THE EFFECT OF RAIN ON THE DEVELOPMENT OF *ERWINIA AMYLOVORA* AND *E. HERBICOLA* POPULATIONS ON APPLE FLOWERS

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SUMMARY

The development of epiphytic populations of the fire blight bacterium, *Erwinia amylovora*, and an antagonist, *E. herbicola*, on apple pistils and flowers with pistils removed was monitored in the spring of 1991. Heavy rain which occurred 2 days after inoculation significantly reducing the populations of both *E. amylovora* and *E. herbicola* on the pistils but there was a concurrent increase in the populations on the rest of the flower. These results agree with reports from overseas that *E. amylovora* develops primarily on the pistils and is readily spread to the hypanthia by rain where infections may occur.

Keywords: fire blight, *Erwinia amylovora*, *Erwinia herbicola*, apple, rainfall

INTRODUCTION

Epiphytic colonization of flowers by the fire blight bacterium, *Erwinia amylovora*, occurs primarily on the stigmata rather than on other flower parts (Thomson 1986). Under natural conditions it appears that small populations of bacteria arrive on flower parts via bees, other insects, splashing rain and drift of air-borne bacteria and occasionally bacterial strands. These low populations may multiply to populations of 10^3 to 10^6 colonies/flower on apparently healthy stigmata. Rain or dew probably plays a significant role in the movement of these epiphytic populations to other flower parts including the hypanthium where infection can occur through the nectarhodes (Thomson 1986). Antagonists such as *E. herbicola* may also be present on the stigmata but their epiphytic growth and fate is unknown. This study was undertaken to determine the destiny of *E. amylovora* and *E. herbicola* when sprayed on the entire flower and the effect of rain on epiphytic populations.

MATERIALS AND METHODS

The site selected for this study was a 15-year-old apple cv. 'Royal Gala' orchard near Hamilton, New Zealand. The re-isolation of the inoculated strains, Ea8865 and Eh318, was facilitated by selecting for resistance to the antibiotics rifampicin and nalidixic acid each at 100 µg/ml. Wild strains were streaked on media with the individual antibiotics incorporated and resistant colonies were selected and confirmed to be identical to the wild type strains in all other respects. Inoculum was grown overnight in shake culture of nutrient broth at 21°C and diluted in tap water to a final concentration of 1×10^6 CFU/ml and 3×10^6 for Ea8865 and Eh318 respectively. Five trees in full bloom were sprayed until runoff with each bacterium with a backpack sprayer.

Three individual flowers from each treated tree were transported to the laboratory in kiwifruit trays and processed within 4 h. Flowers were sampled within 1 h of inoculation and at 1, 2, 3, 4, 5 and 7 days. Sterile forceps were used to remove the five pistils from each flower and place them in 1 ml sterile saline in a 10 ml disposable tube (17 x 100 mm). The pistils were mixed (vortex) for 1 min and a 0.1 ml aliquot was spread on King's medium B (KB) (King *et al* 1954) plus 100 mg/ml of both nalidixic acid and rifampicin (KBnr). The remaining flower portion with the pistils removed was vortexed for 1 min in 1 ml of sterile saline and a 0.1 ml aliquot spread on KBnr.

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Plates were incubated at 28°C for 24–36 h and the colonies typical of Ea8865 and Eh318 were counted. Values are the means of three flowers per tree and five single-tree replications.

RESULTS AND DISCUSSION

There were no wild type bacteria detected in the orchard with resistance to both nalidixic acid and rifampicin nor were there any natural populations of *E. amylovora* or *E. herbicola* detected.

Rain fell on every day after inoculation except day 6 or 144 h (Fig. 2). On most days the accumulation was light but on day 3 there was 29.2 mm of rain. Temperatures averaged between 11 and 13°C during the bloom period; too cool for natural epiphytic populations of *E. amylovora* to develop (Thomson 1986).

The populations on the flower (with pistils removed) immediately after inoculation were 841.4 CFU/flower and 263 CFU/flower for Ea8865 and Eh318 respectively whereas the populations on the pistils were 139.9 CFU/pistil and 132.0 CFU/pistil. However, within 24 h the populations on the flower portion declined to 31.3 CFU/flower and 24.9 CFU/flower for Ea8865 and Eh318 respectively while pistil populations increased to 363.7 CFU/pistil and 346.7 CFU/pistil. The populations of both Ea8865 and Eh318 continued to show an increase on the pistils in the 48 h sample while populations on flowers remained the same or declined slightly when compared to the 24 h sample (Fig. 1).

Fig. 1: Effect of rain on populations of *E. amylovora* and *E. herbicola* on flowers and pistils.

Fig. 2: Daily rainfall (mm) during the trial.

Conditions appeared to be more favourable for multiplication of bacteria on pistils than on the flowers and their populations increased dramatically even under the unfavourable temperatures. The decline in populations on the flower portion may reflect an inhibition of bacterial multiplication but was more likely to be due to a lack of free moisture. The stigmatic surface of the pistils is hygroscopic and contains a hydrophilic compound that maintains the relative humidity in excess of 98% (Ferrari *et al* 1981).

The rain on day 3 caused a dramatic shift in the populations on the pistils and flowers. Flower (without pistils) populations of Ea8865 increased by 62 times and Eh318 by 4.2 times and pistil populations of Ea8865 declined by 38 times and Eh318 by 29.8 times. After the heavy rain the populations reversed again with pistil populations increasing and flower (without pistils) populations generally declining over the next 4 days. The light rains may not have been sufficient to move the bacteria from pistils to flower portions. It should also be noted that flowers selected on days 5 and 7 may not have been inoculated on the first day since no effort was made to remove unopened flowers at the time of inoculation. The populations on these flowers probably originated from natural spread of Ea8865 by bees, other insects or rain.

Rain appeared to have a greater effect on the movement of Ea8865 compared to Eh318 but this study would have to be repeated to confirm this observation.

This study clearly shows that *E. amylovora* and *E. herbicola* multiply preferentially on the pistils when compared to other flower parts and that heavy rain was necessary to relocate the bacteria from the stigmata to other flower parts. It also demonstrated that substantial populations of bacteria are distributed onto the pistils when the entire flower is sprayed. Previous research (Thomson 1986) has shown that when specific populations were specifically placed on either the flower or pistil with a micro-syringe, the populations declined on the flower and increased on the pistil. However, after rain the pistil population declined and the bacteria were found on the flower.

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