Phytoprotection

Evaluation of host resistance inducers and conventional products for fire blight management in loquat and quince
Évaluation d'inducteurs de résistance et de produits conventionnels pour lutter contre la brûlure bactérienne chez le néflier du Japon et le cognassier

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Article abstract

Fire blight disease is one of the most destructive diseases of pome fruits. Due to the lack of effective, non-phytotoxic and publicly acceptable materials for controlling fire blight in pome fruit trees, new strategies to manage *Erwinia amylovora* fire blight are being sought. The resistance-inducing compounds prohexadione-Ca, harpin protein and benzothiadiazole (acibenzolar-S-methyl), the fertilizer humic acid, the bactericides streptomycin and copper salts, and combinations of copper with chemicals were evaluated for their ability to control fire blight on quince and loquat cultivars. Prohexadione-Ca was applied at a rate of 125 mg L^{-1} at two shoot lengths (6-12 cm and 15-20 cm), while benzothiadiazole + metalaxyl (135 mg L^{-1}) and harpin (50 mg L^{-1}) were applied when the shoots measured between 15-20 cm, and again at 30-35 cm. On loquat cv. Cukurgobek, benzothiadiazole + metalaxyl showed about 60% effectiveness. The addition of copper salts reduced the effectiveness of benzothiadiazole + metalaxyl. On quince cultivars, streptomycin ($P \leqslant 0.05$) was the most effective treatment during both years, followed by the harpin protein alone and in combination with copper salts. Prohexadione-Ca, benzothiadiazole + metalaxyl, and harpin protein applications reduced disease severity on inoculated shoots compared with copper and untreated controls. Prohexadione-Ca reduced both shoot length and shoot blight on the two hosts. Humic acid applications were ineffective in controlling fire blight on loquat and quince cultivars. Quince cv. Eşme showed lower disease severity than cv. Ekmek ($P \leqslant 0.05$). The use of resistance-inducing substances during the early phase of shoot growth may offer a means of managing the shoot blight phase of fire blight disease on quince and loquat.

Evaluation of host resistance inducers and conventional products for fire blight management in loquat and quince

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Fire blight disease is one of the most destructive diseases of pome fruits. Due to the lack of effective, non-phytotoxic and publicly acceptable materials for controlling fire blight in pome fruit trees, new strategies to manage *Erwinia amylovora* fire blight are being sought. The resistance-inducing compounds prohexadione-Ca, harpin protein and benzothiadiazole (acibenzolar-S-methyl), the fertilizer humic acid, the bactericides streptomycin and copper salts, and combinations of copper with chemicals were evaluated for their ability to control fire blight on quince and loquat cultivars. Prohexadione-Ca was applied at a rate of 125 mg L^{-1} at two shoot lengths (6-12 cm and 15-20 cm), while benzothiadiazole + metalaxyl (135 mg L^{-1}) and harpin (50 mg L^{-1}) were applied when the shoots measured between 15-20 cm, and again at 30-35 cm. On loquat cv. Cukurgobek, benzothiadiazole + metalaxyl showed about 60% effectiveness. The addition of copper salts reduced the effectiveness of benzothiadiazole + metalaxyl. On quince cultivars, streptomycin ($P \leq 0.05$) was the most effective treatment during both years, followed by the harpin protein alone and in combination with copper salts. Prohexadione-Ca, benzothiadiazole + metalaxyl, and harpin protein applications reduced disease severity on inoculated shoots compared with copper and untreated controls. Prohexadione-Ca reduced both shoot length and shoot blight on the two hosts. Humic acid applications were ineffective in controlling fire blight on loquat and quince cultivars. Quince cv. Eşme showed lower disease severity than cv. Ekmek ($P \leq 0.05$). The use of resistance-inducing substances during the early phase of shoot growth may offer a means of managing the shoot blight phase of fire blight disease on quince and loquat.

Keywords: *Cydonia, Eriobotrya, Erwinia amylovora*, growth regulation, systemic acquired resistance.

[Évaluation d'inducteurs de résistance et de produits conventionnels pour lutter contre la brûlure bactérienne chez le néflier du Japon et le cognassier]

La brûlure bactérienne est l'une des maladies les plus néfastes chez les fruits à pépins. En l'absence de produits efficaces, non phytotoxiques et socialement acceptables pour lutter contre cette maladie causée par *Erwinia amylovora* chez les pomoïdées, de nouvelles stratégies sont recherchées. La capacité de certains composés pouvant induire de la résistance (prohéxadione-Ca, protéine harpine et benzothiadiazole (acibenzolar-S-méthyle)), de l'acide humique utilisé en tant que fertilisant, de bactéricides (streptomycine et sel de cuivre), ainsi que des combinaisons de cuivre et de produits chimiques à lutter contre la brûlure bactérienne chez des cultivars de néflier du Japon et de cognassier a été évaluée. La prohéxadione-Ca a été appliquée à un taux de 125 mg L^{-1} sur deux longueurs de pousses (6-12 cm et 15-20 cm), tandis que le benzothiadiazole + métalaxyl (135 mg L^{-1}) et l'harpine (50 mg L^{-1}) ont été appliqués sur des pousses alors qu'elles mesuraient entre 15 et 20 cm, puis à nouveau alors qu'elles mesuraient entre 30 et 35 cm. Chez le néflier du Japon cv. Cukurgobek, un taux d'efficacité d'environ 60 % a été obtenu avec le benzothiadiazole + métalaxyl; cependant, l'ajout de sel de cuivre en a réduit l'efficacité. Sur les cultivars de cognassier, la protéine harpine, utilisée seule et en combinaison avec le sel de cuivre, s'est avérée le traitement le plus efficace durant les deux années de l'étude, après la streptomycine ($P \leq 0.05$). La prohéxadione-Ca, le benzothiadiazole + métalaxyl et les protéines harpines ont réussi à réduire la gravité de la maladie chez des pousses inoculées comparativement aux témoins traités au cuivre et aux témoins non traités. La prohéxadione-Ca a réduit à la fois la longueur et la brûlure des pousses chez les deux hôtes. Les applications d'acide humique n'ont pas réussi à réduire l'incidence de la brûlure bactérienne chez les cultivars de néflier du Japon et de cognassier. Le cognassier cv. Eşme a été moins gravement affecté par la maladie que le cv. Ekmek ($P \leq 0.05$). L'utilisation de substances pouvant induire de la résistance durant la phase initiale de croissance des pousses peut être une façon de lutter contre la brûlure des pousses causée par la brûlure bactérienne chez le cognassier et le néflier du Japon.

Mots clés : *Cydonia, Eriobotrya, Erwinia amylovora*, régulation de la croissance, résistance systémique acquise.

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INTRODUCTION

Turkey is an important world producer of loquat, *Eriobotrya japonica* (Thunb.) Lindl. After the economic value of loquat was realized, demand for commercial production rapidly increased. Total production increased more than four-fold between 1980 (3000 t) and 2003, when it reached about 12 000 t produced from 246 000 trees (Polat and Caliskan 2007). Turkey supplies 27% of the world's total quince (*Cydonia* spp.) production, with 150 000 t produced annually (Gunes and Dumanoglu 2005).

Fire blight caused by the bacterium *Erwinia amylovora* (Burr.) Winslow *et al.* is the most devastating bacterial disease of apple (*Malus domestica* Borkh), pear (*Pyrus communis* L), quince and loquat trees worldwide (Jones and Aldwinkle 1990; van der Zwet and Keil 1979; Vanneste 2000). The disease significantly limits the area for successful pome fruit production. It is probable that fire blight will remain a concern as the pathogen may be introduced into new pome fruit growing areas (Jock *et al.* 2002; van der Zwet 2002; Vanneste 2000). There are several distinct phases of the disease, including blossom blight, shoot blight and rootstock blight. The diversity of host tissues susceptible to infection, combined with the limited number of management tools available to control the disease, has made it difficult to prevent the progress of fire blight epidemics.

Severe fire blight symptoms were observed on quince trees (*Cydonia vulgaris* (Pers.) cultivars Eşme and Ekmek) in different orchards in Turkey in 2003. The disease had spread to all quince-growing areas located in the Mediterranean, Aegean, Marmara and Middle Anatolia regions of Turkey (Saygili *et al.* 2006).

The most commonly applied bactericides for controlling fire blight in pome fruit trees are copper compounds and antibiotics (Psallidas and Tsiantos 2000). However, russeting of fruits often results from copper treatments. Furthermore, the authorities in many countries do not permit the use of antibiotics for fire blight control because of potential risks of promoting the development of antibiotic resistance in human pathogens (McManus *et al*. 2002). Due to the lack of publicly acceptable, effective and non-phytotoxic preparations to control fire blight, there has been an interest in novel control strategies that trigger defense mechanisms in the host plants. Such effects have been reported for the harpin protein, benzothiadiazole (acibenzolar-S-methyl) and prohexadione-Ca (Aldwinckle *et al.* 2002; Bazzi *et al.* 2003; Maxson and Jones 2002; McManus *et al.* 2002; Momol *et al.* 1999; Norelli *et al.* 2003; Steiner 2000).

One of the most important factors determining host susceptibility to fire blight shoot infections is the intensity of vegetative shoot growth on the fruit trees (Byers and Yoder 1997). An alternative and promising approach to control fire blight is the use of growth-regulating acylcyclohexanediones, such as prohexadione-Ca. The most notable effect of prohexadione-Ca is the control of shoot elongation growth by inhibiting the formation of growth-active gibberellins from inactive precursors (Evans *et al.* 1999; Rademacher 2000). Prohexadione-Ca-treated apple and pear trees are significantly less infected by *E. amylovora* (Aldwinckle *et al.* 2002; Bazzi *et al.* 2003; Buban *et al.* 2002, 2003; Cline and Hunter 2002; Costa *et al.* 2001a; Deckers and Schoofs 2002; Fernando and Jones 1999; Maxson and Jones 2002; Momol *et al.* 1999; Norelli *et al.* 2003; Sobiczewski *et al.* 2001; Winkler 1997; Yoder *et al.* 1999) and *Venturia inaequalis* (Cooke) G. Wint. (Costa *et al.* 2001b; Spinelli *et al.* 2002). Another new approach lies in the induction of systemic acquired resistance (SAR), a self-defense mechanism in plants. The SAR response correlates with the accumulation of pathogenesis-related (PR) proteins. The PR proteins can be induced by some plant activators such as benzothiadiazole and harpin protein (Anonymous 2002; Brisset *et al.* 2000; Fontanilla *et al.* 2005; Jones 2001; Wei and Beer 1996). In addition, humic acid is reported by the manufacturer to improve plant vigour and natural resistance to plant diseases (Anonymous 2000; Freeman 1969).

In this study, the effectiveness of host resistance inducers (prohexadione-Ca, benzothiadiazole and harpin protein) and a fertilizer (humic acid) was investigated on two hosts that have not been studied so far (quince and loquat cultivars) on the shoot blight phase of fire blight disease.

Table 1. Active ingredients, formulation and application rate of the chemical compounds used in the experiment

Active ingredient and percentage		Commercial name / Firm	Formulation	Application rate (100 L^{-1} water)
Prohexadione-Ca	10%	Apogee / BASF	WG[1]	125 g
Benzothiadiazole + Metalaxyl	4% 40%	BION MX 44 / Syngenta	WG	135 g
Harpin protein	3%	Messenger / Eden Bioscience	Powder	50 g[2] + 20 mL adjuvant[3]
Humic acid + Fulvic acid + Potassium hydroxide	55% 30% 8%	K-humate / Hektas A.S.	Powder	20 mL
Streptomycin sulfate	100%	Streptomycine / I.E. Ulagay	Powder	59 g
Copper salts	51.4%	Tenn Cop 5E / Hektas A.S.	Liquid	250 mL

[1] WG: wettable granule.
[2] Prepared in distilled water.
[3] KINETIC® (non-ionic adjuvant) manufactured for Helena Chemical Company.

MATERIALS AND METHODS

Plant material and growth conditions

A loquat cultivar, Cukurgöbek, and two quince cultivars, Eşme and Ekmek, grown extensively in Turkey, were used in this experiment. The test plants were selected among 3-yr-old saplings showing uniform growth. These saplings were transplanted into plastic pots of 25 cm diam filled with 8 kg of soil, and they were grown for 20 d at 25 ± 5°C, 60-75% RH, and under 12 000-14 000 Lux from tungsten-filament lamps for a 16-h photoperiod. The potted plants were placed on the ground 1 m apart at a field location in the Konya province and were watered regularly throughout the growing season.

After transplantation, the trees were fertilized once a week (each pot) with 25 g ammonium sulfate, 25 g diammonium phosphate, 25 g potassium sulfate, and 50 mL of a liquid fertilizer having 0.05% Mn, Cu, Zn, B, and Mo (Kacar and Katkat 1999). In addition, sulfur dust was applied once (4 g L^{-1} water) for powdery mildew control.

Erwinia amylovora strain

After conducting virulence tests on cv. Ankara pear trees, a virulent strain of *E. amylovora* (EAI) was selected for all inoculations (Norelli *et al.* 1984). Stock cultures were preserved at 4°C on the nutrient agar (NA) medium and transferred to new tubes every 3 mo.

Bacterial suspensions were prepared from growing colonies on NA at 23-25°C and were diluted in sterile distilled water (SDW) to give an absorbance of 0.15 at 660 nm. This represented 10^8 cfu mL^{-1} based on viable plate counts. Inoculum was maintained on ice and was used for plant inoculation within 2 h of dilution.

Chemical compounds used in the experiments and their applications

The chemical compounds used in the experiment were: prohexadione-Ca, benzothiadiazole + metalaxyl, harpin protein, humic acid, copper salts and streptomycin. These compounds and their properties are shown in Table 1. Chemical application timing and schedule were based on Momol *et al.* (1999) (Table 2). Prohexadione-Ca was applied twice when the shoots were 6-12 cm and 15-20 cm long. Benzothiadiazole + metalaxyl and harpin protein were applied when the shoots measured 15-20 cm and 30-35 cm. Copper salts and humic acid were applied three times when the shoots were 6-12 cm, 15-20 cm and 30-35 cm long. Streptomycin was applied twice, 1 d before and 1 d

Table 2. Date of the chemical applications and date of inoculation with *Erwinia amylovora* to potted loquat and quince trees

	Application time and shoot length (cm)				
Treatment	June (6-12 cm)[2]	June (15-20 cm)[3]	July (30-35 cm)[4]	July (40-45 cm)[5]	July (40-45 cm)[6]
Prohexadione-Ca					
Inoculated[1]	x	x			
Uninoculated	x	x			
Inoculated + Cu	x	x			
Benzothiadiazole + Metalaxyl					
Inoculated		x	x		
Uninoculated		x	x		
Inoculated + Cu		x	x		
Harpin protein					
Inoculated		x	x		
Uninoculated		x	x		
Inoculated + Cu		x	x		
Humic acid					
Inoculated	x	x	x		
Uninoculated	x	x	x		
Inoculated + Cu	x	x	x		
Copper salts					
Inoculated	x	x	x		
Uninoculated	x	x	x		
Streptomycin					
Inoculated				x	x
Uninoculated				x	x
Control					
Inoculated	x	x	x		
Uninoculated	x	x	x		

[1] Inoculated with *Erwinia amylovora* after application of chemical, on 16 July 2002 and 8 July 2003.
[2] Treatments applied on 15 June 2002 and 9 June 2003.
[3] Treatments applied on 25 June 2002 and 20 June 2003.
[4] Treatments applied on 10 July 2002 and 2 July 2003.
[5] Treatments applied on 15 July 2002 and 7 July 2003.
[6] Treatments applied on 17 July 2002 and 9 July 2003.

after inoculation (Momol *et al.* 1999). Streptomycin and copper salts are known to be effective against fire blight and were applied to allow comparison with the host resistance inducer treatments.

Experimental design and set-up

The experiment was set up in a completely randomized block design with three replicates. A single replicate was a mean from nine shoots on three saplings (Duzgunes *et al.* 1987). Each treatment was applied to five groups of plants (Table 3). The first three groups of plants were treated by the chemicals and inoculated with *E. amylovora* to see the effects of chemicals on disease severity (first group treated with chemicals + *E. amylovora* inoculation, second group treated with chemicals + copper salts + *E. amylovora* inoculation, third group as control 1 treated only with *E. amylovora* inoculation). The fourth group was treated only with the chemicals, and the fifth group, as control 2, was treated with water to see the effects of treatments on shoot growth of loquat and quince. The different combinations of treatments were all analyzed as separate treatments. The experiment was conducted during two growing seasons, 2002 and 2003.

Inoculation of the shoots

Actively growing shoot tips of plants were inoculated by inserting a 0.46-mm-diam (26-gauge) hypodermic needle through the stem just above the youngest unfolded leaf. A suspension of 10^8 cfu mL^{-1} *E. amylovora* was introduced to fill the wound and leave visible drops at both ends of the wound. The treated shoots were labeled with flagging tape for evaluation purposes (Norelli *et al.* 1986).

Evaluation of disease severity and shoot growth

The length of visible fire blight lesions and of the current season's shoot growth was recorded after all lesions had ceased to extend, as indicated by the formation of a determinate margin between diseased and healthy tissues.

Disease severity was calculated using the following formula: Disease severity (%) = (a / b) x 100; where **a** is the length of the blighted part of the shoot (cm), and **b** is the whole length of the shoot (cm) (Fernando and Jones 1999).

Percent effectiveness of the applications (**A**) was calculated according to the following formula: A = 100 x (B - C) / B; where **B** is the percent disease severity in the controls, and **C** is the percent disease severity in treated shoots.

Percent effectiveness of the treatments on reduction of shoot growth (**D**) was calculated in a similar way: D = 100 x (E - F) / E; where **E** is the mean shoot length in the controls, and **F** is the length of treated shoots (Anonymous 1996).

MINITAB (State College, PA, USA) was the statistical program used. The means (expressed as percent disease) were used to determine significant treatment differences. Data were analyzed using MSTAT software (Michigan State University, MI, USA) and the differences between treatments were determined by Duncan's New Multiple Range Test at $P \leq 0.05$.

RESULTS

Control of shoot blight on loquat

Shoot blight caused by *E. amylovora* was destructive on loquat with a disease severity of 91.06 and 82.29% in control plots in 2002 and 2003, respectively (Table 4). Streptomycin was the most effective treatment in preventing shoot blight. It was followed by benzothiadiazole + metalaxyl with 59.48 and 46.62% effectiveness, which was more ($P \leq 0.05$) than all the other treatments during the 2 yr. Prohexadione-Ca, harpin protein and copper salts lacked sufficient efficacy.

The addition of copper to the various treatments did not increase the effectiveness of the plant growth regulator and activators. Copper compound alone was not effective and only reduced shoot blight by 2.49 and 5.71% in 2002 and 2003, respectively. Disease severity in humic acid applications was 95.32 and 80.88% in 2002 and 2003, respectively. It did not differ significantly from the uninoculated control (Table 4).

Reduced shoot growth on loquat

A 2 to 12% reduction in shoot growth was observed in the treatments including streptomycin, copper, BTH and harpin protein. However, the most important reduction (30%) was only obtained with the Prohexadione-Ca treatment (Table 5). Shoot lengths of loquat saplings treated with Prohexadione-Ca were significantly shortened, measuring 16.36 cm and 19.63 cm in 2002 and 2003, respectively, in comparison with the untreated control (23.55 cm and 27.70 cm in 2002 and 2003, respectively).

Control of shoot blight on quince

After the streptomycin treatment, applications of harpin protein alone or in combination with the copper compound were the most effective treatments for the shoot blight phase of fire blight for both years. The effectiveness of harpin protein in disease control on cv. Eşme was 25.83 and 23.06% in 2002 and 2003, respectively, and 11.89 and 14.82% on cv. Ekmek. This was greater than copper compound effectiveness, which was 11.87 and 7.34% on cv. Eşme, and 8.00 and 6.42% on cv. Ekmek ($P \leq 0.05$). Copper salts did not increase the effectiveness of the chemicals (Table 6).

Copper salts were less effective than prohexadione-Ca and benzothiadiazole + metalaxyl applications in reducing the severity of fire blight on shoots inoculated with *E. amylovora* in 2003. Disease severity was numerically lower than with the untreated control, but there were no statistical differences (Table 6). Disease severity values resulting from humic acid treatments did not differ from the untreated control saplings (Table 6).

When the quince cultivars were evaluated independently, disease severity on cv. Ekmek was 56.42 and 60.80% in 2002 and 2003, respectively, and 47.83 and 51.57% on cv. Eşme. These results show that disease severity was less important on 'Eşme' than on 'Ekmek'. The data also show that there are numerical differences in disease severity between the cultivars, but these differences are not significant ($P \leq 0.05$).

Table 3. Experimental design for applications on loquat and quince cultivars

Plant groups	Applications
First group	Chemicals (PC[1], BTHM, HRP, HA, CU, STR) + *E. amylovora* inoculation
Second group	Chemicals (except Copper and Streptomycin) + Copper salts + *E. amylovora* inoculation
Third group (Control$_1$ = for disease severity)	*E. amylovora* inoculation
Fourth group	Chemicals
Fifth group (Control$_2$ = for shoot growth)	Water

[1] PC: Prohexadione-Ca; BTHM: Benzothiadiazole + Metalaxyl; HRP: Harpin protein; HA: Humic acid; CU: Copper salts; STR: Streptomycin.

Table 4. Effectiveness of the treatments on disease severity caused by *Erwinia amylovora* on loquat cv. Cukurgöbek in 2002 and 2003

	2002		2003	
Treatment[1]	Disease severity (%)	Effectiveness (%)	Disease severity (%)	Effectivene (%)
Prohexadione-Ca	88.54 bcd[2]	2.76	75.02 cd	8.83
BTHM	36.89 g	59.48	43.92 g	46.62
Harpin protein	83.62 de	8.17	69.00 e	16.15
Humic acid	95.32 a	0.00	80.88 a	1.71
Copper salts	88.79 bcd	2.49	77.59 bc	5.71
Streptomycin	1.27 h	98.60	2.83 h	96.56
PC + CU	84.84 cde	6.83	74.04 d	10.02
BTHM + CU	61.43 f	32.53	49.39 f	39.98
HRP + CU	82.61 e	9.27	70.00 e	14.93
HA + CU	89.20 bc	2.04	79.89 ab	2.91
Control	91.06 ab	0.00	82.29 a	0.00

[1] BTHM: Benzothiadiazole + Metalaxyl; PC: Prohexadione-Ca; CU: Copper salts; HRP: Harpin protein; HA: Humic acid.
[2] In a column, values followed by the same letter are not significantly different ($P \leq 0.05$) as determined by Duncan's New Multiple Range Test.

Table 5. Effectiveness of the treatments on reducing shoot growth of loquat cv. Cukurgöbek in 2002 and 2003

	2002		2003	
Treatment	Shoot length (cm)	Effectiveness (%)	Shoot length (cm)	Effectiveness (%)
Prohexadione-Ca	16.36 d[2]	30.53	19.63 e	29.13
BTHM[1]	21.00 c	10.82	29.03 abc	0.00
Harpin protein	20.53 c	12.82	29.21 ab	0.00
Humic acid	23.64 a	0.00	31.11 a	0.00
Copper salts	22.25 b	4.24	26.07 d	5.88
Streptomycin	23.09 ab	1.95	26.76 cd	3.39
Control	23.55 a	0.00	27.70 bcd	0.00

[1] BTHM: Benzothiadizole + Metalaxyl.
[2] In a column, values followed by the same letter are not significantly different ($P \leq 0.05$) as determined by Duncan's New Multiple Range Test.

Table 6. Effectiveness of the treatments on disease severity caused by *Erwinia amylovora* on quince cultivars Eşme and Ekmek in 2002 and 2003

	cv. EŞME				cv. EKMEK			
	2002		2003		2002		2003	
Treatment[1]	Diseasess severity (%)	Effectiveness (%)	Disease severity (%)	Effectiveness (%)	Disease severity (%)	Effectiveness (%)	Disease severity (%)	Effectiveness (%)
Prohexadione-Ca	53.59 ab[2]	8.42	53.66 cde	16.14	59.69 ab	6.98	64.17 bc	10.36
BTHM	54.12 ab	7.51	54.67 bcde	14.56	62.27 ab	2.96	64.83 bc	9.44
Harpin protein	43.40 c	25.83	49.23 e	23.06	56.54 b	11.89	60.98 c	14.82
Humic acid	56.21 ab	3.94	60.96 ab	4.73	62.88 ab	2.01	69.03 ab	3.57
Copper salts	51.57 b	11.87	59.29 abc	7.34	59.03 ab	8.00	66.99 abc	6.42
Streptomycin	11.56 d	80.24	7.98 f	87.52	13.29 c	79.28	10.25 d	85.68
PC + CU	50.30 b	14.04	51.93 de	18.84	61.30 ab	4.47	63.14 bc	11.80
BTHM + CU	51.19 b	12.52	55.92 bcde	12.61	63.11 ab	1.65	67.32 abc	5.96
HRP + CU	41.72 c	28.70	51.80 de	19.04	57.43 ab	10.50	61.59 c	13.96
HA + CU	53.93 ab	7.84	57.89 abcd	9.53	60.90 ab	5.09	68.96 ab	3.67
Control	58.52 a[2]	0.00	63.99 a	0.00	64.17 a	0.00	71.59 a	0.00

[1] BTHM: Benzothiadiazole + Metalaxyl; PC: Prohexadione-Ca; CU: Copper salts; HRP: Harpin protein; HA: Humic acid.
[2] In a column, values followed by the same letter are not significantly different ($P \leq 0.05$) as determined by Duncan's New Multiple Range Test.

Table 7. Effectiveness of the treatments on reducing shoot growth of quince cultivars Eşme and Ekmek in 2002 and 2003

	cv. EŞME				cv. EKMEK			
	2002		2003		2002		2003	
Treatment	Shoot length (cm)	Effectiveness (%)	Shoot length (cm)	Effectiveness (%)	Shoot length (cm)	Effectiveness (%)	Shoot length (cm)	Effectiveness (%)
Prohexadione-Ca	33.25 e[2]	22.78	33.94 e	26.10	32.18 e	24.28	32.48 e	30.96
BTHM[1]	41.58 d	3.43	46.02 abcd	0.00	43.63 bcd	0.00	48.71 a	0.00
Harpin protein	41.71 d	3.13	48.02 ab	0.00	43.84 bcd	0.00	47.98 ab	0.00
Humic acid	43.82 bcd	0.00	47.16 abc	0.00	44.83 abcd	0.00	46.97 abc	0.17
Copper salts	41.58 d	3.43	44.32 abcd	3.50	41.63 d	2.04	45.17 abcd	3.99
Streptomycin	41.79 d	2.94	45.73 abcd	0.43	41.73 d	1.81	45.05 abcd	4.25
Control	43.06 cd	0.00	45.93 abcd	0.00	42.50 cd	0.00	47.05 abc	0.00

[1] BTHM: Benzothiadizole + Metalaxyl.
[2] In a column, values followed by the same letter are not significantly different ($P \leq 0.05$) as determined by Duncan's New Multiple Range Test.

Reduced shoot growth on quince

Prohexadione-Ca was the only treatment that significantly reduced shoot length on quince cultivars, and its effectiveness was the highest (30.96%) on the susceptible cv. Ekmek in 2003 (Table 7).

DISCUSSION

The shoot blight phase of fire blight caused by *E. amylovora* is highly destructive within the current and subsequent growing seasons, and improved strategies are required for the control of fire blight on pome fruits. Danovan (1991) and Byers and Yoder (1997) reported that the first factor determining the susceptibility of the host plant against shoot infections of fire blight was rapid shoot growth.

The preventive effects of prohexadione-Ca on shoot growth and fire blight development on apples is a well-known phenomenon (Deckers and Daemen 1999). Prohexadione-Ca was tested on two different rosaceous plants, loquat and quince, at the same rates as those applied to apples. The primary effect of prohexadione-Ca is the control of shoot growth

(Evans *et al.* 1997; Greene 1999; Rademacher and Kober 2003; Yoder *et al.* 1999) in apple, pear, plum and sweet and sour cherry trees (Basak and Rademacher 2000; Elfving *et al.* 2003). A suppression of disease incidence was not associated with substantial growth control on pears (Buban *et al.* 2002), which was comparable to the results on loquat and quince in this study. These results indicate that the effects of prohexadione-Ca on the incidence of fire blight are primarily the result of physiological changes (Rademacher 2004). In addition, Unrath (1999) pointed out that varied results were obtained with prohexadione-Ca in climatically different regions.

Benzothiadiazole (acibenzolar-S-methyl) is known to induce systemic acquired resistance (SAR) against fire blight. Benzothiadiazole mimics the role of salicylic acid in defense reactions, and treated plants produce pathogenesis-related proteins, which are able to degrade some of the bacterial cell walls (Kessmann *et al.* 1996; Oostendorp *et al.* 2001; Thomson *et al.* 1999a, b). Weekly applications of benzothiadiazole (Actigard 50 WG, Novartis) provided 81% control compared with 97.6% with streptomycin on apples (Maxson and Jones 1999). In this study, benzothiadiazole + metalaxyl provided on average 53% disease control for the 2 yr on the loquat cultivar Cukurgobek, but its effectiveness was very low on the two quince cultivars tested. This suggests that the mode of action of the products does not work in the same way on different hosts. The other aspect that should be considered is the very low rate of benzothiadiazole in the mixture we used (4%). A higher degree of control could be expected with a higher concentrated form of the chemical, provided that there is no synergistic effect of metalaxyl. This hypothesis should be further tested on loquat and quince.

Harpin protein provided broad-spectrum protection of plants against fungal, bacterial and viral pathogens (Anonymous 2002; Fontanilla *et al.* 2005; Jones 2001; Momol *et al.* 1999; Wei and Beer 1996). However, it was not as effective on loquat compared to our previous studies on other pome fruits, notably pears (Bastas and Maden 2004). On quince cultivars, harpin protein generally provided better control than the other treatments, except streptomycin.

Streptomycin was effective in preventing the shoot blight phase of the disease on quince and loquat; however, the use of this chemical must be limited to high disease pressure conditions. In the control of fire blight, copper compounds can be effective only at low and medium disease severities (van der Zwet and Keil 1979), and the rate of control is lower on susceptible host pears (Dimova 1990). These alternative chemicals to copper have shown some promise for controlling the shoot blight phase of fire blight on quince and loquat.

We obtained very low disease control of the shoot blight phase of fire blight with the copper compound alone or in combination with benzothiadiazole and harpin protein on quince. The addition of copper to benzothiadiazole reduced the effectiveness of the latter on loquat. Romero *et al.* (2001) also found that the addition of a copper compound to plant activators did not affect the performance of the plant activators. In contrast to our data from this study, some researchers obtained increasing yield and lower disease onset with the application of plant activator + fungicide mixtures (Anonymous 1997). Addition of copper salts did not improve the effectiveness of some of the chemicals but rather reduced it. All the chemicals except humic acid were more effective than copper compound alone.

Even when they were more effective than copper salts, the low disease control obtained from treatments with prohexadione-Ca, benzothiadiazole and harpin protein can be attributed to the inoculation method, high inoculation density, and host/cultivar susceptibility. When these situations are taken into consideration, improved performance may occur in natural infections with repeated applications during the growing season. Therefore, repeated applications should be considered in situations where disease epidemics are anticipated. Humic acid applications were ineffective as a fire blight disease control method both on loquat and quince cultivars. Humic acid should not be used as foliar application on loquat and quince during the growing season. Its negative effect should be further tested under different climatic conditions and with different application doses.

It is important to note that host resistance inducers have to be applied prophylactically against pathogen infections; they should be used 1-3 wk prior to a possible infection risk or inoculation by *Erwinia amylovora*. It will be necessary to find the right strategy for the application of these compounds in different areas. Prohexadione-Ca, benzothiadiazole and harpin protein application should be seen as a complementary action in the whole process of fire blight control measures.

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