RESISTANCE TO STREPTOMYCIN BY BACTERIAL PATHOGENS OF STONE-FRUIT

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Summary

Isolates of *Pseudomonas syringae*, resistant to high concentrations of streptomycin, have been obtained from apricot from orchards in Central Otago. Isolates of *Xanthomonas pruni*, tolerant to low concentrations of streptomycin have been obtained from nectarine from field trials in Havelock North and Auckland. The possible role of streptomycin resistant pathogens in disease outbreaks is discussed.

INTRODUCTION

Bacterial blast, caused by *Pseudomonas syringae* van Hall 1902, and bacterial spot, caused by *Xanthomonas pruni* (Smith 1903) Dowson 1939, are the most serious bacterial diseases of stone-fruits in New Zealand. Standard recommendations for control include spray applications of streptomycin from bud movement during the period that the pathogen is active.

Concern has been expressed that the effectiveness of streptomycin could be vitiated by the development of resistance in populations of the pathogens. A survey of possible resistance to streptomycin was conducted.

METHOD

Epiphyte populations of *P. syringae* from fifteen orchards of apricot in Central Otago showing blast, and of *X. pruni* from single 'spot' affected nectarine trial blocks in Havelock North and Auckland, were examined for resistance. Trees which had not been sprayed with streptomycin in the season, and trees which had been sprayed one or more times, were sampled.

Leaf samples were washed in flasks, and the washings plated onto separate media which encouraged distinctive colony forms of *P. syringae* and *X. pruni*. The media were prepared containing 0, 10, 20, 50 and 100 ppm streptomycin sulphate (745 international units streptomycin/mg streptomycin sulphate) and colonies which developed at the highest concentrations of streptomycin were subcultured for further testing. The identity of colonies as *P. syringae* and *X. pruni* was confirmed using a number of determinative tests.

Isolates of the pathogens were screened for resistance to streptomycin on a medium containing a range of concentrations 0-1000 ppm streptomycin sulphate.

Resistant and susceptible isolates of *P. syringae* were inoculated into peach cv 'Golden Queen' to test the pathogenicity of the isolates. The methods above are described in detail elsewhere (Young 1977).

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The competitive ability of streptomycin resistant *P. syringae* isolates was tested against wild type (susceptible) isolates by co-inoculating equal populations (one resistant isolate with one susceptible isolate) in broth culture. Counts of the total population and the resistant population were made by plating the broth culture onto nutrient agar and onto nutrient agar containing 100 ppm streptomycin sulphate. At 24 h intervals the mixed population was sampled to determine any change in proportion of resistance to susceptible cells.

Doubling times of susceptible and resistant isolates were estimated by the method of Young *et al* (1977).

RESULTS

Large numbers of *P. syringae* and *X. pruni* susceptible to streptomycin were regularly isolated from apricot orchards and nectarine trial blocks, indicating the presence of populations of these pathogens in the trees which were surveyed.

a. *P. syringae*. From six orchards which had been sprayed with streptomycin at a rate of 100 ppm, *P. syringae* resistant to 1000 ppm streptomycin was regularly isolated. Numbers of cells present varied considerably, but commonly exceeded 100 cells/leaf. In general, streptomycin resistant *P. syringae* was obtained from orchards where one or more streptomycin sprays had been applied. In one case, streptomycin resistant *P. syringae* was obtained from an orchard reported to have had no streptomycin sprays that season.

Pathogenicity tests showed that the proportion of pathogenic isolates in the resistant population was not significantly different from the proportion of pathogens in the population of wild type organisms.

Within 24 h of co-inoculation with the wild type isolates the proportion of streptomycin resistant organisms declined from 50% to 0.01%. The mean doubling time of wild type *P. syringae* was 1.5 h and for the resistant organisms was 3.4 h.

b. *X. pruni*. Isolates of this pathogen resistant to 10 ppm and 20 ppm streptomycin were occasionally obtained from the nectarines sprayed with 100 ppm streptomycin. However, these were only capable of very slow growth compared with the wild type.

DISCUSSION

Resistance to streptomycin developed in populations of *P. syringae*. It has not been established whether resistance arises *de novo* in each season or whether a small resident resistant population survives from the previous season. Although the resistant organisms are pathogenic, their slow growth rate suggests that they are less likely than wild type pathogens to induce a serious disease outbreak unless there are prolonged conditions favourable for disease and unless sustained programmes of streptomycin are maintained.

X. pruni resistant to streptomycin was not obtained. Occasionally, isolates tolerant to concentrations lower than that used in recommended spray programmes were obtained, but these would not be expected to be effective in causing disease outbreaks.

Fruit Crops

The small number of streptomycin sprays applied to stone-fruit do not appear to induce the high populations of virulent pathogens resistant to streptomycin necessary to induce serious outbreaks of disease. Heavy spray protocols might lead to disease by resistant populations.

REFERENCES

- Young, J.M., 1977. Resistance to streptomycin in *Pseudomonas syringae* from apricot. *N.Z. Jl. Agric. Res.*: 20 (in press).
- Young, J.M., Luketina, R.C. and Marshall, A.M., 1977. The effects of temperature on growth *in vitro* of *Pseudomonas syringae* and *Xanthomonas pruni*. *J. Appl. Bact.*: 42 (in press).