EVALUATION OF A SIMPLE METHOD FOR DETECTION OF FIRE BLIGHT

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Cultural methods have traditionally been used to monitor the epiphytic populations of *Erwinia amylovora* on apple flowers. This involves washing a sample of flowers or selected floral parts in saline buffer and plating an aliquot of the washing on a culture medium for growth of the bacterium. However, the method can be tedious when processing a large number of samples. A simple stigma-blot technique was shown to be effective for detection of *E. amylovora* (Thomson 1992). *Erwinia amylovora* multiplies preferentially on the stigmas, so by streaking the stigmas on a selective medium, the bacterium can be detected. This technique was used in 1991 and found to have potential for use in New Zealand (Thomson pers. comm.). Another trial in the spring of 1992 further evaluated the use of this technique and compared quantitative detection of *E. amylovora* using stigma-blot and washing techniques.

Gala apple trees in full bloom were sprayed to runoff with a suspension of *E. amylovora*, strain Ea8865 at approximately 106 colony forming units/ml (CFU/ml). After the spray droplets had dried, four flowers were collected from each of five trees for processing using the stigma-blot and the washing methods. The same number of samples was collected for bulk wash to determine the total inoculum loading on the flowers. Samples were collected four times over 3 days.

In the laboratory, two pistils were aseptically removed from each flower and shaken in 2 ml phosphate-buffered saline (PBS) for 2 minutes using a vortex mixer. The washing was plated on CCT medium (Ishimaru and Klos 1984) amended with 100 ug/ml each of rifampicin and nalidixic acid (CCTnr). The remaining three pistils were streaked lightly on the medium covering a quarter of the surface. With the bulk wash method, samples of four flowers from each tree were shaken by hand in 2 ml PBS for 2 minutes, and the washing plated onto CCTnr.

Out of a total of 80 flowers tested over 3 days, there was no significant difference between the two methods in the percentage number of samples which yielded *E. amylovora* (Table 1). The stigma-blot method did not detect the presence of *E. amylovora* on nine flowers, whereas the washing method showed six of these flowers had 10 - 40 CFU/pistil, and three had 195 - 545 CFU/pistil. The washing method did not detect the presence of *E. amylovora* on five flowers which had 1 - 15 CFU/pistil as indicated by the stigma-blot method. It is not known whether the five pistils of apple flowers may have senesced at different rates which affected the growth of bacteria differently. In practice, all five pistils will be blotted onto the medium which will help to increase the chance of detection, whereas, in this trial only some of the pistils were used. In terms of savings in labour and materials, the stigma-blot method may be suitable for large scale monitoring where an indication of the presence of *E. amylovora* on individual flowers is required.

The mean numbers of bacteria recovered at each sampling period are shown in Table 2. The washing method yielded on average 10.7 times more bacteria per pistil than the stigma-blot method. It is apparent that when the bacterial population is below 10 CFU/pistil, there is a chance that the stigma-blot method may not always detect the bacteria. The washing method is clearly more suitable for quantitative studies. Healthy flowers usually contain large epiphytic populations such as 105 - 107 CFU per flower

(Thomson 1986), which are 102 - 104 fold higher than that found on the inoculated flowers in this study. On that basis, the stigma-blot method may be sufficiently sensitive for indicating the presence of bacteria before the appearance of blossom blight symptoms.

TABLE 1: Number of flowers with detectable *E. amylovora* populations using the stigma-blot and washing methods.

Methods	With *E. amylovora*	No *E. amylovora*
Stigma-blot	69	11
Washing	74	6
Both methods	78	2

TABLE 2: Mean populations of *E. amylovora* recovered from the pistils of apple flowers using the stigma-blot and the washing methods, and from the entire apple flower using the bulk wash method.

Date	Washing	Stigma-blot	Bulk wash
	CFU/pistil		CFU/flower
14/10/92	73.0	9.6	4790.0
15/10/92	49.5	3.4	1220.0
15/10/92	41.5	15.2	1140.0
16/10/92	151.0	1.6	885.0
Mean	79.0	7.4	2010.0
SED	6.1	9.9	785.0

Erwinia amylovora populations on the entire flower (bulk wash) declined over the 3 days, while that on the pistils as detected by the washing method increased during the same period. This is caused by multiplication of bacteria on the stigmatic surface, while the populations on other flower parts declined.

The simple and rapid stigma-blot method will be evaluated further for monitoring epiphytic populations of *E. amylovora* on apple and pear blossoms in commercial orchards. The information will be used to assist interpretation of likely fire blight risk predicted by the Maryblyt model (Steiner 1990) and decision making on control strategy.

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