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Field evaluation of treatments for the control of the bacterial apical necrosis of mango (*Mangifera indica*) caused by *Pseudomonas syringae* pv. *syringae*

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Abstract

Bacterial apical necrosis is a critical disease in the main production area of mango in Europe. It is caused by *Pseudomonas syringae* pv. *syringae*, and produces necrotic lesions on mango buds and leaves, causing severe yield losses due to a decrease of flowering and fruit set. A field study to evaluate control treatments against bacterial apical necrosis was carried out during three seasons on mango trees cv. Tommy Atkins in Huelva (Spain). Experimental treatments included Bordeaux mixture, fosetyl-Al, acibenzolar-s-methyl, gibberelic acid, silicon gel, a mixture between acibenzolar-S-methyl and Bordeaux mixture, and combined applications of fosetyl-Al with Bordeaux mixture or silicon gel. The treatments which caused a consistent reduction in bacterial apical necrosis symptoms at similar levels to the conventional treatment with Bordeaux mixture, were the plant resistance activator acibenzolar-S-methyl and the phosphonate derivative fosetyl-Al applied singly or in combination with other compounds, which could be alternative treatments. These treatments showed a significant decrease in the necrotic buds and/or leaves numbers; however, minor differences in *P. syringae*-like population levels were observed. The analysis of the inhibitory and bactericidal concentrations of cupric compounds against *P. syringae* strains isolated from mango tissues suggests that the commercial copper-based treatments with Bordeaux mixture used in the management of mango crops do not work in a bactericidal mode of action.

Introduction

Bacterial apical necrosis of mango (*Mangifera indica*) is a disease characterized by a rapid expansion of necrotic lesions on buds and leaves. Disease symptoms include necrosis of vegetative and flower buds and bud failure (Cazorla et al., 1998). Necrotic lesions on buds sometimes extend through the leaf petiole to the stem. Flower panicles are also affected, which results in severe economic losses due to a decreased fruit set. Lesions on leaves start as intervenial, angular, water-soaked spots which may coalesce, becoming black and slightly raised. Fruit lesions have not been observed (Cazorla et al., 1998). When disease incidence is high, vegetative growth of trees is delayed. Then, trees become alternate bearers annually. These symptoms are similar to those described for blossom blast of pear and stone fruits (English et al., 1980).

The disease has caused severe production losses in southern Europe in some years. Certain mango

cultivars, especially young trees of cv. Tommy-Atkins, Lippens and Manzanillo are very susceptible, whereas other cultivars, such as Sensation or Keitt are less susceptible. The aetiology of the disease has been described and Koch's postulates have been completed for *Pseudomonas syringae* pv. *syringae* (Cazorla et al., 1998; Torta et al., 2003). This bacterium was related to symptom development, especially 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). Rain or dew is essential for inoculum dissemination to other buds and leaves, and wind facilitates disease development by causing microinjuries. Some authors consider *P. syringae* pv. *syringae* as a weak pathogen which causes disease only when the host is stressed, for example, during tree dormancy and budbreak (Hattingh et al., 1989). This agrees with the observation of a large number of *P. syringae* cells colonizing mango tissues before the initiation of apical necrosis symptoms (Cazorla et al., 1998).

Due to the economic impact of the disease, mango growers need control methods to restrict its negative effect on production. No comparison studies of control methods have been reported, but there is considerable experience from growers and extension services which indicates failures in the efficacy of available control methods. Sprays of copper compounds (mainly Bordeaux mixture) applied during autumn-winter, as the typical treatment used for control of several bacterial diseases of fruit trees, were the most commonly used method. However, their efficacy is often limited, and it may be related to the selection of copper-resistant strains of *P. syringae* pv. *syringae* in mango orchards, where intensive copper spraying was used for disease control (Cazorla et al., 2002). Furthermore, high levels of copper have also been reported to be toxic to plant roots because copper interferes with the uptake of iron and other nutrients, especially in acidic soils (Alva and Graham, 1991). For this reason, several attempts have been made to reduce the application of copper compounds to crops and to find alternatives to such control treatments (Ninót et al., 2002). In addition, the European Union countries have introduced legislation limiting the use of copper compounds by regulation n° 473/2002 (Anon, 2002).

The purpose of this study was to evaluate several control methods for management of the bacterial apical necrosis of mango and to test their effect on population levels of *P. syringae* in commercial orchard plots over a three-year-period.

Materials and methods

Disease control trials

Field trials were performed in experimental plots located in commercial orchards in the fruit-tree growing area of Huelva, in the southwest of Spain, where the disease has affected mango production for several years. The study was performed on mango trees of the susceptible cultivar Tommy-Atkins, grafted onto cv. Espada seedlings rootstocks. Trees were grown on acid sandy loam soils. Three independent experiments were carried out during three different growing seasons, using a different orchard each season to avoid cumulative effects of the experimental treatments. In the first season (1997/98), the orchard consisted of 10 year-old trees, at 4×4 m spacing and with a canopy diameter of approximately 2 m. In 1998/99 and 1999/2000, the trees were 12 and 13 years old respectively, spaced 4×6 m, and with a canopy diameter of approximately 3 m. They were drip irrigated at 1–2 day intervals during the summer and in dry periods during the winter. Temperature (°C) and rainfall (mm^3) during the trials were recorded daily at a meteorological station located in the orchards.

Trees to be used in the experiment were selected from those present in the commercial field by uniformity in size and disease incidence. Each experimental treatment was applied on separate individual trees located randomly on the field (ten trees per treatment in the first experiment and twelve trees per treatment in the second and third experiments). Sets of trees treated only with water, and trees treated only with the adjuvant were used as controls (Table 1).

Source, trademark, standard schedule of application and rates of the products used in this study are summarized in Table 1. Assayed treatments for the three seasons included the following chemical compounds: Bordeaux mixture (20% Cu), fosetyl-Al (80%) and gibberelic acid (1.6%). Acibenzolar-S-methyl (50%) and a silicon gel

Table 1. Assayed treatments and bacterial apical necrosis incidence in mango trees recorded in three seasons. Disease incidence was assessed in February as percentage of terminal buds showing necrotic symptoms and in June as percentage of leaves with necrotic areas

Treatments	Rate litre^{-1}	Trademark (Manufacturer)	Necrotic buds in February(%)[a]			Leaves with necrotic areas in June (%)[a]		
			97/98	98/99	99/00	97/98	98/99	99/00
Controls								
No treatment			52.0 a	70.4 a	41.6 a	35.8 ab	43.1 ab	11.6 ab
Water				67.0 ab	36.0 ab		52.5 a	12.7 ab
Adjuvant singly (di-p-menthene 96%)	2.0 ml	Vaporgard® (Lefroy Valley, Australia)	41.2 b	63.2 b	23.7 bc	20.1 bc	45.0 ab	5.6 bc
Compounds (% a.i.[b])								
Acibenzolar-S-methyl (50%)	1.5 g	Bion (Syngenta S.A., Spain)		56.4 c	12.6 d		23.3 bcd	2.6 c
Bordeaux mixture (20% Cu)	3.0 g	Caldo bordelés del Vallés (Industrias Químicas del Vallés S.A., Spain)	38.1 bc	56.3 c	18.2 d	14.6 c	19.7 cd	3.5 c
Fosetyl-Al (80%)	4.0 g	Aliette (Aventis CropScience S.A., Spain)	29.7 c	50.1 d	25.6 b	24.4 bc	25.6 bcd	8.2 b
Gibberelic acid (1.6%)	3.1 ml	Polirend GI LS (Kenogard S.A., Spain)	44.7 ab	55.5 cd	33.4 ab	14.0 c	18.2 cd	6.1 bc
Silicon gel (potassium silicate 34%)	1.4 g	Foret S.A., Spain		63.5 b	27.3 b		37.9 ab	3.3 c
Mixtures								
Acibenzolar-S-methyl + Bordeaux mixture	1.5 g + 3.0 g			46.8 d	13.9 d		15.2 d	3.6 c
Combined treatments								
Fosetyl-Al + Bordeaux mixture	4.0 g + 3.0 g			57.2 c	26.9 b		24.4 bcd	8.9 b
Fosetyl-Al + silicon gel	4.0 g + 1.4 g			55.0 cd	22.0 c		35.6 abc	7.7 b

[a] Within each column, values followed by the same letter are not significantly different ($P > 0.05$) according to the analysis of the variance followed by Fisher's least significant difference test.
[b] Percentage of active ingredient.

(soluble potassium silicate 34%) were also assayed in the second and third seasons, as well as mixed and combined treatments (Table 1). Treatments were applied with an engine operated 15 l hand sprayer (Stihl model SR400, Waiblingen, Germany) to the point of run-off (approximately 15 l per 12 trees), starting the applications in October after harvest and finishing before April (after budbreak). The standard programme of applications consisted of six-seven monthly sprays of each treatment during the second week of each month, except applications for gibberelic acid that were sprayed twice (October and December). In the combined programmes, fosetyl-Al at standard rate was sprayed twice (October and November), and four other applications of a different compound (Bordeaux mixture or silicon gel) in the following four months (December–March). For the mixed treatments, acibenzolar-S-methyl and Bordeaux mixture were mixed prior to every application (Table 1) and applied by the standard programme. Di-p-menthene (96%) at 2.0 ml l^{-1} was used in all applications as an adjuvant, because it is a water emulsifiable organic compound which favours the maintenance of active ingredients on plant surfaces by forming a film, retarding run-off and evaporation.

All the assayed treatments were previously tested for phytotoxicity on 2 year-old mango plants growing in a greenhouse in summer at the Experimental Station 'La Mayora' (CSIC, Algarrobo-Costa, Málaga, Spain). Registered chemicals were applied at manufacturer's recommended rates. The experimental treatment with silicon gel was applied at a non-phytotoxic rate (1.4 g l^{-1}).

Assessment of bacterial populations on mango buds

During the three seasons study plots were monitored for the effect of treatments on bacterial population levels in relation to disease incidence. To estimate bacterial populations on mango trees, mango buds under each treatment were collected aseptically to determine the total bacteria and *P. syringae*-like population levels as previously described (Cazorla et al., 1998). In every season, four to seven samplings were carried out about one week after the monthly treatment application. Briefly, two independent bulked samples of five-six terminal buds were randomly collected from the different trees under the same treatment, one bud per tree, regardless of the aspect of the buds. Samples were placed in sterile plastic bags and transported to the laboratory. Ten ml of sterile phosphate buffer (10 mM, pH 7.2) per gram of fresh weight of plant material were added and homogenized in a lab blender (Colworth Stomacher-400, Seward Ltd, London, UK) for 3 min. The resultant suspension was used for 10-fold serial dilutions in sterile phosphate buffer. Then, 100 μl of each 10-fold serial dilution were plated onto nutrient agar (NA, Difco, Detroit, MI) and King's B medium (KB, King et al., 1954) amended with cycloheximide (100 μg ml^{-1}) to prevent fungal growth. Plates were incubated at 22 °C for 48–72 h. Total bacterial populations were estimated from NA and 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 which were fluorescent and oxidase negative were tested for arginine dihydrolase activity (ADH). *Pseudomonas syringae*-like populations were estimated from colonies which were fluorescent, oxidase negative, and ADH negative (Cazorla et al., 1998). Ice-nucleation activity was also estimated following a multiple-tube ice nucleation test described previously (Cazorla et al., 1995). Results were expressed as colony-forming units (CFU) per gram of fresh weight of bud tissue. In every sampling, several *P. syringae*-like colonies were isolated and selected to confirm the identification. A total of sixty-six isolates from mango trees during this and other studies were characterized and confirmed as *P. syringae* pv. *syringae*, following procedures described previously (Cazorla et al., 1998).

Disease assessment in commercial orchard plot trials

Bacterial apical necrosis symptoms on mango trees were recorded in buds and leaves separately. To evaluate the disease incidence in buds, all the terminal buds (70–200) from every individual mango tree were evaluated for the presence of apical necrosis symptoms. Disease incidence was recorded 4–7 times between August and June as the percentage of necrotic buds in every tree. Observations after April included new growing shoots which reduced the apparent incidence of the disease. The disease index was obtained

independently from ten trees (experiment carried out in 1997/98) or twelve (experiments carried out in 1998/99 and 1999/2000) per assayed treatment, and the mean disease incidence for all the trees for every sampling under the same treatment was calculated. The data means per each treatment, summarized in Table 1, correspond to those data obtained in February before appearance of the new growing shoots. Disease incidence on leaves was also recorded as the percentage of leaves with necrotic areas present in ten arbitrary selected shoots per tree.

Data treatment and statistical analysis

Effect of the treatments was determined by analysis of variance followed by Fisher's least significant difference test at the 0.05 probability level. For this SPSS software (SPSS Inc., Chicago, IL) was used.

In vitro *antibacterial assays*

To reveal the possible antimicrobial effects of the active ingredient of some assayed treatments on *P. syringae* strains from mango, resistance levels to copper compounds (copper sulphate and copper chloride) were determined against 66 *P. syringae* pv. *syringae* strains isolated from mango trees in orchards in this and in previous work (Cazorla et al., 1998, 2002). The sensitivity to ethylphosphonate (active ingredient of fosetyl-Al) was also assayed. The minimal inhibitory concentrations (MIC) of these active ingredients were determined by inoculating the selected bacterial strains onto mannitol–glutamate–yeast extract medium (MGY; Bender and Cooksey, 1986), containing different concentrations of the assayed compound, and evaluating the bacterial growth after 72 h at 22 °C (Cazorla et al., 2002). These experiments were repeated at least three times for each isolate.

Resistance to three commercial compounds used to control bacterial plant diseases, as ZZ Cuprocol® (copper oxychloride 70%, Syngenta, Madrid, Spain), Caldo Bordelés del Valles® (Bordeaux mixture, cupric calcium sulphate 20%, Industrias Químicas del Vallés, Barcelona, Spain) and Aliette® (fosetyl-Al 80%, Aventis Cropscience SA, Valencia, Spain) was also directly evaluated against 25 *P. syringae* pv. *syringae* strains randomly selected. The MIC of these compounds was determined following a similar manner to that described above to test the active ingredients. In addition, the concentration of compound necessary to kill the bacteria was estimated as the minimal bactericidal concentration (MBC). The MBC were evaluated by inoculating the bacterial strains on liquid nutrient broth amended with different concentrations of the assayed compounds, ranging from 0.25 to 50 mg ml^{-1}. After 24 h at 27 °C with shaking, 10 μl aliquots of these cultures were transferred onto KB agar plates to evaluate after 72 h at 22 °C whether the bacterial cells were inactivated or survived after the incubation in the presence of the assayed compounds.

Results

Population levels of total bacteria and P. syringae

The mean temperature and total rainfall of every month during the different experiments are summarized in Figure 1. The period with higher rainfall was November and December of season 1997/98. The season 1997/98 was very warm and wet, and 1998/99 was unusually cool and with moderate rainfall. The only freezing events during the whole experimentation period occurred in December 1998 and February 1999.

The bacterial population densities and the disease levels in terminal mango buds were monitored during the three seasons in the experimental orchards. The bacterial levels of untreated trees in each season are shown in Figure 2. The total bacterial counts obtained on AN and KB were very similar, and the *P. syringae*-like counts were slightly lower (Figure 2). The highest levels of *P. syringae*-like populations (above 10^6 and reaching 10^8 CFU g^{-1} bud) and disease incidence in buds were always recorded in February during the three experimental seasons. In February, most of the bacterial colonies detected belonged to the *P. syringae*-like group (Figure 2). The lowest levels of bacterial populations and disease symptoms in buds were recorded in August (around 10^2 CFU g^{-1} bud). The bacterial populations fluctuated, gradually increasing during the cool and wet autumn and winter months. In general, the bacterial counts obtained from the mango trees under the different treatments were very similar to

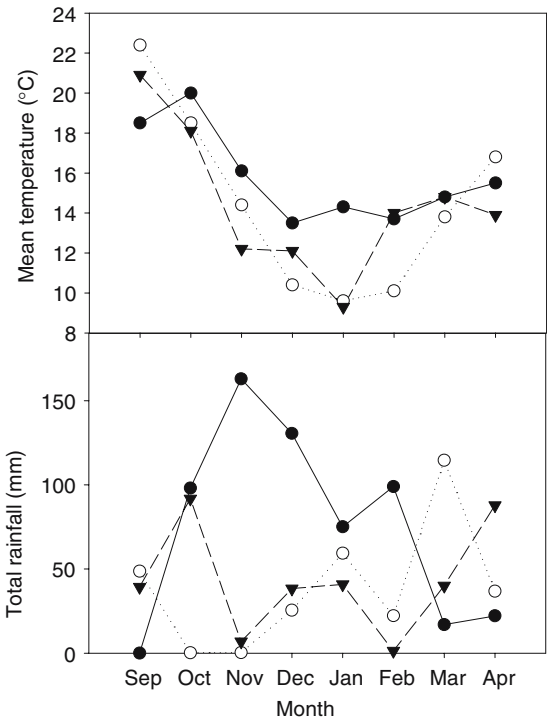

Figure 1. Mean air temperatures (°C), and monthly total rainfall (mm) from September to April for seasons 1997/98 (●), 1998/99 (○) and 1999/00 (▼) in experimental mango fields in Huelva (Spain).

those displayed by the mango trees under conventional treatment with copper compounds.

Average data of bacterial population counts obtained in February (corresponding with the highest disease incidence) from the three independent experiments, were used to construct a summary figure (Figure 3). This figure showed that *P. syringae*-like organisms constituted a main proportion of the total bacterial population on mango buds, regardless of the applied treatment. Interestingly, the levels of *P. syringae*-like and total bacteria recorded from treated and untreated mango trees were similar, and in general close to 10^7 CFU g^{-1} bud (Figure 3). All the selected *P. syringae*-like isolates used to confirm the identification ($n = 66$) were characterized as *P. syringae* pv. *syringae* (data not shown).

Effect of treatments on disease incidence

The effect of the assayed treatments to control the apical necrosis of mango is summarized in Table 1. Different treatments showed a different efficacy in the different years. A statistically significant difference was not observed in all seasons among some of the treatments. The results in Table 1 show that, in general, the disease incidence on buds in February during the second season was the highest, reaching 70.4% of necrotic buds on untreated trees. Treatments with the adjuvant (di-p-menthene) applied singly, provided a slight, but significant protection on buds, when compared with the untreated control in the three experimental seasons. In the first experiment, fosetyl-Al showed the best protection levels. This compound significantly reduced bud necrosis when compared with the adjuvant applied singly or compared with untreated trees. Bordeaux mixture slightly reduced disease symptoms but differences with the adjuvant were not significant (Table 1).

During the coldest second season, the highest reduction in bud necrosis was obtained with the

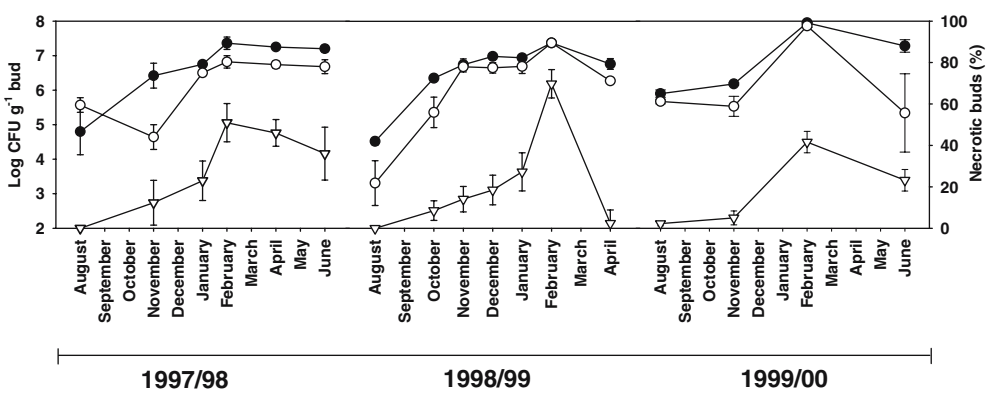

Figure 2. Total bacteria (●), *P. syringae*-like (○) bacterial counts (CFU g^{-1} of fresh weight of buds) and percentage of necrosis incidence on mango buds (∇) on untreated mango trees during the three seasons of study.

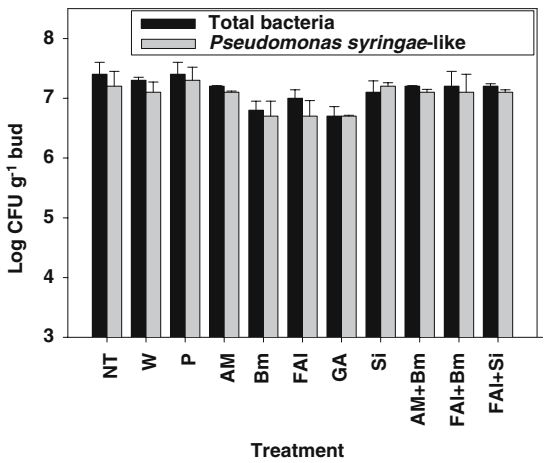

Figure 3. Total bacteria and *P. syringae*-like bacterial counts on buds from mango trees subject to different experimental treatments against bacterial apical necrosis. Each value is the average of the counts obtained in February of the three seasons for each treatment. The treatments considered were: NT, no treatment; W, water; P, adjuvant; AM, acibenzolar-S-methyl; Bm, Bordeaux mixture; FAl, Fosetyl-Al; GA, gibberelic acid; Si, silicon gel; AM mixed with Bm (AM + Bm); FAl combined with Bm (FAl + Bm) or Si (FAl + Si). Data for untreated (NT) and treatments P, Bm, FAl and GA are the means from three experimental seasons; remaining treatments are the mean data from two experimental seasons. Bars indicate the standard error of the mean.

application of fosetyl-Al or a mixture with acibenzolar-S-methyl and Bordeaux mixture. All the other treatments also showed a significant decrease of the disease incidence, except the silicon gel (Table 1).

In the third season, the application of acibenzolar-S-methyl or Bordeaux mixture singly, or the treatment with a mixture of acibenzolar-S-methyl and Bordeaux mixture, significantly reduced disease symptoms when compared with control trees (Table 1).

The analysis of leaf necrosis in June also showed that the highest disease incidence was reached in the coldest second season. Treatments with gibberelic acid and Bordeaux mixture singly and a mixture of acibenzolar-S-mehtyl and Bordeaux mixture significantly reduced leaf necrosis, especially in the second season, when compared with control trees (Table 1). In the third season, the incidence of leaf necrosis was very low and differences among treatments were not observed.

Antimicrobial activity from chemical compounds

Percentages of *P. syringae* pv. *syringae* strains resistant to the assayed antimicrobial agents, present as active ingredients in some chemical compounds used as bactericides for management of plant diseases, are summarized in Table 2. More than 50% of the strains were resistant to copper salts (MIC > 0.8 mM), and all the strains grown in the presence of the maximum amount assayed of the active ingredient of fosetyl-Al, ethyl-phosphonate (MIC > 500 μg ml^{-1}). The ranges of MIC and MBC of the assayed commercial chemical compounds are summarized in Table 3, showing similar values to those displayed in Table 2 where the pure active ingredients were directly assayed.

Discussion

Bacterial apical necrosis of mango is currently managed in southern Europe by four to six applications of copper compounds from September until the start of the budbreak in April. In this study, the efficacy of treatments with the copper-based compound Bordeaux mixture is reported. Five or six applications of Bordeaux mixture generally showed protection against the bacterial

Table 2. Resistance of *Pseudomonas syringae* pv. *syringae* strains from mango (n = 66) against the active ingredients of some chemical compounds used for the control of bacterial plant diseases. The results are presented as the percentage (%) of strains which showed values of minimal inhibitory concentrations (MIC), corresponding to susceptibility or resistance against the assayed compound

Sensitive/Resistant to copper	$CuSO_4$		$CuCl_2$		Ethyl-phosphonate	
	MIC (mMCu)[a]	%	MIC (mMCu)[b]	%	MIC(μg ml^{-1})	%
S	≤0.8	45.4	≤0.8	37.9	100	0
R	≥1.2	54.6	≥1.2	62.1	> 500	100

[a]1 mM Cu = 159.6 μg ml^{-1} $CuSO_4$; [b]1 mM Cu = 134.5 μg ml^{-1} $CuCl_2$.

Table 3. Resistance of *Pseudomonas syringae* pv. *syringae* strains from mango (n = 25) against commercial products used for the control of bacterial diseases. The results are presented as the range of minimal inhibitory concentrations (MIC) and minimal bactericidal concentrations (MBC) of compound which inhibit or inactivate the assayed bacterial strain

	ZZ Cuprocol (g l^{-1}) (mM Cu as active ingredient)	Bordeaux Mixture (g l^{-1}) (mM Cu as active ingredient)	Aliette (g l^{-1}) (mg ml^{-1} ethyl-phosphonate as active ingredient)
MIC	0.25–1 (0.7–3.3)	0.5–2 (1.5–6.1)	1–10 (0.8–8)
MBC	1–2 (3.3–6.5)	0.5–2 (1.5–6.1)	5–10 (4–8)

apical necrosis symptoms, but inconsistently in some cases (Table 1). Furthermore, applications of the resistance activator acibenzolar-S-methyl at standard rate, also resulted in a reduction of the bacterial apical necrosis incidence on mango trees (Table 1), probably due to the enhancing of the tree tolerance as has been reported for other plant-pathogen systems (Oostendorp et al., 2001). The treatment with phytohormone gibberelic acid was carried out in an attempt to break the dormancy of mango trees (Samson, 1986; Agrios, 2005), consequently activating some plant defences. Gibberelic acid sprays only showed significant protection during the coldest 98/99 season and its application would be considered as a complementary treatment in the management of bacterial apical necrosis (Table 1). In the other hand, foliar sprays with fosetyl-Al also showed significant protection during the first two seasons. Fosetyl-Al, as in the case of the Bordeaux mixture treatment, was effective but inconsistent in the control of bacterial apical necrosis of mango, as well as for other bacterial diseases such as fire blight of pear (Paulin et al., 1990), citrus canker (McGuire, 1988), and some bacterial diseases of ornamental plants (Chase, 1993). In addition, Fosetyl-Al and other phosphonates were effective at high doses for control of infections of *P. syringae* pv. *syringae* on pear in controlled environments, showing a better disease protection when the active ingredient dose was increased (Moragrega et al., 1998). Silicon gel applications (Belanger et al., 1995) had no better effects than the adjuvant applied singly, but the bud protection level was improved when it was applied combined with fosetyl-Al treatments (Table 1). Furthermore, monthly applications of a mixture of acibenzolar-S-methyl with Bordeaux mixture reduced the bacterial apical necrosis incidence, showing at least similar values to those displayed by the application of the two compounds separately (Table 1). Treatment with combined applications of fosetyl-Al with Bordeaux mixture or silicon gel, showed similar results to the trees treated only with fosetyl-Al.

Some of the effective compounds used to control apical bud necrosis were also effective on leaf necrosis in the 98/99 season (Table 1). Bordeaux mixture showed a significant protection of mango leaves, probably due to the formation of a superficial film (Becerra, 1995), which could be also improved by the additional effect of the adjuvant used, preventing the production of small injuries on the leaf surface. Gibberelic acid also reduced leaf necrosis. Other treatments, such as mixtures of acibenzolar-S-methyl and Bordeaux mixture, also showed protection, probably by the combination of different mechanisms as has been previously observed (Roemmelt et al., 1999; Louws et al., 2001). It has been reported previously that the severity of apical necrosis disease is associated with cool and wet winters following the colonization of mango tissues by *P. syringae* pv. *syringae*, and this is why a more efficient management of bacterial apical necrosis of mango is required during the cool and/or very wet seasons (Cazorla et al., 1998). The highest incidence of bacterial apical necrosis in the present work was observed during the second season, both in treated and untreated trees (Figure 2 and Table 1), when temperatures reached values below 0 °C for a few nights. Similar to other tropical trees, mango trees become dormant below 15 °C, and with temperatures below 0 °C, tissue damage becomes important (Samson, 1986), which could be favoured by the bacterial ice-nucleation activity of the pathogen *P. syringae* pv. *syringae* (Cazorla et al., 1995, 1998). These differences in climatic conditions as well as the bacterial ice-nucleation activity during every season could be responsible for the different efficacy showed by some treatments (e.g. gibberelic acid, fosetyl-Al) during the years of this study, showing climatic conditions have in general an important

role in the development of apical necrosis symptoms, as previously described (Cazorla et al., 1998). Therefore the climatic parameters in every season should be taken into account for future evaluation and selection of different control compounds against bacterial apical necrosis of mango.

Population levels of *P. syringae* in apical buds were similar in untreated and treated trees, regardless of the time of the year (Figures 2 and 3), which suggests that the treatments used to control bacterial apical necrosis did not reduce the *P. syringae* population at least in short-term field trials. Bacterial counts in February during the three seasons showed a maximum difference of approximately one order of magnitude for all treatments, revealing an apparent absence of bactericidal activity in the assayed treatments. Copper compounds, usually considered as bactericides, were also ineffective for reducing bacterial population levels in the field (Figure 3), as has been observed previously (MacGuire, 1988; Cazorla et al., 2002). Treatment with Bordeaux mixture failed to reduce the bacterial populations on mango tissues but reduced disease levels, suggesting that the possible mode of action of this compound could be other than a direct antimicrobial effect of the copper. Furthermore, *in vitro* assays (Table 2) demonstrated that a high proportion of *P. syringae* pv. *syringae* strains isolated from mango were resistant to the copper salts as the active ingredient. This resistance was also observed against the commercial compounds (Table 3). This fact strengthens the hypothesis that the compounds containing these active ingredients do not work by bactericidal mechanisms.

The application of the adjuvant singly did not have an effect on the bacterial population levels; however it reduced slightly but significantly, the bud necrosis symptoms (Table 1). Adjuvants are generally inert products which improve the accuracy of delivering the chemical to its target by reducing drift and run-off, improving uptake and spread (Ziv and Zitter, 1992). Adjuvant films help to maintain the sprayed substances longer on plant surfaces, extending their action (Gottwald et al., 1997). As mentioned above the most common field mango treatment in southern Europe is cupric calcium sulphate (commonly Bordeaux mixture), but apparently this does not act only as a bactericide in the protective action against bacterial apical necrosis. An additional mode of action could be similar to the adjuvant di-p-menthene, because Bordeaux mixture forms a thick film on plant surfaces due to the calcium it contains (Becerra, 1995), but this hypothesis has to be proved. However, the maximum rates of Bordeaux mixture applied on Spanish commercial mango fields are around $3\ g\ l^{-1}$ and the MIC of *P. syringae* pv. *syringae* strains to this compound are around $2\ g\ l^{-1}$ (Table 3); this could be explained by a failure in the contact of the compound with the bacteria, which could be protected in some parts of the plant such as the buds, where the levels of Bordeaux mixture are probably lower than on leaves or in *in vitro* experiments.

In conclusion, none of the assayed treatments, as expected, conferred complete protection against bacterial apical necrosis of mango (Table 1), probably due to the favourable climatic conditions for disease development (Figure 1 and Table 1) and the high inoculum pressure (Figures 2 and 3). Also, the climatic conditions seem to modulate the efficacy of some control compounds. However, the results from this study suggest that applications of Bordeaux mixture could be an appropriate treatment for the management of bacterial apical necrosis of mango. Furthermore, if it is found necessary to reduce copper applications (because of the selection of bacterial resistance to copper), alternatives to copper compound sprays could be found. Treatments with Fosetyl-Al were efficient in the two first seasons; sprays of phosphonate derivatives have also proved to be a useful tool to control *P. syringae* pv. *syringae* in other field experiments (Montesinos and Vilardell, 2001). Treatments based on the plant defence activator (acibenzolar-S-methyl) applications also gave significant protection levels, and its combination with other compounds could permit efficient management of bacterial apical necrosis of mango using a lower number of cupric compound applications. The promising effects of some of the assayed compounds against bacterial apical necrosis indicate that these compounds could be interesting alternatives to traditional chemical disease control.

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