Brazilian Journal of Microbiology (2004) 35:224-229
ISSN 1517-8382

BIOLOGICAL CONTROL OF FIRE BLIGHT IN PEAR ORCHARDS WITH A FORMULATION OF *PANTOEA AGGLOMERANS* STRAIN *EH 24*

Hatice Özaktan*; Tayyar Bora

Department of Plant Protection, Faculty of Agriculture, University of Ege, Bornova, Ýzmir, Turkey

Submitted: March 12, 2003; Approved: July 05, 2004.

ABSTRACT

Biological control by using epiphytic bacteria against *Erwinia amylovora* has been considered as an alternative method for controlling the disease. Talc-based formulation of *Pantoea agglomerans* strain *Eh-24* was applied at 30% and 100% bloom on two pear orchards which were selected from different locations in the Aegean Region in Turkey. Pear orchard trials were replicated for two years (1999 and 2000) in each place. Talc-based formulation of *P. agglomerans* strain *Eh-24* was sprayed on pear trees which were naturally infected with *E. amylovora*. In the orchard trials conducted in 1999 and 2000, talc-based formulation of *P. agglomerans* strain *Eh-24* reduced the percentage of blighted blossoms on pear orchards by 63% to 76%, approximately. Copper oxychloride+maneb was less effective in reducing the incidence of blossom infection by *E. amylovora* in each pear orchard than the bioformulation treatment. *P. agglomerans* strain *Eh-24* labelled with Str^{R+} was applied at 30% and 100% bloom to monitor the colonization and population dynamics of *P. agglomerans* on pear blossoms. The population size of *P. agglomerans* strain Eh-24 $^{strR+}$ on pear blossoms increased from $2x10^4$ to $1.3x10^6$ cfu per blossom over 18 days.

Key words: biological control, fire blight, antagonistic bacteria, *Pantoea agglomerans (Erwinia herbicola)*, *Erwinia amylovora*

INTRODUCTION

Fire blight caused by *Erwinia amylovora* has threatened pear cultivation in Turkey since 1985 (17). Suppression of the blossom-blight phase of fire blight is a key point in the management of this destructive and increasingly important disease of apple and pear (21). Chemical control of fire blight is difficult because there are few effective bactericides registered and streptomycin, which is effective, and other antibiotics are not registered worldwide. Additionally the pathogen, *E. amylovora*, has developed resistance to streptomycin in several important production areas (8,14,25). Streptomycin also may be used as a medicine for therapeutical purposes, and concerns have arisen that use of streptomycin in orchards may lead to increased a risk of resistance of human pathogens to this antibiotic. The World Health Organization does not recommend its use in plant protection (28). Consequently, streptomycin is banned in Turkey for non-medical use. Copper sprays during flowering have been recommended for fire blight control, but these treatments often cause undesirable phytotoxic effects on blossoms and fruits (14,24,29).

Alternative control strategies are urgently needed for fire blight. Biological control with epiphytic bacteria against *E.amylovora* has been considered as an potential method for controlling the disease (2). The bacterial strains belonging to the species *Pantoea agglomerans* (syn. *Erwinia herbicola*) and *Pseudomonas fluorescens* have been extensively studied as potential biological control agents of fire blight (7,8,26,31). Spraying apple and pear blossoms with suspensions of *P. agglomerans* on occasion has given control of fire blight equivalent to that achieved with streptomycin (32).

Commercial use of antagonistic bacteria for control of fire blight depends upon the development of a formulation in which the bacteria can survive for a considerable length of time. One of the antagonists, *P.fluorescens* strain A506, is now available commercially as afreeze-dried formulation for biological control

*Corresponding author. Mailing address: University of Ege, Faculty of Agriculture, Department of Plant Protection. 35100 Bornova, İzmir, Turkey. Tel. (+90 232) 388-4000/1410. Fax (+90 232) 374-4848. E-mail: ozaktan@ziraat.ege.edu.tr

of fire blight and frost injury in USA (Blight-Ban) (7). In the United States and New Zealand, several strains of *P. agglomerans*, are in various stages of commercial development (7).

In a previous research project, 167 epiphytic bacterial strains including *P. agglomerans*, fluorescent pseudomonads, and some Gram(+) bacteria were obtained from tissues of healthy pear trees in the Aegean Region. *P. agglomerans* strains Eh-24 and 1B significantly reduced the percentage of blighted blossoms in the pear blossom assay by 82% to 98% compared to the untreated control (18).

The objectives of this research were to develop a talc-based formulation of *P. agglomerans* strain Eh-24, to investigate the capacity of bioformulation to control infection of blossoms by *E. amylovora* under commercial pear orchard conditions, and to monitor colonization of pear blossoms by *P. agglomerans* strain Eh-24.

MATERIALS AND METHODS

Antagonistic Bacterium

Approximately 167 bacterial strains were screened for biological control potential using the pear slice assay and detached blossom assay (10). *P. agglomerans* strain Eh-24, which was isolated from healthy pear blossoms in Ýzmir province was the most effective strain by preventing the pathogen at rate 100% on pear slices and 85% on detached pear blossoms (18).

Development of Bioformulation of *P. agglomerans* strain Eh-24

P. agglomerans strain Eh-24 was grown at 24ºC for 20 hours in sucrose (5%) nutrient broth medium on a rotary shaker at 150 rpm. Bacterial suspension was centrifuged for 20 min at 6.000 rpm. Bacterial pellet was resuspended in 0.1M $MgSO_4$ in a 1:1 (w/v) ratio and then combined with either. 15% (v/v) glycerol or 15%(w/v) sorbitol, which were included to stabilize the bacterial cells. Thereafter, the suspension was mixed with an equal volume of autoclaved 1.5% Na-Alginate (1,4). The bacteria – Na Alginate mixture was combined with sterilized talc at the ratio 1:4 (v/v) (11). A wetting agent (Ca-lignosulphate) was added (7%, w/w) to the mixture. The resulting mixture was spread thinly over a glass sheet and air dried in a laminar air-flow cabinet at 24ºC for 1 hour to form a slightly moistened powder (15% moisture content). After drying, the bacterial formulation was powdered in a Waring Blendor and stored in glass bottles with lids as small volumes (3,19,23). The talc-based formulations of strain Eh-24, were stored at 4ºC for 180 days. Survival of *P. agglomerans* strain Eh-24 in the formulations was assessed at 60 day intervals by a dilution plate method (27). There were three replications for each analysis.

Orchard Trials

All experiments were conducted in two commercial pear orchards, which were located in the Aegean (Ýzmir) and Mediterranean (Burdur) Region of Turkey in 1999 and 2000. Trials were conducted on 14 year-old trees of susceptible pear cultivars "Santa Maria" and "Akça" in Burdur and Ýzmir, respectively. In 1999 and 2000, the talc-based glycerol formulation of *P. agglomerans* strain Eh-24 was sprayed at 10^8 CFU/ml onto pear trees in 20-30% and 70-90% bloom. The formulation was freshly prepared each year. Water treated trees were included as non-protected controls. Additional trees were treated with the reference chemical copper-oxychloride + maneb (0.2%,37.5%+20% a.i.). Each treatment was applied to six trees in randomized complete blocks. Between 3 and 4 litres were applied to each tree, sufficient to cause run-off (13,15). Because these assays were carried out in the orchards with active fire blight cankers, the orcherds were not artificially inoculated with *E. amylovora*. The incidence of infected blossom clusters was recorded two weeks after the last application of the bioformulation. In each treatment 240 clusters (40 blossom clusters in six replications) were examined and recorded for their blossom blight incidence. The data were analyzed by ANOVA using SPSS version 9.01 statistical software (SPSS Inc.Chicago, Illinois). Before the analyses were carried out, data on percentage of infected blossoms were transformed using the log transformations. Differences between treatments were determined by Duncan's Multiple Range Test (DMRT) at 5% significance level.

Monitoring the population dynamics of *P. agglomerans* strain *Eh 24* in pear blossoms

A spontaneous streptomycin-resistant mutant of P. agglomerans (strain *Eh-24^{strR+}*) was selected to follow populations during the bloom. A bacterial suspension (10^8CFU per ml) was sprayed to run off on marked branches of pear trees in 20-30%, and 70-90% bloom in Santa Maria pear orchard of Agricultural Faculty of Ege university. The experimental design was a completely randomized block with 3 single tree replications. The day following the second application of strain *Eh-24^{strR+}*, nine blossoms per sampling date from three trees were collected in sterile flasks. To each flask 100 ml of 0.1 M Phosphate Buffer, pH:7.1 was added. Flasks were placed on a rotary shaker at 150 rpm for 10 min. Samples were diluted and spread on each of two petri dishes containing SNA (sucrose nutrient agar) medium, supplemented with streptomycin (100 µg/ml). Bacteria werte enumerated on blossoms 1, 3, 5, 8, 10, 12 and 18 days after application (22).

"In vitro" compatibility of *P. agglomerans* strain *Eh 24* with copper and fungicides

Mancozeb, Copper oxychloride+Maneb and Copper hydroxide are the most commonly used fungicides on pear orchards. In "*in vitro*" tests, these compounds at (3,10,30 and 100 µg active ingredient per ml) were incorporated in SNA medium cooled to 40ºC. Approximately10^5 CFU of A *P.agglomerans strain Eh 24* was spread onto the surfaces of the solidified media. There were seven replication plates for each chemical

dose. After 4 days colonies of Eh24 were counted on each plate. The chemicals, which were not inhibited the colonial development of Eh24 were also tested against the pathogen *E. amylovora* "in vitro".

RESULTS

Survival of *P. agglomerans strain Eh 24* in wettable powder formulation

In an experiment measuring long-term survival of *P.agglomerans* strain *Eh 24*, initial population of strain *Eh 24* in the talc-based formulation was ca. 10^9 CFU per gram. Cells treated with glycerol as a stabilizer maintained the highest populations (10^9 CFU per gram) for 120 days at 4ºC, although the population size decreased to 10^8 CFU per gram during the subsequent 60 days (Table 1). Populations of Eh24 decreased more rapidly when sorbitol was used as a stabilizer compared to glycerol. The population size measured over six months in preparations treated with sorbitol were similar to those in the non-supplemented control (Table 1). We concluded that glycerol increased long-term survival of Eh24 in a talc based formulation.

Table 1. Survival of *P. agglomerans* strain *Eh 24* in talc-based WP formulations using various stabilizers at 4ºC for 180 days.

Stabilizer	Population (CFU per g dried formulation) at various days of storage*			
	0	60	120	180
Sorbitol	$1.0x10^9$	$3.2x10^8$	$1.2x10^7$	$2.0x10^6$
Glycerol	$4.0x10^9$	$5.0x10^9$	$6.0x10^9$	$1.0x10^8$
Control (non supplemented)	$1.7x10^9$	$1.0x10^9$	$1.0x10^7$	$1.0x10^5$

*There were three replication plates for each chemical dose for each analysis.

Orchard Trials

Talc-based WP formulation of *P.agglomerans* strain *Eh 24* was applied twice in two commercial pear orchards in two years. Environmental conditions for natural fire blight were favorable during bloom in Dikili and Burdur in 1999 and high levels of blossom cluster infection occured in orchard trials (59%-76% in untreated controls) (Table 2). In orchard trials conducted in 1999, talc-based formulation of *P. agglomerans* strain *Eh 24* significantly reduced the percentage of blighted blossoms on commercial pear orchards in Dikili and Burdur by 63% to 76%, respectively, compared to the water-treated control .

Treatment with copper oxychloride+maneb aprovided moderate disease control (29% - 39%),which was significantly less effective than the bioformulation treatment (Table 2).

In commercial pear orchard trials in Burdur and Dikili in 2000, the incidence of fire blight on water-treated trees was moderate at 41% and 50%, respectively (Table 3). As in 1999, treatment with the talc-based WP formulation of *P.agglomerans* strain *Eh 24* signiifcantly reduced the incidence of fire blight by 65 to 69% compared to the water-treated control (Table 3). In these trials, treatment of blossom clusters with copper oxychloride+ Maneb did not significantly reduced the incidence of fire blight compare to treatment with water (Table 3).

Table 2. Efficacy of a talc-based formulation of *P.agglomerans* strain *Eh 24* for suppression of blossom blight by indigenous populations of *E.amylovora* in two commercial pear orchards in 1999.

Treatments	Percentage of infected blossoms (%)*		Efficacy (%)**	
	Dikili	Burdur	Dikili	Burdur
Eh-24 talc-based WP formulation	21.87 a***	18.12 a***	63.16	76.25
Copper oxychloride +maneb(%0.2)	36.25 b	54.37 b	38.94	28.69
Untreated Control	59.37 c	76.25 c	—	—

* Mean values for six replications of 40 blossom clusters in each replicate;

** Percentage of reduction in blossom blight compared to blossoms treated with water alone;

*** Means followed by the same letter are not significantly different according to Duncan's Multiple Range Test (DMRT) at 5% significance level.

Table 3. Efficacy of a talc-based formulation of *P.agglomerans* strain *Eh 24* for suppression of blossom blight by *E.amylovora* in two commercial pear orchards in 2000.

Treatments	Percentage of infected blossoms (%)*		Efficacy (%)**	
	Dikili	Burdur	Dikili	Burdur
Eh-24 talc-based WP formulation	17.50 a***	12.50 a***	64.70	69.38
Copper oxychloride +maneb(%0.2)	35.00 b	29.58 b	29.40	27.55
Untreated Control	49.58 b	40.83 b	—	—

* Mean values for six replications with 40 blossom clusters in each replicate;

** Percentage of reduction in blossom blight compared to blossoms treated with water alone;

*** Means followed by the same letter are not significantly different according to Duncan's Multiple Range Test (DMRT) at 5% significance level.

Population dynamics of *P. agglomerans* strain *Eh 24* in pear blossoms

At the first sampling time, Eh-24 $^{strR+}$ was recovered at mean detectable population sizes of 10^4 CFU per blossom from nearly all blossoms sprayed with the bacterial suspension. On those blossoms on which was detected, the mean detectable population size varied from $2x10^4$ to $1.3x10^6$ CFU per blossom within 18 days (Fig. 1). The mean population size of Eh-24^{strR+} on pear blossoms in the orchard increased 100-fold within 18 days after application.

"In vitro" compatibility of *P. agglomerans* strain *Eh 24* with copper and fungicides

*U*nder the conditions of this assay, although ca. 10^5 CFU of *Eh 24* was added to the medium, only 107 colonies were enumerated on plates that were not amended with copper or fungicides (Table 4). Low (10 µg per ml) concentrations of mancozeb reduced growth and survival *Eh24* "in vitro". The addition of copper oxychloride+Maneb completely inhibited the growth of Eh 24 (Table 4). Interestingly, copper hydroxide, even at a dose of 100 µg per ml did not reduce survival of *Eh24* in vitro (Table 4). Copper hydroxide at 10 µg per ml reduced survival of *E. amylovora* (Table 5).

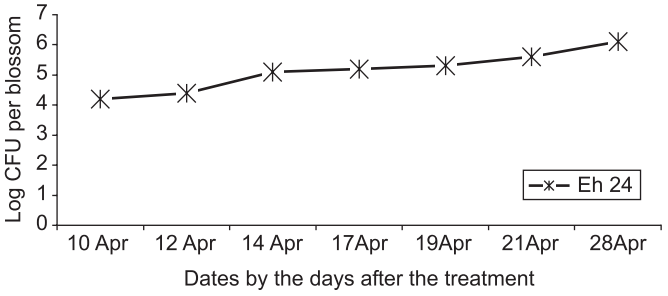

Figure 1. Mean population sizes of *P.agglomerans* strain *Eh 24^{StrR}* in pear blossoms.

Table 4. The effect of copper and fungicides on growth of *P. agglomerans* strain *Eh 24* in vitro.

Tested Chemicals	Colony Counts (CFU per petri dishes)*				
	0 µg/ml (Control)	3 µg/ml	10 µg/ml	30 µg/ml	100 µg/ml
Mancozeb	107	110	27	0	0
Copper oxychloride +maneb	107	0	0	0	0
Copper hydroxide	107	102	98	115	106

*There were seven replication plates for each chemical dose.

Table 5. The effect of copper hydroxideon the colonial development of *E. amylovora* in vitro.

Tested Chemicals	Colony Counts (CFU per petri dishes)*				
	0 µg/ml (Control)	3 µg/ml	10 µg/ml	30 µg/ml	100 µg/ml
Copper hydroxide	260	221	25	0	0

*There were seven replication plates for each chemical dose.

DISCUSSION

Prevention of blossom infections by *E. amylovora* is a key in fire blight management because the cankers and bacterial ooze originating from blossom infections provide much of the inoculum for secondary phases of the disease, including the infection of shoots, fruits, and roostocks (22). Application of copper fungicides during bloom period can cause phytototoxicity on flowers and fruits. Certain strains of epiphytic bacteria, including strains of *P. fluorescens* and *P. agglomerans*, can reduce significantly the severity of fire blight (7,10). If applied to blossoms at early to mid-bloom stage, the biocontrol agents can proliferate on pear and apple stigmas (8,31,16) and exclude the pathogen from infection sites (5,7,31).

Under field conditions, Johnson *et al.*(8) found that early establishment of populations exceeding 10^5 CFU per blossom of *P. fluorescens* Pf A-506 and *P. agglomerans* Eh C9-1 on pear blossoms suppressed establishment and growth of *E. amylovora*, thereby decreasing disease incidence. The incidence of fire blight on blossoms was reduced by about 60% with two applications of bacterial antagonists in experimental plots in the Pacific Northwest (16) and California (12). The efficacy of biological control approached or equaled levels obtained with chemical control in many of the field trials (12).

In this research, the incidence of fire blight on blossoms was reduced by about 63-76% with two applications of talc-based formulation of *P. agglomerans* strain *Eh 24* in two commercial pear orchards in Turkey over two consecutive years. The chemical control reduced the blossom infection of pear trees by only 29-39% in two orchards in 1999 and did not reduce incidence of fire blight in 2000; thus it can be clearly claimed that the biocontrol formulation was more effective against fire blight disease (Table 2 and 3).

To be effective, a biocontrol agent has to be able to multiply in the same ecological niche as the pathogen. This results in competition for space and nutrients which in itself, in some cases, is enough to confer protection (20,31). It was previously determined that competition for sites and growth-limiting nutritional substrates was main mechanism by which *P. agglomerans* strain *Eh 24* suppressed growth of *E. amylovora* on stigmas (18). *P.*

agglomerans strain *Eh 24* was well adapted to compete with the fire blight pathogen for space and nutrients (18).

Large populations of biocontrol bacteria, for example, *P. agglomerans* and *P. fluorescens* on surfaces of pear blossoms in an orchard may reduce establishment of *E. amylovora* (6,10). For biological control to be effective, most stigmatic surfaces in an orchard must be colonized by the bacterial antagonist (7,12), and the population size of the antagonists on stigmas must approach the carrying capacity of population size of the tissue (10^5 to 10^6 CFU/blossom) (30,31). In this research, the mean detectable population size of *Eh-24*$^{strR+}$ varied from 10^4 to 10^6 CFU per blossom under field conditions within 18 days following inoculation (Fig. 1). The population size of strain *Eh-24*$^{strR+}$ increased about 100-fold during bloom. This results showed that *P. agglomerans* strain *Eh 24* colonized blossoms and suppressed the development of fire blight on pear blossoms effectively.

Furthermore, in vitro results indicated that copper hydroxide sprays could be applied to dormant pear trees as protective during winter treatment prior to antagonist application in an Integrated Pest Management program.

Commercial application of *P. agglomerans* strain *Eh 24* for control of fire blight disease depends upon the development of appropriate formulations in which the bacterium can survive for a considerable length of time. Current formulation technology may allow for the development of commercial preparations that optimize the capacity of bacterial antagonists to survive the conditions encountered immediately upon inoculation of aerial plant surfaces (22,27). One challenge in formulation technology is that during rehydration, bacterial cells may die due to osmotic shock. Gelled formulations, using polymers such as polyacrylamide polysaccharides such as Xanthan (9,11), or alternatively alginate (1,4) solve this problem. We used 1.5% Na-Alginate, and observed that alginate seemed to provide some protection to bacterial cells. The present study demonstrated that bacteria could survive in talc-based formulation for more than 120 days at 4ºC. Amending cell preparations with glycerol before drying in talc apppeared to increase lon-term survival of Eh 24 (Table 1). One of the key factors in survival is the preservation of residual hygrometry of about 15% in dried formulation (4). The moisture content in the bioformulation developed also was measured as 15% after drying and presumably maintained this level of hydration in sealed jars.

The use of talc-based formulation of strain *Eh24*, at a concentration of 10^8 CFU/ml significantly reduced the amount of disease development in the pear blossoms under field conditions. The results of this research indicated that talc-based formulations of strain *Eh24* could be effectively applied as blossom treatments to control fire blight. These observations are in agreement with those obtained by commercial bioformulations such as Blightban which are applied to fire blight in USA (7,15).

ACKNOWLEDGMENTS

This research was supported in part by the Scientific and Technical Research Council of Turkey (TUBITAK) and by the Ege University Science-Technology Research and Application Center (EBILTEM).

RESUMO

Controle biológico de fire blight em pereiras empregando uma formulação *de Pantoea agglomerans* EH 24

Controle biológico de *Erwinia amylovora* através do uso de bactérias epifíticas tem sido considerado um método alternativo para o controle de "fire blight". Uma formulação de *Pantoea agglomerans* Eh 24 em talco foi utilizada em pereiras a 30% e a 100% de floração, em duas plantações selecionadas na região Aegean da Turquia. Os experimentos foram repetidos duas vezes (1999 e 2000) em cada plantação. A formulação de *P. agglomerans* foi aspergida nas pereiras naturalmente infectadas com *E. amylovora.* Nos experimentos de 1999 e 2000, a redução da porcentagem de ocorrência de "fire blight" foi reduzida aproximadamente em 63% e em 76%, respectivamente. Oxicloreto de cobre + maneb foi menos eficiente na redução da infecção por *E. amylovora* do que o tratamento com *a* bioformulação. *P. agglomerans* Eh 24 marcada com Str^{R+} foi aplicada a 30% e a 100% de floração para monitorar a colonização e a dinâmica da população de *P. agglomerans* nos brotos das pereiras. Após 18 dias, a população de *P. agglomerans* aumentou de $2x10^4$ para $1,3 \times 10^6$ UFC/broto.

Palavras-chave: controle biológico, fire blight, bactérias antagonistas, *Pantoea agglomerans, Erwinia amylovora*

REFERENCES

1. Bashan, Y. Migration of rhizosphere bacteria *Azospirillum brasiliense* and *Pseudomonas fluorescens* towards wheat roots in the soil. *J. Gen. Microbiol.* 132:3407-3414, 1986
2. Beer, S.V.; Rundle, J.R.; Wodzinski, R.S. Interaction between *E. amylovora* and *E. herbicola* "*in vitro*", in immature pear fruits and in apple blossoms. *Acta Horticulturae,* 151:203-204, 1984.
3. Connick, W.R. Jr. Formulation of living biological control agents with alginate. *ACS-Symposium Series*, 371:241-250,1988.
4. Digat, B. A new encapsulation technology for bacterial inoculants and seed bacterization. 1990, pages 383-391, in: Plant Growth-Promoting Rhizobacteria: Progress and Prospects, 2nd Workshop on PGPR. C. Keel, B. Koller, and G. Defago, eds. Interlaken, Switzerland.
5. Hattingh, M.J.; Beer, S.V.; Lawson, E.W. Scanning electron microscopy of apple blossoms colonized by *Erwinia amylovora* and *E. herbicola. Phytopathology,* 76:900-904, 1986.
6. Jones, A.L.; Schnabel, E.L. Streptomycin and oxytetracycline resistance determinants detected among bacteria from Michigan appleorchards and their potential importance, *Acta Horticulturae,* 489:673-674, 1999.

7. Johnson, K.B.; Stockwell, V.O. Management of Fire Blight:. A case study in microbial ecology. *Annu. Rev. Phytopathol.*, 36:227-248, 1998.
8. Johnson, K.B.; Stockwell, V.O.; McLaughlin, R.J.; Sugar, D.; Loper, J.E.; Roberts, R.G. Effect of antagonistic bacteria on establishment of honey bee-dispersed *E. amylovora* in pear blossoms and on fire blight control, *Phytopathology,* 83:995-1002, 1993.
9. Jung, G.; Mugnier, J.; Diem, H.G.; Dommergues, Y.R. Polymer entrapped Rhizobium as an inoculant for legumes. *Plant and Soil* 65:219-231, 1982.
10. Kearns, L.P.; Hale, C.N. Biological control of fire blight by *E. herbicola:* Survival of applied bacteria in orchard and glasshouse trials. *Acta Horticulturae*, 338:333-336, 1993.
11. Kloepper, J.W.; Schroth, M.N. Development of a powder formulation of rhizobacteria for inoculation of potato seed pieces. *Phytopathology,* 71:590-592, 1981.
12. Lindow, S.E.; Mc Gourty, G.; Elkins, R. Interactions of antibiotics with *Pseudomonas fluorescens* strain A506 in the control of fire blight and frost injury to pear, *Phytopathology*, 86:841-848, 1996.
13. Lindow, S.E.; Wilson, M. Population dynamics of *Pseudomonas fluorescens* strain A506 in pear flowers following inoculation in relation to strategies of biological control of fire blight and frost injury., *Acta Horticulturae*, 338:331-332, 1993.
14. Loper, J.E.; Henkels, M.D.; Roberts, R.G.; Grove, G.G.; Willett, M.J.; Smith, T.J. Evaluation of streptomycin, oxytetracycline, and copper resistance of *E.amylovora* isolated from pear orchards in Washington State, *Plant Dis.,*75:287-290, 1991.
15. Norelli, J.; Aldwinckle, H. Orchard evaluation of chemical and biological spray materials to control fire blight, *Acta Horticulturae,* 338:363-368, 1991.
16. Nuclo, R.; Johnson, K.B.; Sugar, D.; Stockwell, V.O. Importance of secondary spread of bacterial antagonists in the biological control of *E.amylovora, Acta Horticulturae,* 411:297, 1996.
17. Öktem, Y.E.; Benlioðlu, K. Investigations on fire blight (*Erwinia amylovora*) of pome fruits (Abstr.). *J. Turk. Phytopathol.,* 17:106, 1988.
18. Özaktan, H.; Bora, T.; Sukan, F.V.; Sukan, S.; ve Sargýn, S. Studies on determination of antagonistic potential and biopreparation of some bacteria against the Fireblight pathogen. *Acta Horticulturae,* 489:663-668, 1999.
19. Petrolini, B.; Quaroni, S.; Saracchi, M.; Sardi, P. Formulations in alginate of a strain of Streptomyces: Survival and growth in the soil. *Annali di Microbiologiaed Enzimologia* 38:75-83, 1998.
20. Pusey, P.L. Crab apple blossoms as a model for research on biological control of fire blight. *Phytopathology,* 87:1096-1102, 1997.
21. Schroth, M.N.; Thomson, S.V.; Hildebrand, D.C.; Moller, W.J. Epidemiology and control of fire blight. *Annu. Rev. Phytopathol.,* 12:389-412, 1974.
22. Stockwell, V.O.; Johnson, K.B.; Loper, J.E. Establishment of bacterial antagonists of *E. amylovora* on pear and apple blossoms as influenced by inoculum preparation, *Phytopathology,* 88:506-513, 1998.
23. Suslow, T.V.; Schroth, M.N. Rhizobacteria of sugar beets: Effects of seed application and root colonization on yield. *Phytopathology,* 72:199-206, 1982.
24. Teviotdale, B.L.; Viveros, M. Fruit russetting and tree toxicity symptoms associated with copper treatments of Granny Smith apple trees, *Acta Horticulturae*, 489:565-571, 1999.
25. Vanneste, J.L.; Voyle, M.D. Genetic basis of streptomycin resistance in pathogenic and epiphytic bacteria isolated from apple orchards in New Zealand, *Acta Horticulturae,* 489:671-672, 1999.
26. Vanneste, J.L.; Yu, J. Biological control of fire blight using *E. herbicola* Eh252 and *Pseudomonas fluorescens* A506 separately or in combination. *Acta Horticulturae,* 411:351-353, 1996.
27. Vidhyasekaran, P.; Muthamilan, M. Development of formulations of *Pseudomonas fluorescens* for control of chickpea wilt. *Plant Dis.* 79:782-786, 1995.
28. WHO. World health Organization, Technical report No 430, Geneve, 1969.
29. Wilson, M.; Epton, H.A.S.; Sigee, D.C. Biological control of fire blight of Hawthorn, *Acta Horticulturae,* 273:363-365, 1990.
30. Wilson, M.; Epton, H.A.S.; Sigee, D.C. Interactions between *Erwinia herbicola* and *Erwinia amylovora* on the stigma of hawthorn blossoms. *Phytopathology,* 82:914-918, 1992.
31. Wilson, M.; Lindow, S. Interactions between the biological control agents *Pseudomonas fluorescens* A 506 and *Erwinia amylovora* in pear blossoms. *Acta Horticulturae,* 338:329-330, 1993.
32. Wodzinski, V.S.; Umholtz, T.E.; Beer, S.V. Mechanism by which the antibiotic of EH 318 inhibits *Erwinia amylovora* Ea 273, in: Plant Pathogenic Bacteria Edited by: Klement, Z, Budapest, 1990, p.2645-2666.