

Efficacy of Oxolinic Acid and Other Bactericides in Suppression of *Erwinia amylovora* in Pear Orchards in Israel

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The efficacy of oxolinic acid (at 200 and 300 $\mu\text{g a.i./l}$) and of several antibiotic compounds (streptomycin sulfate at 100 $\mu\text{g a.i./l}$, glycoside B at 700 $\mu\text{g a.i./l}$, kasugamycin at 80 $\mu\text{g a.i./l}$ and gentamicin sulfate at 30 and 60 $\mu\text{g a.i./l}$) against *Erwinia amylovora*, the causal agent of fire blight in pears, was evaluated in 43 orchard experiments in 1997–2000 in Israel. In addition to the above orchard experiments, the efficacy of the bactericides was tested in five experiments with artificial inoculation. Natural fire blight symptoms were observed in 16 of the 43 experiments; in 13 of them, disease intensity and its distribution among the experimental plots provided a basis for data analysis, leading to reliable conclusions concerning the efficacy of the tested bactericides. Oxolinic acid at 300 $\mu\text{g a.i./l}$ was highly effective against *E. amylovora* and reduced disease severity significantly in all experiments, as compared with the untreated plots; however, a concentration of 200 $\mu\text{g a.i./l}$ was not effective in some cases. Among the tested antibiotics, only gentamicin sulfate was as effective as oxolinic acid. Results of the artificial inoculation experiments corroborated those obtained in the naturally infected orchards. The pre-infection activity of oxolinic acid was determined on blossom clusters that were sprayed with the bactericide before inoculation. Control efficacy on blossom clusters sprayed 1–4 days before inoculation ranged from 68% to 80%, a level which did not differ significantly from that observed on blossom clusters sprayed on the day of inoculation (80% control). The postinfection activity of oxolinic acid was determined on blossom clusters that were sprayed with the compound after inoculation. Oxolinic acid was as effective when applied 1 or 2 days after inoculation as when it was applied on the day of inoculation; however, application of the bactericide 3 days after inoculation no longer resulted in significant disease suppression. Oxolinic acid has been used commercially in Israel since 1998 with appreciable success.

KEY WORDS: *Erwinia amylovora*; fire blight; disease management; bactericides.

INTRODUCTION

Fire blight, caused by the bacterium *Erwinia amylovora* (Burrill) Winslow *et al.*, is the most destructive pathogen of pears and other pome fruit trees worldwide (8,14,22,23). The disease was first detected in Israel in 1985 and within 2 years it had spread to all pear production areas of the country (15,24). In the 10 years that followed, infections were

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sporadic. Although epidemics in some orchards were occasionally severe, leading to death and uprooting of trees, the intensity of the disease countrywide was mild. The situation was different in the Samaria production area in 1995 and in northern Israel in 1996. In those years severe epidemics developed in these regions, leading to substantial damage and to uprooting of numerous orchards. In Samaria ~85% of the pear orchards area (~100 ha) was uprooted following the 1995 epidemic.

Management of fire blight is difficult and it is necessary to combine several different measures to eliminate the source, reduce the bacterial inoculum, limit its spread, prevent plant infection and reduce plant susceptibility (19,23). Chemicals which can be used against bacterial pathogens of plants belong to three categories: heavy metals, antibiotics, and chemicals triggering a defense reaction in plants. Heavy metals (most often copper compounds) are quite good bactericides, but their use may have phytotoxic effects on leaves and fruits; therefore, they are employed primarily before bloom. Antibiotics often provide the best control of fire blight. The first experiments on fire blight control with streptomycin were performed in the USA in the early 1950s and by the end of that decade, streptomycin was widely used in apple and pear orchards. During the 1960s orchards in California sometimes received ten or more streptomycin sprays per season. Other antibiotic compounds such as oxytetracycline, kasugamycin and gentamicin sulfate are also effective against *E. amylovora* (1,13,20).

The use of antibiotics has, however, two major drawbacks. Antibiotics, especially streptomycin, are used in human and animal medicine, and therefore their use for plant protection is under strict regulation and in some countries is not allowed at all (for example, in France, Spain, Italy and Switzerland). Secondly, repeated and frequent use of antibiotics often leads to the selection of antibiotic-resistant strains of *E. amylovora*. Such strains were detected in the early 1970s in some areas of the USA, and later they were also found in other regions of the USA, and in New Zealand, Greece and other countries (11,12).

In Israel, since 1986 streptomycin has been the only bactericide registered for fire blight management during bloom. It has been widely used by pear growers, with up to 12 sprays being applied per season. Streptomycin-resistant *E. amylovora* strains were first detected in 1991 in an isolated pear orchard in the northern Negev. Following failure to control fire blight with streptomycin in 1995 and 1996, the distribution of streptomycin-resistant strains of *E. amylovora* in Israel was surveyed, and 107 pear, apple, loquat and quince orchards were monitored during 1994–1997. Streptomycin-resistant strains of the bacterium were recovered from flowers and from infected branches collected from most growing regions in the country (10).

The development of populations of *E. amylovora* resistant to streptomycin created a serious threat to the pear industry in Israel, since it was the only registered bactericide; therefore, there was an urgent need to find and register an effective alternative compound. The number of potential candidates was limited, but one, for which there was some documented evidence of efficacy, was oxolinic acid. Oxolinic acid (Starnar, previously coded as S-0208; 5-ethyl-5,8-dihydro-8-oxo[1,3]dioxolo[4,5-g]quinoline-7-carboxylic acid), is a bactericide developed by Sumitomo Chemicals Corporation in Japan. It is a synthetic quinoline-family compound with high antibacterial activity against some gram-negative bacteria (7). Oxolinic acid inhibits DNA-gyrase, resulting in inhibition of DNA replication, and the bacteria treated with the compound lose the ability to multiply. The efficacy of oxolinic acid (at 300 μg a.i./l) in suppression of *E. amylovora* was evaluated in England

(9), Belgium (4), Cyprus (5), Poland (18), Egypt (6), Greece (21) and Israel (Zilberstaine, unpublished). Control achieved by the compound ranged from 70% to 98%, and in all cases efficacy was at least as good as that of the standard antibiotics used for comparison (which most often was streptomycin). Based on this evidence, an emergency registration certificate was issued in 1997 by authority of the Israel Ministry of Agriculture and Rural Development, covering the use of oxolinic acid against *E. amylovora*. However, for permanent registration and to support the adequate and rational use of the product, there was a need for more comprehensive information concerning its activity. In a nationwide research program initiated in 1997, the efficacy of oxolinic acid and of various other bactericides in the suppression of fire blight in pear was tested, and the pre- and postinfection activity of oxolinic acid were determined. A preliminary report on some of the data was published previously (16).

MATERIALS AND METHODS

Efficacy of oxolinic acid and other bactericides against *E. amylovora* Forty-three orchard experiments were conducted during the years 1997–2000: 11 each in 1997 and in 1998, 12 in 1999, and 9 in 2000. All experiments were conducted in commercial orchards spread over most pear production areas in the country. The experiments were located in northern Israel (the Galilee region, the Golan Heights and the Hula Valley), in the center of the country (Sharon and Lakhish regions) and in the south (the northern Negev) (Fig. 1). Trees in the experiments were not artificially inoculated, and therefore natural infections were expected. The efficacy of the following bactericides was evaluated: oxolinic acid (20% WP, Starner, Sumitomo Chemicals, Japan), applied at 300 and 200 μg a.i./l; streptomycin sulfate (20% WP, Agrept, Nihon Nohyaku, Japan), applied at 100 μg a.i./l; glycocide B (70% EC, A. Shizer, Israel), applied at 700 μg a.i./l; kasugamycin (2% WP, Hokko, Japan), applied at 80 a.i./l; and gentamicin sulfate (10% WP, Agry-Gent, Quimica Agronomica de Mexico, Mexico), applied at 30 and 60 μg a.i./l. Details of the treatments included each year are presented in Table 1. The bactericides were applied with a hand gun sprayer in 1000 l water/ha. The first spray in each experiment was applied soon after the initiation of flowering; subsequent sprays were applied at 4–6-day intervals until the termination of flowering. The number of sprays in each experiment varied according to the duration of flowering and ranged from three to seven. The experimental plot size was four to six pear trees in a row, and there were four or five replicates per treatment. The experimental design (completely randomized or complete block design) was determined in each experiment according to the local conditions.

Disease intensity was recorded only in the experiments where epidemics developed at intensities sufficient to enable reliable data analysis and interpretation in terms of the efficacy of the compounds. Disease was assessed visually on the two or three trees that were located in the center of each experimental plot. Two individuals inspected the trees from both sides, and counted the number of infected blossom clusters (*i.e.*, disease severity). Infected blossom clusters were not removed. Assessments were made several times after the first observation of disease symptoms, until there was no further increase in disease severity. In this report the counts from the last assessments are presented. Data were analyzed by appropriate ANOVA tests at $P < 0.05$. Trained employees made the observations in the orchards and, in some cases, infected blossom clusters, shoots or branches were taken to the laboratory for identification of the causal agent(s) by polymerase

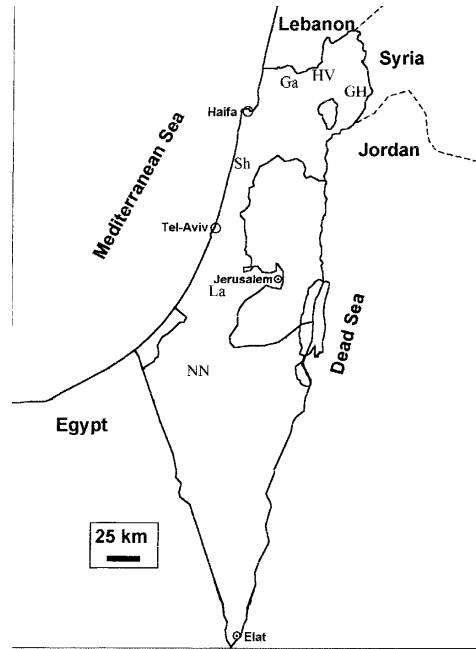


Fig. 1. Pear-production regions in Israel where experiments were conducted in this study, during the years 1997–2000. Ga = Galilee; HV = Hula Valley; GH = Golan Heights; Sh = Samaria; La = Lakhish; NN = northern Negev.

chain reaction (PCR). In all cases, the causal agent was confirmed to be *E. amylovora*.

In addition to the above set of experiments, the efficacy of the various bactericides was tested in 1997 and 2000 with artificial inoculation in five experiments, conducted in orchards located in the Samaria region. The bactericides were applied in the morning (0600–0800 hours): in 1997, pear trees were sprayed by means of a hand gun sprayer; in 2000, individual blossom clusters were sprayed with an air-pressure hand sprayer (model 4023, Hozelock Ltd., Bucks, UK). In both years individual blossom clusters were marked with colored, numbered plastic bands. The marked blossom clusters were inoculated to runoff with bacterial suspension, with a hand-held air-pressure sprayer. Inoculation was done on the same day, in the late afternoon (1700–1830 hours) with a mixture of 1:3 streptomycin-sensitive and streptomycin-resistant strains of *E. amylovora* (10^7 bacteria/ml). In 1997 there were four replicates per treatment, each consisting of ten blossom clusters; in 2000 there were two replicates per treatment, each consisting of 40 blossom clusters. The incidence of infection (percentage of diseased blossom clusters out of the total number inoculated) was recorded 10–14 days after inoculation.

Pre- and postinfection activity of oxolinic acid The duration of the pre- and postinfection activity of oxolinic acid was determined in four artificial inoculation experiments conducted in an experimental orchard. One experiment was done in the spring of 1998, two in the autumn of 1998/99 and one in the spring of 1999. (Pear trees occasionally bloom in early autumn in Israel.) The designs of all experiments were similar: Blossom-clusters were sprayed with oxolinic acid (at a concentration of $300 \mu\text{g a.i./l}$) during five (autumn experiments) or eight (spring experiments) consecutive days. Different clusters

TABLE 1. Summary of the treatments and the number of experiments conducted yearly for determining the efficacy of various bactericides against *Erwinia amylovora*, the causal agent of fire blight in pears, in Israel

| Bactericides and rates ($\mu\text{g a.i./l}$) | Artificial inocula- tion experiments | Natural infection experiments | | | | Total num- ber of experiments |
|--|---|-------------------------------|--------|--------|-------|-------------------------------------|
| | | 1997 | 1998 | 1999 | 2000 | |
| Untreated control | 5 | 11 (2) ^z | 11 (5) | 12 (5) | 9 (1) | 43 (13) |
| Oxolinic acid (300) | 5 | 11 (2) | 11 (5) | 12 (5) | 9 (1) | 43 (13) |
| Oxolinic acid (200) | 2 | 2 (2) | 9 (3) | - | - | 11 (5) |
| Streptomycin (100) | 1 | 10 (2) | - | - | - | 10 (2) |
| Glycocide B (700) | 1 | 5 (2) | - | - | - | 5 (2) |
| Kasugamycin (80) | 1 | 5 (2) | - | - | - | 5 (2) |
| Gentamicin sul- fate (30) | 1 | - | - | 1 (1) | 6 (1) | 7 (2) |
| Gentamicin sul- fate (60) | 1 | - | - | 1 (1) | 2 (1) | 3 (2) |

^zFigures in parentheses indicate the number of experiments in which disease intensity and its distribution among the experimental plots enabled the data to be analyzed and reliable conclusions to be drawn, concerning the efficacy of the tested bactericides.

were sprayed every day; thus, individual blossom clusters were sprayed only once. The spray was applied to runoff in the morning (0600–0800 hours) by means of a hand-held air-pressure sprayer, pointed directly at each marked blossom cluster. At the time of spraying, there were at least two open blossoms in each cluster; the rest had not opened yet. Sprayed blossom clusters were marked with colored, numbered bands. On the third day (autumn experiments) or the fifth day (spring experiments) after the initiation of each experiment, all the marked blossom clusters (including those that had not yet been sprayed with the bactericide) were inoculated with the streptomycin-sensitive strain of *E. amylovora* (10^7 bacteria/ml) as described above. Inoculation day will be referred to hereafter as ‘day 0’. Thus, blossom clusters that were sprayed with oxolinic acid before inoculation were treated on days -1, -2, -3 and -4. Those that were sprayed with oxolinic acid 1, 2 or 3 days after inoculation were treated on days +1, +2 and +3, respectively. Every day, there were also blossom clusters that were marked with colored bands but were not sprayed with oxolinic acid. These clusters were inoculated on day 0, and served as controls. Each treatment consisted of four replicates, each including ten different blossom clusters. Thus, 400 and 640 blossom clusters were inoculated in the autumn and the spring experiments, respectively. Fire blight symptoms were apparent 5–8 days after inoculation, and disease incidence (the percentage of inoculated blossom clusters which was infected) was determined for each replicate. Disease incidence in the non-treated blossom clusters was 92–100% in all experiments. For data analysis, control efficacy (CE, %) was calculated for each treatment (application date), from the disease incidence records in the untreated control (D_c) and the bactericide-treated (D_t) plots: $CE = 100 - (D_t / D_c * 100)$. Since the effects of the treatments in the four experiments were similar, the data were pooled and the mean control efficacy values (\pm SE) for each treatment are presented in this report.

TABLE 2. Effect of various bactericides on *Erwinia amylovora* in naturally infected pear orchards. Figures indicate the severity of fire blight symptoms (number of infected blossom clusters) per tree

| Bactericides and rates ($\mu\text{g a.i./l}$) | 1997 | | | 1998 | | | | | 1999 | | | | | 2000 | |
|---|---------------------|------------|---------------------|-----------|------------|-----------|-----------|-----------|-----------|-----------|-----------|------------|-----------|------------|------------|
| | Exp. 1 ^z | Exp. 2 | Exp. 1 ^z | Exp. 1 | Exp. 2 | Exp. 3 | Exp. 4 | Exp. 5 | Exp. 1 | Exp. 2 | Exp. 3 | Exp. 4 | Exp. 5 | Exp. 1 | Exp. 5 |
| Untreated control | 29.4 ab | 17.4 a | 157.0 a | 14.1 a | 30.0 a | 14.8 a | 42.7 a | 42.7 a | 14.0 a | 18.5 a | 34.1 a | 162.3 a | 42.7 a | 217.5 a | 217.5 a |
| Oxolinic acid (300) | 5.0 c | 0.8 b | 25.0 b | 2.3 b | 13.7 b | 4.2 b | 17.7 b | 17.7 b | 4.5 b | 4.4 b | 4.2 b | 17.4 b | 17.7 b | 16.2 b | 16.2 b |
| Oxolinic acid (200) | 5.0 c | 4.2 b | 125.1 a | 2.0 b | 22.0 ab | - | - | - | - | - | - | - | - | - | - |
| Streptomycin (100) | 12.2 bc | 22.4 a | - | - | - | - | - | - | - | - | - | - | - | - | - |
| Glycocide B (700) | 34.5 a | 13.8 ab | - | - | - | - | - | - | - | - | - | - | - | - | - |
| Kasugamycin (70) | 28.1 ab | 3.9 b | - | - | - | - | - | - | - | - | - | - | - | - | - |
| Gentamicin sulfate (30) | - | - | - | - | - | - | - | - | - | - | 10.9 b | - | - | 23.7 b | 23.7 b |
| Gentamicin sulfate (60) | - | - | - | - | - | - | - | - | - | - | 12.0 b | - | - | - | - |

^z Within columns, data followed by a common letter do not differ significantly ($P < 0.05$) as determined by Fisher's protected LSD test.

RESULTS

Efficacy of oxolinic acid and other bactericides against *E. amylovora* Fire blight symptoms from natural infections were observed in 16 experiments, in 13 of which (two in 1997, five in 1998, five in 1999 and one in 2000) the disease intensity and its distribution among the experimental plots were such as to enable the data to be analyzed and reliable conclusions to be drawn concerning the efficacy of the tested bactericides. In the three other experiments disease intensity was very low and *E. amylovora* infections were unevenly distributed among experimental plots. Such a situation precludes the drawing of reliable conclusions concerning the effects of the treatments. Oxolinic acid at a concentration of 300 $\mu\text{g a.i./l}$ was highly effective against *E. amylovora* and reduced disease severity significantly in all experiments, as compared with the untreated plots (Table 2). The efficacy of the compound was related to the severity of the disease in untreated plots (which provided an indication of the disease pressure in the experiment). When disease severity in the untreated plots was low (up to 25 infected blossom clusters per tree), there were 0–5 infected blossom clusters in plots treated with oxolinic acid. When disease severity in untreated plots was moderate (25–50 infected blossom clusters per tree), there were 4–17 infected blossom clusters in the treated plots. When disease severity in untreated plots was high (more than 150 infected blossom clusters per tree), 15–25 infected blossom clusters were observed in the treated plots (Table 2). Results of the artificially inoculated experiments corroborated these findings. However, since inoculum quantity in artificially inoculated tests is higher than in natural infections, the control efficacy of 300- $\mu\text{g a.i./l}$ sprays was more variable and ranged from 52% to 90% (Table 3).

TABLE 3. Effect of various bactericides on *Erwinia amylovora* in artificially inoculated pear trees. Figures indicate the incidence of fire blight (as %) on individually inoculated blossom clusters^z

| Bactericides and rates ($\mu\text{g a.i./l}$) | Exp. 1 Cv. Spadona ^y | Exp. 2 Cv. Costia | Exp. 3 Cv. Costia | Exp. 4 Cv. Jantil | Exp. 5 Cv. Spadona |
|--|---------------------------------------|-------------------------|-------------------------|----------------------|--------------------------|
| Untreated control | 100 a | 80 a | 93 a | 82 a | 100 a |
| Oxolinic acid (300) | 30 c | 38 b | 45 b | 25 b | 9 b |
| Oxolinic acid (200) | 30 c | - | 30 b | - | - |
| Streptomycin (100) | 55 b | - | - | - | - |
| Glycocide B (700) | 100 a | - | - | - | - |
| Kasugamycin (80) | 90 a | - | - | - | - |
| Gentamicin sulfate (30) | - | - | - | - | 54 ab |
| Gentamicin sulfate (60) | - | - | - | - | 36 ab |

^zThe bactericides were applied ~ 10 h before inoculation. Exps. 1–4 were conducted in 1997; exp. 5 was conducted in 2000. Forty blossom clusters were inoculated in each treatment in expts. 1–4 and 80 blossom clusters were inoculated in each treatment in exp. 5.

^yWithin columns, data followed by a common letter do not differ significantly ($P < 0.05$) as determined by Fisher's protected LSD test.

Use of a reduced rate of oxolinic acid (200 $\mu\text{g a.i./l}$) resulted in adequate disease suppression in most, but not all, experiments. In two of the five orchard experiments in which this rate was tested, disease severity in treated plots did not differ significantly from that recorded in untreated control plots (Table 2). In the other experiments, as well as in

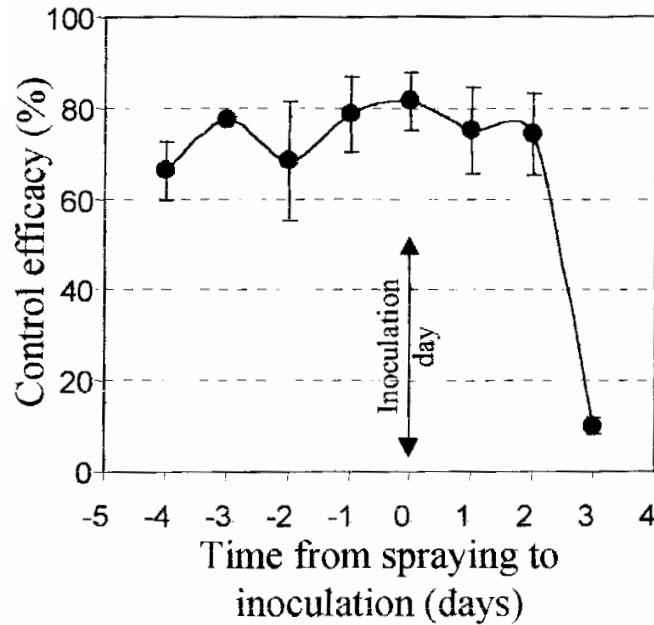


Fig. 2. Pre- and postinfection activity of oxolinic acid in suppression of fire blight, caused by *Erwinia amylovora*, in pears. Blossom clusters were sprayed with the bactericide before and after the day of inoculation (day 0). Results are means (\pm SE) of four experiments. Values of control efficacy express the degree of reduction in disease incidence, in the bactericide-treated vs the non-treated blossom clusters. Disease incidence in the non-treated blossom clusters ranged from 92% to 100% in all experiments.

the two artificial inoculation tests, disease severity in plots treated with the reduced rate of oxolinic acid was significantly lower than that in the untreated plots (Tables 2 and 3).

The efficacy of the various antibiotic compounds that were tested in the trials varied. As compared with the untreated control, streptomycin reduced disease severity only in the artificial inoculation tests, in which a streptomycin-sensitive strain of *E. amylovora* was part of the inoculum mixture used for inoculation. However, streptomycin was not effective in the orchard experiments, where natural infections occurred. Kasugamycin and glycoside B were not effective in at least two of the three experiments in which they were tested. On the other hand, gentamicin sulfate was as effective as oxolinic acid in the two orchard experiments but less effective in the artificial inoculation test (Tables 2 and 3).

No phytotoxicity was recorded on flowers or any other parts of the trees sprayed with any of the compounds, at any of the tested rates.

Pre- and postinfection activity of oxolinic acid The pre-infection activity of oxolinic acid was determined on blossom clusters that had been sprayed with the bactericide before the day of inoculation. Control efficacy on blossom clusters sprayed 1–4 days before inoculation ranged from 68% to 80% (Fig. 2). This level of control efficacy did not differ significantly from that observed on blossom clusters sprayed on the day of inoculation (day 0, 80% control). The postinfection activity of oxolinic acid was determined on blossom clusters that were sprayed with the compound after inoculation. Oxolinic acid was as

effective when applied 1 or 2 days after inoculation as when it was applied on the day of inoculation, but application of the bactericide 3 days after inoculation no longer resulted in significant disease suppression (Fig. 2).

DISCUSSION

Epidemics of *E. amylovora* occur sporadically. It is not uncommon that the disease does not develop even in orchards that were severely infected in the preceding season. Moreover, disease in infected orchards is often unevenly distributed and disease foci are frequently formed. All this poses serious difficulties in fire blight experimentation, since the likelihood of occurrence of natural infections with uniform distribution of the disease at an experimental site is not high. There are two possible ways to overcome this difficulty. The first is to conduct a large number of experiments, under diverse climatic conditions, in the hope that reliable estimation of treatment effects would be possible in at least some of them. The problem is that a vast amount of effort would be needed. The other possible solution is to use artificial inoculation tests. The probability of success is high, but artificial inoculations do not always imitate natural infections adequately. In the present study we used both methods to increase the probability of success. During the years 1997–2000, 43 orchard experiments were conducted in all pear-growing regions of the country. Natural infections developed in 16 of the experiments, and in 13 of them (31% success) it was possible to analyze the data and detect significant differences among the applied treatments. In addition, five artificial inoculation experiments were done and, in general, the results of these tests corroborated those derived from the natural infection experiments.

In all the experiments conducted in this study, application of oxolinic acid at 300 μg a.i./l significantly reduced disease severity as compared with the untreated control. Significant control was achieved regardless of the disease pressure in the experiment. In three experiments disease pressure was high, *i.e.*, the number of infected blossom clusters in untreated trees exceeded 150 per tree. In these experiments, control efficacy in oxolinic acid-treated trees ranged from 84% to 92% (Table 2). Control efficacy in the artificial inoculation experiments was more variable but also high (Table 3). This level of control is seldom observed in orchard experiments treated with streptomycin. Our results concerning the efficacy of oxolinic acid are in good agreement with those reported by numerous authors from several countries. Nevertheless, none of those studies involved such a wide range of severities and so many experiments (4,5,6,9,18,21). A reduced rate of oxolinic acid (200 μg a.i./l) was effective in some experiments. However, in two experiments (nos. 1 and 3, 1998) disease severity in plots treated with oxolinic acid at the reduced rate did not differ significantly from that recorded in untreated plots (Table 2). Disease pressure in one of the experiments was high. Thus, it is possible that the lower rate of oxolinic acid would be insufficient under extreme epidemic situations.

Postinfection activity of bactericides is rare. In most studies published so far it was found that bactericides were effective against *E. amylovora* only if applied prior to, or up to 24 h after, inoculation (3). Oxolinic acid is a protectant compound and we were surprised to find that spraying 2 days after inoculation still prevented infections and provided adequate suppression of the disease (Fig. 2). These results may be interpreted by means of the model suggested by Beattie and Lindow (2) for colonization of the phyllosphere by pathogenic and non-pathogenic bacteria. They described a multi-step procedure, starting from multiplication of the bacteria on the plant surface, to modification

of the local environment by the bacteria, creation of micro-colonies, entry of the bacteria into the internal spaces of the host through natural openings (such as stomata, hydathodes or flower nectaries) or wounds (created, for example, by hail), and multiplication of the bacteria within the openings or wounds. Bacteria which have not yet penetrated into the intercellular spaces are still sensitive to external influences, and it is possible that in our experiments bacterial multiplication in the blossom openings lasted 48 h after inoculation. Thus, molecules of oxolinic acid were still able to reach the bacteria cells before the latter had penetrated into the host tissue and to prevent their multiplication. However, 3 days after inoculation, the bacteria had already penetrated the intercellular spaces, where the bactericide could not affect them. Regardless of whether or not this is the reason why postinfection application of oxolinic acid was effective, the fact is that the phenomenon exists: 2-day postinfection efficacy was observed in the four experiments, conducted over three different seasons. The practical implication of this finding is that oxolinic acid can be used, and can be effective, up to 48 h after the occurrence of infection. This is an important feature of the compound, because an infection is often identified only after it has already occurred. This may be done by analysis of weather data.

The efficacy of various antibiotics (*i.e.*, streptomycin sulfate, glycoside B, kasugamycin and gentamicin) was tested in this study; only gentamicin sulfate was effective in most experiments (Tables 2 and 3). Registration of an additional compound for fire blight control would be extremely important to pear growers, enabling them to alternate between bactericides and reduce the risk of development of resistant *E. amylovora* populations.

Results of the present study suggest that oxolinic acid is highly effective against *E. amylovora*, and experience gained in the use of this compound in commercial orchards in Israel confirms our findings. In a comprehensive survey conducted during the years 1996–1999, data on fire blight intensity and related subjects were collected, and observations were made yearly in one-third to one-half of the pear production area in Israel (17). Analysis of disease onset records and weather data enabled the dates of infections to be identified and it was found that only one to three infection episodes occurred in any individual orchard each year. The survey enabled the efficacy of management actions taken by the growers to be evaluated. Most pear orchards were sprayed with bactericides during bloom in the years 1996–1999, the number of applications in individual orchards ranging from one to five. In 1996 most orchards were sprayed with streptomycin but in the subsequent years only oxolinic acid was used (because of resistance to streptomycin). Control efficacy in commercial orchards, as reflected in the survey, was not related to the number of sprays applied during bloom but to the timing of spraying. When sprays were timed properly (that is, up to 4 days before, or 2 days after, the time of infection [Fig. 2]), control efficacy was high. If sprays were not applied at all, or if spraying was improperly timed (which is equivalent to not spraying at all), symptoms were not prevented and the resultant intensity of the disease was high (17).

One of the major problems in chemical control is the ability of the pathogens to develop populations resistant to the pesticides. The problem is more pronounced when pathogenic bacteria are concerned, as the populations are larger and the frequencies of mutation are higher than in pathogenic fungi. Streptomycin-resistant populations of *E. amylovora* have developed in many countries in the world, and there is a risk of resistance developing against oxolinic acid as well. To reduce this risk, an anti-resistance strategy was developed and implemented. It incorporates the following components. (i) Avoidance of using sub-

optimal doses of the at-risk compound. Although the efficacy of oxolinic acid at a rate of 200 $\mu\text{g a.i./l}$ did not differ significantly (in most experiments) from that of 300 $\mu\text{g a.i./l}$, the recommended rate is 300 $\mu\text{g a.i./l}$. (ii) Alternation of the at-risk compound with compounds having different modes of action. Efforts were devoted to identify additional bactericides that are effective against *E. amylovora*; among them, gentamicin sulfate was found effective and may be used, after registration, in alternation with oxolinic acid. (iii) Avoidance of using the at-risk compound when large populations of the bacteria exist. Growers are strictly advised not to apply oxolinic acid when infected blossom clusters are observed in the orchard; application is recommended only after all infected tissues have been removed and burned. Moreover, although oxolinic acid was found effective when applied up to 48 h postinfection, such use of the compound should be avoided, if possible. *E. amylovora* populations increase rapidly when conditions are conducive to the pathogen, and by 48 h after infection large populations of the bacteria may develop. (iv) Reduce the number of applications of the compound at risk. Since the number of infection episodes in individual orchards is only one to three per year (17), a few, properly timed applications of bactericides will protect the blossoms adequately. However, for such a practice to be effective, a reliable forecast system is necessary. Recently, a decision support system, Fire Blight Control Advisory (FBCA), was developed (16) and tested in orchard experiments during the years 1998–2000, and also in limited commercial use in 2000. Use of FBCA enabled the time of infections to be identified properly and fire blight to be suppressed adequately, with an average of 2.5 sprays per season. Implementation of these measures can not guarantee that strains of *E. amylovora* resistant to oxolinic acid will not develop, but it may lower the probability of their occurrence.

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