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The effect of inoculum concentration and time of application of various bactericides on the control of fire blight (*Erwinia amylovora*) under artificial inoculation

JOHN TSIANTOS[1] and PETROS PSALLIDAS[2]

[1]NAGREF, Plant Protection Institute of Volos, P.O. Box. 1303, 380 01 Volos, Greece
[2] Benaki Phytopathological Institute, 14561 Athens, Greece

Summary. The efficacy of various bactericides for the control of fire blight (*Erwinia amylovora*) in pear flowers was tested. Bactericides were applied preventively one day before, or curatively one or three days after artificial inoculation with pathogen concentrations of 10^4, 10^5 and 10^7 cfu ml^{-1}. The results indicated that a) preventive sprays were more effective than curative sprays; b) the effectiveness of curative sprays decreased progressively from the first to the third day; c) the infection was proportional to inoculum concentration, both in the water–sprayed (control) and in the bactericide–sprayed flowers. There was also significant interaction between bactericide and inoculum concentration. Best results were achieved with streptomycine (Agrept) at 0.5 g and 1 g l^{-1} H_2O; oxolinic acid (S–0208) 1.5 g l^{-1} H_2O; and flumequine (Firestop) 2 ml l^{-1} H_2O. Kasugamycin (Kasumin), phosetyl–Al (Aliette) and copper hydroxide (Kocide) were less effective.

Key words: pear, chemical control, bacterial diseases.

Introduction

Fire blight of pomaceous trees caused by *Erwinia amylovora* was first recorded in Greece in 1984 (Psallidas, 1987; Tsiantos, 1987). Since then, damage caused has varied from year to year and was particularly severe in 1987 (Tsiantos, 1987).

Current control strategies in Greece are mainly the pruning of the infected parts of the trees during the winter, and applying bactericides during blooming, which is the most sensitive stage for infections to develop. The bactericides that are registered and used are streptomycin (Agrept), which is permitted only during the blooming period (Psallidas and Tsiantos, 2001), at a dosage of 1 g l^{-1} H_2O, copper compounds e.g. copper hydroxide (Kocide) and to a less extend flumequine (Firestop). The effectiveness of these bactericides vary from year to year and from orchard to orchard.

Earlier experiments with natural and artificial infections showed that Agrept, Firestop and S–0208 (oxolinic acid) applied during flowering give satisfactory protection (Koistra and Langeslag, 1981; Koistra and Gruyter, 1984; Jones and Byrde, 1987; Paulin *et al*., 1987; Clarke *et al*., 1993; Tsiantos and Psallidas, 1993; Shtienberg *et al*., 2001). Reports on other bactericides, such as phosetyl–Al (Aliette), kasugamycin (Kasumin) and copper compounds, are contradictory (Paulin *et al.,* 1990; Larue and Gaulliard, 1993; Norelli and Aldwinckle,1993; Sugar *et al.,* 1993; Tsiantos and Psallidas,

Corresponding author: J. Tsiantos
Fax: +30 24210 68921
E-mail: instfyt@hol.gr

1993). It seems that inoculum concentration and the time of bactericide application affect the effectiveness of bactericides. Garret (1990) suggested guidelines for experiments on chemical control of the pathogen in the E.U.

Since there were no reports dealing with the above parameters together, experiments were carried out to study these factors after artificial inoculation. The experiments were performed in 1993–1995 in the framework of the European research project FAIR–0203. Part of this work was presented at the 6th Panhellenic Phytopathological Conference.

Materials and methods

The experiments were conducted in the Magnessia prefecture. Pear trees of the main local variety Krystalli, 7–12 years old and in a free shape, were pruned to remove any old infections. The flowers of preselected twigs at the 80–90% bloom stage were spray–inoculated after removing any unopened flowers. The inoculum was a water suspension of a mixture of 4 local strains of *Erwinia amylovora* at concentrations of 1–2$\times$10^4 (1994 and 1995), 1–2$\times$10^5 and 1–2$\times$10^{7}cfu ml^{-1}. The flowers were sprayed with the bactericides at three times, one day before inoculation (preventive spray), one day after and three days after inoculation (curative sprays). Flowers treated with water were used as control. The bactericides, their active ingredients and dosages are listed in Table 1.

Aliette was used only as a preventive because in previous experiments it had not show any curative effect (Tsiantos and Psallidas, 1993), probably because of its mode of action (Larue and Gaulliard, 1993).

Because the disease is highly destructive, precautions have to be taken when using fire blight inoculum in bactericide screening tests in the open orchard. The flowers were sprayed to run off using a low-pressure hand sprayer. In order to avoid dispersal of the bactericides, and particularly of the inoculum, to neighboring twigs during spraying, the parts of twigs to be sprayed were kept inside a plastic tube cut in half longitudinally. After spraying, the twigs were enclosed in plastic bags, which were torn open at the bottom to maintain high humidity and avoid dispersal of the disease by rain or insects. Each of the four replicates per treatment consisted of 6–8 twigs bearing approximately 100 flowers (total 400 flowers). After 15 days the treated twigs were cut and the disease was evaluated in the lab and expressed as the percentage of healthy flowers remaining. Analysis of variance was performed on the percentages after arcsine transformation (Beer, 1976, 1978) and the means were compared using Tukey's multiple range test at P=0.05.

Results

Table 2 gives the analysis of variance for 1995. There were significant differences between bactericides, times of application and inoculum concentrations. The interaction between bactericides and inoculum concentration was also significant, but that between chemicals and time of application was only of border significance, and the interaction between inoculum concentration and time of application was not significant. The results for the other two years were similar.

Agrept 0.5 g, Agrept 1 g l^{-1} H_2O, S–0208 and Firestop showed the best preventive control regardless of inoculum concentration. The percentages of healthy flowers with these sprays ranged from 60 to 97% and were significantly different from the control (Table 3, 4, 5). There was no significant dif-

Table 1. Bactericides used in the experiments.

Bactericides and source	Active ingredient (%)	Dosage l^{-1} H_2O
Agrept (Nihon)	Streptomycin (20)	1 g
Agrept (Nihon)	Streptomycin (20)	0.5 g
Firestop (3 M)	Flumequine (15)	2 ml
S–0208 (Sumitomo)	Oxolinic acid (20)	1.5 g
Aliette (Rhone–Poulenc)	Phosetyl-Al (80)	3 g
Kasumin (Hokko)	Kasugamycin (2)	4 ml
Kocide 10 (Griffin)	Copper hydroxide (50)	0.9 g

ference between these bactericides. Kasumin, Kocide and Aliette showed significant preventive action but only at low inoculum concentration.

Agrept at both concentrations, S–0208 and Firestop gave curative action up to 3 days after infection, but Kocide and Kasumin did not show any significant curative effect. Although the percentage of healthy flowers was greater in trees sprayed with Kocide and Kasumin, the difference was not significant.

The effectiveness of all sprays was greater when used preventively than when used curatively. When applied curatively, spray effectiveness decreased progressively from the first to the third day after infection.

The percentage of infected flowers was almost always proportional to inoculum concentration, although the difference was not always significant. The same was found with trees sprayed with water (control).

Table 2. Analysis of variance on chemical control of Fireblight in 1995.

Source of variation	DF	F	Significance level
Bactericides	3	83.76	0.000
Time of spraying	2	23.09	0.000
Concentration of inoculum	2	52.12	0.000
Interactions			
Bactericides × concentration of inoculum	6	3.451	0.000
Bactericides × time of spraying	6	1.970	0.075
Concentration of inoculum × time of spraying	4	0.564	0.680

Table 3. Effectiveness of preventive (Prev.) and curative (Cur.) sprays on pear flowers (var. Kristalli) artificially inoculated with *Erwinia amylovora* at concentrations of 10^5 and 10^7 cfu ml^{-1} in 1993.

Bactericide, Dosage ml^{-1} H_2O, Application time	Healthy flowers (%)	
	10^5 cfu ml^{-1}	10^7 cfu ml^{-1}
Firestop, 2 ml, Prev.	79 fg[a]	70 e
Firestop, 2 ml, Cur. 1 day	65 cdefg	62 de
Firestop, 2 ml, Cur. 3 days	51 bcdef	48 cde
Kasumin, 4 ml, Prev.	28 bcd	12 ab
Kasumin, 4 ml, Cur. 1 day	22 abc	8 ab
Kasumin, 4 ml, Cur 3 days	11 a	5 ab
Agrept, 0.5 g, Prev.	75 efg	60 de
Agrept, 0.5 g, Cur. 1 day	66 cdefg	21 abc
Agrept, 0.5 g,□Cur3 days	56 bcdef	6 ab
Agrept, 1 g, Prev.	93 g	57 de
Agrept, 1 g, Cur. 1 day	70 defg	46 cde
Agrept, 1 g, Cur. 3 days	61 cdefg	17 abc
S-O208, 1.5 g, Prev.	78 efg	63 de
S-O208, 1.5 g, Cur. 1 day	56 bcdef	57 de
S-O208, 1.5 g, Cur. 3 days	55 bcdef	29 bcd
Kocide, 0.9 g, Prev.	36 bcde	7 ab
Kocide, 0.9 g, Cur. 1 day	26 abcd	4 a
Kocide, 0.9 g, Cur. 3 days	13 ab	2 a
Aliette, 3 ml, Prev.	37 bcde	2 a
Control (water)	11 a	2 a

[a] Means within columns with the same letter are not significantly different (P=0.05).

Table 4. Effectiveness of preventive (Prev.) and curative (Cur.) sprays on pear flowers (var. Kristalli) artificially inoculated with *Erwinia amylovora* at concentrations of 10^4,10^5 and 10^7 cfu ml^{-1} in 1994.

Bactericides, Dosage ml^{-1} H_2O, Application time	Healthy flowers (%)		
	10^4 cfu ml^{-1}	10^5 cfu ml^{-1}	10^7 cfu ml^{-1}
FireStop, 2 g, Prev.	87 ef[a]	78 efgh	65 de
FireStop, 2 g, Cur. 1 day	68 bcde	61 cdef	60 de
FireStop, 2 g, Cur. 3 days	55 abcd	62 cdef	52 de
Agrept, 0.5 g, Prev.	83 def	82 fgh	81 ef
Agrept, 0.5 g, Cur. 1 day	68 bcde	74 defg	49 de
Agrept, 0.5 g, Cur. 3 days	62 abcde	46 bcde	52 de
Agrept, 1 g, Prev.	90 ef	87 fgh	81 ef
Agrept, 1 g, Cur. 1 day	88 def	92 gh	76 ef
Agrept, 1 g, Cur. 3 days	81 cdef	84 fgh	48 cde
S-O208, 1.5 g, Prev.	97 f	97 h	91 f
S-O208, 1.5 g, Cur. 1 day	89 def	90 fgh	71 def
S-O208, 1.5 g, Cur. 3 days	78 cdef	88 fgh	14 ef
Kocide, 0.9 g, Prev.	44 bc	32 bc	14 ab
Kocide, 0.9 g, Cur. 1 day	25 a	20 ab	4 a
Kocide, 0.9 g, Cur. 3 days	28 ab	21 a	6 a
Aliette, 3 ml, Prev.	76 cdef	74 defg	15 abc
Control, (water)	25 a	10 a	8 ab

[a] See Table 3.

Table 5. Effectiveness of preventive (Prev.) and curative (Cur.) sprays on pear flowers (var. Kristalli) artificially inoculated with *Erwinia amylovora* at concentrations of 10^4 ,10^5 and 10^7 cfu ml^{-1} in 1995.

Bactericides, Dosage ml^{-1} H_2O, Application time	Healthy flowers (%)		
	10^4 cfu ml^{-1}	10^5 cfu ml^{-1}	10^7 cfu ml^{-1}
Agrept, 0.5 g, Prev.	95 c[a]	93 d	86 c
Agrept, 0.5 g, Cur. 1 day	86 bc	90 d	80 bc
Agrept, 0.5 g, Cur. 3 days	82 bc	70 bcd	60 b
Firestop, 3 g, Prev.	92 c	91 d	80 bc
Firestop, 3 g, Cur. 1 day	81 bc	78 cd	72 bc
Firestop, 3 g, Cur. 3 days	71 abc	67 bcd	55 b
Kasumin, 4 ml, Prev.	73 abc	66 bcd	24 a
Kasumin, 4 ml, Cur. 1 day	70 abc	37 abc	17 a
Kasumin, 4 ml, Cur.3 days	63 abc	40 abc	22 a
Kocide, 0.9 g, Prev.	68 abc	43 abc	19 a
Kocide, 0.9 g, Cur. 1 day	66 abc	46 abc	20 a
Kocide, 0.9 g, Cur. 3 days	51 abc	32 ab	15 a
Aliette, 3 ml, Prev.	76 abc	68 bcd	20 a
Control (water)	41 a	27 a	17 a

[a] See Table 3.

Discussion

The significant variations that this kind of experiment usually shows (Clarke *et al.,* 1993) prevented the statistical separation of the treatments. As a result, differences as great as 40% were not significant. The amount of detectable variation is decreased if the number of replicates is increased.

However, the preventive spray at the blossoming stage was more effective than the curative sprays, so it should be preferred. The efficiency of the chemicals was increased depending on the time of application. Prediction systems for fire blight epidemics (Billing, 1980) can help in timing applications. The computerized systems "Firescreens" (Jacquart and Paulin, 1991) and "Maryblyt" (Lightner and Steiner, 1990) were developed for this purpose. These systems have been evaluated in Greek climatic conditions (Tsiantos and Psallidas, 2000) but they should be improved so that they can be applied in practice.

The bactericides Agrept (both doses), S–0208 and Firestop gave the best preventive results. These results are in agreement with the results of Koistra and Gruyter (1984); Paulin and Lachaud (1984); Jones *et al*. (1987); Paulin *et al*. (1990); Tsiantos and Psallidas (1993); and Shtienberg *et al*. (2001), even though Moller *et al*. (1973), reported that sprays with copper (Kocide) were better than those with streptomycin. Maybe this was due to the occurrence of natural infection, so that the amount of actual inoculum was not precisely measured. Pathogen strains resistant to streptomycin could also have arisen. Maybe for this last reason Shtienberg *et al*. 2001 did not report good results with streptomycin in experiments with natural infections. Sprays with Kasumin, Kocide and Aliette had limited preventive effect, and only when inoculum pressure was low. Moreover, Aliette and Kasumin showed some toxicity to the Krystalli variety, probably because of the high humidity inside the plastic bags.

Under the conditions of the experiment, Agrept (both doses), S–0208 and Firestop showed curative action when applied up to three days after infection. Their effectiveness decreased progressively from the first to the third day. Probably this does not happen to the same degree under natural conditions. In previous experiments under natural conditions, when bactericides were applied four days after a heavy rain (possible onset of infection), there was no blossom protection even with streptomycin (unpublished data). Shtienberg *et al.* (2001) reported curative control for only two days after artificial inoculation. Nevertheless, when the sprays cover the entire surface of the flowers, some degree of curative action can be expected. This can be due either to local systemic action, as with streptomycin (Beer, 1978) or to some washing off of inoculum during spraying. The curative action of the bactericides was higher when inoculum concentration was low, even though the difference was not always significant. Under the experimental conditions, Kasumin and Kocide did not show any curative action.

Inoculum concentration affected the effectiveness of bactericides both as preventive and as curative sprays. When inoculum pressure was low (10^4 or 10^5 cfu ml^{-1}) even Aliette, Kasumin and Kocide gave significant preventive protection and in some cases some curative action, though it was not significantly different from the control. This effect is reflected in the significance of the interaction between bactericides and inoculum concentration (Table 2).

A useful conclusion is that there were no significant differences in flower protection between Firestop, S–0208 and Agrept (both doses). This means that significant protection of the flowers was ensured at the lower concentrations, with less pollution of the environment and at lower cost. To reduce the risk of forming resistant strains the bactericides S–0208 (when and if it is registered), Firestop and Agrept should be used alternatively since they have different ways of action, and generally an anti–resistance strategy (Shtienberg *et al.,* 2001) should be followed. Also Kocide (in non–toxic concentrations), Kasumin and Aliette offer significant protective action when inoculum pressure is low, and can replace Firestop (which is still very expensive) and the antibiotics. Even though new bactericides have been tried and new methods of control have been tested in recent years, the findings of the experiment–which should be verified under natural infection conditions–can be very useful in determining the best strategy to control the disease. Growers will achieve better disease control if they reduce inoculum pressure by cutting out the infected parts of the trees in their orchards and in neighboring sources of the inoculum (deserted orchards, wild hosts) and if they spray at the right time with the right compound.

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