THE COMBINED USE OF TWO BIOLOGICAL CONTROL AGENTS, *PSEUDOMONAS FLUORESCENS* A506 AND *ERWINIA HERBICOLA* EH252, AS A NEW STRATEGY TO CONTROL FIRE BLIGHT

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SUMMARY

Pseudomonas fluorescens A506 and *Erwinia herbicola* Eh252, are effective biological control agents, respond differently to climatic condition, and reduce the incidence of fire blight by different mechanisms. In this study these two strains did not inhibit each other when sprayed together on apple flowers, and when together they controlled fire blight as well as either strain sprayed separately.

Keywords: biological control, fire blight, *Erwinia herbicola*, *Pseudomonas fluorescens*, apple

INTRODUCTION

Fire blight is an economically important bacterial disease of apple and pears that can be controlled only by spraying streptomycin throughout flowering. Streptomycin resistant strains of *E. amylovora* have recently been isolated in New Zealand (Thomson *et al.* 1993; Vanneste and Yu 1993) and therefore alternative treatments will soon be needed. Biological control offers the possibility to reduce fire blight incidence; however, due to climatic variations, results are usually variable from year to year. This problem may be avoided by using a combination of biological control agents that respond differently to climatic conditions. *Pseudomonas fluorescens* A506 and *Erwinia herbicola* Eh252, are among the most effective strains to control fire blight, and come from different selection programs; Eh252 was isolated from apple and selected after experiments in New York (Beer *et al.* 1984), and France (Vanneste and Yu 1990), while A506 was isolated from pear and selected for its efficacy in controlling fire blight in California (Lindow 1982). More importantly they inhibit *Erwinia amylovora* the fire blight pathogen, by different mechanisms. One of the mechanisms used by Eh252 to inhibit *E. amylovora* is production of a peptide antibiotic (Vanneste *et al.* 1990, 1992), whereas strain A506 does not produce any inhibitory compound, but reduces fire blight incidence by displacing populations of the pathogen following competition for nutrients and space (Wilson and Lindow 1993). *In vitro* Eh252 and A506 do not inhibit each other (Vanneste unpublished results).

Since the level of control provided by both strains is dependent on their population level on flowers, it is essential that these two strains do not inhibit each other *in vivo*. Thus, we compared the population dynamics of these two strains when sprayed together or separately on apple flowers. Since *E. amylovora* multiplies on the stigma and most probably enters the plant tissue by the nectaries (Vanneste 1994), the pistils and the rest of the flower, called nectaries, were considered separately. In parallel to this population study, we compared the reduction of fire blight incidence when blossoms were treated with each strain separately or together.

METHODS

All the experiments were conducted on apple cultivar Granny Smith, in a research orchard at Pukekohe. For the experiments on the populations of biological control

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agents, the bacterial strains of Eh252 and A506 were grown separately at 25°C in Roux bottle on Luria medium for 24 h and were diluted before application with de-ionized water to 3.4×10^7 colony forming units (cfu) per ml for Eh252 and to 2.7×10^7 cfu/ml for A506. When these strains were used together, equal volumes of the previous bacterial suspensions were mixed together resulting in a suspension containing 1.7×10^7 cfu/ml of Eh252 and 1.3×10^7 cfu/ml of A506. On 30 October, bacterial suspensions were sprayed to run off onto at least 15 newly opened blossom clusters on one or two trees. The day following treatment, five flowers per day and per treatment were dissected; pistils and nectaries were washed separately in a 10 mM MgSO_4 solution. This was continued daily for a further 7 days. Serial dilutions were sprayed on selective media allowing determination of the populations of Eh252 and A506 on pistils and nectaries. Flowers that were sprayed with water only were also processed to determine the natural population of *E. herbicola* and of *P. fluorescens* strains; these are the base-line populations.

The same bacterial suspensions as described above were used to compare the ability of Eh252 and A506 applied separately or together to control fire blight. Each treatment was applied to at least one tree randomly selected in each of the five blocks. On 30 October, bacterial suspensions were sprayed to run off onto 9 to 26 newly opened blossom clusters, with an average of 16 clusters per tree. In some cases, to increase the number of clusters treated, two trees in the same block were used for the same treatment; this has been taken into consideration in the data analysis. Water and streptomycin (100 ppm) were used as a negative and as a positive control, respectively. The following day, the same blossom clusters were sprayed to run off with a suspension of *Erwinia amylovora* Ea273 at 1.1×10^6 cfu/ml. Suspension of the pathogen was prepared by dilution of an overnight culture grown under the same conditions as those described for the biological control agents. Five weeks after inoculation, the numbers of healthy and diseased clusters were recorded. The statistical analysis of the percentage of infection and of the angular transformation of the percentage of infection was weighted by the number of clusters treated per tree.

Fig. 1 A: Populations of *E. herbicola* Eh252 Fig. 1B: Populations of *P. fluorescens* A506

- Pistils Eh252 or A506 alone
- Pistils Eh252 and A506 in combination
-▲..... Pistils not inoculated
- Nectaries Eh252 or A506 alone
- Nectaries Eh252 and A506 in combination
-▲..... Nectaries not inoculated

Figure 1: Populations of *E. herbicola* Eh252 and *P. fluorescens* A506 on pistils and nectaries of apple flowers inoculated with each strain separately or in combination.

RESULTS

The population level of Eh252 on pistils was similar whether this strain was inoculated alone or in mixture with A506 (Fig. 1A). However, populations of Eh252 on nectaries was consistently slightly lower when applied in combination with A506 than when applied alone (Fig. 1A). Populations of A506 on pistils or nectaries were similar whether it was sprayed alone or in combination (Fig. 1B), indicating that the growth of A506 was not affected by the presence of Eh252.

Infection of apple blossom clusters by *E. amylovora* was reduced significantly and to similar level when Eh252 or A506 were applied separately or together (Table 1). However, treatment with streptomycin was significantly better than any of the treatments with the biological control agents.

TABLE 1: Incidence of fire blight on apple flowers treated with different biological control agents before inoculation.

Treatment	% infection ¹	Angular transformation of % infection ²
water	58.15	49.90
Eh252	31.07	32.22
A506	29.09	32.23
A506 + Eh252	32.96	34.63
streptomycin	8.8	17.02

¹ Weighted mean of the percentage of infection per tree.

² Angular transformation of the percentage of infection. The angular transformation used in this study, is the arcsine of the square root of the percentage of infection divided by 100. The standard error of the difference (SED) varied for each pair of treatments considered, because of the different number of clusters used per treatment. The highest value of the SED for the means of the transformed data was 5.56.

DISCUSSION

To assess the benefit of using a combination of bacterial strains to reduce the variation in biological control of fire blight, we followed the population of the two biological control agents Eh252 and A506 when sprayed separately or together. We also compared the level of infection of apple blossoms treated with these two strains separately or together.

The population levels reached on apple blossoms by Eh252 or A506 was comparable whether these strains were sprayed separately or together. The only exception was the population of Eh252 on nectaries of flowers treated with both strains of biological control agents, which was always slightly lower than the population of flowers treated with Eh252 alone. This was unexpected since such a difference was not observed in similar experiments carried out in an apple orchard in the Waikato (Vanneste *et al.* 1994). Since the level of control is dependent on the level of populations, and since the population level was similar when Eh252 and A506 were sprayed separately or together, then these two strains sprayed together should control fire blight at least as well as the best of these strains used separately. This year there was no significant difference in the control of fire blight using either Eh252 or A506 alone. Thus, not surprisingly the control obtained using these two strains together was not significantly different from that obtained using either strain separately.

This is the first report of biological control of fire blight in New Zealand using Eh252 or A506; the results obtained are consistent with those previously obtained in Europe (Vanneste and Yu 1990) or in the U.S.A (Beer *et al.* 1984; Wilson and Lindow 1993). This is also the first report of Eh252 and A506 being used together for the control of fire blight. These two strains do not inhibit each other on apple flowers, and when used in combination they have the potential to control fire blight more consistently and under a wider range of climatic conditions than either strain alone.

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