STREPTOMYCIN RESISTANCE IN *ERWINIA AMYLOVORA* FROM APPLE AND PEAR ORCHARDS

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SUMMARY

Streptomycin resistant strains of *Erwinia amylovora* (fire blight) were isolated in seven out of 43 properties in the second season (1992/93) of monitoring New Zealand apple and pear orchards. All strains were resistant to 100 mg/ml streptomycin in amended KB medium. Diseased shoots from three nurseries were also screened, but *E. amylovora* was not present. Combined with the findings of 1991, resistant isolates have been detected in 13 out of 68 Hawkes Bay orchards or nurseries surveyed. With one exception the distribution of orchards with streptomycin resistance appears to be restricted to an area of 3 km radius north of Hastings. Samples from orchards in the Waikato and Otago regions yielded only streptomycin sensitive strains of *E. amylovora*. Five surveyed orchards in the Waikato were free of the problem. Strains of *Pseudomonas syringae* resistant to streptomycin were also isolated from 21.4% of samples. Resistant *E. amylovora* strains were identified using a DNA probe.

Keywords: *Erwinia amylovora*, apples, pears, streptomycin resistance.

INTRODUCTION

Control of fire blight of apples and pears during the growing season has traditionally relied on the application of streptomycin. Depending on the geographical location, some orchards which are prone to fire blight may apply up to 10 streptomycin sprays in spring. The intensive streptomycin application is likely to exert selection pressure on the bacterial population and result in development of streptomycin resistance (Str^r). Strains of *Erwinia amylovora* resistant to streptomycin were first detected in New Zealand in 1992 (Thomson *et al.* 1992). It was also found that the resistance was not plasmid borne but probably due to chromosomal mutation. Presence of Str^r appeared to be restricted to the Hawkes Bay area, within two pipfruit localities. Monitoring for the presence of streptomycin resistance was continued in the 92/93 season in order to determine both presence and spread of streptomycin resistant strains.

METHOD

Shoots of apple, pear and nashi with fire blight symptoms were collected from various pipfruit growing districts in New Zealand. The majority of the samples were collected from Hawkes Bay and Waikato because of the presence of fire blight. Small pieces of infected fruit pedicel, blighted flowers, leaf petiole or stem were shaken in 10 ml sterile distilled water. The washings were streaked onto King's medium B (KB) (King *et al.* 1954) and CCT (Ishimaru and Klos 1984) medium. A Sensi-Disk (Difco Corp.) impregnated with 10 mg of streptomycin sulphate was placed at the beginning of the streak and incubated at 28°C for 24-48 h.

Colonies which grew within 20 mm of the disk were streaked onto KB medium amended with 100 µg/ml streptomycin to confirm resistance. The colonies were also sub-cultured on KB and CCT media for identification. Identification of *E. amylovora*

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was based on formation of craters on the colony surface on CCT medium, and orange colonies on Miller and Schroth's sorbitol medium (1972). Fluorescent isolates of *Pseudomonas* spp. on KB medium were identified tentatively using LOPAT tests of Lelliot *et al.* (1966). The identity of Str^r isolates of *E. amylovora* was confirmed using a DNA probe to type strain ICMP1540 (Clark *et al.* 1992).

RESULTS AND DISCUSSION

A total of 168 samples with symptoms similar to fire blight were tested for the presence of Str^r in *E. amylovora* (Table 1). Altogether 43 properties were surveyed of which 33 were in Hawkes Bay, six were in the Waikato, one in Whangarei and three in Otago. *Erwinia amylovora* was recovered from 48.8% of the samples. Of these 39% were Str^r while the remaining isolates were sensitive to streptomycin. The Str^r strains were isolated from seven orchards in the Hawkes Bay area. With one exception all of the samples from these seven orchards yielded Str^r *E. amylovora* strains (Table 2). One of these orchards had been tested and found to have Str^r also in the 91/92 survey. Diseased shoots from three nurseries in Hawkes Bay were also tested, but *E. amylovora* was not isolated. An unexpectedly high number of the samples had Str^r strains of *Pseudomonas syringae* which causes bacterial blast (Table 1). This constituted 48.8% of the properties surveyed. The high recovery of *P. syringae* was probably caused by a high incidence of bacterial blast in the 92/93 season with the symptoms not being easily distinguished from those of fire blight. Fire blight and bacterial blast occurred together in 10 of the properties. In addition, Str^r strains of *E. herbicola*, a common saprophytic bacterium on apples, were frequently isolated.

Together with the results of the 1991/92 survey (Thomson *et al.* 1992), a total of 13 out of 90 orchards in New Zealand have now been found to have Str^r *E. amylovora*. Half of these orchards with Str^r are located around the Pakowhai area. Except for an orchard at Ongaonga, which is around 26 km south-west of Hastings, most of the orchards with Str^r remain located within a fairly restricted area approximately 5 km to the north of Hastings. Str^r strains of *E. amylovora* were not detected from orchards in the Waikato for the second year.

Up to two sprays of streptomycin had been applied during blossom on most of the affected orchards (Table 2). So far, growers have not reported any loss of control of fire blight. An orchard which was found to have Str^r in the previous survey had ceased using streptomycin sprays this season. In the previous survey one of the orchards surveyed had nine streptomycin sprays applied. With the limited information available, it is not possible at this stage to relate the development of Str^r to the intensity of streptomycin application.

TABLE 1: The incidence of streptomycin resistance in *E. amylovora* and *P. syringae* in apple and pear orchards in the 1992/93 season.

	<i>E. amylovora</i>		<i>P. syringae</i>	Total number
	Str ^s	Str ^r	Str ^r	tested
Number of orchards	19	7	21	43
Number of samples	50	32	36	168

Str^s — sensitive to streptomycin.

Str^r — resistant to streptomycin.

The presence of Str^r in *P. syringae* has been reported in New Zealand (Young 1977). In the United States, development of Str^r strains of *P. syringae* pv. *papulans*, which causes blister spot of Mutsu apples, has rendered use of streptomycin for control ineffective (Burr *et al.* 1988; Norelli *et al.* 1990; Jones *et al.* 1991). As many Hawkes Bay orchards tend to have mixed plantings of pipfruit and stonefruit, application of streptomycin sprays on one crop may inadvertently exert selection pressure on other

pathogenic bacteria present in the vicinity. The rapidity of spread of Str^r may be governed by the genetic mechanism regulating development of resistance. Overseas, rapid spread of Str^r was attributed to transposition and conjugation of genetic determinants which are plasmid borne (Norelli *et al.* 1990). Depending on the mechanism of spread of Str^r in *P. syringae* in New Zealand, the situation will need to be closely monitored.

TABLE 2: The recovery of Str^r *E. amylovora* and spray regimes of orchards with streptomycin resistance.

Orchard	No. of samples tested	No. of Str ^r isolates	No. of streptomycin sprays	Host
OL	7	2	2	pear
MP	6	6	2	apple
RS	3	3	2	pear
KG	4	4	0	pear
KF	7	7	2	pear
NR	3	3	*	pear
DD	7	7	*	pear

* Data not available.

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