SHORT COMMUNICATION

COMPETENCE OF PEAR SHOOT AND FRUIT WOUNDS FOR *ERWINIA AMYLOVORA* INFECTION

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SUMMARY

Pruning and artificial 'hailstone wounds' on pear shoots and needle wounds in fruitlets were contaminated every 6 hours, starting from a few seconds after injury, and up to 66 h with known inoculum doses of a virulent strain of *Erwinia amylovora*. A single wound was made on each shoot and fruitlet. Cankers on shoots and brown areas on fruits caused by successful infection were observed, respectively, 20 and 8 days after contamination at 25±2°C. The shoot wounds were progressively less competent for infection as the interval increased between wounding and contamination. The largest reduction in competence occurred in the first 24 h after wounding (from 80-85% successful infections to approximately 10-20%). The pear shoot pruning wounds were still competent after 48 h and up to 60 h, although less than 5% of inoculations were successful. Acropetal cankers developed above the 'hailstone wounds' between 35 and 66 h after wounding. Wounds on fruits kept in a damp chamber remained competent longer than those on young pear tree shoots kept at 50-90% relative humidity. The variation in competence in the pear shoot and fruitlet wounds is discussed and compared with other bacterial host-pathogen systems.

Key words: competence, pruning wound, hailstone wound, fire blight, wound repair, wound healing.

Fire blight is destructive when it affects susceptible pear cultivars under environmental conditions favourable to infection and spread of the causal organism *Erwinia amylovora* (*E. a.)* (Van der Zwet and Beer, 1995). A fire blight epidemic occurred in pear orchards of Emilia Romagna, in northern Italy, in the summer of 1997 when there were numerous thunderstorms accompanied by hail (Calzolari *et al.,* 1999). Wounds caused by hailstones provide ideal penetration sites for *E. a.* spread by rain (Van der Zwet and Beer, 1995). Wounds caused by summer pruning, wind and other events can also provide penetration sites.

Plants actively repair wounds which breach their outer protective layers (Baron and Zambryski, 1995). The local response around the dead cells is accompanied by systemic responses in tissues remote from the initial injury (Farmer and Ryan, 1992). Over time, a wound becomes increasingly less competent for inoculation by a pathogen and this competence disappears once repair is complete (Bostock and Stermer, 1989). Ambient temperature is the most important factor affecting competence, although relative humidity and CO_2 and O_2 concentrations play a role. Knowledge of wound competence in various plant organs can be useful to set up effective, low cost, environmentally-friendly measures for integrated control of wound pathogens (Bostock and Stermer, 1989). This communication reports the results of a study on the competence of pear shoot hailstone and pruning wounds for inoculation with *E. a.* The competence of pear fruit wounds was also studied.

At the end of June, we used 2 year-old pear trees cv. 'Abate Fetel', 80-100 cm high, with 6-7 shoots each, grown in individual pots (diameter 30 cm) in the open, and transferred to climatic chambers at 25±2°C in the greenhouse at 50-90% relative humidity, 4 days before the experiment. The immature pear fruitlets cv. 'Conference' used had a diameter of 3-4 cm. The fruitlets were detached in the morning and used on the same day.

Inoculation was performed with the virulent strain OMP-BO 1077/7, routinely grown on YDC-agar slants for 24 hours at 27°C. Ten µl of a 24 hour-old cell suspension were placed on each wound. The inoculum dose was 10,000 bacteria for each pruning wound and 2000 bacteria for each hailstone wound. For the wounds in the pear fruitlets, 5 µl of suspension containing approximately 100 cells were used. The different inoculum doses were calculated on the basis of preliminary experiments. The inoculum doses were prepared by making tenfold dilutions of a 0.060 A_{660} suspension.

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At the start of the experiment (time 0) half of the pear trees were wounded by pruning and half as if by hailstones. A single wound per shoot was made. The wounds were made on shoots of similar length at the internode between the 5^{th} and 6^{th} leaf counted from the tip. The pruning wound consisted of a transverse cut made with secateurs. The hailstone wound consisted of a vertical irregular scratch in the bark of 1-2 cm, often exposing the cambium. The hailstone was simulated by hitting the bark with the rounded tip of a cylindrical metal bar, pushed by a spring from the barrel of a specially designed piston. The percussion was standardized.

At the same time the immature pear fruitlets were wounded with a sterile needle (diameter 0.2 mm) at the equator, down to a depth of approximately 5 mm. The pear fruitlets were placed on individual 1.5 cm high cylinders on the bottom of Plexiglas containers designed for this purpose. Before closing, a thin film of water was poured on the bottom to ensure a high humidity. The pear containers were kept closed in the same chambers as the pear trees.

Starting from the beginning of the experiment (time 0), at 6 h intervals up to 66 h, shoots chosen at random, 7 with pruning wounds and 7 with hailstone wounds, one from each tree, were treated with the bacterial suspension. The same number of shoots chosen at random, with the two types of wounds, were treated with water and used as controls. The suspensions were prepared freshly every 6 hours, to ensure suspensions with the same number of cells, of similar age and physiological state.

The presence and the length (in cm) of the cankers in each group of wounds was monitored 20 days after contamination starting from the pruning wound or the lower edge of the 'hailstone scratch' and moving downwards. At certain times, for the 'hailstone' wounds, the cankers were measured upwards, starting from the upper edge of the scratch. In the pear fruitlets the brown areas were observed 8 days after contamination on the surface around the inoculation hole associated with brownish areas of the pulp with a glassy, water-soaked margin. The competence of the wound at each time was assessed as the ratio between number of successful inoculations and total number of inoculations for each type of wound.

Wound competence decreased progressively from 0 to 48 h for all three types. In the shoots, the competence of 'hailstone wounds' dropped to approximately 15% after 24 h (Fig. 1A); between 0 and 48 h there was an exponential decrease in the percentage of successful inoculations ($\ln y = 0.228 - 0.003 \cdot x^2$; $R^2 = 0.95$). After 30 h and up to 66 h, no wound gave rise to basipetal cankers, but only acropetal cankers causing apical necrosis of the shoots (Fig. 2). Already after 20 days, callus ridges around the 'hailstone wounds' could be clearly seen in control shoots and their apices were vigorous. Obviously, the severity of the 'hailstone wounds'

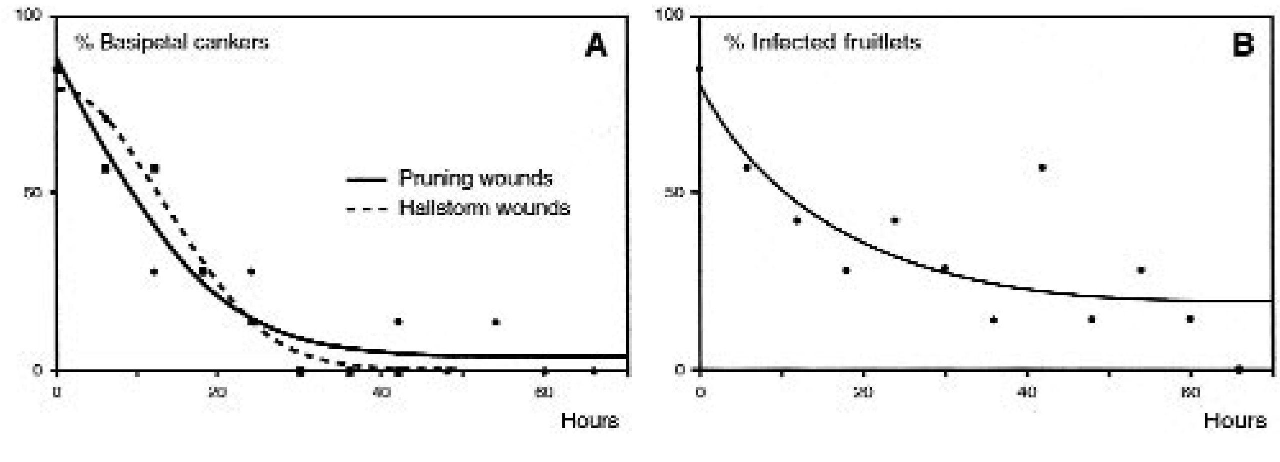

Fig. 1. A. Variation over time in the percentage of basipetal cankers caused by *E. amylovora* following contamination of pear shoot pruning and artificial hailstone wounds made every 6 hours starting from a few seconds (0 h) after wounding. The inoculum dose was 10,000 bacteria for each pruning wound and 2000 bacteria for each 'hailstone wound'. Squares and black circles indicate mean values for hailstone and pruning wounds respectively. The cankers were observed 20 days after contamination. The pear trees were kept in a greenhouse at 25±2°C and a relative humidity of 50-90%. **B.** Variation between 0 and 66 h in the percentage of pear fruitlets infected by *E. amylovora* following contamination of needle wounds made every 6 hours starting from a few seconds (0 h) after wounding. The inoculum dose was 100 bacteria for each wound. The infected areas were screened 8 days after contamination.

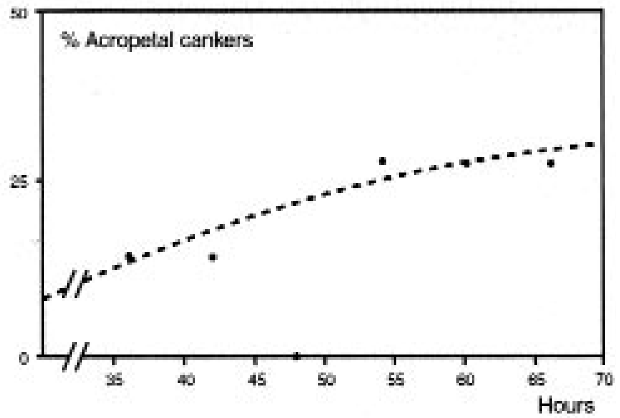

Fig. 2. Variation between 30 h and 66 h in the acropetal cankers caused by *E. amylovora.*

was not sufficient to cause irreparable damage to the distal part of the shoots. Between 36 and 66 h, the percentage of acropetal cankers increased slightly reaching maximum values of around 30% (ln y = 0.858 - $1517.88/x^2$; R^2 = 0.61). These results indicate that the tissues above a 'hailstone wound' remain competent infection sites for longer and, following inoculation, offer less effective barriers to bacterial colonisation. This agrees with previous observations that the tissues between the canker and the shoot tip are more easily colonised (Rosen, 1929; Shaw, 1934).

The competence of pruning wounds also decreased to approximately 15% after 24 h (Fig. 1B); between 0 and 66 h the decrease was exponential [y = 0.036 + 1.421/(1 + exp - (x - 3.025)/-8.61); R^2 = 0.91] with values of just a few percent after 66 h.

In the pear fruitlets, wound competence decreased over time with an exponential curve [y = 0.179 + 0.627 exp(-x/15.46); R^2 = 0.67], and after 24 h was approximately 30%. The decrease was less than that in the shoot wounds and after 66 h the percentage of successful infections remained around 20%.

As expected, these results indicate that the percentage of successful *E. amylovora* inoculations decreases as time between wounding and contamination increases in both shoots and fruits. This agrees with the findings in the same system (Crosse *et al.*, 1972; Van der Zwet and Keil, 1972) and in others (Hewitt, 1938; Kiryu *et al.*, 1954; Goto, 1990). The largest decrease in competence occurred within 24 h. In pear tissues this may be due to cell wall modification, associated with accumulation of tannin, callose, pectic substances, gums and suberin, but not to periderm formation (Spotts *et al.*, 1998). On the other hand, wound competence for *Agrobacterium tumefaciens* infection varies with a bell-shaped curve (Lipetz, 1966); in this case, however, it is related to tumour transformation (Sheng and Citovsky, 1997) as well as bacterial colonisation of the apoplast. Under our conditions, after 48 h, the 'hailstone' and pruning wounds still showed a low competence. This is in agreement with the variations in competence over time of apical excision wounds in apple shoots (Crosse *et al.*, 1972) but not with those in sandblasted pear shoots (Van der Zwet and Keil, 1972).

The wounds on the fruits offered over time a higher number of successful infection sites than those on the shoots. This higher risk of infection is emphasised by the fact that the inoculum dose for the fruitlets was 100 bacterial cells, *i.e.* 20 and 100 times less than those used for the 'hailstone' and pruning wounds. A dose of 100 cells is equivalent to 10 times the minimum inoculum threshold on fruits using the same technique (data not shown). The higher competence over time of the fruit wounds may be explained by the prolonged period in the moist chamber. This favours endophytic bacterial growth (Shaw, 1935), and slows down the repair process (Lipetz, 1970; Bostock and Stermer, 1989). This may explain the discrepancy between our data and those from orchard inoculations, where fruit wounds are no longer penetration sites by 48 h (Van der Zwet and Keil, 1972).

Thunderstorms with hail or other wounding events in *E. amylovora* host plants require immediate antibacterial treatment, or at least within 24 h (Van der Zwet and Beer, 1995). Our results confirm the potential efficacy of early treatment. After 24 h and up to at least 66 h, our results indicate that there is still a risk of infection although the percentages are low in both the shoots and in the fruits. If antibacterial treatment is delayed, the active principle used should have a curative as well as a preventive action.

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