A BIOASSAY TO MEASURE IN THE LABORATORY THE INHIBITION OF FIRE BLIGHT BY DIFFERENT BIOLOGICAL CONTROL AGENTS AND THEIR DERIVATIVES

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Fire blight is an economically important bacterial disease of apple and pear, for which we are developing a biological control strategy (Vanneste *et al.* 1995b). Studies on biological control agent / pathogen interactions have been hindered by the lack of a simple and reliable assay that would allow, in the laboratory, the assessment of a large number of biological control agents or of their derivatives. The current assay was developed more than 10 years ago and consists of pipetting into a well, bored in the cheek of a pear fruit cut in half, a suspension of the biological control agent followed by a suspension of the pathogen (Beer and Rundle 1983). These fruits were then incubated in a humid chamber for up to 6 days. This assay had several limitations, the major one being that only pears incubated in the same humid chamber could be compared (Vanneste *et al.* 1992). No more than four treatments could be compared in one experiment, and, since only six pear halves per treatment could be used, the statistical analysis of the results was practically prevented. Furthermore, detecting the first signs of infection, presence of exudate in the well, could sometimes prove quite difficult leading to a false negative. A false positive might also be recorded because not all the surface of the well was protected with the biological control agent before inoculation.

By using cores of immature pear fruit (ca. 2 mm thick and ca. 4 mm diameter) we were able to overcome all these shortcomings. Pears were surface disinfected with ethanol 70%, sliced and cored using a sterile cork borer number 1. The cores were placed on a wet paper towel in a large tray used as a humid chamber. Twenty µl of a biological control agent suspension or of MgSO₄ 10 mM, used as a negative control, were pipetted over the entire surface of the core and allowed to dry before the cores were inoculated with 10 ml of a suspension of the fire blight pathogen. The flat surface of the core was examined under a magnifying glass for presence of exudate, which proved very easy and reliable. As described this technique eliminated the false positive and false negative results found in the previous assay. Furthermore, cores were small enough such that it was possible to routinely compare twelve different treatments (biological control agents, bacterial metabolites or chemicals), using 12 cores per treatment allowing statistical analysis of the results.

Using this bioassay we compared the ability of Eh252, a strain of *Erwinia herbicola* that reduces fire blight incidence in apple and Asian pear orchards (Beer *et al.* 1984; Vanneste and Yu 1990, 1993; Vanneste *et al.* 1995a), to protect pear fruit from fire blight, with that of other biological control agents. Analysis of the data using generalised linear models with logit link and errors proportional to the error structure for a binomial model, showed that Eh252 and E1 were significantly better ($P < 0.001$) than other strains of biological control agent isolated so far including Eh318, and Eh112Y (Fig. 1). Using the same analysis, we were also able to confirm the role of an inhibitory peptide produced by Eh252 in the control of fire blight. Derivatives of Eh252 that had lost the ability to produce this inhibitory compound (10:12, 10:12

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FIGURE 1: Control of fire blight on pear cores by different biological control agents.

pCPP25 and C5) were significantly ($P < 0.001$) less able to protect pear from fire blight than derivatives that produce this peptide (Eh252, 10:12 pANT3 and 10:12 pANT5) (Fig. 2). Pear cores were also used to assess the pathogenicity and the virulence of different strains of the fire blight pathogen.

This new bioassay will be used to identify the best biological control agents for fire blight and to study their mode of action.

FIGURE 2: Effectiveness of different derivatives of Eh252 for control of fire blight on pear cores.

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