MERIWA PROJECT : M 227

RAPID IDENTIFICATION OF SPECIES OF *PHYTOPHTHORA*

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QUARTERLY REPORT NO. 3

OCTOBER - DECEMBER

INTRODUCTION

In M 227 Quarterly Report No. 2 a work programme for the third quarter was outlined.

Work programme for the third quarter :

Stage	Type of Work	Time (Weeks)
1.1.2	The *Phytophthora* Culture Collection	1
2.1.1	Comparison of Local Isolates with Standards	1.5
2.1.2	Intraspecific Isozyme Variation	7
2.2	Interspecific Differences	2
3.1	Introduce Technical Staff to Methodology	1*
	Third MERIWA Report	0.5
	Total	13

* Staff training was scheduled for completion in the fourth quarter.

Work to be completed in Stages 1, 2 and 3 were to run concurrently.

What follows is an account of progress made in the third quarter on each of the sections listed above. Any incomplete or new business arising from those areas of research are also discussed.

WORK DONE DURING THE THIRD QUARTER

In M227 quarterly Report No. 1 a *Phytophthora* species was considered to be a priority species if it occurred locally.

Priority Species : *Phytophthora cinnamomi*
P. citricola
P. cryptogea
P. drechsleri
P. megasperma
P. nicotianae

Stage 1

1.1 CULTURES TO BE PREPARED

1.1.2 The *Phytophthora* Culture Collection

In this section our objective was to acquire, from various collections, the isolates required to research the rapid identification technique. In the second quarter 92% of the required isolates had been prepared, and it became apparent that more *P.drechsleri* samples from Western Australia were needed.

Our isozyme analysis of samples of the various priority species (see section 2.1.2) identified more variation in the Western Australian samples than had previously been reported for Australia (Oudemans & Coffey 1991, Mills *et. al.* 1991, Forster & Coffey 1993, and Oudermans *et. al.* 1994). Consequently it was decided that additional reference isolates from California were needed.

Procedure

See section 1.1.2 of M 227 Quarterly Report No. 1.

Results

All of the Western Australian samples required for the project have been prepared. Action has been taken to acquire the additional necessary reference isolates from California. Half a week is required to complete the culture collection.

Stage 2

2.1 ISOZYME PROFILES OF *PHYTOPHTHORA* SPECIES

2.1.1 Comparison of Local Isolates with Standards

One of the objectives of this project, as outlined in the 'Application for Research and Development Assistance' to MERIWA, was to compare isozyme profiles of local isolates with standards obtained from Californian and ACT collections with the view to establishing the reliability of the electrophoresis technique.

When Western Australian isolates of *P. cinnamomi* were compared with 10 Californian and 3 ACT standards, we were able to identify local isolates with the same profiles as those of the standards (M 227 Quarterly Report No. 2). This result, we believe, establishes credibility in our identifications of unknown cultures of this species using the isozyme technique.

Procedure

The isozyme patterns of local isolates of *P. citricola* (n = 12), *P. cryptogea* (n = 110), *P. drechsleri* (n = 42), *P. megasperma* (25) and *P. nicotianae* (40) were compared with those of Californian standards.

Results

Western Australian isolates of *P. nicotianae* produced isozyme profiles similar to those of 4 Californian standards currently available to us. This result assurres us that, with a high degree of confidence, we can identify unknown cultures of this species by their isozyme profiles.

A significant proportion of the local isolates of *P. citricola, P. cryptogea, P. drechsleri* and *P. megasperma* that we tested produced isozyme profiles unlike those of the Californian standards currently available to us. More standards of these species are expected to arrive from California soon (see section 1.1.2 above). A further 1.0 week's work is required to complete this area of research.

2.1.2 Intraspecific Isozyme Variation

The primary objective of this project is to identify isozyme banding pattern differences between the local species of *Phytophthora*. In order to achieve this goal one must first describe the range of banding patterns that occur within each of the priority species.

In M 227 Quarterly Report No. 2 we reported on the various isozyme profiles we observed in *P. cinnamomi*, and how our results compared with those of other researchers. Our objective in this quarter was to document the various isozyme profiles of the remaining 5 priority species.

Procedure

Crude protein samples of local isolates of *P. citricola* (n = 12), *P. cryptogea* (n = 110), *P. drechsleri* (n = 42), *P. megasperma* (25) and *P. nicotianae* (40) were assessed electrophoretically with 13 enzyme systems. The isozyme profiles thus generated were subjected to a preliminary analysis.

Results

W believe that sufficient numbers of *P. cryptogea*, *P. drechsleri*, *P. megasperma* and *P. nicotianae* samples were assessed in the third quarter to adequately document the variation in these species (see section 1.1.1 of M 227 Quarterly Report No. 1).

The variation in local isolates of *P. citricola* was not documented to our satisfacion in the third quarter, and will require 0.5 week's work to finish this area of research.

2.2 Interspecific Differences

As pointed out in 2.1.2 above a primary objective of this research is to determine whether or not isozymes discriminate between the priority *Phytophthora* species. In order to do this, bands that are 'fixed' (monomorphic i.e. lack variation) and different between species need to be identified. If fixed differences can be recognized, an isozyme key will be generated. The efficiency and cost effectiveness of identifying unknown cultures to species by isozymes will then be tested against the morphological method currently in use by the Vegetation Health Service (VHS).

Procedure

Isozyme variants of *P. citricola* identified in section 2.1.2 above were compared with *P. megasperma*, in a side-by-side trial of 12 isozymes, and those isozymes that showed fixed differences between these species were recorded.

A similar trial was performed comparing *P. cryptogea* with *P. drechsleri*.

Results

Four fixed differences were recognized between *P. citricola* and *P. megasperma*, and we recognized five fixed diferences between *P. cryptogea* and *P. drechsleri*. To complete this area of research a further 13 trials have to be performed, requiring 1.5 week's work.

Stage 3

3.1 INTRODUCTION OF TECHNICAL STAFF TO ISOZYMES

If it is shown that it is more efficient and cost effective to identify unknown *Phytophthora* cultures to species by isozymes than by morphology, our objective is to have CALM staff trained to perform this service on a regular basis.

Procedure

Two CALM technicians from the VHS attended individual training sessions in the use of the isozyme laboratory and isozyme protocols for a day a week for 3 weeks, and then for half a day a week over the 3 succeeding weeks during the third quarter.

Outcome

Both staff members now have sufficient experience to work in an isozyme laboratory and carry out the various cellulose acetate gel electrophoresis protocols required for the identification of local *Phytophthora* species. It was our intention to have the two technicians train together as a team in the isozyme laboratory, which would require a further 2 half day sessions. The isozyme protocols are varied (see section 1.3 of M 227 Quarterly Report No. 1) and require that technicians perform them on a regular basis in order to achieve best results.

References

Oudemans P & MD Coffey. 1991. Mycol. Res. **95** : 19-30.

Mills S D, H Forster and M D Coffey. 1991. Mycol. Res. **95** : 31-48.

Forster H & M D Coffey. 1993. Mycol. Res. **97** : 1101-1112.

Oudermans P, H Forster and M D Coffey. 1994. Mycol. Res. **98** : 189-199.

PROGRAMME FOR THE FINAL QUARTER

The fourth and final quarter is of 10 weeks duration. The work to be done in this quarter is scheduled as follows :

Stage	Type of Work	Time (weeks)
1.1.2	The *Phytophthora* Culture Collection	0.5
2.1.1	Comparison of Local Isolates with Standards	1
2.1.2	Intraspecific Isozyme Variation	0.5
2.2	Interspecific Differences	1.5
3.1	Introduce Technical Staff to Methodology	0.5
4.1	Comparison of Isozyme & Morphology Techniques for Efficiency and Cost	3
	Final MERIWA Report	3
	Total	10

Work in the above 4 stages is to run concurrently.

Final Comment

The above work schedule for the final quarter is in accordance with that proposed for the third quarter as presented and justified in Quarterly Report No. 2. Otherwise all work areas are progressing in accordance with the research application programme, and no obstacles have been encountered at this stage.