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Determination of Application Time for Chemical Control of Fire Blight Disease in Pear Varieties

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ABSTRACT

Fire blight, caused by the bacterium *Erwinia amylovora*, is a serious disease of pear, apple, and other plants of the Rosaceae family. In this study, from the point of view of continuousness of protection of fire blight disease and shoot growth in growing season, application times and effectiveness of host resistance inducers, harpin protein, benzothiadiazole, prohexadione-Ca as alternatives to conventional products, streptomycin, copper and maneb+copper were evaluated on susceptible pear varieties in greenhouse and field conditions. Type 1 and Type 2 applications for prevention of the disease and Type 3 and Type 4 applications for evaluation of shoot growth were performed. Type 2 application of harpin protein gave remarkable effectiveness on prevention of the disease about 49% and 65% in greenhouse and field, respectively. After Type 1 and 2 applications by prohexadione-Ca and benzothiadiazole, disease severity significantly decreased comparing to applications of copper and maneb+copper and, controls. Only prohexadione-Ca applications significantly reduced shoot lengths and plants were highly affected by the application Type 4 of this chemical. According to findings, applications of Type 2 provided better results than Type 1 on all of pear varieties in greenhouse and field conditions and use of resistance inducing substances during the production season is proposed in managing of shoot blight phase of fire blight disease.

Keywords: Resistance inducers; Susceptible host; Chemical control; *Erwinia amylovora*

Armut Çeşitlerinde Ateş Yanıklığı Hastalığının Kimyasal Mücadelesinde Uygulama Zamanının Belirlenmesi

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ÖZET

Erwinia amylovora' nın sebep olduğu ateş yanıklığı, armut, elma ve diğer Rosaceae familyası bitkilerinin ciddi bir hastalığıdır. Bu çalışmada, ateş yanıklığı hastalığına karşı, yetiştirme sezonu içerisinde korunmanın devamlılığı ve sürgün gelişimi bakımından, geleneksel ürünlere, streptomisin, bakır ve maneb+bakır, alternatif olarak konukçu dayanıklılığını teşvik edicilerin, harpin protein, benzothiadiazole, prohexadione-Ca etkililiği ve uygulama zamanları hassas armut çeşitlerinde, sera ve arazi koşullarında değerlendirilmiştir. Tip 1 ve Tip 2 uygulamaları hastalığın engellenmesi, Tip 3 ve Tip 4 uygulamaları sürgün gelişimi değerlendirilmesi için yapılmıştır. Harpin proteinin Tip 2 uygulaması hastalığın önlenmesinde sırasıyla sera ve arazide %49 ve %65 oranlarında dikkate değer etkililik vermiştir. Prohexadione-Ca ve benzothiadiazole ile Tip 1 ve Tip 2 uygulamalarından sonra, hastalık şiddeti bakır, maneb+bakır uygulamalarına ve kontrollere kıyasla önemli ölçüde azalmıştır. Sürgün uzunluklarını sadece prohexadione-Ca uygulamaları önemli olarak azaltmış ve bitkiler, bu kimyasalın Tip 4 uygulamasından büyük ölçüde etkilenmişlerdir. Elde edilen bulgulara göre, tüm armut çeşitlerinde, sera ve arazi koşullarında Tip 2 uygulamaları, Tip 1'den daha iyi sonuçlar sağlamıştır ve ateş yanıklığı hastalığının sürgün yanıklığı evresi kontrolünde, dayanıklılığı teşvik eden kimyasalların kullanımı önerilmektedir.

Anahtar sözcükler: Dayanıklılık teşvik ediciler; Hassas konukçu; Kimyasal mücadele; *Erwinia amylovora*

1. Introduction

Erwinia amylovora is the causal agent of fire blight in most species of the family Rosaceae (Vanneste 1995) and may cause substantial economic damage. Most economically relevant apple and pear cultivars are highly susceptible to fire blight, a risk even more intensified in modern high-density orchards, which mainly consist of susceptible cultivar-rootstock combinations (Longstroth 2001). Because of favorable weather conditions and cultivar susceptibility, the disease spread quickly (Zwet 1996) and as a result, fire blight has become a major limiting factor for successful pome fruit production.

The most important chemicals for controlling fire blight caused by *E. amylovora* on pome fruit trees are copper compounds and antibiotics (Psallidas & Tsiantos 2000). However, russeting of fruits often results from copper treatments. Furthermore, using antibiotics in plant production is highly controversial due to the potential risk 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 combat fire blight, there has been much interest in recent times in novel control strategies. This situation has directed so many researchers to control the disease in ecologically sound methods based on disturbing the host-pathogen relations. Plant activators and growth regulators are sought as the most promising chemicals (Tosun & Ergun 2002).

A first factor determining the host susceptibility for fire blight shoot infections is the intensity of the vegetative shoot growth on the fruit trees (Beyers & Yoder 1997). Prohexadione-Ca is a plant growth regulator that is used to control the vegetative growth of especially apple and pear trees (Evans et al 1997; Evans et al 1999). Prohexadione-Ca has been shown not only to suppress apple shoot growth but also to reduce the incidence of secondary shoot blight infections (Breth et al 1999; Bastas & Maden 2004) and suppress the extension of lesions (Beyers & Yoders 1997; Rademacher 2004).

Another new way is the systemic acquired resistance (SAR), a self defense mechanism of the plants. The SAR response correlates with the accumulation of certain pathogenesis related (PR) proteins. The PR proteins can be induced by some plant activators such as benzothiadiazole and harpin protein. Benzothiadiazole mimics the role of salicylic acid in defense reactions, and treated plants produce pathogenesis-related proteins, which are able to degrade bacterial cell walls (Kessmann et al 1996; Thomson et al 1999 a, b; Oostendorp et al 2001).

Harpin protein which is isolated from *E. amylovora* initiates a complex set of metabolic responses in the treated plant, causing natural gene expression and eliciting a plant's natural defense and growth systems (Wei et al 1992; Wei & Beer 1996; Momol et al 1999a). These chemicals activate natural growth systems, improving crop yield, quality and food safety while simultaneously triggering defense systems to protect against diseases and some pest damages. Mixture of maneb+copper gives satisfactory disease control with no phytotoxicity after blossom period (Momol et al 1991).

The aim of this work was to determine the best chemical control strategy for fire blight disease according to application times of some resistance inducers and conventional bactericides on susceptible pear varieties.

2. Materials and Methods

2.1. Plant material and growth conditions

The pear cultivars, Santa Maria, Williams and Ankara which are severely affected by fire blight in Turkey (Momol & Yegen 1993) and other important cultivars; Deveci and Rıza Bey, which are grown extensively, were used in the experiments in 2007. In greenhouse experiments, test plants of 3 years old saplings and in orchard experiments, the trees of 14 years old showing uniform growth were selected. Saplings were transplanted into plastic bags of 25 cm diameter, filled with 8 kg of soil and they were grown at 25 ± 5°C, at 60-75% RH, in the light intensity of 12000-14000 lux illuminated by tungsten-filament lamps to give 16-h photoperiod for 20 days. After transplantation, the saplings were fertilized with ammonium sulphate 25 g pot^{-1}, diammonium phosphate 25g pot^{-1}, potassium sulphate 25 g pot^{-1} twice a week; by supplying 50 ml of a liquid fertilizer having Mn, Cu, Zn, B, Mo at less than 0,05% once a week (Kacar & Katkat 1999) and the potted plants were watered when necessary throughout the growing season. In addition, sulphur dust (4 g l^{-1}) and Dicofol (0,2 m*l* l^{-1}) active ingredient were applied for powdery mildew and mite control, respectively. In the beginning of growing season, pear trees were pruned, fertilized and sprayed to prevent insect injury for a healthy growth in the orchard.

2.2. Erwinia amylovora strain (EAI)

The strain (EAI) used in the experiments was selected from one of the 5 collected strains causing 88% disease severity, based on the virulence test cited by Norelli et al (1984). The virulence was tested on cv. Ankara pear trees. Stock cultures were preserved on the Nutrient Agar (NA) medium in tubes at 4 °C in a refrigerator. The bacteria were transferred every 3 months to new tubes. Bacterial suspensions prepared from growing colonies on Nutrient Agar (NA) at 23–25°C and were diluted in sterile distilled water to give an absorbance of 0.15 at 660 nm. From viable plate counts this represented 10^8 cfu ml^{-1}. Inoculum was maintained on ice and was used for plant inoculation within 2 h of dilution.

2.3. Chemical compounds and applications

The chemicals used in the experiments are given in Table 1. Application of chemicals was planned according to Momol et al (1999b) and modified as shown in Table 2.

To determine disease severity, before *E. amylovora* inoculation (type 1 applications), prohexadione-Ca was applied when the shoot lengths were 6-12 cm and 15-20 cm, benzothiadiazole+metalaxyl and harpin protein were applied two times when the shoot lengths were 15-20 cm and 30-35 cm, copper salts of fatty and rosin acids and maneb+copper were applied

Table 1-Active ingredients, trade names, formulations and application rates of chemicals
Çizelge 1-Kimyasalların aktif maddeleri, ticari isimleri, formulasyonları ve uygulama oranları

Active Ingredient and Percentage		*Commercial Name / Firm*	*Formulation*	*Application Rate* (100 *l water*)
Prohexadione-Ca	10%	BAS 125 10 W / BASF	WG	125 g
Benzothiadiazole + Metalaxyl	4% 40%	BION MX 44 / Syngenta	WG*	135 g
Harpin protein	3%	Messenger / Eden Bioscience	Powder	50 g** +20 m*l* adjuvant***
Maneb + Copper oxychloride	20% 37.5%	Herkul / Hektaş Company	Powder	400 g
Streptomycin sulfate	100%	Streptomycine / I.E. Ulagay	Powder	59 g
Copper salts of fatty and rosin acids	51.4%	Tenn Cop 5E/ Hektaş Company	Liquid	250 m*l*

* WG: wettable granule
** It was diluted with distilled water
*** Non ionic adjuvant, KINETIC® was manufactured by Helena Chemical Company (225 Schilling Blvd.Collierville, TN 38017, USA)

Table 2-Application times of chemicals and *Erwinia amylovora* inoculation based on shoot length

Çizelge 2-Sürgün uzunluklarına göre kimyasalların uygulama zamanları ve Erwinia amylovora inokulasyonu

Chemicals	*Application times and shoot lengths of plants*							
	May 31 (6-12 cm)	June 10 (15-20 cm)	June 20 (30-35 cm)	June 25	June 26[5]	June 27	July 6	July 16
Prohexadione-Ca[1]	x	x			+			
Prohexadione-Ca[2]	x	x			+		x	x
Prohexadione-Ca[3]	x	x			-			
Prohexadione-Ca[4]	x	x			-		x	x
Benzothiadiazole+Metalaxyl[1]		x	x		+			
Benzothiadiazole+Metalaxyl[2]		x	x		+		x	x
Benzothiadiazole+Metalaxyl[3]		x	x		-			
Benzothiadiazole+Metalaxyl[4]		x	x		-		x	x
Harpin protein[1]		x	x		+			
Harpin protein[2]		x	x		+		x	x
Harpin protein[3]		x	x		-			
Harpin protein[4]		x	x		-		x	x
Maneb+Copper[1]	x	x	x		+			
Maneb+Copper[2]	x	x	x		+		x	x
Maneb+Copper[3]	x	x	x		-			
Maneb+Copper[4]	x	x	x		-		x	x
*Copper salts of fatty…[1]	x	x	x		+			
Copper salts of fatty….[2]	x	x	x		+		x	x
Copper salts of fatty….[3]	x	x	x		-			
Copper salts of fatty….[4]	x	x	x		-		x	x
Streptomycin[1]				x	+	x		
Streptomycin[2]				x	+	x	x	x
Streptomycin[3]				x	-	x		
Streptomycin[4]				x	-	x	x	x
Water Control[1]	x	x	x	x	+	x	x	x
Water Control[2]	x	x	x	x	-	x	x	x

* Copper salts of fatty….: Copper salts of fatty and rosin acids

[1] Application of the chemical before the date of inoculation with *E. amylovora* (Type 1)

[2] Application of the chemical after the date of inoculation with *E. amylovora* (Type 2)

[3] Dates of application of the chemicals (Type 3)

[4] Dates of application of the chemicals (Type 4)

(Type 1 and Type 2 applicationswere conducted to determine the effects of the chemicals on disease severity)

(Type 3 and Type 4 applications were conducted to determine the effects of the chemicals on shoot growth)

[5] *Erwinia amylovora* inoculation date

Water control[1]: bacterial inoculation with *E. amylovora*

Water control[2]: no *E. amylovora* inoculation

three times when the shoot lengths were 6-12 cm, 15-20 cm and 30-35 cm and streptomycin was applied twice, one day before and one day after the *E. amylovora* inoculation (Momol et al 1999b). In Type 2 applications, after *E. amylovora* inoculations second group of pear plants were treated twice with ten day intervals by the chemicals, in addition to chemicals that were applied before *E. amylovora* inoculation.

To evaluate the effects of the chemicals on shoot growth, the plants were sprayed twice or three times, depending on the chemicals. In this application /Type 3) no *E. amylovora* inoculation was performed. In Type 4 applications the plants were sprayed 4 or 5 times depending on the chemicals. In Type 4 applications no *E. amylovora* inoculation was performed. In addition, streptomycin was applied to plants one day before and after inoculation with Erwinia amylovora (Type 1). In Type 3 application with streptomycin, the chemical was applied twice. No Erwinia amylovora inoculation was performed with the Type 3 streptomycin application. In Type 2 application with streptomycin, the chemical was applied once before the inoculation with *E. amylovora* and applied 3 times after the bacterial inoculation. In Type 4 application with streptomycin, the chemical was applied 4 times and no E. amylovora inoculation was performed. Water controls were also performed with and without *E. amylovora* inoculation.

2.4. Experimental design and setup

The experiment was set up as a full factorial arrangement of treatments in a completely randomized plot design with 3 replications in the greenhouse and 5 replications in the orchard. For each treatment, mean of the nine shoots at three saplings for greenhouse experiments and the fifteen shoots at three trees for the orchard were counted as one replication (Duzgunes et al 1987). Every treatment was applied to six groups of plants; the three group of plants were treated by the chemicals and inoculated with *E. amylovora* to see the effects of the treatments on fire blight disease severity:
first group; chemicals + *E. amylovora* inoculation (two or three treatments depending on the chemicals, before inoculation (type 1),
second group; chemicals + *E. amylovora* inoculation (totally four or five treatments depending on the chemicals, before and after inoculation (type 2),
third group (water control$_1$); only *E. amylovora* inoculation).
Other three groups; only treated by the chemicals to see the effects of the treatments on shoot growth of pear varieties, in these groups no *E. amylovora* inoculation was performed;
first group; only treatment by chemicals (two or three treatments, depending on the chemicals, (type 3),
second group only treatment by chemicals (totally four or five treatments, depending on the chemicals) (type 4),
third group (water control$_2$); no inoculation with *E. amylovora*, no application with chemicals, only water applied.

In addition, streptomycin was applied to plants one day before and after inoculation with *E. amylovora* (Type 1). In Type 3 application with streptomycin, the chemical was applied twice. No Erwinia amylovora inoculation was performed with the Type 3 streptomycin application. In Type 2 application with streptomycin, the chemical was applied once before the inoculation with *E. amylovora* and applied 3 times after the bacterial inoculation. In Type 4 application with streptomycin, the chemical was applied 4 times and no E. amylovora inoculation was performed.

The shoots (Type 1 and Type 2 plants) were inoculated using bacterial suspension of 10^8 cfu ml^{-1} by hypodermic injection method (Norelli et al 1986). The treated shoots were labeled with flagging tape for evaluation purposes.

2.5. Evaluation of disease severity and shoot growth

Disease severity (DS, %) was calculated by the following equation;

$$DS = (a / b) 100 \quad (1)$$

where a is the length of the blighted part of the shoot (cm); 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;

$$A = (B - C / B) 100 \quad (2)$$

where B is the percent disease severity in the controls; C is percent disease severity in treated shoots.

Percent effectiveness of the treatments on reduction of shoot growth (D) was calculated in a similar way,

$$D = (E - F / E) 100 \quad (3)$$

where E is the mean shoot length in the controls; 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 Least Significant Difference (LSD) Test at $P < 0.01$.

3. Results

3.1. Effects of the chemicals on shoot blight

Shoot blight phase of fire blight disease was best controlled by streptomycin on all of the pear varieties and in application types where *E. amylovora* inoculated, and there was no statistically significant difference between the application types (Types 1 and 2) of streptomycin. Streptomycin gave the highest effectiveness about 97% in the two application types at $P<0.01$ (Table 3).

Harpin protein was the most effective and hopeful chemical following streptomycin in both application types (1 and 2). The lowest disease severity was obtained as 40.64% and 25.88% on cv. Ankara by harpin protein in greenhouse and field, respectively. Mean effectiveness of the chemical was 49.76% in greenhouse and 65.30% in field by type 2 applications on all of the cultivars. Type 2 applications of harpin protein were the most successful within all of the chemicals (Table 3).

The third successful chemical prohexadione-Ca caused less shoot blight together reducing of shoot lengths (Table 3 and 4). Considerable results were obtained from the use of this chemical with type 2 applications (means 57.59% in greenhouse and 49.36% in field). In comparison to control plants (control means in all of the cultivars, 84.67% in greenhouse and 75.67% in field). In addition, prohexadione-Ca provided rather better results on disease severity than benzothiadiazole + metalaxyl and copper compounds by the type 1 and 2 applications. Effectiveness of the chemical was determined as 31.98% in greenhouse and 34.76% in field by type 2 applications (Table 3).

The disease severity was also reduced by benzothiadiazole + metalaxyl, maneb + copper and copper salts of fatty and rosin acids, respectively. Better results were obtained with type 2 applications as compared to type 1 applications. Copper applications were not effective at the expected level and their values were almost close to the control values. Disease severities were 76.92% and 73.00% in greenhouse and 67.48% and 64.77% in field by copper applications of type 1 and type 2, respectively. Benzothiadiazole showed higher effects than commonly used copper compounds for fire blight control. In particular, type 2 applications of benzothiadiazole provided reasonable effectiveness (Table 3).

It appears that Ankara cultivar showed the lowest disease severity (55.68% and 46.06%, in greenhouse and field, respectively) as compared to other cultivars (Table 3).

3.2. Effects of the chemicals on shoot growth

Harpin protein, benzothiadiazole + metalaxyl, maneb + copper, copper salts of fatty and rosin acids and streptomycin applications gave remissible effects in comparison to control plants on shoot growth of pear cultivars and application types in greenhouse and field conditions (Table 4). From the point of view of reduction of shoot length, applications of prohexadione-Ca clearly were the most effective than the other chemicals. When the application types were taken into consideration, there were statistically significant differences between the application types and type 4 had higher reduction than type 3 (Table 4).

Precisely related results between the reduction of shoot length and disease severity were obtained (Tables 3 & 4). The reduction of shoot growth was the highest by type 4 application (25.34 cm in greenhouse and 50.31 cm in field) which provided lower disease control (61.13% and 52.61% in greenhouse and field, respectively) as compared to control treatments (Table 3 & 4).

4. Discussion

Research and development of alternatives to antibiotics and copper compounds for the control of fire blight are necessary to prevent potential economic losses. Because of the lack of publicly acceptable, effective and non phytotoxic preparations to control fire blight, there has been much interest in recent times in novel control strategies which trigger defense mechanisms in the host plants. Such effects can be achieved by benzothiadiazole, harpin protein, prohexadione-Ca (Kessman et al 1994; Sticher et al 1997; Jensen et al 1998; Momol et al 1999b; Rademacher 2000; Steiner 2000; Aldwinckle et al 2002; Maxson & Jones 2002; McManus et al 2002; Norelli et al 2003).

Danovan (1991) and Beyers & Yoder (1997) reported that the first factor determining the susceptibility of the host plant against shoot infections of fire blight was rapid shoot growth. In the control of fire blight, copper compounds can be effective only at low and medium levels of disease severity (Zwet & Keil 1979) and the rate of control is lower on pears (Dimova 1990). We obtained very low disease control of shoot blight phase of fire blight from copper compounds.

The results indicated that for both application types (Type 1 & 2) of testing the chemicals,

Table 3-Effects on fire blight disease severity of application types of the chemicals in greenhouse and field

Çizelge 3-Sera ve arazide kimyasalların uygulama tiplerinin ateş yanıklığı hastalığı üzerindeki etkileri

Chemicals	Disease Severity, %					*MEANS*	Effects of Application Types		Effectiveness of Chemicals for Application Types[1]	
	Ankara	Santa Maria	Williams	Deveci	Rıza Bey		1	2	1	2
				Greenhouse Experiments						
Cont.	82.78±1.12 c*	87.04±0.74 a	84.06±0.90 bc	86.47±1.08 ab	83.02±1.04 c	84.67±0.52 A	84.67±0.75 a	84.67±0.75 a	-	-
Copper	72.91±1.38 efgh	75.52±1.05 de	74.59±0.96 ef	78.16±1.45 d	73.62±1.18 efg	74.96±0.60 B	76.92±0.75 b	73.00±0.64 c	9.15	13.78
Ma+Cop.	65.54±1.15 kl	71.87±0.79 ghi	70.35±0.44 hi	72.57±0.98 fgh	69.79±1.04 ij	70.03±0.59 C	71.40±0.62 cd	68.65±0.89 e	15.6	18.92
BTH+Met.	65.20±1.34 kl	69.85±1.28 ij	67.30±0.90 jk	70.94±1.27 hi	66.89±1.29 kl	68.04±0.63 D	69.81±0.63 de	66.27±0.88 f	17.55	21.73
Pro-Ca	57.32±1.77 n	64.30±2.23 l	59.11±1.61 mn	64.53±2.05 l	60.39±1.71 m	61.13±0.94 E	64.66±1.08 f	57.59±0.86 g	23.63	31.98
Hrp	40.64±3.35 r	52.77±2.51 o	48.84±2.76 pq	50.92±2.37 op	48.05±2.74 q	48.24±1.38 F	53.96±1.07 h	42.53±1.44 i	36.27	49.76
Streptomycin	5.36±0.53 s	2.95±0.17 st	2.50±0.25 t	1.94±0.30 t	3.74±0.43 st	3.30±0.26 G	3.53±0.45 j	3.06±0.28 j	95.83	96.38
MEANS	*55.68±3.77 C***	*60.61±4.00 A*	*58.11±3.92 B*	*60.79±4.11 A*	*57.93±3.83 A*	-	*60.71±2.46 A*	*56.54±2.47 B*		
				Field Experiments						
Control	71.92±1.09 de	79.77±0.97 a	75.60±0.59 bc	77.21±1.61 ab	73.88±0.70 cd	75.67±0.66 A	75.67±0.95 a	75.67±0.95 a	-	-
Copper	61.88±1.12 hi	70.08±0.78 ef	67.12±1.28 g	67.54±1.17 fg	64.01±1.5 h	66.12±0.72 B	67.48±0.95 b	64.77±1.00 c	10.82	14.40
Ma+Cop.	56.71±1.31 mn	60.46±1.38 ijk	60.07±2.05 ijkl	61.24±1.89 hij	58.26±1.65 klm	59.35±0.76 C	62.24±0.82 d	56.46±0.73 f	17.74	25.38
BTH+ Met.	58.49±1.23 jklm	60.54±1.19 ijk	58.50±1.84 jklm	58.95±1.54 jklm	57.42±1.69 lm	58.78±0.65 C	60.27±0.94 e	57.29±0.76 f	20.35	24.28
Pro-Ca	46.38±1.78 q	54.55±1.52 no	50.74±1.94 p	57.89±1.78 klm	53.47±1.60 op	52.61±1.01 D	55.86±1.18 f	49.36±1.17 g	26.17	34.76
Hrp	25.88±2.77 u	33.37±2.15 s	29.68±2.71 t	36.24±1.81 r	30.31±2.27 t	31.10±1.17 E	35.94±0.88 h	26.25±1.27 i	52.50	65.30
Streptomycin	1.18±0.16 v	1.29±0.13 v	0.95±0.12 v	1.66±0.33 v	0.77±0.15 v	1.17±0.09 F	1.43±0.14 j	0.9±0.10 j	98.11	98.81
MEANS	46.06±3.58 C	51.44±3.83 A	48.95±3.75 B	51.53±3.68 A	48.30±3.62 B	-	51.27±2.30 A	47.24±2.33 B		

Ma+Cop.: Maneb+Copper. BTH+Met: Benzothiadiazole+Metalaxyl. Pro-Ca: Prohexadione-Ca. Hrp: Harpin protein

[1] Effectiveness of chemicals for application type was calculated by application type (control)–application type (effects of the chemical) / application type (control)×100

***Means followed by different letters are statistically significant $P<0.01$

Significance *P* Values		Application Types (T)	Chemicals (C)	Varieties (V)	C × T	C × V	T × V	C × T × V
Disease Severity								
	Greenhouse	0.001	0.001	0.001	0.001	0.001	0.8659	0.9992
	Field	0.001	0.001	0.001	0.001	0.001	0.1298	0.1550

Table 4-Effects on shoot lengths of application types of the chemicals and in greenhouse and field

Çizelge 4-Sera ve arazide kimyasalların uygulama tiplerinin sürgün uzunlukları üzerindeki etkileri

Application Types	Effects of Chemicals on Shoot Lengths, cm							*MEANS*
	Control	Copper	Ma.+Cop.	BTH+ M.	Pro-Ca	Hrp	Streptomycin	
				Greenhouse Experiments				
3	42.01±0.47 abc*	40.68±0.47 d	41.01±0.49 cd	42.65±0.40 a	30.15±0.52 e	42.25±0.42 ab	41.19±0.53 bcd	*39.99±0.43 A*
4	42.01±0.47 abc	40.56±0.43 d	40.39±0.49 d	42.97±0.47 a	25.34±0.45 f	42.52±0.37 a	40.77±0.36 d	*39.22±0.58 B*
MEANS	*42.01±0.32 A***	*40.62±0.31 B*	*40.70±0.34 B*	*42.81±0.30 A*	*27.74±0.56 C*	*42.38±0.27 A*	*40.98±0.32 B*	-
				Field Experiments				
3	76.57±0.67 abcde	75.35±0.71 efg	75.63±0.59 defg	77.46±0.59 ab	55.77±0.52 h	76.86±0.66 abcd	76.09±0.51 cdefg	*73.39±0.74 A*
4	76.57±0.67 abcde	74.76±0.53 g	74.91±0.68 fg	77.88±0.56 a	50.31±0.52 i	77.37±0.53 abc	76.17±0.55 bcdef	*72.57±0.92 B*
MEANS	*76.57±0.46 BC*	*75.06±0.44 E*	*75.27±0.45 DE*	*77.67±0.40 A*	*53.04±0.62 F*	*77.11±0.42 AB*	*76.13±0.36 CD*	-

Ma+Cop.: Maneb+Copper. BTH+Met: Benzothiadiazole+Metalaxyl. Pro-Ca: Prohexadione-Ca. Hrp: Harpin protein

***Means followed by different letters are statistically significant $P<0.01$

Significance *P* Values	Application Types (T)	Chemicals (C)	Varieties (V)	C × T	C × V	T × V	C × T × V
Shoot Length							
Greenhouse	0.0007	0.001	0.001	0.001	0.2227	0.6219	0.9948
Field	0.001	0.001	0.001	0.001	0.6988	0.8804	0.9997

streptomycin was the most effective compound. The curative effect of streptomycin may be due to the fact that the pathogen requires several days to become established on the plant and/or it has limited systemic activity (Psallidas & Tsiantos 2000).

Since harpin is clearly required for the pathogenicity of *E. amylovora*, interference with harpin or its activity may provide new bases for the control of fire blight (Beer et al 1993). Harpin protein was shown to provide broad spectrum protection of plants against fungal, bacterial and viral pathogens (Wei & Beer 1996; Momol et al 1999b) and similarly it was highly effective on fire blight disease on the pears as cited by other authors on the other hosts (Wei & Beer 1996; Momol et al 1999a,b).

Best results were obtained when prohexadione-Ca was applied approximately 2 weeks prior to infection. According to its physiological mode of action, prohexadione-Ca has to be used prophylactically against pathogen infections, i.e. it needs to be applied 5-21 days prior to a possible infection risk (or inoculation) by *E. amylovora* (Bazzi et al 2003). Post infection treatments with prohexadione-Ca are not of practical value in reducing fire blight (Schupp et al 2002).

Preventive effects of prohexadione-Ca on shoot growth and fire blight development on apples is a well known phenomenon (Deckers & Daemen, 1999), and the usual proposed rate is 125 g 100 l^{-1} in two applications. The chemical was tested on the most susceptible host, pears, in the same rates applied to apples. It reduced shoot length about 35% on pear cultivars however reduction of the disease percentage was around 10% (Bastas & Maden 2004). This shows that reduction in shoot length does not correlate with the disease reduction on other hosts. Unrath (1999) pointed out that different results were obtained from prohexadione-Ca in the climatically different regions. In apple trees treated with prohexadione-Ca, growth of vegetative shoots slows down within 10 to 14 days after application. The reduced growth rate makes the growing shoot tips less susceptible to infection by *E. amylovora*.

A compound known to induce systemic acquired resistance (SAR) against fire blight is benzothiadiazole (acibenzolar S-methyl, ABM). Benzothiadiazole mimics the role of salicylic acid in defense reactions, and treated plants produce pathogenesis-related proteins, which are able to degrade bacterial cell walls (Kessmann et al 1996; Thomson et al 1999 a, b; Oostendorp et al 2001). Weekly applications of benzothiadiazole (Actigard 50 W) were shown to reduce fire blight 81 percent compared to 97.6 percent of streptomycin (Maxson and Jones, 1999). Spinelli et al (2006) informed that under field conditions, ABM reduced fire blight incidence up to 40%. In our experiments, benzothiadiazole + metalaxyl mixture (Bion) gave average 20% effectiveness on the tested pear cultivars. A higher rate of disease control could be expected from the more concentrated form of the chemical. No synergistic effect of metalaxyl was observed. Oosterndorp et al (2001) reported that SAR in plants is characterized by protection against a broad range of pathogens, the induction of a set of proteins and dependence on salicylic acid (SA). They reported that Bion does not show any antimicrobial activity *in vitro* but instead it activates resistance against pathogens. Bion is translocated systemically in plants and can take the place of SA in the natural SAR signal pathway. Also Brisset et al (2000) reported that Bion protected apple seedlings from infection when applied before artificial inoculation. This protection was associated with the activation of defense related enzymes such as peroxides and β-1, 3-glucanases. These enzymes were active for 17 days. They noted though, that there could be other defense reactions and compounds that are involved in the observed protection. Protection against fire blight by Bion has also been reported by Zeller & Zeller (1999). One result in our investigation that is in agreement with other reports (Momol et al 1999b; Thomson et al 1999a, b) is that Bion is not as effective as streptomycin, especially when the inoculum pressure is high. The efficacy of Bion would be greater if the host plants were apples instead of pears (Thomson et al 1999b).

The obtained low disease control than expected can be attributed to artificial inoculation, infiltration of high inoculum density and the use of susceptible plants in the experiments. Better results might be obtained in the natural infections. Besides, continuity of applications with the resistance inducers after infection of the bacterium should be considered. These positive effects should be further tested under natural conditions in pome fruit orchards.

It is important to mention that these treatments that interfere with host susceptibility (growth regulation and/ or SAR) should be applied some weeks before the real infections occur. This allows the plants to switch on their natural defense system in time. It will be required to find the right strategy for the applications of these compounds in different areas. Maybe harpin protein should be seen as a complementary action in the whole process of fire blight control measures.

Till now, we did not find any study about continuousness of these resistance inducers in current season. In this study, especially four times applications of harpin protein and prohexadione-Ca gave important and successful results.

The timing of chemical sprays during the period of host susceptibility to infection should be the main concern of growers and advisers, since it is very important for the efficacy of sprays and the optimization of applications. This may result in saving for the grower as well as in preventing environmental problems. One advantage of the chemicals that induce SAR, if their effects last as long as is reported (Brisset et al 2000), is that the time of application is not as critical as it is with antibiotics and copper compounds. Thus trees will be protected for a longer time than with the usual bactericides. Even so it would be worthwhile to determine the optimum time of application (Tsiantos et al., 2003). Although not more effective than streptomycin they should have a role in Integrated Pest Management as they can reduce the applications of streptomycin and thus lessen the possibility of development of streptomycin-resistant strains. All of the above results should be verified under natural infection conditions in the orchards, in different places, years and hosts.

5. Conclusion

Fire blight disease is particularly important on pear and apple trees. Sprays of streptomycin provide the best control currently available, but they are limited in protective and eradicative action. Resistance inducers as alternative chemicals to streptomycin and copper have shown some promise for controlling the shoot blight phase of fire blight on pears. Continuous application of harpin protein and prohexadione-Ca gave important and successful results in the growing season. The timing of chemical sprays during the period of host susceptibility to infection should be the main concern of growers.

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