COLONISATION OF APPLE AND PEAR LEAVES BY DIFFERENT STRAINS OF BIOLOGICAL CONTROL AGENTS OF FIRE BLIGHT

J.L. VANNESTE[1], D.A. CORNISH[1], F. SPINELLI[2] and J. YU[1]

[1]*HortResearch, Ruakura Research Centre, Private Bag 3123, Hamilton, New Zealand*
[2]*Department of Arboriculture, University of Bologna, Viale Fanin 46, 40127 Bologna, Italy*

Corresponding author: jvanneste@hortresearch.co.nz

ABSTRACT

The ability of biological control agents of fire blight to establish and colonise the surface of apple and pear leaves was investigated. Two commercially available biological control agents, *Pantoea agglomerans* P10c and *Pseudomonas fluorescens* A506, and two strains of *P. agglomerans* isolated from apple orchards in New Zealand were used. All of these strains are able to reduce fire blight incidence on apple and pear flowers. After spray application the percentage of leaf surface colonised by the biological control agents was measured by imprinting leaves on fresh agar plates. *Pseudomonas fluorescens* A506 did not survive for more than two days in the field, while the strains of *P. agglomerans* survived for several days but the percentage of leaf area colonised decreased rapidly after 48 hours. These strains might not have the characteristics necessary to control fire blight infections on shoots.
Keywords: *Pantoea agglomerans, Pseudomonas fluorescens*, biological control, shoot blight.

INTRODUCTION

Fire blight is the most destructive bacterial disease of apple and pear trees (Vanneste 2000). During spring, infection occurs when *Erwinia amylovora,* the fire blight pathogen, enters the plant through the flowers (Thomson 2000). These infections result in the loss of the year's crop and in some cases can result in the death of the tree. Very importantly, these early infections also provide the inoculum that will lead to shoot infections during summer. Most strategies for the control of fire blight rely on the prevention of flower infection during spring, to protect the year's crop and to eliminate the inoculum for further infections. However, in years when climatic conditions are not favourable to fire blight infection during spring and when few symptoms are visible on flowers, orchardists would like to be able to omit protection during spring and focus on controlling shoot infections during summer.

Control of shoot infection using copper-based products or streptomycin would require regular spraying over a long period of time. The long-term risks associated with this strategy are phytotoxicity and development of strains of the pathogen resistant to these compounds (Vanneste & Voyle 2001, 2003). The non-pathogenic epiphytic bacteria *Pantoea agglomerans* P10c and *Pseudomonas fluorescens* A506 are able to reduce fire blight incidence on apple and pear flowers (Vanneste et al. 2002; Lindow 1982), and are available commercially in New Zealand and the USA respectively. The potential of these strains to limit incidence of fire blight on shoots is dependent on their ability to establish and colonise the surface of apple and pear leaves.

This paper reports the survival of P10c, A506 and two other strains of *Pantoea agglomerans* on apple and pear leaves in the laboratory and in the field.

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MATERIALS AND METHODS

Bacterial strains and media

The biological control agents used in this study were *Pseudomonas fluorescens* A506, commercially available in the USA as BlightBan™ (Plant Health Technologies), and *Pantoea agglomerans* P10c, commercially available in New Zealand as Blossom Bless® (Gro-Chem NZ Ltd). Two strains of *P. agglomerans* isolated in New Zealand from apple orchards, JLV07 and JLV08, and a rifampicin resistant derivative of the *E. amylovora* strain ICMP8865, called 8865nr, were also used. The strains were grown on complete medium of Luria Agar (Invitrogen™ Life Technologies) and incubated at 28°C. Bacterial concentration in each suspension was determined just before use.

Leaf imprint experiments

Fully developed apple or pear leaves free of any disease symptoms were randomly collected from the canopy between December and February. They were sprayed to run off on both sides with a freshly prepared bacterial suspension made in 10 mM $MgSO_4$ or with 10 mM $MgSO_4$ by itself and left to dry naturally. In the field experiments the leaves were left on the tree until they were required for imprinting. Imprinting consists of pressing the leaf onto a freshly poured plate of Luria agar medium (Leben et al. 1970). When possible both sides of the leaves were imprinted on the same plate. All the agar plates were supplemented with cycloheximide (50 µg/ml) to suppress fungal growth. Leaves sprayed with *P. agglomerans* P10c, JLV08 or *E. amylovora* 8865nr were imprinted on plates supplemented with rifampicin (20 µg/ml). Leaves sprayed with *P. fluorescens* A506 were imprinted on plates supplemented with streptomycin (100 µg/ml). Leaves sprayed with 10 mM $MgSO_4$ alone were imprinted on each of the above media. The percentage of leaf area that was colonised by the strain of interest was determined after 48 hours of incubation for the field experiments and 24 hours of incubation for the experiment in the laboratory. The percentage area colonised by the different bacterial strains was visually estimated by comparing the leaf imprint to patterns of known percentage of colonisation.

Colonisation on pear leaves in the laboratory

Leaves collected in February from pear trees cv. Conference were placed on damp paper towels, and arranged in eight groups of 10. A suspension of P10c containing $2.3x10^9$ cfu/ml was diluted in 10 mM $MgSO_4$ to give a series of bacterial suspensions ranging from $2.3x10^9$ cfu/ml to $2.3x10^3$ cfu/ml. For each concentration, 10 leaves were sprayed on both sides with a hand held sprayer and placed onto paper towels. Five leaves from each treatment were imprinted 3 and 24 hours after spraying.

Colonisation on pear leaves in the field

In an experimental orchard at Ruakura, potted pear trees of cv. Williams Bon Chrétien were sprayed in December with P10c at $6x10^8$ cfu/ml, JLV07 at $4x10^8$ cfu/ml, JLV08 at $2x10^9$ cfu/ml and A506 at $2x10^8$ cfu/ml. Five leaves per treatment were collected for imprinting 1, 24, 48, 72 and 96 hours after spraying.

Colonisation on apple leaves in the field

Potted apple trees of cv. Pink Kiss held in an experimental orchard, were sprayed with P10c at $9x10^8$ cfu/ml, JLV07 at $4x10^8$ cfu/ml, JLV08 at $3x10^8$ cfu/ml and A506 at $1x10^8$ cfu/ml in December. Five leaves per treatment were collected for imprinting 1, 24, 48, 72 and 96 hours and 7 days after spraying. In addition, a suspension of A506 at $2x10^8$ cfu/ml, P10c at $4x10^7$ cfu/ml or 8865nr at $9x10^7$ cfu/ml was sprayed in January onto leaves of apple trees cv. Pink Kiss planted in an experimental orchard at Ruakura. Twelve leaves per treatment were imprinted 0.5, 7, 24, 48, and 96 hours after spraying.

RESULTS

The results of the experiment carried out in the laboratory on pear leaves are presented in Figure 1. The higher the concentration of P10c used to spray the leaves, the higher the percentage of leaf area colonised by this bacterium. However, even when using a suspension containing $2.3x10^9$ cfu/ml not all the surface of the leaves was colonised; the maximum leaf area colonised was 95%. The percentage of area colonised seemed stable

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over the first 24 hours following spraying, since in most cases there was no significant difference between leaves imprinted at 3 and 24 hours.

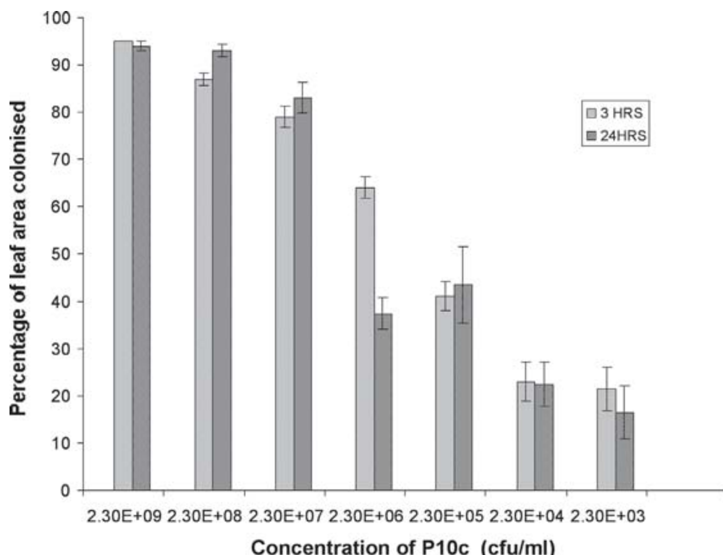

FIGURE 1: Mean areas of colonisation of pear leaves in the laboratory by *Pantoea agglomerans* P10c, applied at a range of concentrations and assessed 3 and 24 hours after application by imprinting leaves onto agar. Bars represent the standard error of the means.

In all field experiments the leaves treated with 10 mM $MgSO_4$ only did not show any bacteria similar to those that were sprayed. The results of the field experiments on apple and pear leaves are presented in Tables 1-3. In all cases the percentage of area of the leaf colonised by any of the biological control agents decreased considerably over a period of a few days. The bacterium that survived for the least amount of time was in all cases *P. fluorescens* A506. *Pantoea agglomerans* P10c seemed to survive less than the other strains of *P. agglomerans* but the initial level of colonisation was also slightly lower. Interestingly, all the strains of *P. agglomerans* tested survived longer than the pathogen *E. amylovora* (Table 3).

TABLE 1: Mean percentages of pear leaf area colonised by *Pseudomonas fluorescens* A506 and three strains of *Pantoea agglomerans* (P10c, JLV07 and JLV08) at different times after application to potted pear trees in an experimental orchard.

	Hours after application				
Strains	1 h	24 h	48 h	72 h	96 h
A506	12	0	0	0	0
P10c	72	46	29	3	0
JLV07	92	72	50	33	14
JLV08	92	63	46	40	21

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TABLE 2: Mean percentages of apple leaf area colonised by *Pseudomonas fluorescens* A506 and three strains of *Pantoea agglomerans* (P10c, JLV07 and JLV08) at different times after application to potted apple trees in an experimental orchard.

	Hours after application					
Strains	1 h	24 h	48 h	72 h	96 h	168 h
A506	32	16	0	0	0	0
P10c	61	51	0	11	9	0
JLV07	74	80	14	20	21	9
JLV08	78	81	21	29	24	8

TABLE 3: Mean percentages of apple leaf area colonised by *Pseudomonas fluorescens* A506, *Pantoea agglomerans* P10c, or *Erwinia amylovora* 8865nr at different times after application to apple trees in an experimental orchard.

	Hours after application					
Strains	0.5 h	7 h	24 h	48 h	72 h	96 h
A506	45	0	0	0	0	0
8865nr	78	26	16	3	1	0
P10c	87	47	41	24	19	2

DISCUSSION

The leaf-colonising ability of different bacterial strains, initially isolated for their ability to reduce fire blight infection on apple and pear flowers, was tested in the laboratory and in the field. None of the strains tested seemed to be able to establish and to colonise the surfaces of apple or pear leaves under the conditions and within the limited time of the experiments. Indeed, it is likely that the bacteria were just surviving, since their numbers was usually declining steadily over the course of the experiments. The exception is the experiment carried in the laboratory on pear leaves where it seems that at least in the first 24 hours the level of colonisation by P10c was stable or even increasing. However, the ability of the bacterium to colonise pear leaves for longer than 24 hours in the laboratory was not tested. The decrease in leaf area colonised in the field over time may reflect the effects of the environment on the ability of a strain to establish as an epiphyte. Sabaratnam & Beattie (2003) reported that the ability of a strain of *P. agglomerans* to colonise the surface of maize and bean leaves was strongly influenced by the environmental conditions; it was particularly dependent on the presence of a high relative humidity. The protected environment of the laboratory most probably allowed the better establishment of P10c on pear leaves.

Although *P. agglomerans* and *P. fluorescens* are two species described as epiphytes, none of the strains tested were originally isolated from leaf surfaces. The inability of the strains tested to survive for a long period of time in the field might reflect that to be able to colonise leaf surfaces, a bacterial strain requires some unique characteristics. These characteristics could be the ability to survive in an environment where water and some nutrients are lacking, where UV levels are high and where there is competition for the resources provided by the plants (Beattie & Lindow 1999). *E. amylovora* did not survive on apple leaves in the field, which was expected, since in contrast to the other strains tested, *E. amylovora* is not an epiphytic bacterium (Thomson 2000). Since every bacterium successfully transferred onto the agar medium during imprinting gives rise to a colony, the percentage of the leaf area calculated after imprinting is always larger than

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the area taken up by the initial bacterium which give rise to the colony. Interestingly, even when sprayed at high concentrations the leaves were not entirely covered by the epiphytic bacteria.

The ability of P10c and A506 to control fire blight on blossom has been linked to the ability of these two strains to colonise the stigmatic surfaces of apple and pear flowers (Vanneste et al. 2002; Lindow 1982). It seems logical to expect that similarly the ability of a bacterial strain to prevent fire blight infections on shoots will be dependent on its ability to colonise the leaf surface. Based on the results obtained it is not expected that any of these strains would be able to control fire blight during summer. Whether a strain isolated from leaf surfaces could colonise apple and pear leaves to such an extent as to be able to reduce the incidence of fire blight on shoots has yet to be demonstrated.

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