

INCIDENCE OF *ERWINIA HERBICOLA* IN NEW ZEALAND APPLE ORCHARDS: IMPLICATIONS FOR BIOLOGICAL CONTROL

L.P. KEARNS and C.N. HALE

*Dept. Plant and Microbial Sciences, University of Canterbury
and DSIR Plant Protection, Auckland*

SUMMARY

Erwinia herbicola populations were monitored on apple trees during flowering in selected orchards in Canterbury (1989) and Hawkes Bay (1990) to determine the possible role of this species in the biological control of fire blight. In all four orchards surveyed *E. herbicola* populations remained negligible (less than 50 cfu/blossom) throughout flowering, increasing rapidly at petal drop to become one of the dominant species present. This suggests that *E. herbicola* is a poor epiphyte on apple blossoms and that biological control strategies using direct spray applications of viable bacteria onto blossoms may not be of value.

Keywords: fire blight, *Erwinia herbicola*, survey, apple blossom, incidence

INTRODUCTION

Fire blight is a disease of pipfruit and other rosaceous plants causing significant economic losses in many fruit-growing countries overseas. This disease, caused by the bacterium *Erwinia amylovora*, blights flowers and twigs, destroying subsequent fruit development.

Disease control includes winter applications of copper sprays and timed applications of antibiotic streptomycin during flowering. These applications are expensive and must be made prior to infection, creating the need for accurate disease forecasting. In addition, the use of antibiotics can lead to the development of pathogen resistance, and streptomycin-resistant *E. amylovora* has been isolated (Miller and Schroth 1972; Moller *et al* 1972; Coyier and Covey 1975).

Epiphytic bacteria isolated from host plants have been shown to be antagonistic to *E. amylovora* both *in vitro* and in plant bioassays. To date, most research attention has been paid to *E. herbicola*, a closely associated bacterium and a known antagonist of *E. amylovora*, which has been used in orchard trials overseas (Riggle and Klos 1972; Thompson *et al* 1976; Beer *et al* 1984, 1987; Nicholson *et al* 1990). More recently, inhibitory pseudomonads have been investigated as potential antagonists of *E. amylovora* (Wilson *et al* 1990).

Although the fire blight pathogen is present in New Zealand, incidence of the disease is less than expected using standard prediction models (Thompson and Hale 1987). The low incidence of disease outbreaks in this country, despite high pathogen populations and conducive climatic conditions, suggests that *E. amylovora* may be subject to some form of biological control. This paper reports on monitoring of *E. herbicola* at two apple orchards in Canterbury and two in Hawkes Bay.

METHODS

In 1989 mature apple trees (*Malus x domestica* Borkh. cv. Golden Delicious) were sampled in two orchards in Loburn, Canterbury. One orchard had a previous history of fire blight infection and the other no previous history of fire blight. In 1990 cv. Gala and cv. Royal Gala apple trees in Hawkes Bay were sampled from two orchards with no previous history of fire blight. Streptomycin sprays were not used in the orchards surveyed.

Proc. 44th N.Z. Weed and Pest Control Conf. 1991: 91-94

Twenty blossom samples were collected daily at random from each orchard throughout the flowering period (Canterbury, October 5 — November 11, 1989; Hawkes Bay, October 10 — November 8, 1990). Entire blossom clusters (5-7 flowers) were aseptically removed into a sterile plastic bag and stored on ice (2-4 hours) until further processing. Blossoms were washed for 2 minutes with vigorous shaking in 10 ml sterile saline, 0.85% (w/v) + peptone, 1.5% (w/v). Dilutions, 1:100 and 1:1000, or 1:500 and 1:5000, of the blossom wash were plated onto: a) Luria-Bertani agar (Miller 1972) + cycloheximide at 50 mg/litre (Sigma), to estimate total bacterial populations and b) Miller and Schroth (MS) agar (Miller and Schroth 1972), to estimate populations of *Erwinia* spp. Plates were incubated at 25 °C for 3 days.

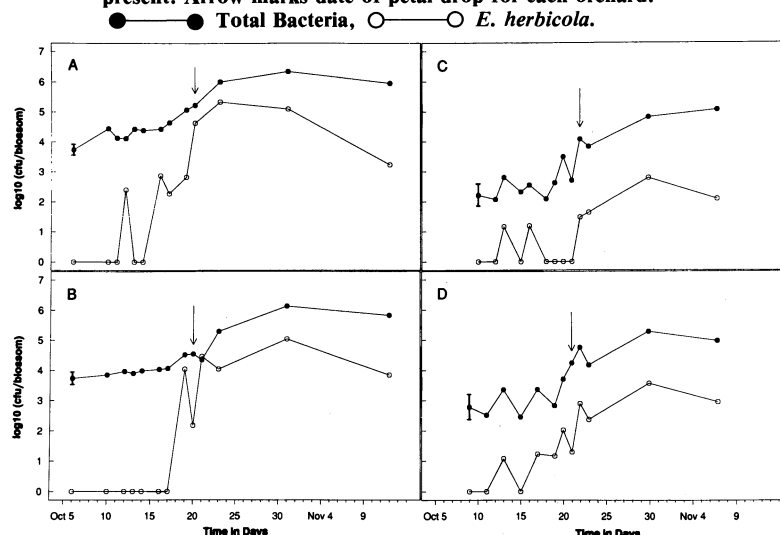
Yellow colonies growing on MS agar were blotted onto nylon membranes (Hybond N +, Amersham) for hybridisation with a radiolabelled *E. amylovora* total genomic DNA probe (Hale and Clark 1990) to enable differentiation between *E. amylovora* and *E. herbicola* colonies.

RESULTS AND DISCUSSION

In all four orchards surveyed *E. herbicola* populations remained negligible (less than 50 cfu/blossom) throughout most of the flowering season, increasing at petal drop to levels of $5 \times 10^2 - 1 \times 10^3$ cfu/blossom (Hawkes Bay) and 5×10^3 cfu/blossom (Canterbury) (Fig. 1). *E. herbicola* populations decreased slowly thereafter as immature fruit developed. Low overall *E. herbicola* populations during flowering suggest that the bacterium is a poor epiphyte on apple blossoms during the period apple trees are most susceptible to fire blight infection.

Artificial inoculation of host plants with excess *E. herbicola* (10^8 cfu/ml, sprayed until runoff) has shown that the bacterium can colonise the blossom in the same way as the pathogen (Rundle and Beer 1987; Wodzinski *et al* 1987). However, natural populations of *E. herbicola* are low (this study; Manceau *et al* 1990). *E. herbicola* populations studied in a pear orchard in France (Manceau *et al* 1990) showed a similar distribution in time as seen in this survey, with the bacterium only appearing on blossoms at the end of flowering.

Fig. 1: Bacterial populations on apple blossoms in selected orchards in Canterbury (A) and (B) and Hawkes Bay (C) and (D). *E. herbicola* populations remain low until petal drop, then increase to become a major bacterial species present. Arrow marks date of petal drop for each orchard.



Variable protection has been reported from orchard trials with *E. herbicola* which have employed direct spray applications of viable bacteria onto trees (Riggle and Klos 1972; Thompson *et al* 1976; Beer *et al* 1984, 1987). Survival of the applied bacteria on trees was not confirmed in these trials. Our study suggests that poor survival of *E. herbicola* may have contributed to the variable results obtained.

Hawkes Bay orchards supported lower total bacterial populations and lower relative *E. herbicola* populations than Canterbury orchards. *E. herbicola* populations at petal drop represented 1% of the total bacterial population in Hawkes Bay orchards compared with 30-50% of the total bacterial population in Canterbury orchards. Although further monitoring would be necessary to assess the significance of this, it is worth noting that low *E. herbicola* populations were found in orchards that had no previous history of fire blight infection, although located in an area where fire blight is frequently found.

Pseudomonads constituted the majority of bacteria on trees throughout flowering in both Canterbury and Hawkes Bay orchards. Given the high epiphytic capability of pseudomonads these bacteria may be more suitable as potential biological control agents for fire blight, provided strains antagonistic to *E. amylovora* can be found. Further to the present study isolates collected from the orchards surveyed were assayed for antagonism to *E. amylovora* both *in vitro* and in plant bioassays. No *Pseudomonas* isolated were found that were strongly inhibitory to *E. amylovora* on plants, although many were inhibitory *in vitro*. Fluorescent pseudomonads which protect plants from fire blight infection have been isolated from hawthorn and pear (Wilson *et al* 1990). The inability to detect inhibitory pseudomonads in our study may have been due to the bioassay methods employed, rather than a true absence of antagonistic strains.

ACKNOWLEDGEMENTS

J. Maindonald for carrying out the statistical analyses of the orchard data. L. Kearns was supported by a Ph.D. study grant from D.S.I.R.

REFERENCES

- Beer, S.V., Rundle, J.R. and Norelli, J.L., 1984. Recent progress in the development of biological control of fire blight — a review. *Acta Hort.* 151: 195-201.
- Beer, S.V., Rundle, J.R. and Norelli, J.L., 1987. Orchard evaluation of five strains of *Erwinia herbicola* for control of blossom infection. *Acta Hort.* 217: 219.
- Coyier, D.L. and Covey, R.P. 1975. Tolerance of *Erwinia amylovora* to streptomycin sulphate in Oregon and Washington. *Plant Dis. Reporter* 59: 849-852.
- Hale, C.N. and Clark, R.G., 1990. Detection of *Erwinia amylovora* from apple tissue by DNA hybridisation. *Acta Hort.* 273: 51-55.
- Manceau, C., Lalonde, J.C., Lachaud, G., Chartier, R. and Paulin, J.-P., 1990. Bacterial colonisation of flowers and leaf surfaces of pear trees. *Acta Hort.* 273: 73-81.
- Miller, J.H., 1972. Experiments in Molecular Genetics. Cold Spring Harbour New York.
- Miller, T.D. and Schroth, M.N., 1972. Monitoring the epiphytic population of *Erwinia amylovora* on pear with a selective medium. *Phytopathol.* 62: 1175-1182.
- Moller, W.J., Beutel, J.A., Reil, W.O. and Zoller, B.G., 1972. Fireblight resistance to streptomycin in California. *Phytopathol. (Abs.)* 62: 779.
- Nicholson, S.L., Sigee, D.C. and Epton, H.A.S., 1990. Biological control of fire blight of perry pear: comparative evaluation of antagonists on immature fruit slices, micropropagated shoots and orchard blossom. *Acta Hort.* 273: 363-365.
- Riggle, J.H. and Klos, E.J., 1972. Relationship of *Erwinia herbicola* to *Erwinia amylovora*. *Can. J. Bot.* 50: 1077-1083.
- Rundle, J.R. and Beer, S.V., 1987. Population dynamics of *Erwinia amylovora* and a biological control agent, *Erwinia herbicola*, on blossom parts. *Acta Hort.* 217: 221-222.
- Thompson, S.V. and Hale, C.N., 1987. A comparison of fire blight incidence and environment between New Zealand and western United States. *Acta. Hort.* 217: 93-98.

- Thompson, S.V., Schroth, M.N., Moller, W.J. and Reil, W.O., 1976. Efficacy of bactericides and saprophytic bacteria in reducing colonisation and infection of pear flowers by *Erwinia amylovora*. *Phytopathol.* 66: 1457.
- Wilson, M., Epton, H.A.S. and Sigeo, D.C., 1990. Biological control of fire blight of hawthorn. *Acta Hort.* 273: 363-365.
- Wodzinski, R.S., Sobiczewski, P. and Beer, S.V., 1987. Survival of an introduced strain and natural populations of *Erwinia herbicola* on apple. *Acta Hort.* 217: 245-251.