

DEVELOPMENT OF FIELD STRATEGIES FOR FIRE BLIGHT CONTROL INTEGRATING BIOCONTROL AGENTS AND PLANT DEFENSE ACTIVATORS IN MOROCCO

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SUMMARY

The bacterial antagonists (*Bacillus subtilis* GB03, *B. subtilis* QST713, *B. subtilis* Y1336 and *Pantoea agglomerans* P10c) and plant defense activators [acibenzolar-S-methyl (ASM), fosetyl aluminium (F-Al), potassium phosphites (PH) and prohexadione-Ca (ProCa)] were evaluated individually and in combination for efficacy in controlling fire blight in Morocco. Under laboratory conditions, on detached blossoms of apple and pear, only biocontrol treatments based on *P. agglomerans* P10c and their mixture with *B. subtilis* QST713 showed a significant reduction in the incidence of the disease when compared to other treatments. Under field conditions, both the above mixture of biocontrol agents as well as all other strains were tested alone or combined with plant defense activators using a split-split-plot design. The treatments were applied on the trees at timings based on their modes of action. Results showed that, when used alone, *P. agglomerans* P10c, *B. subtilis* QST713, their mixture (1:1), *B. subtilis* GB03 and *B. subtilis* Y1336 reduced blossom infection under field conditions by 66, 63.8, 61.7, 64.2, and 53%, respectively. For plant defense activators this reduction was 61.5, 56.6, 50 and 49% for ASM, ProCa, F-Al and PH, respectively. On shoots, it ranged from 40 to 80% for biocontrol agents, but for plant defense activators it varied from 46 to 96.5%. Two individual applications of ProCa were the most effective treatment for reducing shoot blight incidence. The combination of plant defense activators and biocontrol agents allowed the highest protection rate against blossom and shoot blight ranging from 76 to 98.2%. The greatest protection was insured by *B. subtilis* QST713, *P. agglomerans* P10c or their mixture combined with ASM or ProCa.

Keywords: fire blight, integrated control strategy, biocontrol, *Pantoea agglomerans*, *Bacillus subtilis*, acibenzolar-S-methyl, fosetyl aluminium, potassium phosphites, prohexadione-Ca.

INTRODUCTION

The bacterium *Erwinia amylovora*, which causes fire blight in pear (*Pyrus communis*), apple (*Malus domestica*) and quince (*Cydonia oblonga*) and some other members of the family Rosaceae, is one of the most challenging pathogens of pome fruit production worldwide (Momol and Aldwinckle, 2000). This disease has been detected in Morocco in 2006, in a commercial orchard in the rural area of Ain Orma in the vicinity of Meknes (Fatmi *et al.*, 2008). Since then, *E. amylovora* has spread throughout the region of Meknes (Morocco) and caused important losses in pear, apple and quince crops. Currently, fire blight affects the orchards of the entire region of the Middle Atlas, which is the stronghold of the national production of apple and pear (Hannou *et al.*, 2013).

Traditionally, the most commonly used control strategy for the management of fire blight was pruning of diseased twigs and branches combined with application of copper-containing formulations or antibiotics. Unfortunately, pruning can be inefficient at high disease pressure and copper materials are often phytotoxic, while antibiotics are only effective against blossom infections (Johnson and Stockwell, 2000). Furthermore, the use of antibiotics is currently outlawed in several countries, including Morocco. As a result, research was directed toward identifying alternative solutions for fire blight management. Thus, new approaches have been explored in plant protection technologies that could be used effectively in integrated disease management programs. Use of biological control agents has been reported as effective alternative tool for disease control (Kunz *et al.*, 2008; Stockwell *et al.*, 2010). Several biological control agents have been developed. They are authorized or in the process of registration in different countries (Sundin *et al.*, 2009). Additionally, there has been also much interest in recent years in novel control strategies which trigger defense mechanisms in the host plants. Such effects can, for instance, be achieved by fosetyl aluminium (F-Al), potassium phosphites (PH), harpin protein, acibenzolar-S-methyl (ASM) and prohexadione-Ca (ProCa) (Steiner, 2000; Spinelli *et al.*, 2007; Aćimović *et al.*, 2015; Johnson and Temple, 2016).

Table 1. Biological control agents and plant defense activators tested for fire blight control

Material	Active ingredient (a.i.)	Moroccan registration	Rate	
			Per liter or per hectare	a.i. in suspension ^(x) or per tree
Biological products				
COMPANION	1.5 × 10 ⁷ cfu/ml of <i>B. subtilis</i> GB03	UNIVERS HORTICOLE	5 ml/l	7.5 × 10 ⁴ cfu/ml
SERENADE AS	1 × 10 ⁹ cfu/g of <i>B. subtilis</i> QST713	BAYER SA	10 ml/l	1 × 10 ⁷ cfu/ml
BIOBAC	1 × 10 ⁹ cfu/g of <i>B. subtilis</i> Y1336	SAOAS	3g/l	3 × 10 ⁶ cfu/ml
BLOSSOM BLESS	10 ¹¹ CFU/g of <i>P. agglomerans</i> P10c	AGRIFUTUR S.r.l. ^(y)	1g/l	1 × 10 ⁸ cfu/ml
Plant defense activators				
ALEXIN® 95 PS	95% of mono and bi-potassium salt of phosphorus acid	ARD UNIFERT Morocco	2.5 kg/ha	2.16 g/tree ^(z)
ALIETTE FLASH	80% of Fosetyl Aluminium	BAYER SA	3.75 kg/ha	2.73 g/tree
BION 50 WG	50% of Acibenzolar-S-méthyl	Syngenta Morocco	0.150 kg/ha	0.068 g/tree
REGALIS	10% of Prohexadione-calcium	BASF Morocco	1.5 kg/ha	0.137 g/tree

^xBased on concentration stated in the product label.

^yThis product is not registered in Morocco, it was supplied as sample in lyophilized form by AGRIFUTUR S.r.l.

^zThe number of trees per hectare is 1100.

Several mechanisms were proposed to explain the inhibitory effect of different bacterial antagonists on *E. amylovora*, including the production of toxic secondary metabolites and competition for nutrients and space (Cabrefiga *et al.*, 2007). However, plant defense activators do not have any bactericidal activity, but they interfere with plant metabolism, either triggering the plant defense mechanism (ASM, F-AI and PH) leading to systemic acquired resistance (SAR), or suppressing shoot growth (ProCa), thus lowering shoot susceptibility to infection (Psallidas and Tsiantos, 2000). Since plant defense activators (PDAs) and biological control agents (BCAs) reduce the incidence of blossom blight and the consequent shoot blight by different modes of action, their combination may result in an enhanced level of control.

The purpose of this study was to evaluate the effect of BCAs and PDAs, alone or in combination, against fire blight in Morocco, in an effort to develop more sustainable and integrated strategies for reducing fire blight incidence on rosaceous fruit trees. Thus, we investigated various combinations of BCAs and PDAs in experiments to test (i) the efficacy of BCAs against *E. amylovora* in laboratory on detached blossoms of pear and apple; (ii) the efficacy of BCAs against fire blight on blossom clusters of pear trees in the field; (iii) the usefulness of integrated control of fire blight in commercial pear orchard with different combinations of BCAs and PDAs.

MATERIALS AND METHODS

Source of the bacterial pathogen strain of *E. amylovora*, the BCAs and PDAs used. The local strain of *E. amylovora* isolated in 2008 from a diseased *Pyrus communis* (Ait Bahadou *et al.*, 2016) was used as pathogen inoculum in the laboratory trials. The biological control agents and the plant defense activators evaluated in this study are shown in Table 1.

Biocontrol efficacy of BCAs on attached flower assays.

Laboratory experiments were performed with pear and apple flowering branches. Antagonist strains (*Bacillus subtilis* GB03, *B. subtilis* QST713, *B. subtilis* Y1336 and *Pantoea agglomerans* P10c) were tested individually and in mixtures (1:1) for their ability to suppress growth of *E. amylovora* on flowers as described previously (Ait Bahadou *et al.*, 2016). Branches of “Coscia” pear and “Golden” apple were collected at pink petals stage (BBCH 57; Stauss, 1994) from the area of Meknes and Imouzzar-Kandar. In the laboratory, the collected twigs were placed in bottles of distilled water and were held in a growth chamber at a day/night temperature of 22/10°C, 90 ± 10% relative humidity and daylight time of 17 h. Blossoms with senescent stigmas and no opened flowers were removed before inoculation, leaving only 30 newly opened blossoms per twig. Treatments were arranged in a randomized complete block design with three replications. BCAs were prepared by dilution of formulated products in water as recommended in the data sheet of each product and were applied on blossoms using a micro-sprayer. Table 2 summarizes the combinations of all antagonists in pear and apple. After 24 h of incubation at 26°C on Levane medium, the pathogen was resuspended in sterile water at a concentration of 5 × 10⁸ CFU/ml (optical density of 0.2 at 660 nm) and was applied to flowers using a small brush by touching the stigmas. Disease incidence was measured by the percentage of blighted blossoms (number of blighted blossoms divided by the total number of blossoms per branch).

Testing of BCAs against blossom blight under field conditions. To test the efficacy of various antagonist strains and the mixture of QST713 and P10c strains, experiments were conducted under natural high disease pressure in a commercial pear orchard (var. ‘Coscia’ on BA29 rootstock) planted in 2004 at the Toulal location in the Meknes region. Treatments were arranged in a randomized complete block design with five single trees

Table 2. Antagonists and their mixtures evaluated for fire blight control on apple and pear (laboratory trials)

Bacterial strain	Mixture (1:1)							
	<i>B. subtilis</i> GB03		<i>B. subtilis</i> QST713		<i>B. subtilis</i> Y1336		<i>P. agglomerans</i> P10c	
	Pear	Apple	Pear	Apple	Pear	Apple	Pear	Apple
<i>B. subtilis</i> GB03	X ^(*)	X	X	--	X	--	X	--
<i>B. subtilis</i> QST713			X	X	X	--	X	X
<i>B. subtilis</i> Y1336					X	X	X	--
<i>P. agglomerans</i> P10c							X	X

(*) Mixture of the same strain means that this was tested alone.

replicates per treatment in 2013 and four single trees replicates per treatment in 2015 and 2016. BCAs were prepared according to the manufacturer's instructions (Table 1) and the applications on trees were carried out using a knapsack sprayer with constant pressure until runoff. Two applications of the antagonist were performed (Stockwell *et al.*, 2010), one at 10% bloom and the second one week later (60% bloom) under a temperature of 20°C and low wind. Disease evaluation was carried out one week after the second application of the antagonist. Trees were rated for disease incidence measured as a percentage of blighted blossoms observed in ten branches that were randomly chosen on each tree. In total, 40 to 50 branches were considered by treatment with an average of 30 blossoms per branch.

Integration of BCAs and PDAs to control blossom and shoot blight under field conditions. Field experiments were conducted in 2015 and 2016, in the same pear orchard mentioned previously under natural high disease pressure. Treatments were arranged in split-split-plot design with three blocks, with five main-plot treatments for BCAs, a sub-plot for injection or foliar application of PDAs and sub sub-plots for PDAs. The main plot treatments included negative control, GB03, QST713 and Y1336 strains of *B. subtilis*, P10c strain of *P. agglomerans* and the mixture of QST713 and P10c strains. The sub sub-plot treatments included control, ASM, F-Al, PH and ProCa.

Application of BCAs and PDAs on pear trees. The rates of all treatments applied in the experiment are provided in Table 1. Trees were treated twice with ASM, F-Al, PH or ProCa, the first application was between flower-bud break (BBCH 55) and pink bud stage (BBCH 57) and the second was performed at BBCH 71. Plants treated with water served as a negative controls. Formulations of BCAs were applied first at BBCH 61 and a week later at BBCH 65. All treatments were applied to near runoff (1000l/ha) with backpack sprayers (Model super 16, MATABI, Spain) in early morning when wind conditions were calm. For trunk injections, four cardinally oriented injection ports per tree, inclined at 45° and positioned approximately 10-15 cm above the ground level, were created by drilling 25 mm into the xylem tissue and 10 mm in diameter, with a cordless drill (BRICOTEAM BT 2898SF, 50 Hz 18V 0-550 rpm). On each port, a 2.5 ml syringe was placed

and the contours were chewed to prevent infections. The required rate per tree of each plant defense activator was injected with a solution of 20 ml prepared using sterile distilled water at BBCH 51 and at BBCH 69.

Disease assessment. The numbers of infected flower clusters on each experimental unit were counted during 2-3 weekly surveys that extended through early-/mid-April. Diseased flower clusters were removed immediately after counting. The total number of blighted flower clusters per experimental unit was converted to incidence (%) as described earlier. For shoot blight incidence, four cardinally main branches were randomly examined for each tree; the incidence was measured by calculating the number of blighted shoots relative to the total number shoots of the observed main branch.

Statistical analysis. The percent incidence data were transformed by the arcsine square root function before analysis of variance and mean separation by Fisher's least significant difference (LSD) test at P=0.05. The analysis of variance (ANOVA)/General Linear Model procedure of SPSS (version 17.0 for Windows, Chicago, SPSS Inc.) was used.

RESULTS

Biocontrol efficacy of BCAs on attached flower assays. The fire blight symptoms were observed in the different experimental units and the confirmation of the visual flow-ers symptoms was performed by plating bacterial suspen-sions recovered from infected flowers on Levane medium. The incidence of the disease on apple and pear blossoms for different treatments varied throughout the trial period. However, this incidence was higher on the pear than on the apple blossoms. Indeed, on apple blossoms the mix-ture of QST713 and P10c strains and P10c strain allowed a significant disease reduction. Eight days after inocula-tion with *E. amylovora*, the incidence was only 17.78% for P10c strain treatment and 10% for the mixture (Fig. 1A). Treatments with Y1336, GB03 and QST713 strains of *B. subtilis* allowed a slight reduction of the disease, reaching 61.11, 70 and 76.67%, respectively, at the end of the test. On pear blossoms, the lowest incidence was obtained with

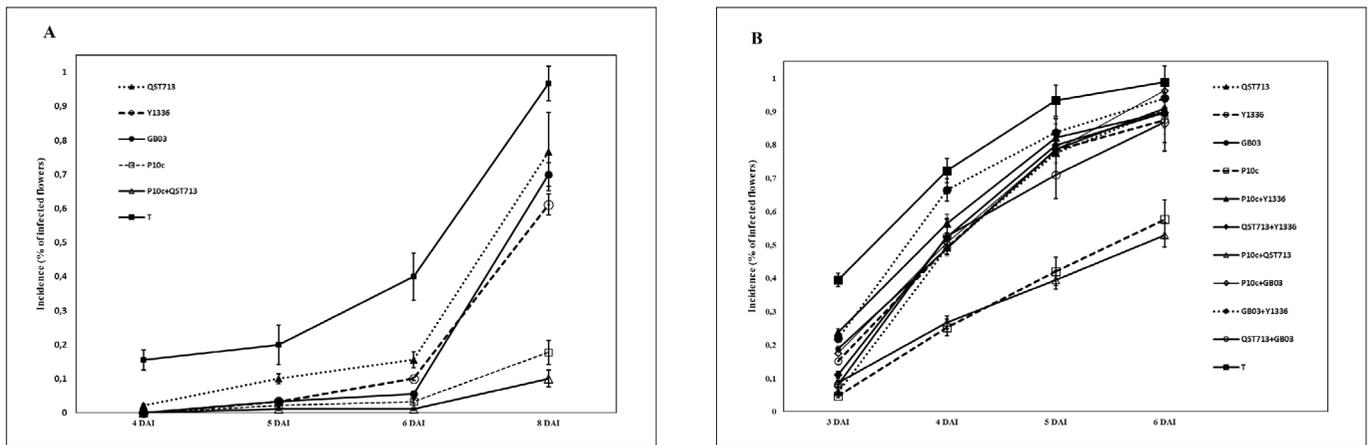


Fig. 1. Incidence (% of infected flowers) of fire blight on apple (A) and pear (B) blossoms that were treated with *Bacillus subtilis* GB03, *B. subtilis* QST713, *B. subtilis* Y1336, *Pantoea agglomerans* P10c or their mixture (1:1) and inoculated after 24 h with *E. amylovora*. Antagonists were prepared by dilution of formulated products in water as recommended in data sheet of each product and the concentration of *E. amylovora* was 5×10^8 cfu/ml.

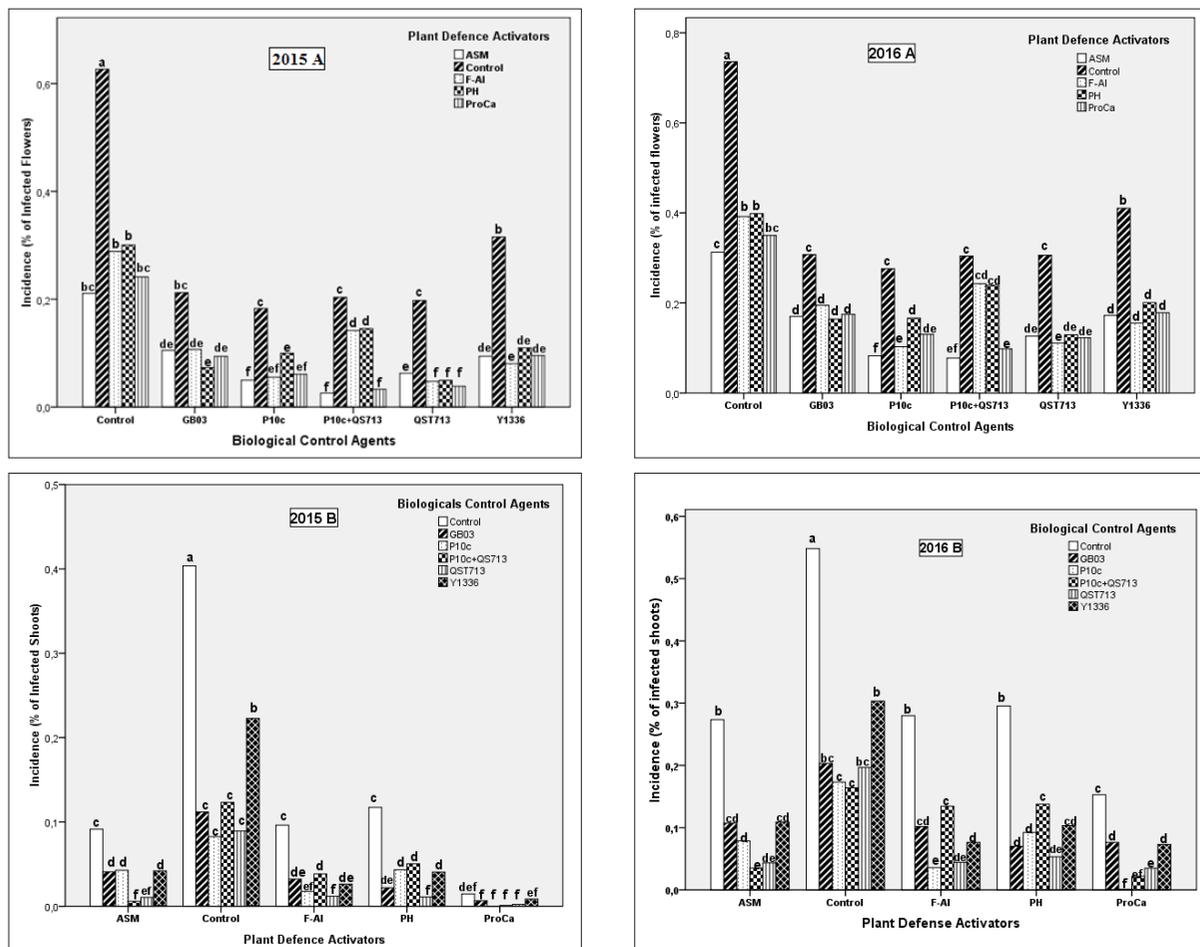


Fig. 2. Incidence of fire blight on pear trees treated with water, biocontrol agents alone or in combination with plant defense activators. The incidence was rated on blossoms (A) and shoots (B). Experiments were conducted in 2015 and 2016 under natural high disease pressure in a commercial pear orchard (var. ‘Coscia’ on BA29 rootstock). Trees arranged in spit-split plot in a randomized complete block design with three blocks, with five main-plot treatments (BCAs), a split-plot (injection or foliar application of PDAs) and split-split plot (PDAs). The main plot treatments included control, GB03, QST713 and Y1336 strains of *B. subtilis*, P10c strain of *P. agglomerans* and the mixture of QST713 and P10c strains. The split-split plot treatments included control, acibenzolar-S-methyl (ASM), fosetyl aluminium (F-AI), potassium phosphites (PH) and prohexadione-Ca (ProCa). Treatments were sprayed twice with water or biocontrol agents at BBCH 61 and BBCH 65 and with plant activators at BBCH 57 and BBCH 71. Trunk injections were performed at BBCH 51 and BBCH 69.

Table 3. Blossom blight control on pear trees cv. "Coscia" treated with bacterial antagonists in orchard trials conducted under high disease pressure in Meknes region from 2013 to 2016

Treatment	2013		2015		2016	
	Infected flowers (%)	Disease control (%)	Infected flowers (%)	Disease control (%)	Infected flowers (%)	Disease control (%)
Control	65.5 a		62.7 a		73.6 a	
GB03	20.9 b	68.2	21.2 c	66.2	30.7 c	58.3
P10c	23.2 b	64.7	18.3 c	70.8	27.6 c	62.5
QST713	23.4 b	64.4	19.7 c	68.6	30.6 c	58.4
Y1336	23.0 b	65.0	31.5 b	49.8	41.0 b	44.3
QST713+P10c	27.0 b	58.9	20.3 c	67.6	30.4 c	58.7

Single-tree plots arranged in a complete randomized block design with four to five replications per treatment were sprayed twice with GB03, P10c, QST713, Y1336 or the mixture of P10c and QST713 at 10 and 60% bloom. Year of trial. Within a column, means followed by the same letter are not significantly different according to the least significant difference test at $P=0.05$. The arc-sine square root transformation was applied to incidence data prior to analysis of variance; incidence on each tree was computed by dividing the number of blighted blossoms by the prebloom count of total number blossoms of observed branch. Ten branches were randomly observed for each tree.

treatments based on P10c and the mixture of P10c and QST713, *i.e.* 57.68% for P10c and 52.95% for the mixture. By contrast, other treatments did not reduce the incidence of the disease (Fig. 1b).

Biological control of fire blight on pear trees in the field conditions. Results from orchard trials in 2013, 2015 and 2016, which were carried out under natural infection conditions, are listed in Table 3. The BCAs GB03, P10c, QST713, Y1336 and the mixture (1:1) of QST713 with P10c significantly reduced the proportion of infected flowers compared to the water control. In fact, this reduction was statistically similar for all treatments in 2013. However, in 2015 and 2016 significant differences in efficacy were observed between different BCAs. Y1336 was less effective than other BCAs in reducing blossom blight incidence.

Effect of integration of BCAs and PDAs on reduction of blossom and shoot blight incidence. Fire blight incidence was monitored over two consecutive years in "Coscia" pear orchard (Fig. 2). Generally, blossom and shoot blight incidences were higher in 2016 compared to 2015. On water-treated control trees, the incidence recorded in 2016 was slightly above 70% on blossoms and 50% on shoots and only about 60% on blossoms and 40% on shoots in 2015.

Treatments of BCAs and PDAs significantly reduced blossom and shoot blight incidence. In fact, on blossoms (Fig. 2A), this reduction ranged approximately from 50% to 70% in 2015 and from 44% to 62.5% in 2016 for BCAs, but it varied only from 52% to 66.3% in 2015 and from 46% to 57.4% in 2016 for PDAs. In these two years, comparable blossom blight control was obtained with P10c, QST713, their mixture, GB03 strains and ASM. Statistically, it was more than that obtained with Y1336 strain, ProCa, F-Al and PH treatment. On shoots (Fig. 2B), the reduction of the disease incidence ranged from 44% to 80% in 2015 and from 40% to 70% in 2016 for BCAs. However, for PDAs, this reduction ranged from 71 to 96.5% in 2015 and from 46 to 72% in 2016. When used

alone, ProCa was the most effective treatment in reducing shoot blight incidence in 2015. In contrast, in 2016 the efficacy of ProCa was similar to all BCAs except Y1336 strain and significantly higher than other PDAs. No significant differences in controlling of both blossom and shoot blight were observed between foliar and injection applications of PDAs.

During these two year trials, the efficacy of integration of BCAs with PDAs was evaluated for blossom and shoot blight control. Increases in efficacy were observed for all treatments tested in this study (Fig. 2). The performance of each used combination varied greatly among trials. Indeed, GB03 and Y1336 combined with all PDAs reduced equally the incidence of both blossom and shoot blight in both growing seasons. In 2015, the most effective treatments on blossom were the combinations of QST713 or P10c with all PDAs and the combinations of ASM or ProCa with the mixture of QST713 and P10c. However, the combinations of ProCa with all BCAs, the combination of QST713 with F-Al or PH and the combination of ASM with the mixture of QST713 and P10c were most effective in reducing shoot blight incidence. In 2016, all combinations provided statistically the same level of blossom blight control, with the exception of the combination of ASM with P10c or with the mixture of QST713 and P10c, which significantly provided the highest protection rate, whereas the integration of ProCa with P10c or the mixture of QST713 and P10c provided the best reduction of shoot blight development.

DISCUSSION

Fire blight is still a challenging disease for commercial production of apple and pear. To date, finding a reliable control strategy for this devastating disease is a difficult task as the available control practices are inadequate or ineffective in controlling the natural disease outbreaks. During the last two decades, researchers interested in fire blight investigated the use of plant resistance activators

and BCAs as potential alternatives to antibiotics and copper (Zeller and Zeller, 1999; Johnson and Stockwell, 2000; Norelli *et al.*, 2003; Johnson and Temple, 2013; Ait Bahadou *et al.*, 2016). However, both strategies showed inconsistent efficiency for fire blight control in some environments (Ngugi *et al.*, 2011). Therefore, integrated control strategy, which includes all of these practices, is an alternative to enhance the level of disease control and the complementarity of their mode of actions may have a synergistic effect that can overcome the inconsistent efficacy.

This study contributes new knowledge on fire blight management. It is one of the few studies dealing with the use of BCAs in integration with PDAs to control fire blight disease (Ozaktan *et al.*, 2011; Phillion *et al.*, 2011; Spinelli *et al.*, 2012). Other studies aimed to integrate antibiotics with BCAs or PDAs (Lindow *et al.*, 1996; Momol *et al.*, 1999; Stockwell *et al.*, 1996, 2008; McGhee and Sundin, 2011; Johnson *et al.*, 2016) and/or integrate BCAs with lime sulfur (Kunz *et al.*, 2008; Johnson and Temple, 2013).

In laboratory trials involving branches with attached blossoms, BCAs were evaluated alone or in mixture against blossom blight. The successful biological treatments were the mixture of QST713 with P10c and P10c alone in both pear and apple whereas other BCAs and mixtures were not effective in reducing the incidence of the disease. Johnson and Stockwell (1998) reported that the effect of BCAs seems to be strongly affected by the inoculum density. Moreover, Stockwell *et al.* (2011) demonstrated that in mixture certain BCAs are mechanistically incompatible, in that one strain interferes with the mechanism by which a second strain suppresses plant disease. Nevertheless, mixtures composed of mechanistically compatible strains of BCAs can suppress disease more effectively than individual BCAs. Vanneste and Yu (1996) reported that the populations of A506 or Eh252 on pistils or nectaries were similar whether they were sprayed alone or in combination, indicating that these two strains are not antagonistic to each other on flowers. Similar results were obtained on Asian pear flowers by Vanneste and Yu (1996).

Under field conditions, foliar sprays or trunk injection of ASM, F-AI, PH and ProCa alone were effective in reducing the incidence of blossom and shoot blight during the two consecutive years of the study. Previously, it was reported that these compounds are mainly effective in protecting pear and apple from the shoot blight stage of the disease. However, they have also been shown to protect against the blossom blight stage (Psallidas and Tsiantos, 2000; Spinelli *et al.*, 2007; Aćimović *et al.*, 2015). In contrast, BCAs prevent only flower infection (Johnson and Stockwell, 2000). Nevertheless, Ülke and Çınar (1999) and Mikiciński *et al.* (2016) demonstrated that on shoots six strains of *P. agglomerans* (*E. herbicola*) and strain 49M of *Pseudomonas graminis* showed a higher (up to 80%) efficacy in controlling fire blight when a 24 h time interval between the application of antagonists and pathogen inoculation was used. In the present study, we demonstrated also

that two spray applications of BCAs GB03, P10c, QST713, Y1336 and the mixture (1:1) of QST713 and P10c reduced the blossom blight incidence by an average rate ranging from 44.3 to 71% compared to water treatments in orchard trials. Similar results were also achieved in fire blight control with two spray applications of bacterial antagonists *Pseudomonas fluorescens* strain A506 and *Pantoea agglomerans* strain C9-1 (Johnson and Stockwell, 2000; Ozaktan *et al.*, 2011), *P. agglomerans* strain Pa21889, *Bacillus subtilis* strain BsBD170 and *Rabnella aquatilis* strain Ra39 (Laux *et al.*, 2003), *B. subtilis* strain BS-F3 (Alexandrova *et al.*, 2002). The slightly higher fire blight incidence observed in 2016 compared to 2015 is probably due to rain and dew, which occurred during bloom in this season (Steiner, 2000).

The results of our study highlighted that the combination of PDAs and BCAs generally increased the protective effect of the two single treatments against blossom and shoot blight. In contrast, in a study on apple trees ('Cortland') on seedling rootstocks, Phillion *et al.* (2011) found that the application of Actigard (acibenzolar-S-methyl) in combination with the *P. agglomerans* C9-1 strain results in a negative interaction and possibly even complete suppression of disease control. However, our results corroborate the previously findings of Schoofs *et al.* (2015), who indicated that *B. subtilis* QST713 exhibited a high activity against *E. amylovora*, which resulted in reducing disease progression as necrosis and limited ooze formation on the infected tissue. In addition, a combination of *B. subtilis* QST713 with the PDE activity of fosetyl aluminium seems promising for the protection of blossoms. Such results might be true for the integrated application of P10c strain with the bio-regulator ProCa. Spinelli *et al.* (2012) concluded that in the field, the combination of ProCa and P10c generally increased the protective effect of the two single treatments against blossom blight on apple and pear. Similar findings were demonstrated for the control of shoot blight on pear under conditions of natural infection after application of *P. vagans* strain C9-1 followed by ProCa treatment (Ozaktan *et al.*, 2011). Earlier studies showed also that the combination sprays of the BCAs *Pseudomonas fluorescens* A506 and *B. subtilis* QST713 (Serenade) with ProCa (Apogee) were significantly effective against fire blight and their use appeared enhanced by surfactants (Adaskaveg *et al.*, 2006). In general, the higher protective effect observed against the pathogen by using a combination of PDAs and BCAs might be the result of broad and complementary modes of action: the competition and antibiosis provided by BCAs and the induction of resistance by PDAs. Therefore, undoubtedly, the results obtained from this study highlighted the feasibility of using a combination of BCAs and PDAs in controlling fire blight disease under laboratory and field conditions. These results are of great importance and may further contribute to the development of a reliable integrated control strategy for this disease.

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