



Mana Kai Rangahau

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***Phytophthora infestans; New Zealand
mating types and fungicide sensitivity***

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1 *Executive summary*

This project aimed to characterise field populations of *Phytophthora infestans*, which causes late blight of potato. A total of six isolates of *P. infestans* were obtained from potato crops, three in the 2002/03 growing season and three in 2003/04. The mating type of the isolates was determined and the sensitivity of the 2003/04 isolates to metalaxyl was assessed. Metalaxyl has been widely and successfully used for control of potato late blight in this country. Mating type and metalaxyl sensitivity were determined with assistance from researchers in Finland and the USA.

1.1 *Mating type characterisation*

All of the *P. infestans* isolates were of the A1 mating type, suggesting that sexual reproduction may not occur in New Zealand. This may have implications relating to the evolution and adaptability of the pathogen in this country. Furthermore, if oospores of the pathogen are not produced (the outcome of sexual reproduction), the over-wintering stage of the pathogen may not be important in the epidemiology of late blight in New Zealand. Epidemics are probably instigated from inoculum produced on over-wintering plants or from infected tubers in waste tuber dumps. This is why it is important to eliminate volunteer plants and exposed dumps of waste tubers, thus preventing production of new season primary *P. infestans* inoculum that could initiate new late blight epidemics.

Isolates of *P. infestans* obtained in this study were different from isolates commonly found overseas. The 2002/03 isolates were different pathotypes from the most commonly occurring pathotype in Europe, and the 2003/04 isolates had DNA profiles not previously recorded in international DNA databases. The results from this study are preliminary because of the small number of isolates evaluated, but our results suggest that current New Zealand populations of *P. infestans* may be unique.

1.2 *Pesticide resistance characterisation*

All three *P. infestans* isolates obtained in the 2003/04 growing season were insensitive (resistant) to the phenylamide chemical metalaxyl. This suggests that phenylamide resistance management may not have been effective, at least in the Manawatu where the isolates were obtained. Appropriate resistance management strategies should continue to be applied throughout the New Zealand potato industry to maintain the usefulness of this important pesticide group. These strategies include:

- using products with appropriate mixtures of chemicals (all currently available metalaxyl-containing products are mixtures);
- utilising effective late blight prediction models to target chemical applications to periods of high disease risk;
- applying high rates of active ingredients that are alternatives to phenylamides;
- applying mixture products at intervals not exceeding 14 days;
- using no more than four consecutive applications of mixture products;
- applying phenylamide containing products as preventative rather than curative treatments; and
- applying non-chemical disease control methods (e.g. plant resistance, appropriate cultural controls) for late blight management.

1.3 *Late blight prediction models*

A related FRST-funded programme is assessing late blight prediction models for improved efficiency of late blight control. This has indicated that these models have promise for improving pesticide management to target chemical applications to periods of high disease risk rather than using calendar-based spray programmes. Reductions in pesticide applications are likely to result from accurate disease prediction. As well as improved economic and practical efficiency of late blight control, reductions in applications of phenylamide chemicals will assist management of pathogen resistance to these pesticides.

2 Introduction

Phytophthora infestans causes late blight of potatoes, a disease that is generally regarded as the most important malady of potato crops throughout the world. Kirk (1905) first officially recorded potato late blight and the pathogen *P. infestans* in New Zealand, noting that the disease had been seen in this country as early as 1892. Late blight was probably introduced into New Zealand on infected plant material, most likely on seed potato tubers imported from North America or Great Britain, and may have been present much earlier than the official first record indicates. This disease has been a serious threat to potato production in New Zealand since the time of the first record.

2.1 Mating types of *P. infestans*

Phytophthora infestans is a member of the Chromista, the kingdom of living organisms that includes "pseudofungi" and a closely related group of algae. In the field, the pathogen requires living host plant tissue for rapid development, and warm, high humidity environmental conditions both for dispersal and for infection of host plants. The organism is heterothallic; that is, it requires two mating types (designated mating types A1 and A2) before sexual reproduction can take place. Sexual reproduction results in the development of oospores, which are resistant structures that survive for long periods where host tissue is unavailable, and where environmental conditions are unfavourable for infection and growth. Sexual reproduction is also well recognised in biology as allowing compatible individuals in populations to mix their genetic material, providing the potential for evolution of new types. For plant pathogens such as *P. infestans*, new types in populations may possess new virulence capabilities, or different sensitivities to environmental factors, including chemicals used by growers for disease control.

The A1 and A2 mating types of *P. infestans* were originally thought to exist together only in Central America. Populations of the pathogen in other areas of the world were only of the A1 type, consisting of a single clonal lineage designated US-1 (Goodwin et al. 1994). This suggests that movement of the pathogen around the world had been from very limited sources (Fry & Goodwin 1997). In the early 1980s, the A2 mating type was found in Europe (Hohl & Iselin 1984), and has subsequently has been identified in many other areas of the world. These findings indicate that a new phase of migration of the pathogen has occurred since about 1980 (Fry & Goodwin 1997).

2.2 Chemical control of late blight

Several chemicals are currently registered in New Zealand for control of late blight in potatoes. Of these, the phenylamide chemical metalaxyl has been commonly and successfully used for a number of years. Insensitivity (resistance) to this chemical was recognised in *P. infestans* in Europe in the early 1980s,

soon after the chemical became widely used for late blight control (Morton & Urech 1988). Resistance management strategies were quickly introduced after insensitivity was confirmed. A component of these strategies is to include non-phenylamide chemicals in mixture products with metalaxyl to control pathogen strains that are insensitive to these chemicals (Urech 1988). The phenylamides are still an important component of late blight control, but they are now always made available as mixture products.

2.3 *Characterisation of P. infestans in New Zealand*

The population of *P. infestans* attacking potato crops in New Zealand has not been fully characterised. Because adequate characterisation is likely to affect the choice of late blight control strategies, a study was instigated, firstly, to determine the mating type(s) of the pathogen in this country, and secondly, to ascertain if New Zealand strains of *P. infestans* are insensitive to metalaxyl. This report outlines the results of the study.

3 *Methods*

3.1 *2002/2003 growing season*

Late blight-affected plant material was collected from potato crops in the Pukekohe region in November 2002. Tissue pieces from heavily affected leaf material were taken to the laboratory and placed in high humidity conditions. Attempts were made to isolate *P. infestans* into agar culture from sporulating plant material. Three isolates were successfully established. Two of these were collected from cv. Russet Burbank, while the cultivar yielding the third isolate was unknown. The isolates were sent for pathotyping to Asko Hannukkala, MTT Agrifood Research Finland, Jokioinen, Finland.

3.2 *2003/2004 growing season*

Further collections of late blight-affected plant material were made from potato crops in the Pukekohe, Manawatu, Hawke's Bay and South Canterbury regions. As in the previous season, tissue pieces from heavily affected leaf material were taken to the laboratory and placed in high humidity conditions. Attempts were made to isolate *P. infestans* into agar culture from sporulating plant material. Of these collections, the isolates Pi-04/1, Pi-04/2 (both ex cv. Russet Burbank, from Feilding, Manawatu) and Pi-04/3 (ex cv. Russet Burbank, from Opiki, Manawatu) were successfully cultured on agar plates. These isolates were sent to Asko Hannukkala for pathotyping. In addition, cultures of the same isolates were sent to Dr Kenneth Deahl of the USDA, Maryland, USA, for DNA profiling and metalaxyl sensitivity testing.

4 *Results and discussion*

4.1 *P. infestans* isolates from the 2002/03 growing season

Isolates obtained in 2002/2003 were all characterised as mating type A1. Virulence tests showed that the pathotype of two of the isolates was R(1,4,10,11), while that of the third isolate was R(1,3,4,7,8,10,11). The most common pathotype in Europe is the R(1,3,4,7,10,11) pathotype, so all three isolates from New Zealand were different pathotypes from the one that is most common in Europe. Furthermore, the virulence type 8, which was identified in one of the New Zealand isolates, is rare in Europe.

4.2 *Isolates from the 2003/04 growing season*

All three *P. infestans* isolates obtained during 2003/04 were identical to each other according to assessments of their DNA profiles by Dr Kenneth Deahl. The isolates were characterised as mating type A1, and designated as Gpi 86/100. Furthermore, the DNA profile of the New Zealand isolates was different from that of the strain US-1. The New Zealand isolates lacked band 9 in the DNA profile, and have bands 18 and 19 present (see Appendix). Dr Deahl checked the Global Marker database and determined that this profile is not currently recorded in the database. This suggests that these three New Zealand isolates represent *P. infestans* populations with a unique (previously unreported) DNA profile. Dr Deahl has proposed that further collaboration could provide a more comprehensive picture of the New Zealand populations of *P. infestans*, by collection of about 50 isolates of the pathogen from potato and tomato for DNA typing.

All three isolates were resistant to metalaxyl in tests of sensitivity to this chemical.

The results from the assays of the 2003/04 isolates by the Finnish collaborators will be obtained in July 2004, during a visit to MTT Agrifood Research Finland by Dr Viljanen-Rollinson.

4.3 *General discussion*

The number of *P. infestans* isolates obtained in this study was small, and for this reason is unlikely to fully represent the populations of the pathogen in New Zealand. Nevertheless, characterisation of the isolates we obtained gives some important indications about the possible make-up of the New Zealand populations of the late blight pathogen.

All of the isolates were identified as mating type A1. This suggests that the A2 mating type is not yet present in this country. If this is the case, then New Zealand has avoided the recent (since 1980) migrations of the A2 mating type around the world. The present strict quarantine criteria for importation of potatoes into New Zealand have probably been successful in preventing import of strains

of the A2 mating type. The lack of A2 strains would also limit the pathogen's ability to produce oospores, so this finding may indicate that oospore inoculum is not a factor in the epidemiology of late blight in this country. If this is the case, then epidemics of late blight would be instigated from asexual *P. infestans* inoculum produced from infections on volunteer plants, or on discarded tubers in waste dumps or other areas where they are left lying over winter. Cultural control of late blight should therefore aim to eliminate these sources of primary inoculum. Furthermore, if only the A1 mating type is present, then the likelihood of pathogen variation through sexual reproduction in field populations would be reduced.

4.3.1 *Resistance management*

All three of the isolates obtained in the 2003/04 growing season were characterised as resistant to metalaxyl. These isolates were all from the Manawatu region. This suggests that phenylamide resistance management strategies have not been effectively applied, at least in that area. Resistance management strategies for this group of pesticides should include the following components (Urech 1988):

- use of appropriate fungicide mixture products (all metalaxyl-containing products currently available in New Zealand are mixtures);
- utilisation of effective late blight prediction models to target chemical applications to periods of high disease risk;
- application of high rates of alternative active ingredients to phenylamides;
- application of mixture products at intervals not exceeding 14 days;
- use of no more than four consecutive applications of mixture products;
- application of phenylamide-containing products as preventative rather than curative treatments; and
- use of non-chemical methods (e.g. plant resistance, appropriate cultural controls) in an integrated approach to late blight management.

These components should be applied as comprehensively as possible in pesticide management of late blight in New Zealand potato crops.

4.3.2 *The New Zealand situation*

Results from this study suggest that New Zealand populations of *P. infestans* may be unique in the world. This is probably because New Zealand has not been exposed to recently developed strains of the pathogen, is geographically isolated from other potato and tomato growing regions, and has strict and rigidly applied quarantine regulations relating to import of potato material. We strongly recommend that this study is expanded to examine the profiles of a larger number of isolates than studied here, to adequately characterise the population of the pathogen in this country.

5 *Related research funded by the NZ Foundation for Research, Science and Technology*

A related FRST-funded project has assessed the efficacy of late blight prediction models for management of timing of fungicide applications for control of the disease. Promising results have been obtained in the Pukekohe area. Untreated trial plots had more disease than plots where fungicides were applied either by calendar timing or according to different model recommendations. Trials in the 2004/05 growing season will be designed to determine timing of fungicide application to evaluate disease control efficacy as directed by different models in comparison with a calendar spray programme. In addition, historical trap plant data will be analysed and the performance of the models will be compared with these data. If these results are encouraging, we will explore future funding opportunities for testing the usefulness of disease prediction in assisting decisions on timing of fungicide applications for late blight management. Future research on this topic is likely to focus on the Pukekohe and Manawatu regions, where late blight epidemics are usually most severe. This will form the focus of a future MAF SFF proposal for which VegFed support will be requested.

6 *Acknowledgements*

Our collaboration with Asko Hannukkala (MTT Agrifood Research Finland) and Dr Kenneth Deahl (USDA, Maryland, USA) was essential to the completion of this project.

7 *References*

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Appendix

Tabular representation of the DNA profile, strain designation and sensitivity to metalaxyl of three *Phytophthora infestans* isolates obtained from late blight-affected potato crops (cv. Russet Burbank) in the Manawatu region in 2003/04 (courtesy of Dr Kenneth Deahl, USDA, Maryland, USA).

Isolate	DNA Band Number																								
	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25
Pi/04-1	1	0	1	0	1	0	1	0	0	1	0	0	1	1	0	1	0	1	1	1	1	0	0	1	1
Pi/04-2	1	0	1	0	1	0	1	0	0	1	0	0	1	1	0	1	0	1	1	1	1	0	0	1	1
Pi/04-3	1	0	1	0	1	0	1	0	0	1	0	0	1	1	0	1	0	1	1	1	1	0	0	1	1

Strain designation: Gpi 86/100 (all isolates)

Mating type: A1 (all isolates)

Metalaxyl test: Resistant (all isolates)