A total of 75 Erwinia amylovora isolates, collected primarily from fire blighted quince (Cydonia oblonga) and pear (Pyrus syriaca and P. communis) shoots, were tested for copper resistance and streptomycin sensitivity. Sixty eight isolates, representing 90.6% of the total were inhibited by 10 µg/ml streptomycin and were thus regarded as streptomycin sensitive. On the contrary, three isolates that were not inhibited by 2000 µg/ml streptomycin were considered as resistant. Fifteen isolates grew well on casitone yeast extract medium in the presence of 1.2 mM copper sulfate and none survived on the same medium containing 2.4 mM copper sulfate, indicating that most of the Syrian E. amylovora isolates are copper tolerant and could cause severe problems even in copper heavily sprayed orchards. The use of both bactericides in controlling fire blight disease in Syria is discussed.

Key words: Pear, apple, fire blight, Erwinia amylovora, streptomycin, copper sulfate.

Fire blight, caused by the Gram-negative bacterium Erwinia amylovora, is the most destructive disease of many rosaceous plants including apple (Malus domestica), pear (Pyrus communis) and quince (Cydonia oblonga) (Van der Zwet and Keil, 1979). Susceptible plants can be severely damaged or killed in the nursery and commercial orchards (Vanneste, 2000). No single measure is available for controlling fire blight (Momol and Aldwinckle, 2000) but careful plant management and quarantine measures, together with reliable disease detection and monitoring, may help minimizing its spread (Zhang and Geider, 1997). Copper-containing sprays are routinely used against a number of diseases, including fire blight (Loper et al., 1991) because since 1900, copper-based compounds have been established as effective bactericides against fire blight on apples and pears (Van der Zvet and Keil, 1979). Copper ranks after silver and mercury for toxicity, accumulating in the outer membrane or in the cytoplasm of Gram-negative bacteria. Its toxic effect is expressed as a growth inhibition of bacterial cultures (Geider, 1999).

If allowed, the application of an aminoglycoside antibiotic such as streptomycin would represent an additional tool for controlling fire blight (Schatz et al., 1944). Streptomycin limits the normal growth of the cells by interfering with the proper reading of messenger RNAs. Streptomycin-resistant strains of E. amylovora have been detected in several countries (Jones and Schnabel, 2000) and the genetic basis for resistance has been extensively studied (Choiu and Jones, 1991; Amyes and Gemmel, 1992; McManus et al., 2002). To cope with streptomycin, several strategies are deployed by the pathogen, including alteration of the target site and production of streptomycin-degrading enzymes (Amyes and Gemmel, 1992).

E. amylovora is currently present in 46 countries and was reported in 1988 from Lebanon, a country that borders Syria (Saad et al., 1999; Van der Zvet, 2002). In the course of a survey conducted in Syria in 2005 and 2006, 75 bacterial isolates were obtained mainly from fire blighted quince and pears, which were identified as E. amylovora by biochemical and molecular tests (Ammouneh et al., 2008). Consequently, the objectives of this study were to assess the effects of copper sulfate (a compound heavily used by Syrian farmers) on the Syrian E. amylovora population and to determine whether streptomycin-resistant isolates of this bacterium occur in the country.

Bacterial isolates were routinely cultured on sucrose nutrient agar (SNA) plates (Billing et al., 1961), that were incubated at 26±1°C until colonies developed, to be then transferred to 4°C. The isolates were also maintained in 20% glycerol at -80°C.

Screening for resistance to streptomycin was according to Burr et al. (1988). Colonies grown for 48 h on SNA were resuspended in 1x phosphate buffer saline (PBS) and adjusted spectrophotometrically to a cell density of 1x10^8 colony-forming unit (CFU)/ml. A suspension of 100 µl was spread on the surface of SNA
plates. Sterile filter paper disks impregnated with 0, 10, 50, 100, 250, 500, 1000, and 2000 µg/ml streptomycin were placed on SNA-inoculated plates. The presence of an inhibition zone was checked 48 h post incubation at 26±1ºC. The experiment was repeated twice.

Results of these trials showed that the majority of the tested isolates (90.6% of the population) were sensitive to streptomycin, since their growth was inhibited by a concentration of 10 µg/ml (Fig. 1). Four isolates, i.e. 5.3% of the population, were classified as moderately resistant because their growth was inhibited by a streptomycin concentration up to 500 µg/ml. Only three isolates (4%) showed complete resistance for their growth was not inhibited by an antibiotic concentration of 2000 µg/ml.

As to copper, it is well known that, when dissolved, copper sulfate causes a severe pH drop. To circumvent this problem, copper ions were complexed with Tris by dissolving 0.2 M CuSO₄ in 0.8 M Tris base and the pH adjusted to 6.8 with additional Tris base (Geider, 1999). Casitone yeast extract (CYE) medium was used instead of SNA due to its limited copper binding capacity to test the sensitivity of bacterial isolates to copper, following the method of Loper et al. (1991). Bacterial suspensions containing 1x10⁸ (CFU)/ml of E. amylovora isolates were spotted on CYE plates containing different concentrations of CuSO₄, i.e. 0.08, 0.16, 0.32, 0.64, 1.2 and 2.4 mM, that were incubated at 26±1ºC for 72 h, when growth was recorded. The test was repeated twice.

All isolates grew well in the presence of 0.16 mM CuSO₄ but none of them tolerated the 2.4 mM concentration. (Table 1). Of the tested isolates, 14, 29 and 32 were able to grow in the presence of copper sulfate at 1.2, 0.64 and 0.32 mM concentration, respectively (Table 1).

Thus, 19.7% of the Syrian E. amylovora population (14 isolates) was tolerant to 1.2 mM copper sulfate, which corresponds to the rate utilized in field applications (TomLin, 1994). This may largely due to the extensive and frequent use of copper-based compounds to control various plant pathogens in Syria. By contrast, none of 138 of E. amylovora isolates from Washington State (USA) grew on copper sulfate 0.16 mM, although spontaneous mutants with tolerance to a 0.16 mM concentration were observed at a frequency of 1x10⁻⁶ to 1x10⁻⁷ mutant colony per wild type colony (Loper et al., 1991). Consequently, copper based compounds could be used to control this pathogen in Washington State but not in Syria.

It is noteworthy that none of the Syrian streptomycin resistant isolates was also copper sulfate tolerant. The presence of streptomycin-resistant isolates in Syrian orchards in Al-Zabadani region (40 km west of Damascus) and Kafer Hor (50 km south west of Damascus) could be explained by the unauthorized importation of infected plant materials and rootstocks from neighboring countries, possibly Lebanon, which is 6 to 10 km from these Syrian regions and where the disease was detected much earlier (Saad et al., 1999).

Recently, the level of virulence of the 75 E. amylovora isolates used in this study was determined (Ammouneh et al., 2008) but no relationship was found between virulence and sensitivity to streptomycin, since both streptomycin sensitive and resistant isolates were identified among the highly virulent ones. Finally, the persistent search for new chemicals against E. amylovora has resulted in the release of new compounds for replacing

![Fig. 1. Growth inhibition of E. amylovora isolate 37 (EaSy37) by different concentrations of streptomycin.](image)

<table>
<thead>
<tr>
<th>Colony response</th>
<th>Copper sulfate concentration (mM)</th>
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<tr>
<td>Resistant (normal growth)</td>
<td>0.08</td>
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<tr>
<td>Tolerant (reduced growth)</td>
<td>0.16</td>
</tr>
<tr>
<td>Sensitive (no growth)</td>
<td>0.32</td>
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<tr>
<td></td>
<td>0.64</td>
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<td></td>
<td>1.2</td>
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<td>2.4</td>
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Table 1. Effect of copper sulfate on the growth of 75 Syrian isolates of E. amylovora.
copper-based compounds and streptomycin, such as oxolinic acid and flumequin which, however, are of little benefit, either because their use is not permitted in many countries or due to phytotoxicity and resistance problems (Thomson, 2000; Shtienberg et al., 2001).

In Syria, fire blight incidence is still sporadic, thus management strategies should focus on monitoring infected foci to prevent movement of E. amylovora-contaminated plant material and disease spreading throughout the country. Ultimately, the development of fire blight resistant cultivar with desirable characteristics holds the best promise for satisfactory and durable control.

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