*Phytopathol. Mediterr.* (2008) 47, 272–276

# Resistance to *Erwinia amylovora* in immature pears induced by acibenzolar-S-methyl in the orchard

ANTONIO MAZZUCCHI and AGOSTINO BRUNELLI

Dipartimento di Protezione e Valorizzazione Agro-Alimentare (DIPROVAL), Sezione di Patologia Vegetale, Università degli Studi di Bologna, via G. Fanin 46, 40127 Bologna, Italy

**Summary**. A field trial was carried out to determine whether resistance to *Erwinia amylovora* could be induced in unripe pears with acibenzolar-S-methyl (ASM), comparing the effectiveness of ASM with a formulate of copper hydroxide. The random block trial was carried out in a commercial pear orchard between June 15 and 29. The treatments were applied in mid-June. Unripe pears were collected from the trees immediately and 1, 2, 4, 7 and 14 days after treatment, and inoculated in the greenhouse within 2–3 hours with a constant dose of a virulent strain of *Erwinia amylovora*. After inoculation, the pears were stored in a moist chamber at 25±2°C in a greenhouse, and after 8 days the diameter of each surface lesion was measured as well as its width and depth inside the pulp. In the fruits of ASM-treated trees there was a reduction in the lesion diameter of the peripheral tissues around the inoculation holes, but not in the deep pulp tissues. Specifically, the symptoms appeared to be milder in the peripheral tissues even 7 and 14 days after treatment with ASM, especially at the lower concentration. The copper compound gave protection to the immature pears for not more than 4 days. These results justify the use of ASM for the protection of unripe fruit in the spring-early summer period to control bacterial fire blight in pear orchards.

**Key words:** fire blight, plant defence, crop protection, BTH, Bion.

## Introduction

Fire blight caused by *Erwinia amylovora* has become endemic in numerous areas of the central and eastern Po valley (northern Italy). Pear, apple and hawthorn are the host species most affected (Calzolari and Contessi, 2002). In pear trees, besides the characteristic symptoms on the blossoms, inflorescences, leaves, shoots, twigs and branches, symptoms are also frequently found on the fruit.

Irregular brownish spots, with lighter brown borders, and sometimes with dark green haloes, appear on the unripe pears and spread until almost the entire surface is covered. The fruit shrivels and becomes mummified. Infection occurs through microlesions of various kinds or wounds caused by hail during thunderstorms.

From May to the end of July, the purpose of chemical treatments is to protect the shoots and unripe pear fruit. Because of the ban on antibiotics, there are very few active principles available in Italy for the chemical control of fire blight. To protect the fruitlets just after setting, the green fruits during swelling, and the shoots, the phytosanitary service recommends applications of copper compounds during the periods at risk, signalled by the forecasting systems (Bugiani and Govoni, 2002). While the copper compounds have a localized action, the benzothidiazoles are systemic active principles that induce resistance (Tomlin, 2006).

Induced resistance to fire blight has been studied in pear blossoms, leaves and shoots, in the greenhouse and in the field, but never on the fruit (Momol *et al*., 1999; Thompson *et al*. 1999a,b; Zel-

Corresponding author: A. Brunelli
Fax +39 051 2096546
E-mail: brunelli@agrsci.unibo.it

ler and Zeller, 1999; Tsiantos and Psallidas, 2000; Brisset *et al*., 2002; Bazzi *et al*., 2003, 2006; Spinelli *et al*., 2006 ).

The aim of this study was to test whether applications of acibenzolar-S-methyl (ASM) induced resistance to fire blight in immature orchard-grown pears in spring and summer. The effectiveness of ASM was compared with that of a copper compound.

## Materials and methods

### Bacterial strain

The virulent strain OMP-BO 1077/7 of *Erwinia amylovora* was used, after routine growth on YDC-agar for 24 h at 27°C. Aqueous suspensions of young cells with a turbidity of $0.060A_{660}$, equivalent to $1\times10^8$ UFC $ml^{-1}$, were inoculated. The strain had been isolated from pear in 1994 in the Emilia-Romagna region (Italy) and is stored by A. Calzolari in the collection of the Servizio Fitosanitario Regionale, Bologna, Italy.

### Chemical treatments

Bion 50WG (Syngenta Crop Protection, Basel, Switzerland), containing the active principle benzo (1,2,3) thiadiazole-7-carbothioic S-methylester (ASM), was used to induce resistance at concentrations of 0.06 g $l^{-1}$ (~100 g $ha^{-1}$) (Bion *min*) and 0.13 g $l^{-1}$ (~200 g $ha^{-1}$) (Bion *max*) (Leadbeater and Staub, 2007). Kocide DF (40% Cu hydroxide WG, Dupont) was used as a reference compound with a localized action at a concentration of 0.4 g $l^{-1}$ (~60g $ha^{-1}$)

### Experimental orchard

A total of 64 six-year-old pear trees cv. Abate Fétel were used. The trees, trained in an espalier form in a commercial orchard at Tresigallo (Ferrara), were divided into 4 random blocks of 16 trees each. Each block contained 4 plots of 4 trees: control, Kocide, Bion *min* and Bion *max*. The pear trees were sprayed on June 15th. Control trees were sprayed with water.

### Pear samples

Just after spraying, when the droplets had evaporated, (time 0), and 1, 2, 4, 7 and 14 days later, 60 pears were collected from each block, 15 from each plot. The pears, 3–4 cm in diameter, were collected at random from the 4 trees in each plot.

The pears collected in bags were placed in fresh containers (15–20 C°) and transferred within 2 h to the greenhouse (at approximately 25–27 C°), where they were inoculated.

### Pear challenge inoculations

A 5 $\mu$l drop of bacterial suspension was placed at a point on the greatest circumference of the unripe pear and then a 1 mm steel needle was used to penetrate the pulp, through the drop, to a depth of 8 mm. To ensure a constant depth, a metal ring with a hole had been welded to the stem of the needle. The inoculum dose was approximately 5000 bacterial cells. After inoculation, the pears were placed vertically in polystyrene support cells (60 cells per support) under a plastic tunnel in a moist chamber and stored in a greenhouse with natural lighting for 8 days at 25±2 C°.

### Phytopathometry and statistics

Eight days after inoculation of the pears the maximum diameter of the external lesions and the depth and width of the internal lesions were measured. The internal lesions were measured in a cross-section of the fruit, at the site of the inoculation point. The results were subjected to statistical analysis using the SPSS program (version 11.01) and the Duncan test was performed ($P$=0.05).

## Results

Kocide, Bion or water were sprayed in the morning of June 15, at which time no natural fire blight infection was visible on any of the fruits. There were very few *E. amylovora* infections in the orchard at that time. In the afternoon of the same day, the pears collected received the challenge-inoculations for time 0. The pears appeared unripe, green, healthy, with diameters of 3–4 cm. During the trial period, the pear trees did not receive any chemical treatment and did not show any signs of natural infection.

The results of the challenge-inoculations on the pears collected in the pear orchard at the various times are shown in Table 1.

The diameters of the external lesions on the pears from the trees treated with Bion were always smaller than those of the pears from the control trees at all 6 inspection times (Table 1, A). With the higher Bion concentration, these differences became significant only after 1, 4 and 14 days, but

not at time 0 or after 2 and 7 days. With the lower concentration, the differences were significant at time 0 and after 1, 4, 7 and 14 days, but not after 2 days. The pears from the trees treated with Kocide also had lesions with a smaller diameter than the controls at the 6 inspection times, but the differences were only significant at time 0 and after 2 and 4 days. The diameter of the lesions in the pears from the trees treated with Kocide was smaller or significantly similar to those of the pears from the trees treated with both concentrations of Bion at time 0 and after 2 days, but not after 1, 4, 7 and 14 days. The percentage of inoculations exhibiting no effect was 2.8%, including the frequencies of the treated and the control pears.

The maximum depth of the internal lesions of the fruits from trees treated with the higher concentration of Bion was smaller than that of the fruits from the control trees for all 6 inspection times, but the differences were only significant for time 0 and after 7 days, not after 1, 2, 4 and 14 days (Table 1, C). For the lower concentration of Bion the depth of the lesions was similar to that of the pears from the control trees and greater after 0, 1, and 2 days.

The maximum width of the internal lesions in the fruits from trees treated with the higher concentration of Bion was smaller than that of the fruits from the control trees at time 0 and after 1, 2, 4 and 7 days, but the difference was only significant for time 0 (Table 1, B). For the lower concentration of Bion, the width of the lesions was smaller and there was a significant difference with the control fruits only after 4, 7 and 14 days. In the pears treated with Kocide the width was significantly smaller than the control fruits at time 0 and after 7 and 14 days.

Table 1. Average diameters (mm) of the external lesions, maximum width and maximum depth of the internal lesions in pears, cv. Abate Fétel, collected in an orchard and inoculated with a virulent strain of *E. amylovora* within 2–3 hours in the greenhouse. The pears were collected just after spraying (time 0) and 1, 2, 4, 7 and 14 days (d) later. The inoculum dose for each wound was approximately 5000 bacterial cells. The pears were stored in a moist chamber for 8 days at 25±2C° in a greenhouse under natural lighting. Each value is the mean of 60 measures. Control pears were sprayed with water. Letters in the same column distinguish the values that are significantly different.

| Treatment | Rate ($g\ ha^{-1}$) | Size (mm) of external lesion after different spraying times | | | | | |
|---|---|---|---|---|---|---|---|
| | | 0 | 1 d | 2 d | 4 d | 7 d | 14 d |
| A - External lesions: maximum diameter | | | | | | | |
| Control | | 13.08 a | 9.11 a | 9.09 a | 11.73 a | 11.53 a | 9.55 ab |
| Kocide | 60 | 7.76 b | 8.37 ab | 7.43 c | 8.85 b | 11.37 ab | 10.17 a |
| Bion *min* | 100 | 7.40 b | 5.40 bc | 7.82 abc | 6.91 c | 8.49 c | 6.80 c |
| Bion *max* | 200 | 10.64 a | 5.51 c | 7.91 ab | 8.68 bc | 11.04 ab | 6.68 c |
| B - Internal lesions: maximum width | | | | | | | |
| Control | | 11.06 a | 7.91 ab | 8.82 ab | 9.42 a | 9.48 a | 5.96 a |
| Kocide | 60 | 9.64 bc | 7.33 b | 8.18 b | 8.34 ab | 8.18 b | 4.64 b |
| Bion *min* | 100 | 10.47 ab | 8.54 a | 9.43 a | 7.78 b | 7.34 b | 5.13 c |
| Bion *max* | 200 | 8.92 c | 6.91 b | 8.62 b | 8.52 ab | 8.39 ab | 6.83 d |
| C - Internal lesions: maximum depth | | | | | | | |
| Control | | 12.73 a | 11.14 ab | 11.71 ab | 11.10 a | 12.35 a | 8.89 a |
| Kocide | 60 | 12.74 a | 10.30 b | 10.53 c | 10.45 a | 12.11 ab | 7.75 b |
| Bion *min* | 100 | 12.83 a | 11.48 a | 12.20 a | 10.28 a | 10.81 c | 8.56 ab |
| Bion *max* | 200 | 11.78 b | 10.36 b | 11.03 bc | 11.00 a | 11.32 bc | 8.34 ab |

## Discussion

To assess the resistance induced in the fruits, the chemical compounds were sprayed on the trees in the orchard and the challenge-inoculations were made with the same dose of inoculum in unripe pears just after collecting, and at preset times after treatment. The fruits were subsequently stored in a controlled environment for 8 days after inoculation.

The diameters of the external lesions of pears from trees treated with Bion were smaller than those of pears from the control trees at all 6 inspection times between the field treatment and the challenge-inoculation. The differences were significant only after 1, 4, and 14 days for the higher concentration, and after 0, 1, 4, 7 and 14 days for the lower concentration. In certain cases, the non-significance of the differences was a consequence of variability in the measurements, probably because of the irregular borders of the lesions, and the decision to measure the greatest diameter. The lower efficacy of Bion at the higher concentration may be the result of phytotoxicity. Although the recommended concentration (0.15 g $l^{-1}$) for pear and apple trees was close to the higher concentration used in this trial (0.13 g $l^{-1}$), it is possible that in the actively swelling, unripe pears, which are true pools of assimilates during the experimental period, the optimum threshold for inducing resistance, involving signal molecules mainly translocated through the phloem, had been exceeded. In tomato a concentration of 0.2 g $l^{-1}$ causes serious phytotoxicity with a marked reduction or halt of growth and leaf chlorosis (A. Mazzucchi, unpublished data). Overall, these results indicate that in unripe pears from trees treated with Bion the tissue around the challenge-inoculation point exhibited an appreciable degree of resistance to *E. amylovora* 4, 7, and above all 14 days after treatment, but not at time 0, or 1 and 2 days later. This indicates that the resistance induced by Bion had an induction period of less than 4 days. On the other hand Kocide, a compound not inducing resistance, gave the greatest protection to pears at 0, 1, 2 and 4 days after treatment, but not after 7 and 14 days. This confirms the generally accepted opinion that the protection given by copper compounds lasts no more than 8–10 days.

The maximum depth and maximum width of the internal lesions in pears collected from trees treated with Bion were significantly smaller than those of the control only 5 times out of 24. This indicates that the internal tissues of pears treated with Bion did not exhibit an appreciable resistance to *E. amylovora*. After challenge-inoculation, the pears were stored in a moist chamber. The relative humidity of almost 100% and the composition of the atmosphere in the intercellular spaces in the deeper part of the pulp may on the one hand have favoured multiplication of the facultative anaerobic *E. amylovora* cells, and on the other have damaged the defence barriers of the plant. Indeed, when Bion was applied to unripe pear slices subsequently inoculated with *E. amylovora,* the amount of exudate on the surface was significantly reduced as compared with the control, and maximum inhibition was achieved with the lowest concentration (0.06%, as compared with 0.12%) (Zeller and Zeller, 1999). This indicates that the aerobic environment of the slices and the concentration of Bion is critical for the expression of resistance in the pulp tissues. It is probable that the resistance induced in this trial was only expressed in the external tissues and not in the deeper ones. The resistance induced in the external tissues of immature pears fully justified the use of Bion to protect the fruit in June–July, as infection of the fruitlets occurs through small surface wounds, caused by hail, rubbing or insects.

## Literature cited

Bazzi C., G. Sponza, A. Brunelli and P. Gianati, 2003. Aspetti della lotta chimica al colpo di fuoco batterico del pero. *Notiziario Tecnico CRPV* 66, 85–95.

Bazzi C., E. Biondi, R. Berardi and A. Brunelli, 2006. Efficacy of bioagents and chemicals against pear shoot blight. *Acta Horticulturae* 704, 283–287.

Brisset M.N., M. Faize, C. Heinz, S. Cesbron, M. Tharaud and J.P. Paulin, 2002. Induced resistance to *Erwinia amylovora* in apple and pear. *Acta Horticulturae* 509, 335–338.

Bugiani R. and P. Govoni, 2002. Modello Cougarblight. Modelli previsionali nella difesa dalle avversità fungine e batteriche. *Il Divulgatore* XXV (5), 66–69.

Calzolari A. and A. Contessi, 2002. Un controllo più intenso per il colpo di fuoco. *Agricoltura* 30(5), 54–56.

Leadbeater A. and T. Staub, 2007. Exploitation of induced resistance: a commercial perspective. In: *Induced Resistance for Plant Defence* (D. Walters, A. Newton and G. Lyon, ed.), Blackwell Publishing, Oxford,UK, 258 pp.

Momol M.T., J.L. Norelli and H.S. Aldwinckle, 1999. Evaluation of biological control agents, systemic acquired resistance inducers and bactericides for the control of fire blight on apple blossom. *Acta Horticulturae* 489, 553–558.

Psallidas P.G. and J. Tsiantos, 2000. Chemical control of

fire blight. In: *Fire Blight. The disease and its Causative Agent, Erwinia amylovora* (J.L. Vanneste ed.) CABI Publishing, Wallingford, UK, 199–234.

Spinelli F., C. Andreotti, G. Spada, I. Ponti and K. Geider, 2006. Chemical control of fire blight: application of prohexadione-calcium, acibenzolar-S-methyl, and copper preparations in vitro and under field conditions. *Acta Horticulturae* 704, 233–237.

Thompson S.V., M.N. Brisset, R. Chartier and J.P. Paulin, 1999a. Induced resistance in apple and pear seedlings to fire blight by Bion and correlation with some defense-related enzymes. *Acta Horticulturae* 489, 583–588.

Thompson S.V., S.C. Gouk and J-P. Paulin 1999b. Efficacy of Bion (Actigard) to control fire blight in pear and apple orchards in USA, New Zealand and France. *Acta Horticulturae* 489, 589–595.

Tomlin C.D.S., 2006. *The Pesticide Manual*. 14th Ed. BCPC, Alton, Hampshire, UK, 1350 pp.

Zeller W. and V. Zeller, 1999. Control of fire blight with the plant activator Bion. *Acta Horticulturae* 489, 639–645

*Accepted for publication: November 26, 2008*