

DISTRIBUTION OF BENEFICIAL BACTERIA ON NASHI AND APPLE FLOWERS USING HONEY BEES

D.A. CORNISH, M.D. VOYLE, H.M. HAINE, R.M. GOODWIN
and J.L. VANNESTE

*HortResearch, Ruakura Research Centre, Private Bag 3123,
Hamilton, New Zealand*

ABSTRACT

Honey bees have previously been shown to disperse biocontrol agents of fire blight, such as *Erwinia herbicola* Eh252, onto apple flowers. In this study we investigated whether bees could also effectively disperse Eh252 onto nashi flowers. After providing lyophilised bacteria to two bee hives in a commercial nashi orchard for seven days, 83% of the flowers were colonised with Eh252. After removal of bacterial inoculum, 95 % of newly opened flowers were still colonised, indicating transfer of beneficial bacteria from flower to flower. In a similar experiment carried out three weeks later in an apple orchard, the rate of colonisation of apple flowers by Eh252 in the first three days was even greater.

Keywords: Fire blight, honeybees, biological control, nashi, apple.

INTRODUCTION

Fire blight, caused by *Erwinia amylovora*, is a bacterial disease which affects certain commercial crops such as apple (*Malus pumila*) and nashi (*Pyrus pyrifolia*). *Erwinia herbicola* Eh252 is a biological control agent that has been shown to inhibit the growth of *E. amylovora* on plates (Vanneste *et al.* 1992), as well as reducing the incidence of fire blight in field trials (Vanneste 1996).

During the blooming period, flowers are the main point of entry for *E. amylovora*. The bacteria colonise the stigma, move to the nectaries and then enter the plant tissue (Vanneste 1995). Once plant tissue has been infected, disease development can only be stopped by removing the diseased tissue. Limiting the incidence of fire blight is therefore best achieved by preventing *E. amylovora* from entering the plant tissue. This means that biological control agents are most efficient when brought directly to the stigma as soon as the flowers open, preventing *E. amylovora* from colonising the flower.

We have shown previously that honey bees (*Apis mellifera*), currently used in orchards to aid pollination, could be used to disperse beneficial bacteria onto apple flowers (Vanneste 1996). However there are differences between apple and nashi which could limit the dispersal of Eh252 on nashi flowers by honey bees, for example, nashi flowers bloom approximately three weeks earlier than apple flowers, when weather is not as warm, and when bees are not as active. Honey bees also appear to be more attracted to apple flowers than nashi flowers. In this study we conducted a series of experiments to determine whether honey bees could be used to disperse Eh252 onto nashi flowers.

MATERIALS AND METHODS

Strain and media

A spontaneous rifampicin resistant strain of *E. herbicola* Eh252, called Eh252R was used in the experiments. This strain was grown on Luria plates (25 g/l of Gibco BRL Luria broth and 12 g/l bacteriological agar) supplemented with rifampicin (20 µg/ml). This medium was used for selective recovery of Eh252R from field samples.

Preparation of lyophilised powder of *Erwinia herbicola*

Sterile trays (37 x 26 x 6 cm) were filled with approximately 300 ml of Luria agar supplemented with 1% glycerol and rifampicin (20 µg/ml). The trays were then inoculated with 1 ml of overnight culture of Eh252R, wrapped in plastic bags and incubated for two to three days at room temperature. Cells were harvested using a glass spreader and mixed with 18% (w/v) skim milk powder and 1% (w/v) xanthan gum (Sigma) before being freeze-dried and ground into a fine powder through a sieve (mesh no. 68). Lyophilised powder was stored at -20°C. The population of Eh252R was approximately 2.2×10^{11} colony forming units (cfu)/g, as determined by dilution plating onto selective medium.

Orchard trials

The trial was conducted in a commercial nashi orchard in Waikato, using 109 trees of nashi cultivar Kosui distributed in a 4300m² block of mixed varieties. Two bee hives were placed in one corner of the block. A pollen insert, a device which allows bees to pick up pollen or other materials before exiting the hive, was attached to each hive and 5 g of lyophilised bacteria placed into the insert at least once, and up to three times a day between 9.30 am and 3.30 pm. Powder was not loaded during rainfall. A total of 75 g of powder was loaded per hive over a 7 day period.

A second trial was conducted in a commercial apple orchard in Waikato using 184 Royal Gala trees (~2 years old). A hive was placed at one end of the block. A pollen insert was attached to the bee hive and 5 g of lyophilised powder was loaded into the insert up to four times a day between 8 am and 2 pm. A total of 50 g of lyophilised powder was loaded over a 3 day period.

Prior to the trials, the presence of naturally occurring rifampicin resistant bacteria indistinguishable from Eh252R on flowers was determined. One hundred nashi flowers 2-3 days old, were randomly picked. Petals and stems were removed before washing all the flowers together in 54 ml of 10 mM MgSO₄ solution. One hundred µl of the washing solution was spread onto selective medium. After overnight incubation at 28°C, bacterial colonies were counted. One hundred apple flowers were individually washed in 1 ml of 10 mM MgSO₄. Four 20 µl drops of each washing solution were pipetted onto selective media. Plates were incubated overnight at 28°C and bacterial colonies were counted.

To determine the percentage of flowers colonised with Eh252R, two flowers (2-3 days old) were collected from the 184 apple trees each day for three days or from the 109 nashi trees each day for seven days. Petals and stems were removed before washing flowers individually in 1 ml of a 10 mM MgSO₄ solution. Four 20 µl drops of each flower washing solutions were pipetted onto selective medium, incubated overnight at 28°C and bacterial colonies counted.

For the nashi trial, the pollen inserts and powder were removed on the seventh day and the hive entrance cleaned of any visible Eh252R powder. Flowers, 2-3 days old were collected from the 109 nashi trees four and eleven days later and checked for the presence of Eh252R as described above.

RESULTS

Colonisation of nashi flowers

No naturally occurring rifampicin resistant bacteria were found in the control sample of nashi flowers prior to the introduction of *E. herbicola* Eh252R. The percentage of flowers colonised with Eh252R increased steadily to reach 81% on day 7 (Figure 1). On day 11, 4 days after removal of the pollen insert, the percentage of colonisation was slightly lower, but on day 18, had increased to 95%. The population of Eh252R on nashi flowers ranged between 50 and 2.5×10^4 cfu/flower. The mean population of Eh252R present on colonised flowers increased over time (Table 1).

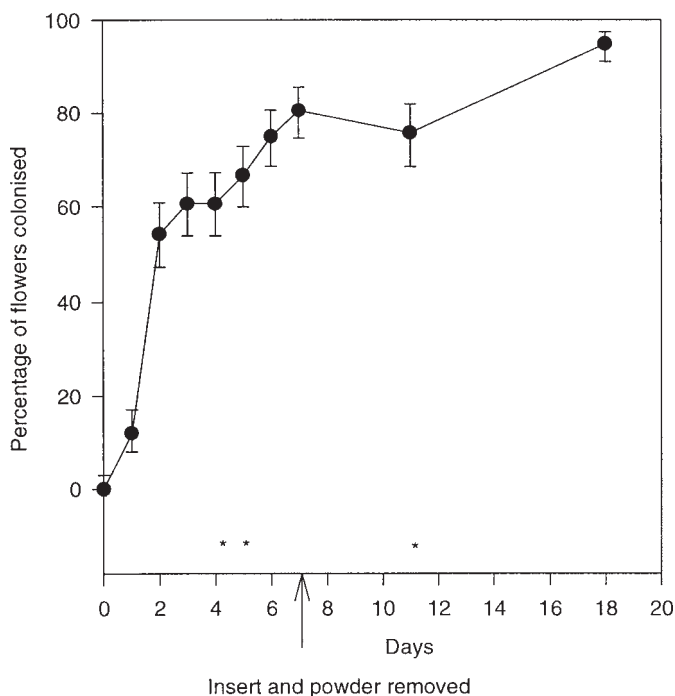


FIGURE 1: Percentage of nashi flowers colonised by *E. herbicola* Eh252R (bars represent the 95% confidence interval).

* = Rainfall between 1.8 and 13.4 mm.

TABLE 1: Mean population of *E. herbicola* Eh252R on colonised nashi flowers.

Day	Geometric Mean	\log_{10} (sem)
1	1.2×10^2 cfu/flower	2.10 (0.156)
7	4.0×10^2 cfu/flower	2.60 (0.067)
11	3.1×10^2 cfu/flower	2.49 (0.068)
18	5.0×10^3 cfu/flower	3.36 (0.052)

Colonisation of apple flowers

No naturally occurring rifampicin resistant bacteria were found in the control sample of apple flowers tested before the introduction of Eh252R. The percentage of flowers colonised by Eh252R was 62.5%, 81% and 83% on days 1, 2 and 3 respectively. The population of Eh252R on apple flowers ranged between 12 and 2.0×10^4 cfu/flower. The mean population of Eh252R found on apple flowers for day 1, 2 and 3 increased slightly (Table 2). The rate of colonisation by Eh252R in these two experiments is significantly different (Figure 2).

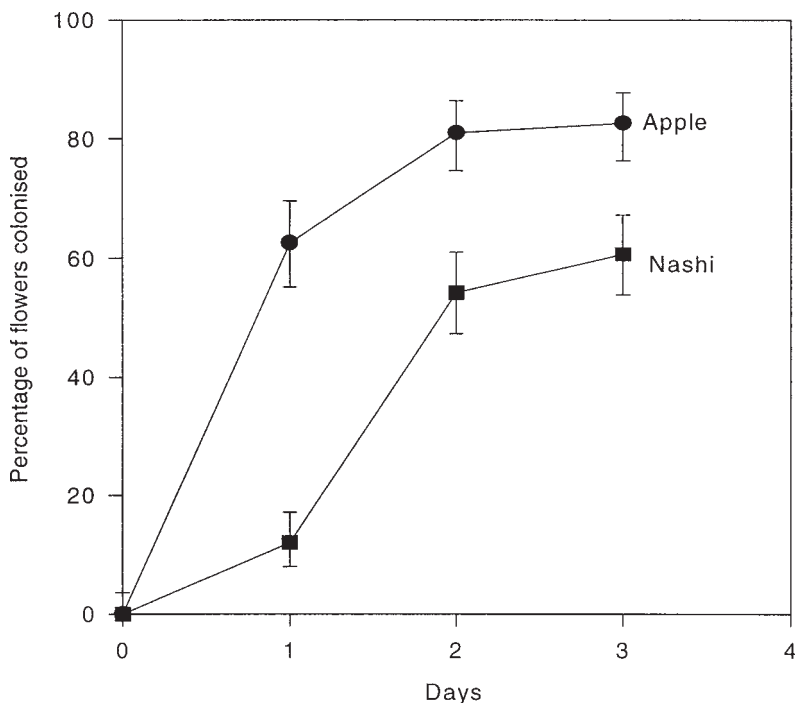


FIGURE 2: Percentage of apple and nashi flowers colonised by *E. herbicola* Eh252R (bars represent the 95% confidence interval).

TABLE 2: Mean population of *E. herbicola* Eh252 on colonised apple flowers.

Day	Geometric mean	\log_{10} (sem)
1	2.2×10^2 cfu/flower	2.33 (0.072)
2	5.5×10^2 cfu/flower	2.74 (0.067)
3	4.8×10^2 cfu/flower	2.68 (0.062)

DISCUSSION

Honey bees have been shown to disperse the biological control agent *E. herbicola* Eh252R on apple flowers (Vanneste 1996). The goal of these experiments was to determine whether bees could also be used to disperse beneficial bacteria onto nashi flowers. The rate of colonisation of flowers by beneficial bacteria is affected by the frequency of bee visits to these flowers. This is affected by different factors such as presence of flowers from other plants, presence of nearby bee hives without pollen inserts, nectar production, temperature, wind and rain. The faster rate of colonisation of apple flowers by Eh252R, compared to that of nashi flowers, as seen in these two experiments, may be due to some of these factors. The apple trial was carried out three weeks after the nashi trial, when the weather was warmer and when bees were more

active. There was no rainfall during the apple trial, while it rained on three different days during the nashi trial. Rain limited the amount of powder loaded into pollen inserts as well as decreasing bee activity. Furthermore, as noted earlier, honey bees appear to be more attracted to apple flowers than nashi flowers.

The presence of flowers on other plants or presence of other food sources would also divert bees from target flowers, impacting directly on their effectiveness to vector biological control agents. Such a situation might have occurred in the apple experiment. After day 3, the insert was still being filled regularly, but very few bees were seen visiting apple flowers, and we observed a concomitant decrease in the percentage of apple flowers colonised by Eh252R after day 3 (unpublished data).

After the removal of the pollen insert and powder, 75 % of nashi flowers were colonised by Eh252R. These flowers were closed when the pollen insert was removed. The presence of Eh252R on these flowers suggest that bacteria were transferred from flower to flower, most probably by bees and other insects. This transfer proved to be efficient leading to 95 % of the flowers colonised 11 days after removal of the insert. This allows further dispersal of biological control agents without the need to load more lyophilised powder into the pollen insert.

ACKNOWLEDGEMENTS

We thank Warren Sexton and Kent Hodgson for the use of their nashi and apple orchard respectively. We also thank Barbara Dow for statistical analysis. This work was partly funded by the Foundation of Research Science and Technology.

REFERENCES

- Vanneste, J.L., Yu J. and Beer S.V., 1992. Role of antibiotic production by *Erwinia herbicola*, Eh252 in biological control of *Erwinia amylovora*. *J. Bacteriol.* 174: 2875-2796.
- Vanneste, J.L., 1995. *Erwinia amylovora*. Pp. 21-46 In: Pathogenesis and Host-Parasite Specificity in Plant Diseases. Vol. 1 K. Khomoto, R.P. Singh and U.S. Singh (Eds); Pergamon Press, Oxford, U.K.
- Vanneste, J.L., 1996. Honeybees and epiphytic bacteria to control fire blight, a bacterial disease of apple and pear. *Biocontrol News and Information.* 17, 67N - 78N.