringae* pv. *syringae* (Pss), is currently the most limiting disease affecting the mango crop in the Mediterranean area. The copper-based compound Bordeaux mixture (BM) is considered to be the conventional treatment against BAN, but it does not act as a bactericide. Alternative experimental treatments to BM that are compatible with organic farming were tested for their ability to control BAN disease. Field trials were conducted over six seasons in different mango orchards with natural infestation of Pss. The experimental treatments included applications of Silicon gel (a commercial formulation of aqueous potassium silicate), dicalcium phosphate, Kaolinite, and Ulmasud B® (bentonite, powder); BM was applied as the conventional treatment. During the first two seasons (small-scale trial, 2002–2004), all these experimental compounds were applied in order to select those treatments providing the greatest reduction of BAN symptoms. In the next three seasons (2005–2008), a semi-commercial scale trial was carried out with the best-performing treatments, resulting in the selection of Silicon gel. Finally, Silicon gel was tested in a commercial scale trial during the last season (2008–2009). Trees treated with Silicon gel showed significantly fewer necrotic buds and leaves, reaching disease levels very similar to those using the conventional treatment with BM. However, minor differences in *P. syringae*-like population levels were observed, as has been described in previous studies. The possible mode of action of the Silicon gel is discussed. Currently, the Silicon gel compound is undergoing the registration process for its commercial use in mango management in Spain.

Keywords: bacterial apical necrosis, Bordeaux mixture, mango, *Pseudomonas syringae*, Silicon gel

Introduction

Mango (*Mangifera indica*) is one of the most important fruit crops in the world. In Europe, most of the mango production is located in southern Spain and Portugal. In southern Spain, the mango crop has expanded in the last 5 years from 800 ha in 2004 to 3000 ha in 2009, of which more than 1000 ha are in full production (Pérez & Morales, 2009). However, the commercial viability of this crop has been threatened by the frequent occurrence of bacterial apical necrosis (BAN), causing reduction in mango yields of 30–50% in years with severe attacks. This disease is caused by the phytopathogenic bacterium *Pseudomonas syringae* pv. *syringae* (Pss) (Cazorla *et al.*, 1998). BAN affects mango crops in subtropical regions such as Spain, Portugal, Italy, Israel and Australia (Kennelly *et al.*, 2007). This bacterium also causes disease in other hosts such as stone fruits, pome fruits and crop plants (Kennelly *et al.*, 2007). BAN is characterized by rapidly expanding necrotic lesions on buds and leaves. Stem and flower panicles can also be affected, as the symptoms can extend from buds through the leaf petiole. The symptoms of this disease develop more strongly in cool years, and it has been suggested that the severity of outbreaks depends to a great extent on winter and spring temperatures; however, other factors may be involved in disease initiation (Cazorla *et al.*, 1998).

Although many compounds have been reported to control bacterial diseases in fruit trees, only one study of control methods has been reported in mango crops. This suggested that the most effective treatment for controlling BAN was the spraying of copper compounds Bordeaux mixture (BM) (Cazorla *et al.*, 2006). Unfortunately, continuous treatment with BM can lead to many problems. One such problem is that copper is a major heavy metal contaminant that accumulates from mining,

*E-mail: adevicente@uma.es

Published online 22 November 2011

metal processing, fertilizers, fungicides, agricultural and municipal wastes, sewage sludge dispersal and traffic emissions (Wang, 1997; Xiong, 1998; Kabata-Pendias, 2001). Another potential problem with excessive copper usage to control BAN could be loss of efficacy due to selection for copper-resistant strains of *P. syringae* (Cazorla *et al.*, 2002; Vanneste *et al.*, 2008). Moreover, the toxicity of copper to roots (Alva & Graham, 1991), young shoots and leaves (Kairu *et al.*, 1985) is well documented. Few studies have described the phenomenon of copper bioaccumulation in fruits, nor have the implications for human health of the migration of this metal into food been addressed. In this regard, it has been reported that the application of copper for olive crop protection has negative effects on the olive ecosystem (Iannotta *et al.*, 2007), and copper bioaccumulation has been described in Chinese cabbage (Xiong & Wang, 2005). The European Union has introduced legislation limiting the use of copper compounds in regulation no. 473/2002 (Anonymous, 2002).

For all of these reasons, several attempts have been made to reduce the application of copper compounds to agricultural crops (Ninót *et al.*, 2002) and to find alternative experimental treatments that are environmentally friendly and compatible with organic farming. Currently, BM is the treatment recommended to control BAN, and it is a film-forming and antimicrobial compound which has been extensively used in agriculture (Agrios, 2005). It forms a thick film on plant surfaces due to the calcium it contains (Becerra, 1995). BM actively controls BAN, but no effect has been observed on *P. syringae* levels, suggesting that antimicrobial activity may not be its main mode of action against BAN (Cazorla *et al.*, 2006). Because different film-forming polymers have been reported to counteract plant diseases (Ziv & Frederiksen, 1983, 1987), the aim of this study was to evaluate alternative experimental treatments to BM mainly based on a film-forming mode of action, that are environmentally friendly and suitable for organic farming. Field experiments were carried out in order to test a selection of film-forming compounds over six seasons with the goal of providing mango growers in the Mediterranean region an alternative to BM treatment for the control of BAN.

Materials and methods

Experimental treatments

Details of experimental treatments selected to test for efficacy against BAN, including sources, trademarks, doses and application regimes are summarized in Table 1. Kaolinite is a natural clay mineral that acts as a repellent/protectant, and forms a barrier film to protect plants from insects, bacterial and fungal diseases. Dicalcium phosphate is a source of calcium and phosphorus for the plant, also used to increase soil fertility. Ulmasud B® is a natural bentonite that prevents plant disease against bacterial and fungal pathogens, activating the plant defences and Silicon gel (a commercial formulation of aqueous potassium silicate) is a natural source of silicon that has been shown to be involved in eliciting the plant induced systemic resistance (ISR) response.

Field disease control assays

Field assays were conducted in experimental plots located in commercial orchards in the fruit-tree growing area of Malaga, in southern Spain, where the BAN disease has limited mango production for several years (Cazorla *et al.*, 1998, 2006). The study was performed on mango trees, in cvs Tommy Atkins and Keitt grafted onto cv. Espada seedling rootstocks, which showed similar levels of susceptibility. The trees were grown on acid sandy loam soil. They were drip irrigated at 1–2 day intervals during the summer and twice per week during the winter. The temperature (°C) and rainfall (mm) during the trials were recorded daily (Fig. 1) at a meteorological station at La Mayora Experimental Station (CSIC, Málaga, Spain) located in the study area. Six independent experiments on three different scales were carried out during six different growing seasons, and different orchards were used depending on the scale of the trial (details summarized in Table 1). Different trees were used in each season to avoid cumulative effects of the experimental treatments. First, two independent experiments were carried out during 2002–2003 and 2003–2004. A small-scale trial was performed using six experimental orchards and evaluating seven different treatments (Tables 1 and 2). Five of the orchards consisted of 15-year-old mango trees of cv. Tommy Atkins at 4 × 4 m spacing and with a canopy diameter of approximately 2 m. The remaining orchard (Sarmiento) consisted of 13-year-old mango trees of cv. Keitt at 4 × 6 m spacing with a canopy of approximately 2·5 m. For this small-scale trial, each commercial orchard had 10 trees per experimental treatment, and all the terminal buds (n = 70–200) on each mango tree were examined for the presence of apical necrosis symptoms to evaluate disease severity.

Secondly, for the semi-commercial scale trial, (seasons 2005–2006, 2006–2007 and 2007–2008), four treatments were assessed in four different orchards using groups of 50–100 trees per orchard. The experimental treatments tested in this trial were Silicon gel, Silicon gel + Nu-film® (di-1-p-menthene 96%), Ulmasud B® and Ulmasud B® + Nu-film®, because these treatments produced the best results in the control of BAN during the first trial. In addition, BM-treated and untreated trees were included as controls. Three orchards consisted of 18-year-old mango trees of cv. Tommy Atkins, and one orchard (Sarmiento) consisted of 16-year-old mango trees of cv. Keitt. In the semi-commercial scale trial 40 buds per tree were counted, 10 from each quadrant of the tree.

Finally, a commercial scale trial (2008–2009) was carried out in one orchard (Sarmiento) consisting of 19-year-old mango trees of cv. Keitt, where 1000 trees were treated with the selected experimental treatment (Silicon gel), 100 trees were treated with BM, and 100 trees remained untreated.

Table 1 Treatments, dose L^{-1} and active material used in this study to control bacterial apical necrosis (BAN) on mango trees. Different experimental treatments were assessed depending on the trial scale

				Seasons on mango trees					
				Small scale		Semi-commercial		Commercial	
Experimental treatments	Active material	Dose L^{-1}	Company	2002–2003	2003–2004	2005–2006	2006–2007	2007–2008	2008–2009
Controls									
Untreated				x	x	x	x	x	x
Assayed compounds									
Bordeaux mixture (2×)	Cuprocalcium sulphate orthophosphoric	6·0 g	Industrias Quimicas del Vallés, S.A. (Spain)	x	x				
Bordeaux mixture	Cuprocalcium sulphate orthophosphoric	3·0 g	Industrias Quimicas del Vallés, S.A. (Spain)			x	x	x	x
Silicon gel + dicalcium phosphate	SiK (34% Si) + $CaHPO_4.2H_2O$	3·1 g + 2·7 g	FMC Foret (Spain) + Quimialmel (Spain)	x					
Kaolinite	$Al_2Si_2O_5(OH)_4$	6·7 g	Quimialmel (Spain)		x				
Silicon gel (1×)	SiK (34% Si)	2·7g	FMC Foret (Spain)	x	x				
Silicon gel (4×)	SiK (34% Si)	10·8 g	FMC Foret (Spain)		x	x	x	x	x
Silicon gel (4×) + Nu-film®	SiK (34% Si) + di-1-p-menthene 96%	10·8 g + 0·4 mL	FMC Foret (Spain) + Miller Chemicals and Fertilizer Corp. (USA)			x	x	x	
Ulmasud B® (1×)	Bentonite, powder	6·7 g	Biofa (Germany)	x	x				
Ulmasud B® (2×)	Bentonite, powder	13·4 g	Biofa (Germany)		x	x	x	x	
Ulmasud B® (2×) + Nu-film®	Bentonite, powder + di-1-p-menthene 96%	13·4 g + 0·4 mL	Biofa (Germany) + Miller Chemicals and Fertilizer Corporation. (USA)			x	x	x	

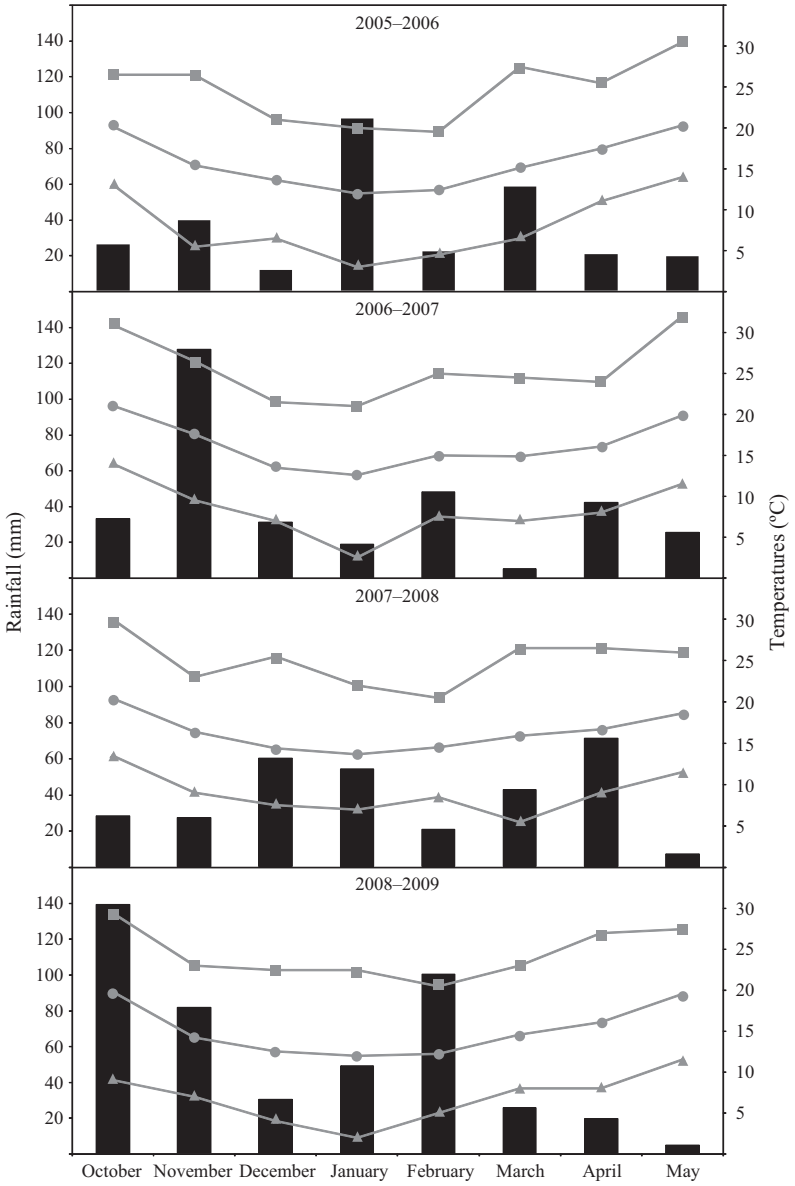

Figure 1 Weather conditions from October to May during the semi-commercial scale trial (2005–2006, 2006–2007 and 2007–2008) and the commercial scale trial (2008–2009). (▲) Monthly absolute minimum temperature, (●) monthly mean temperature, (■) monthly absolute maximum temperature, and rainfall (black columns).

The trees used in all of these experiments were selected from the commercial orchards by their uniformity in size and disease incidence. Each experimental treatment was applied on separate individual trees located in the field. Depending on the type of orchard and trial, treatments were applied with an engine-operated 15 L hand sprayer (Stihl model SR400) or a 300 L trailer sprayer (Rittenhouse model GB 300L). In all cases, the trees were treated to the point of run-off (approximately 15 L per 10–15 trees), with applications starting in October after harvest and finishing before April (after budbreak). The standard treatment application programme was dependent on the scale of the trial. In the first two seasons (small-scale trial), all the treatments, including the control, were applied six times: one per month between October and March. In the semi-commercial scale seasons, the treatments were applied four times between November and March, but these application schedules were determined by the weather, with the treatment being applied when the temperature decreased or after periods of heavy rain. During the semi-commercial scale trial, Nu-film® at 2·0 mL L^{-1} was used as an adjuvant for Silicon gel (4×) and Ulmasud B® (2×) because it favours the maintenance of active ingredients on plant surfaces by forming a film that retards run-off and evaporation. All the experimental treatments and their dosages were previously tested

Table 2 Total *Pseudomonas syringae*-like bacterial counts on buds from mango trees in the different experimental treatments assessed against bacterial apical necrosis and the effect of the experimental treatments in different commercial orchards during the small-scale trial (seasons 2002–2003 and 2003–2004). The data corresponds to February when the disease severity was highest. Disease incidence was assessed as percentage of terminal buds showing necrotic symptoms

		Season 2002–2003		Season 2003–2004	
Orchard	Treatments	CFU g^{-1} Pss-like	% Affected buds[a]	CFU g^{-1} Pss-like	% Affected buds
Sarmiento	Untreated	$3{\cdot}0 \times 10^7$	77·6a	$3{\cdot}4 \times 10^7$	22·1a
	Bordeaux mixture (2×)	$6{\cdot}1 \times 10^7$	77·0a	$1{\cdot}2 \times 10^8$	17·4b
	SiK + dicalcium phosphate	$1{\cdot}7 \times 10^8$	69·7a	NT	NT
	Kaolinite	NT	NT	$1{\cdot}1 \times 10^8$	17·5b
	Silicon gel (1×)	$1{\cdot}7 \times 10^8$	71·4a	$8{\cdot}5 \times 10^6$	19·3ab
	Silicon gel (4×)	NT	NT	$5{\cdot}2 \times 10^7$	12·4c
	Ulmasud B® (1×)	$6{\cdot}8 \times 10^7$	58·4b	$1{\cdot}0 \times 10^8$	16·0b
	Ulmasud B® (2×)	NT	NT	$6{\cdot}3 \times 10^7$	10·2c
Provera	Untreated	$1{\cdot}5 \times 10^7$	48·6a	$3{\cdot}6 \times 10^7$	38·1a
	Bordeaux mixture (2×)	$7{\cdot}6 \times 10^6$	18·3c	$9{\cdot}1 \times 10^3$	16·4c
	SiK + dicalcium phosphate	$2{\cdot}2 \times 10^7$	42·6b	NT	NT
	Kaolinite	NT	NT	$4{\cdot}8 \times 10^7$	38·0a
	Silicon gel (1×)	$4{\cdot}3 \times 10^6$	20·0c	$4{\cdot}6 \times 10^7$	31·4b
	Silicon gel (4×)	NT	NT	$1{\cdot}5 \times 10^8$	16·4c
	Ulmasud B® (1×)	$2{\cdot}1 \times 10^7$	21V3c	$1{\cdot}8 \times 10^7$	21·2bc
	Ulmasud B® (2×)	NT	NT	$3{\cdot}7 \times 10^7$	14·3c
Tío Palomo	Untreated	$1{\cdot}6 \times 10^7$	20·0a	$2{\cdot}3 \times 10^5$	22·2a
	Bordeaux mixture (2×)	$4{\cdot}9 \times 10^6$	9·6b	$2{\cdot}0 \times 10^8$	12·1b
	SiK + dicalcium phosphate	$1{\cdot}0 \times 10^7$	20·0a	NT	NT
	Kaolinite	NT	NT	$1{\cdot}2 \times 10^8$	22·0a
	Silicon gel (1×)	$6{\cdot}4 \times 10^5$	7·0b	$1{\cdot}2 \times 10^8$	11·6b
	Silicon gel (4×)	NT	NT	$1{\cdot}2 \times 10^7$	8·8 b
	Ulmasud B® (1×)	$4{\cdot}3 \times 10^6$	10·8b	$4{\cdot}7 \times 10^6$	14·4ab
	Ulmasud B® (2×)	NT	NT	$2{\cdot}2 \times 10^7$	16·0ab
El Chelín	Untreated	$2{\cdot}0 \times 10^7$	41·1a	$1{\cdot}7 \times 10^7$	12·0a
	Bordeaux mixture (2×)	$2{\cdot}8 \times 10^7$	31·0 a	$9{\cdot}0 \times 10^7$	1·1b
	SiK + dicalcium phosphate	$5{\cdot}3 \times 10^7$	34·9a	NT	NT
	Kaolinite	NT	NT	$1{\cdot}2 \times 10^7$	5·9 ab
	Silicon gel (1×)	$1{\cdot}4 \times 10^7$	33·3a	$3{\cdot}3 \times 10^6$	5·3ab
	Silicon gel (4×)	NT	NT	$7{\cdot}9 \times 10^7$	9·2a
	Ulmasud B® (1×)	$3{\cdot}4 \times 10^7$	18·9b	$2{\cdot}9 \times 10^7$	6·6ab
	Ulmasud B® (2×)	NT	NT	$6{\cdot}7 \times 10^7$	11·9a
Antonio Cano	Untreated	NT	NT	$6{\cdot}2 \times 10^7$	43·2a
	Bordeaux mixture (2×)	NT	NT	$2{\cdot}6 \times 10^7$	21·7c
	SiK + dicalcium phosphate	NT	NT	NT	NT
	Kaolinite	NT	NT	$6{\cdot}3 \times 10^7$	39·2 a
	Silicon gel (1×)	NT	NT	$6{\cdot}1 \times 10^7$	30·6b
	Silicon gel (4×)	NT	NT	$6{\cdot}8 \times 10^7$	27·4b
	Ulmasud B® (1×)	NT	NT	$3{\cdot}6 \times 10^7$	27·2b
	Ulmasud B® (2×)	NT	NT	$3{\cdot}2 \times 10^6$	28·2b
Montero	Untreated	NT	NT	$8{\cdot}1 \times 10^7$	12·1a
	Bordeaux mixture (2×)	NT	NT	$1{\cdot}1 \times 10^8$	8·7a
	SiK + dicalcium phosphate	NT	NT	NT	NT
	Kaolinite	NT	NT	$6{\cdot}8 \times 10^7$	6·2ab
	Silicon gel (1×)	NT	NT	$1{\cdot}0 \times 10^8$	12·0a
	Silicon gel (4×)	NT	NT	$1{\cdot}3 \times 10^8$	9·1a
	Ulmasud B® (1×)	NT	NT	$2{\cdot}1 \times 10^8$	8·6a
	Ulmasud B® (2×)	NT	NT	$2{\cdot}6 \times 10^8$	9·7a

[a]Values followed by the same letter are not significantly different ($P > 0{\cdot}05$) according to the analysis of variance followed by Fisher's least significant difference test.
NT, Not tested.

for phytotoxicity on 2-year-old mango plants growing in a greenhouse in summer at La Mayora Experimental Station, (Cazorla *et al.*, 2006). During the commercial scale trial (2008–2009), only three applications of Silicon gel were applied to the mango trees (15 November, 20 January and 20 February).

Quantification of bacterial populations on mango buds

In the six different seasons (three different scale trials), the effect of the experimental treatments on the bacterial population levels in relation to disease severity was monitored. To estimate bacterial populations on the mango trees, buds under each treatment were collected aseptically to determine the total heterotrophic bacteria and *P. syringae*-like population levels as previously described (Cazorla *et al.*, 1998). Two independent bulked samples of five terminal buds were randomly collected from different randomly selected trees under the same treatment, one bud per tree, regardless of the aspect of the buds. The samples were placed in sterile plastic bags, transported to the laboratory and processed independently. Ten millilitres of sterile phosphate buffer (10 mM, pH 7·2) per gram fresh weight of buds was added, and the buds were homogenized in a blender (Colworth Stomacher-400; Seward Ltd) for 3 min. The resultant suspension was used for 10-fold serial dilutions in sterile phosphate buffer. Then, 100 μL aliquots of each 10-fold serial dilution were plated onto King's B (KB) medium (King *et al.*, 1954) amended with cycloheximide (100 μg mL^{-1}) to prevent fungal growth. The plates were incubated at 22°C for 48–72 h. Total bacterial populations were estimated from KB counts. Colonies obtained from the KB plates were tested for fluorescence under UV light (265 nm) and for oxidase reaction using a plate-size sterile paper disc to make a replica of the growing colonies. Those colonies that were fluorescent and oxidase-negative were tested for arginine dihydrolase activity (ADH). *Pseudomonas syringae*-like populations were estimated from colonies that were fluorescent, oxidase-negative and ADH-negative (Cazorla *et al.*, 1998).

Disease assessment in the different trials

Bacterial apical necrosis symptoms on the mango trees were recorded as the number of terminal buds with necrotic symptoms. To evaluate disease severity, the terminal buds from every individual mango tree were observed for the presence of apical necrosis symptoms using the following scale: 0 = healthy bud; 1 = initial necrotic bud; 2 = advanced necrotic bud and 3 = dead bud. The number of buds rated as 2 or 3 was used to estimate disease severity. The disease assessment was carried out based on the disease severity of the experimentally treated trees and that of the untreated control trees. The Disease Control Index (DCI) was calculated by dividing the mean disease severity for treated trees by the mean disease severity for untreated trees, by using the following formula:

$$\mathrm{DCI} = \sum_{n=1}^{N} \frac{I/i}{J/j}$$

where N is the total number of trees analysed for each treatment; I is the number of necrotic buds; i is the total number of buds in the treated trees (for each treatment); and J and j are the equivalent values but for the control treatment (untreated trees).

Observations made after April included new growing shoots, which creates a bias in favour of reduced disease incidence. The data average for each treatment therefore corresponds to data obtained in February when the disease incidence was higher and before the appearance of new growing shoots.

Data treatment and statistical analysis

In order to classify the treatments based on their effectiveness in the small-scale trial, the Kendall's coefficient of concordance (Codina *et al.*, 1993) was used. Effects of the treatments were assessed by analysis of variance followed by Fisher's least significant difference test at the 0·05 probability level. SPSS software (SPSS Inc.) was used.

Results

Screening experimental treatment alternatives to Bordeaux mixture

Small-scale trial

The first trial (2002–2003 and 2003–2004) was carried out in six different orchards and compared six alternative experimental treatments to no treatment and to BM treatment. The *Pseudomonas syringae*-like population densities and the disease levels in terminal mango buds were monitored. The highest levels of *P. syringae*-like populations (above 10^6 and even reaching 10^8 CFU g^{-1} bud) and the highest disease incidence in buds were recorded in February (Table 2) during both seasons, when the disease severity was highest. Values for the percentage of affected buds followed by the same letter are not significantly different ($P > 0{\cdot}05$) according to the analysis of variance followed by Fisher's least significant difference test. These results were used to obtain a ranking of these experimental treatments according to their effectiveness at controlling BAN by applying Kendall's coefficient of concordance (Table 3). In the first season (2002–2003), the best treatments were BM (2×), Silicon gel and Ulmasud B®, with no significant difference between BM and Silicon gel as their Rj values were the same. In the second season, the best treatments were again BM (2×), Silicon gel (4×), and Ulmasud B®. Based on these results, Silicon gel and Ulmasud B® were selected as experimental treatments for further detailed experimentation in comparison with BM.

Semi-commercial scale trial

In this second trial against BAN (2005–2008), BM was reduced to 1× because at 2×, the concentration is higher than that permitted by regulations (Anonymous, 2002). Also, Silicon gel (4×) and Ulmasud B® (2×) were assessed alone and with an adjuvant (Nu-film®) to extend the presence of the active material on the leaf surface. In this trial, the weather conditions were different in each season, and

Table 3 Ranking of the effectiveness of experimental treatments used during the small-scale trial based on the protective effect against bacterial apical necrosis (BAN). The ranking of the effectiveness was established by the Kendall's coefficient of concordance (Rj). The most effective treatments are highlighted in bold

	Experimental treatments (2002–2003)					Experimental treatments (2003–2004)					
Orchards	Untreated	Bordeaux mixture (2×)	SiK + dicalcium phosphate	Silicon gel (1×)	Ulmasud B® (1×)	Untreated	Bordeaux mixture (2×)	Kaolinite	Silicon gel (1×)	Silicon gel (4×)	Ulmasud B® (1×)	Ulmasud B® (2×)
Sarmiento	4	4	2	3	1	7	4	5	6	2	3	1
Provera	5	1	4	2	3	6	3	6	5	2	4	1
Tío Palomo	4	2	4	1	3	6	3	6	2	1	4	5
El Chelín	5	2	4	3	1	6	1	3	2	5	4	6
Antonio Cano						6	1	6	5	2	2	4
Montero						6	3	1	6	4	2	5
Rj[a]	18	9	14	9	8	37	15	27	26	16	19	22
Ranking	5	2	4	2	1	7	1	6	5	2	3	4

[a]Rj value is obtained by assigning increasing values with decreasing effectiveness of each treatment.

are summarized in Fig. 1. Higher rainfall was recorded in January 2006 and November 2006, whereas the 2007–2008 season was the driest. The 2005–2006 season was unusually cool and wet, with moderate rainfall, and the other seasons (2006–2007 and 2007–2008) were slightly warmer and with reduced rainfall.

The *P. syringae*-like populations increased gradually during the cool and wet autumn and winter months from October to February, and started to decrease when the cool period had finished, with the highest bacterial levels recorded in February. In general, the bacterial counts on mango trees under the experimental treatments were very similar to those under conventional treatment with BM and on untreated control trees (Fig. 2). The highest bacterial counts were obtained in February, and the highest disease severity values were also observed in February, as shown in Figure 3 for untreated trees in the three seasons. With the exception of the Montero orchard (due to a severe pruning by the farmer), the disease severity was highest in the 2005–2006 season due to the most unfavourable environmental conditions among the three seasons of this semi-commercial trial (in particular, low temperatures). Likewise, in the 2006–2007 and 2007–2008 seasons, the disease severity was clearly lower, and this was due to warm temperatures and moderate rainfall. Taking into account that the highest detectable values of necrotic symptoms were always observed in February, the disease severity data from only this month were considered when comparing the effectiveness of the different applications and when estimating the disease control index in the three seasons of the semi-commercial scale field trial (Fig. 4). The untreated control trees always showed the highest disease severity, except for the Montero orchard in 2007–2008, where the trees treated with Ulmasud B® showed higher values of disease than the untreated control trees. Analysis of the DCI independently of orchard or of season (Fig. 4) yielded similar results. Thus, in most of the independent trials in each orchard and each season, Silicon gel (4×) was a more effective treatment against BAN than Ulmasud B® (2×), showing levels of protection against BAN similar to those of the conventional BM treatment. Including all the data together in a statistical analysis revealed that all the treatments were able to control BAN but at different levels, with Silicon gel (4×) being the most protective treatment together with the Silicon gel + Nu-film®, and presenting effectiveness equivalent to BM. The Silicon gel + Nu-film® combination was not selected for the last trial because the adjuvant (Nu-film®) was difficult to remove from the hand- and trailer-sprayer and also because the cost–benefit was considered less favourable for the farmer.

Commercial scale trial

Finally, in a full orchard trial in the 2008–09 season, the treatment selected in the previous trials, Silicon gel (4×), was compared with the conventional treatment (BM) and with no treatment (Table 4). The weather conditions in this trial were cool and wet, with low temperatures

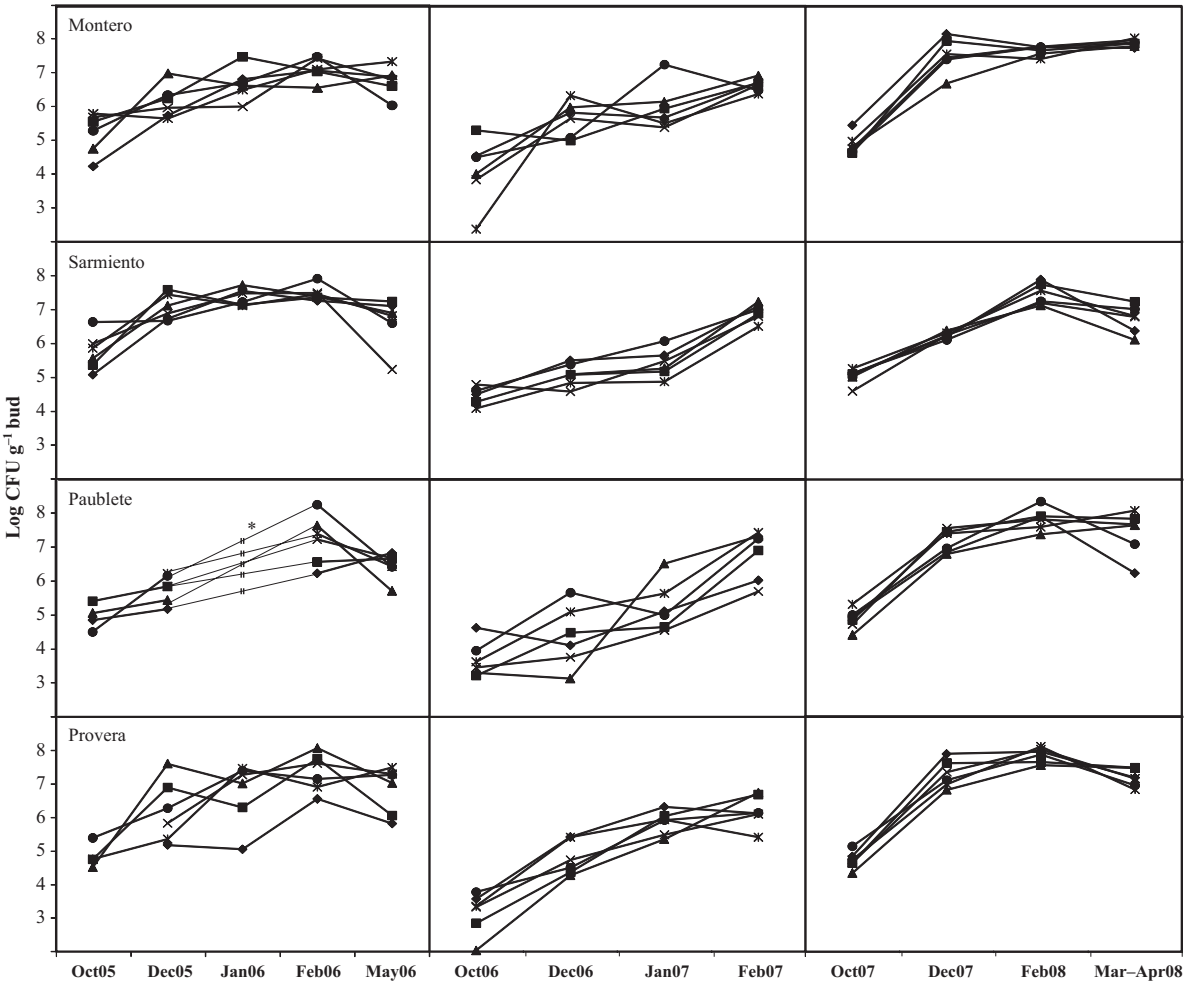

Figure 2 *Pseudomonas syringae*-like populations levels on mango buds (log CFU g^{-1}) during the 2005–2006, 2006–2007 and 2007–2008 seasons in four different orchards and for each experimental treatment. Experimental and control treatments: (♦) Silicon gel, (■) Silicon gel + Nu-film ®, (▲) Ulmasud B®, (✖) Ulmasud B® + Nu-film®, (✱) Bordeaux mixture, (•) Untreated control trees. *Data were not obtained from Paublete orchard in January 2006 due to the weather and field conditions.

during the winter months and high rainfall from October to February (Fig. 1), all of which favours BAN development. This commercial trial was carried out in the Sarmiento orchard due to the uniformity in size and cultivar of the mango trees. Disease severity was monitored from October to May, and Silicon gel and BM provided similar levels of protection against BAN, resulting in a significant reduction in disease severity. These reductions were clearly evident during the winter months, but especially in February when the most severe attack occurred.

Discussion

In this study different alternative treatments (Table 1) to BM (a copper-based compound) that are compatible with organic farming were evaluated for their ability to control BAN. Currently, the most widespread treatment used to control BAN in the Mediterranean area is BM, requiring four to six applications from September to April prior to budbreak (Cazorla *et al.*, 2006). Due to problems such as copper resistance in Pss, the bioaccumulation of copper and toxicity that may affect human health, this study performed different trials at different scales to identify an effective treatment alternative to BM (Alva & Graham, 1991; Cazorla *et al.*, 2002; Xiong & Wang, 2005; Vanneste *et al.*, 2008). Initially, in a small-scale trial, Ulmasud B®, Silicon gel and BM were the most effective treatments, and thus, Ulmasud B® and Silicon gel were selected for a subsequent, more complete trial at a semi-commercial scale (Tables 2 and 3). In these assays, the bacterial counts in February generally showed less than one order of magnitude difference between all treatments and the untreated controls, revealing an apparent absence of bactericidal activity of the treatments (Table 2). Copper compounds are usually considered to be bactericides, but in this study BM was ineffective at reducing bacterial population levels (Table 2), as has been observed previously (McGuire, 1988; Cazorla *et al.*,

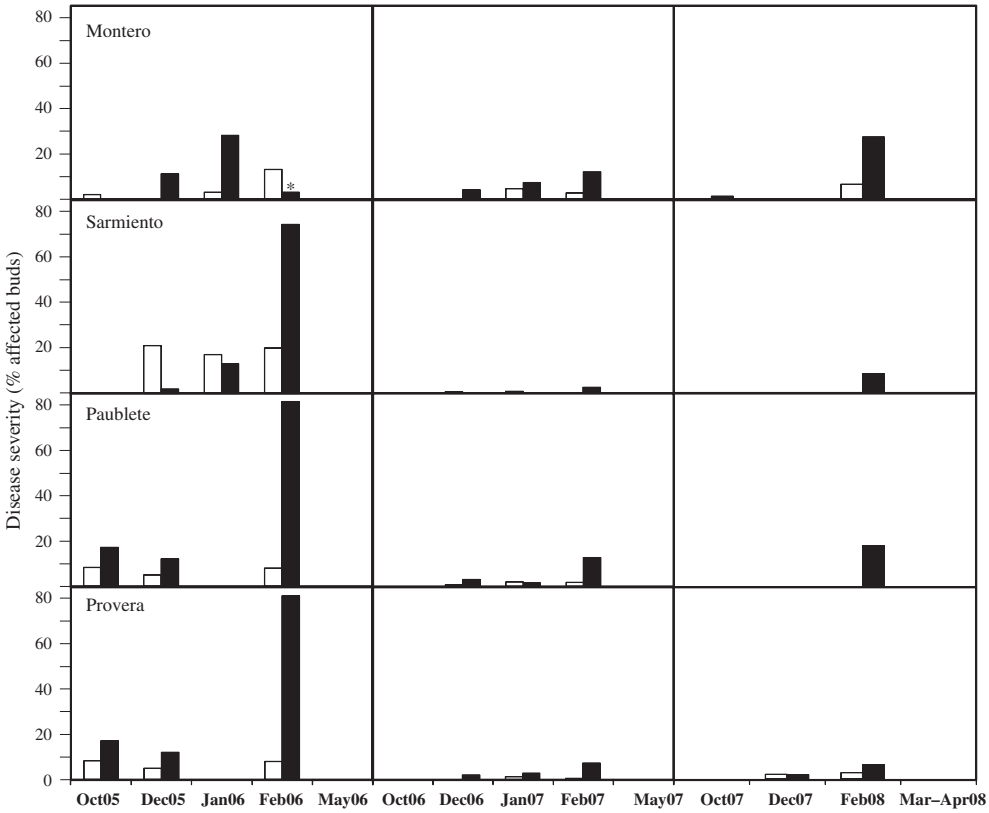

Figure 3 Severity of bacterial apical necrosis disease on mango trees during the semi-commercial scale trial (2005–2008). Disease severity in the untreated control trees (black columns) and trees treated with Bordeaux mixture (white columns) was assessed at every sampling by observing the percentage of terminal buds showing necrotic symptoms. *The percentage of necrotic buds at this point is lower due to severe pruning by the farmer.

2002). Similarly, Silicon gel, Ulmasud B® and BM all failed to reduce the bacterial populations on mango tissues, but they all reduced disease levels, suggesting a non-bactericidal mode of action of these compounds. An alternative mode of action could be by a film-forming activity as has been previously described for BM on mango (Becerra, 1995). In this sense, di-1-p-menthene (an adjuvant used to help in the maintenance of active material on the plant canopy) applied alone also provided a modest level of protection (Cazorla *et al.*, 2006).

It is suggested that Silicon gel and Ulmasud B® may act as a physical barrier preventing the entry of the pathogen as has been previously described for silicon protection of horticultural plants against fungal pathogens (Datnoff *et al.*, 2001; Kim *et al.*, 2002; Liang *et al.*, 2005; Guével *et al.*, 2007; Sun *et al.*, 2010) and bacterial pathogens (Diogo & Wydra, 2007), but not in fruit trees. However, the mode of action could also be related to the induction of ISR as has been reported from root applications and silicon amendments (Rodgers-Gray & Shaw, 2000, 2004; Bélanger *et al.*, 2003; Rodrigues *et al.*, 2003; Fauteux *et al.*, 2005). There are no data in woody plants about the possible influence of Silicon gel on cell wall lignification processes, but this previously described mode of action remains a possibility (Ma *et al.*, 2001; Kim *et al.*, 2002). The role of silicon in inducing systemic resistance in plants has recently been reviewed (Hammerschmidt, 2011). However, the silicon-inducing resistance seems to be a very complex phenomenon, that could involve many other unknown factors.

When the bacterial population levels were monitored during the semi-commercial scale trial, the maximum *P. syringae*-like levels on mango trees were reached in February for all the treatments (Fig. 2) in all three seasons. Similarly, the highest disease severity values were always observed in February for the trees treated with BM, the untreated trees (Fig. 3), and for the rest of the treatments (data not shown). This result was observed under both favourable and unfavourable environmental conditions. Based on these results, it was decided to use the data from February to compare the different treatments against BAN. At this time, due to the weather conditions, the symptoms of BAN and *P. syringae* levels are the highest, as has been described by Cazorla *et al.* (1998). In the 2006–2007 and 2007–2008 seasons, the disease severity was low due to high minimum temperatures (around 10°C) and low rainfall in winter (Fig. 1), leading to a shorter dormancy period. By contrast, in the 2005–2006 season, the opposite occurred, as the highest levels of disease severity were recorded due to a low minimum

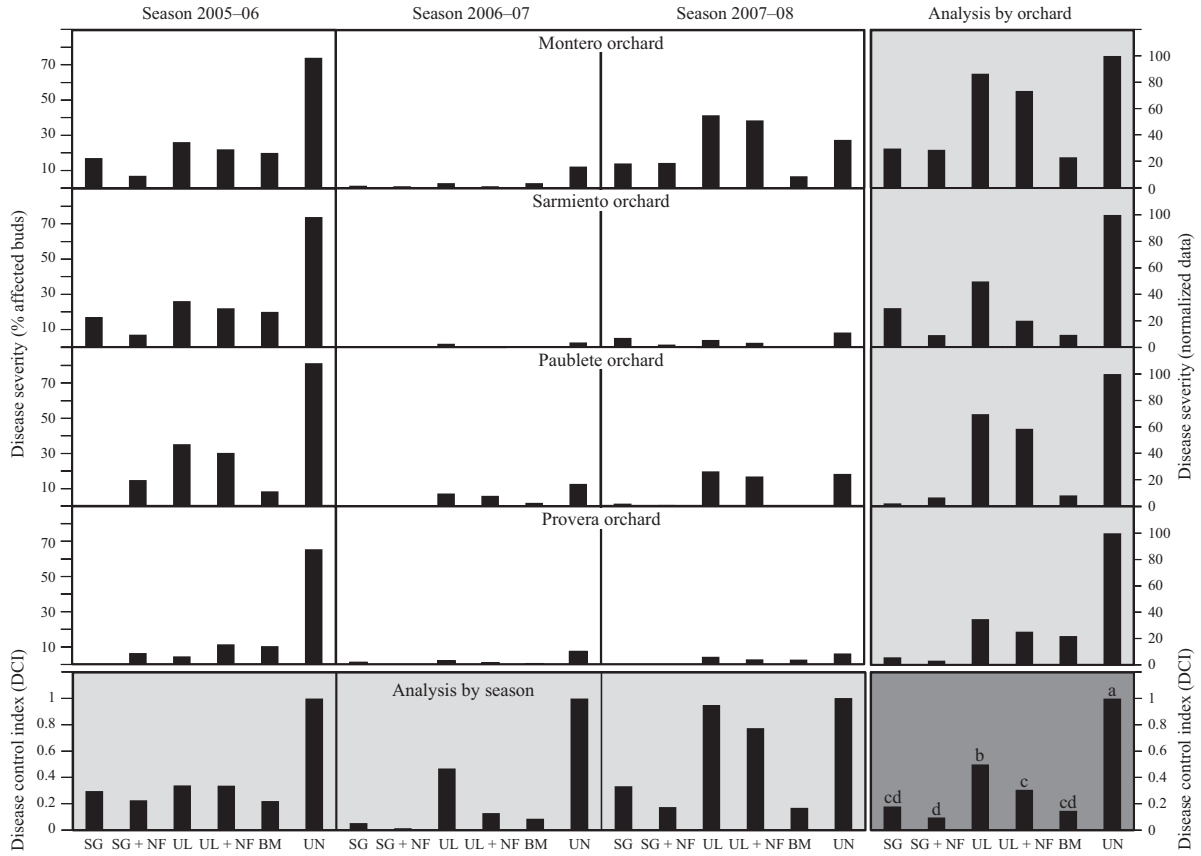

Figure 4 Disease severity and Disease Control Index (DCI) observed in February in the semi-commercial scale trial with the different treatments. Data from the different treatments for each orchard and season are presented in the white panels. Analysis by orchard (normalized data) for the three different seasons (2005–2006, 2006–2007 and 2007–2008) and analysis by season globally considering the four different orchards per season are shown in the light grey panels. Finally, a global analysis of the data from all the orchards and seasons taken together was carried out (dark grey panel). The DCI was calculated for each experimental treatment as the ability to control BAN relative to the disease severity of untreated trees. Values followed by different letters are significantly different ($P > 0{\cdot}05$) according to Fisher's analysis of variance. SG: Silicon gel (4×), SG+NF: Silicon gel (4×) + Nu-film®, UL: Ulmasud B® (2×), UL+NF: Ulmasud B® (2×) + Nu-film®, BM, Bordeaux mixture; UN, Untreated.

Table 4 Disease severity and weather conditions in the commercial trial in Sarmiento orchard (2008–2009), applying Silicon gel in comparison with Bordeaux mixture. Different letters show significant differences

	Disease severity (% affected buds)[a]			Weather conditions	
	Silicon gel	Bordeaux mixture	Untreated	Average temperature (°C)	Monthly rainfall (mm)
October	0·0	0·0	0·0	19·8	138·8
December	4·0b	2·0b	10·0a	12·5	30·1
January	11·0b	12·0b	25·0a	12·0	48·7
February	46·6b	47·4b	67·6a	12·2	100·0
May	0·0[b]	0·0	0·0	19·5	4·3

[a]Values followed by the same letter in each month are not significantly different ($P > 0{\cdot}05$) according to the analysis of variance followed by Fisher's least significant difference test.

[b]The disease severity drops to zero in May due to the appearance of the new shoots, all of them healthy.

temperature (<5°C) and high rainfall. In 2005–2006 when the winter weather was unfavourable (cool and rainy), clear protection was observed against BAN with all the treatments compared to the untreated trees, where the disease severity was around 70–80% in the different orchards (Fig. 4). However, in the 2006–2007 and 2007–2008 seasons when the winter weather conditions were mild, the protective effect of the different treatments was

less evident because a generally low level of symptoms was observed. Nevertheless, the protective effect of Silicon gel was similar to the effect of BM (Fig. 4). A global analysis of all the treatments by orchard or by season in terms of DCI shows that all the treatments reduced the symptoms of BAN under both favourable and unfavourable environmental conditions. Therefore, Silicon gel was the most effective of the alternative treatments, also giving a cost–benefit against BM of around 50%. The Silicon gel can be considered to be an environmentally friendly treatment alternative to BM that provides a similar level of protection (Fig. 4). The effectiveness of Silicon gel observed in these trials was corroborated in one commercial scale trial (2008–2009) wherein the protective effect of Silicon gel also clearly controlled BAN symptoms as effectively as BM. Silicon gel provided disease protection in all seasons, but especially in February (Table 4). In this season, the weather conditions were unfavourable, with minimum temperatures around 5°C in January to February and high rainfall (Fig. 1, Table 4) contributing to the high incidence of BAN symptoms.

In summary, the results presented in this work indicate that Silicon gel is an effective, environmentally friendly treatment for the control of BAN in mango crops that is equivalent in efficacy to BM. Silicon gel may protect via its film-forming activity, but additional effects on ISR through foliar application cannot be discounted. An application programme has been developed for controlling BAN using 4–6 applications of Silicon gel at a dose of 10·8 g L^{-1} between November and April and paying very close attention to rainy periods that can wash the Silicon gel from the mango trees. The effectiveness of Silicon gel has become clear to mango farmers in Spain, and currently, Silicon gel is undergoing authorization procedures for its commercial use in mango crops in Spain.

Acknowledgements

This work has been supported by grants from CICE-Junta de Andalucía, Ayudas Grupo PAIDI AGR-169 and Proyecto de Excelencia (P07-AGR-02471), co-financed by FEDER (EU). We thank especially SAT 2803 TROPS and all collaborating farmers for allowing the use of their orchards. The authors thank J. Rosales for his cooperation and assistance during the preliminary assays. We also thank everybody in our lab who helped in the processing of samples, especially H. Zeriouh, L. García-Gutiérrez and R. Martín.

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