INTERACTIONS BETWEEN AN ANTAGONIST, *ERWINIA HERBICOLA*, AND *E. AMYLOVORA* AND POTENTIAL FOR BIOLOGICAL CONTROL OF FIRE BLIGHT

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SUMMARY

Studies were conducted in a controlled environment and in an apple cv. 'Royal Gala' orchard during the spring of 1991 to evaluate biological control of fire blight, caused by *Erwinia amylovora*, using an antagonist, *E. herbicola*, strain Eh318. The effects of the interactions on populations of *E. amylovora* and *E. herbicola* on apple pistils alone and on remaining flower parts were monitored. In both studies, apple trees pre-inoculated with Eh318 had a lower incidence of fire blight compared with trees not pre-inoculated with Eh318. In a controlled environment, the populations of *E. amylovora* on pistils pre-inoculated with Eh318 declined 24 h after inoculation while populations of *E. amylovora* alone increased. Under both field and controlled environment conditions, the populations of Eh318 on the pistils and flowers were not affected by the presence of *E. amylovora*.

Keywords: fireblight, *Erwinia amylovora*, *Erwinia herbicola*, apple, biological control

INTRODUCTION

Biological control of fire blight has been successful with the use of antagonists such as *Erwinia herbicola* and *Pseudomonas fluorescens* (Beer *et al* 1984). The suggested mechanisms for the protection include the production of antibiotics (Vanneste *et al* 1990), or competition for nutrients (Farabee and Lockwood 1988) by the antagonists. Thomson (1986) described the important role of the stigmata in the epiphytic development and infection of flowers by the pathogen. The growth of *E. amylovora* was significantly inhibited on hawthorn flowers by the presence of *E. herbicola* (Wilson *et al* 1989) and the site of the inhibitory interaction was shown to be the stigmata (Hattingh *et al* 1986; Rundle and Beer 1987). The interactions of the pathogen and the introduced antagonist on the infection site, the flower, may provide some insights into the mechanism of biological control. This study was designed to evaluate the influence of the antagonist, *E. herbicola* strain Eh318, on the multiplication and pathogenesis of *E. amylovora* on specific parts of apple flowers.

MATERIAL AND METHODS

Field study

The field study was conducted on 15-year-old apple trees cv. 'Royal Gala' near Hamilton, New Zealand. *Erwinia amylovora* strain 8865 (Ea8865) and *E. herbicola* strain 318 (Eh318) were selected for resistance to nalidixic acid and rifampicin each at 100 µg/ml, by streaking the bacteria on media with the antibiotics incorporated. Inoculum was prepared from overnight shake cultures grown in nutrient broth at 21°C. The final concentration was adjusted in tap water to 1 x 10⁶ and 3 x 10⁶ colony forming units/ml (CFU/ml) for Ea8865 and Eh318, respectively. Trees were in full bloom when inoculated by spraying the entire tree with the appropriate bacterial suspensions using a Solo backpack sprayer. Treatments included: Ea8865, Eh318, Eh318 followed 24 h later with Ea8865, streptomycin 100 mg/ml followed 24 h later with Ea8865, and a check sprayed with tap water only. The experimental design was a completely randomized block with single tree treatments replicated five times.

Proc. 45th N.Z. Plant Protection Conf. 1992: 295-300

To assess the indigenous populations for *E. amylovora* and other bacteria for resistance to rifampicin and nalidixic acid, 25 flowers were sampled in bulk, placed in a plastic bag and shaken vigorously for 2 min in 25 ml sterile saline to wash off the bacteria. Aliquots (0.1 ml) of the wash water were spread on a selective medium CCT (Ishimaru and Klos 1984), King's medium B (KB) (King *et al* 1954) and CCT supplemented with 100 mg/ml rifampicin and nalidixic acid.

Populations of Ea8865 and Eh318 were determined on flowers and pistils by sampling three individual flowers from each treated tree, 1 h after inoculation and at 1, 2, 3, 4, 5 and 7 days. Flowers were taken to the laboratory where they were processed within 4 h. Pistils were removed from each flower with sterile forceps and washed in 1 ml sterile saline in 10 ml disposable tubes (17 x 100 mm). After mixing (vortex) for 1 min, a 0.1 ml aliquot was spread on KB and CCT plus rifampicin and nalidixic acid (KBnr and CCTnr). The remaining flower portion with the pistils removed was mixed for 1 min in 1 ml of sterile saline and a 0.1 ml aliquot spread on KBnr. Hereafter the flowers with pistils removed will be termed the "flower portion". Plates were incubated at 28°C for 24-36 h and the colonies typical of *E. amylovora* and *E. herbicola* were counted.

The incidence of fire blight on each tree was determined on 27 November, 51 days after the inoculation, by counting all of the fire blight infections. Isolations were made from 10 infected clusters by plating on CCT and CCTnr.

Controlled environment study

A similar study was conducted in a controlled environment room (CE) at 23°C and 75-85% relative humidity using potted apple cv. 'Royal Gala' trees in full bloom. The trees were 1.5 m tall with a trunk diameter of 25 mm. All unopened flowers were removed 1 day before inoculation. Trees were sprayed to run-off with a hand-held sprayer. Treatments included: Ea8865, Eh318, Eh318 followed 24 h later with Ea8865 and a water sprayed check. The inoculum levels for both Eh318 and Ea8865 were adjusted to 1×10^6 CFU/ml. A streptomycin treatment was not included due to a shortage of trees.

There were four trees with approximately the same number of flowers for each treatment. Trees were removed from the CE room at the time of inoculation to avoid the possibility of drift or cross contamination. Ten individual flowers collected from the four trees were sampled and processed, as noted in the field study, within 1 h of inoculation and at 1, 2 and 3 days after inoculation. Fire blight infections were counted on 11 November, 30 days after inoculation.

Data were analysed by ANOVA after $\log_e (X + 1)$ transformation.

RESULTS AND DISCUSSION

The bacterium Eh318, significantly reduced the incidence of fire blight in the orchard study (Table 1). The environmental conditions were not conducive for fire blight with precipitation occurring on almost every day of the study and mean temperatures too low for development of natural epiphytic populations of *E. amylovora*. On day 2, after inoculation, there was heavy rain (over 29 mm). Despite these unfavorable conditions, it was possible to re-isolate the marked bacteria on flowers for up to 1 week after inoculation. There was no consistent reduction of Ea8865 populations on the flower portions or pistils pre-inoculated with Eh318 (Figs. 1, 2). The populations on pistils of Ea8865 alone or when pre-inoculated with Eh318 were quite similar throughout the duration of the study. The population of Ea8865 on the flower portions pre-inoculated with EH318 was 10 to 100 fold higher when compared to flowers inoculated with Ea8865 alone for the first 1-2 days after inoculation. However, from day 3 onward there was very little difference in the two populations on both pistils and flower portions.

The population of Eh318 on pistils was nearly identical whether alone or in combination with Ea8865 (Fig. 3). The situation was the same on flower portions indicating that there was no inhibitory or stimulatory influence of *E. amylovora* on the growth of *E. herbicola*.

Fig. 1: Populations in the field study of *E. amylovora* Ea8865 on apple flowers inoculated with Ea8865 alone or in combination with a pre-inoculation of *E. herbicola*, Eh318. (bars represent daily SED).

Fig. 2: Populations in the field study of *E. amylovora* Ea8865 on pistils inoculated with Ea8865 alone or in combination with a pre-inoculation of *E. herbicola*, Eh318. (bars represent daily SED).

TABLE 1: Incidence of fireblight on apple trees, cv. 'Royal Gala', in the field, 51 days after inoculation of flowers with *Erwinia herbicola*, strain Eh318, and streptomycin for control of *E. amylovora*, strain Ea8865.

Treatment	Mean no. of infections/tree ³
<i>Erwinia amylovora</i> strain Ea8865 ¹	3.69(43.8)
<i>E. herbicola</i> strain Eh318/Ea8865 ²	3.14(26.0)
Streptomycin/Ea8865 ²	2.78(16.0)
Eh318 ³	2.22(9.8)
Check	1.12(3.0)
SED	0.396

¹ Trees were spray inoculated with Ea8865 at 1×10^6 or Eh318 at 3×10^6 ;

² Trees were spray inoculated with Eh318 (1×10^6 CFU/ml) or streptomycin (100 µg/ml) 24 h before inoculation with Ea8865 (1×10^6 CFU/ml);

³ log_e (X+1) transformed with untransformed means in brackets, SED applies to transformed data.

Fig. 3: Populations in the field study of *E. herbicola* Eh318 on flowers inoculated with Eh318 alone or in combination with Ea8865. (bars represent daily SED).

There was a significant reduction in the number of infections on trees grown in the controlled environment conditions which had been pre-inoculated with Eh318 (Table 2). There were no infections on the check trees or those treated with Eh318 alone.

TABLE 2: Incidence of fire blight on potted apple trees, cv. 'Royal Gala' in a controlled environment, 30 days after inoculation of flowers with *Erwinia herbicola*, strain Eh318 and *E. amylovora* strain Ea8865.

Treatment	Mean no. of infections /treatment ³
<i>Erwinia amylovora</i> strain 8865 ¹	2.01(7.3)
<i>E. herbicola</i> strain Eh318/ Ea8865 ²	1.07(2.0)
Eh318 ¹	0
Check	0
SED	0.294

¹ Potted and flowering trees were inoculated with *E. amylovora* strain 8865 or *E. herbicola* strain Eh 318 at 1×10^6 CFU/ml;

² Trees were inoculated with Eh318 on day 1 followed 24 h later with Ea8865, both at 1×10^6 CFU/ml;

³ Values are $\log_e (X+1)$ for the mean number of fire blight infections on the four treated trees/treatment with untransformed means in brackets, SED applies to transformed data.

Similar to the orchard study, the population of Eh318 on pistils and flower portions was not influenced by the presence of Ea8865.

The population of Ea8865 on pistils pre-inoculated with Eh318 was almost 100 fold less than on pistils not pre-inoculated with Eh318 at 24 h after inoculation and remained lower throughout the study (Fig. 4). By 72 h, the pistils pre-inoculated with Eh318 still had populations only one tenth those on pistils not pre-inoculated with Eh318. The flower portions showed a similar trend with populations of Ea8865 on the trees pre-inoculated with Eh318 about one tenth that on trees not pre-inoculated with Eh318 after 48 and 72 h (Fig. 5).

Fig. 4: Populations in a controlled environment of *E. amylovora* Ea8865 on flowers inoculated with Ea8865 alone or in combination with a pre-inoculation of *E. herbicola*, Eh318. (bars represent daily SED).

Fig. 5: Populations in a controlled environment of *E. amylovora* Ea8865 on pistils inoculated with Ea8865 alone or in combination with a pre-inoculation of *E. herbicola*, Eh318. (bars represent daily SED).

In both studies, the apple trees pre-inoculated with Eh318 had a lower incidence of fire blight compared with trees not pre-inoculated with Eh318. In the field study, the degree of protection by Eh318 was not significantly less than that with streptomycin. The biocontrol by *E. herbicola* may be due, in part, to inhibition of *E. amylovora* but this effect was only apparent in the first two days of the study.

The inhibitory effect of *E. herbicola* on the epiphytic growth of *E. amylovora* was not apparent in the field study. This may have been because the cool temperatures in the orchard were not conducive to the growth of *E. amylovora* and the frequent rains washed the bacteria off the pistils. It could be expected that if inhibition had occurred in the orchard it would only persist for a few days. Flowers retain their integrity over a period of about 5 days after which they lose their petals and are not favorable sites for multiplication of epiphytic bacteria. Therefore, the flowers sampled late in the orchard study were probably closed at the time of inoculation and any bacteria on these flowers would be there due to natural spread. Because repression of *E. amylovora* by *E. herbicola*, was not apparent in the field, the significant level of control of fire blight obtained suggests that a mechanism other than the direct inhibition of *E. amylovora* may be in operation. Perhaps the biocontrol bacteria induce a resistance mechanism in the plant.

ACKNOWLEDGEMENTS

To Mr and Mrs A.M. Nobilo for the use of their apple trees; Mrs D. Voyle and Mr S. Hutchings for technical assistance and Ms B. Dow for statistical analysis.

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