MERIWA PROJECT : M 227

RAPID IDENTIFICATION OF SPECIES OF *PHYTOPHTHORA*

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QUARTERLY REPORT NO. 2

JULY - SEPTEMBER

INTRODUCTION

In M 227 Quarterly Report No. 1 a work programme for the second quarter was outlined.

Work programme for the second quarter :

Stage	Type of Work	Time (Weeks)
1.1.2	The *Phytophthora* Culture Collection	3
1.2	The Culture Medium	1
1.3.2	Isozyme Protocols	2.5
2.1	Isozyme Profiles of *Phytophthora* Species	6*
	Second MERIWA Report	0.5
	Total	13

* The isozyme profiles of priority *Phytophthora* species are scheduled for completion in the third quarter.

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

What follows is an account of progress made in the second 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 SECOND 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 first quarter 38% of the required isolates were prepared, and it became apparent that sufficient numbers of 2 priority species (*P. drechsleri* and *P. nicotianae*) were not available.

Procedure

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

Results

92% of the required isolates have been prepared. The 8% balance is represented by *P. drechsleri*, for which we have, as yet, insufficient numbers. Action has been taken to acquire the outstanding isolates. One week is required to complete the culture collection.

1.2 THE CULTURE MEDIUM

A medium for culturing copious quantities of actively growing *Phytophthora* mycelium has been developed. No further work is required in this area of research.

1.3.2 Isozyme Protocols

The four isozyme protocols identified in M227 Quarterly Report No. 1 were :

1. Protein extraction
2. Electrophoresis
3. Assay techniques
4. Photographic record of results

Procedure

See section 1.3.2 of M 227 Quarterly Report No. 1.

Results

Work on the above 4 protocols has been completed.

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.

Procedure

Trial 1. A number of local *P. cinnamomi* isolates were compared isozymically with 10 Californian and 3 ACT *P. cinnamomi* standards.

Trial 2. A small number of local *P. citricola* isolates were compared isozymically with 2 Californian *P. citricola* standards.

Results

In both comparative trials we were able to identify local isolates with the same isozyme profiles as those obtained from the Californian and ACT standards. This result has established, we believe, credibility in our technique. A further 1.5 weeks will be required to complete comparative trials for the 4 other priority species.

2.1.2 Intraspecific Isozyme Variation

The primary objective of this research 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.

Procedure

The Cellulose Acetate Gel Electrophoresis (CAGE) technique we adopted (see 1.3.1 of M 227 Quarterly Report No. 1) was designed to compare the isozyme profiles of 11 samples in a run. 5 runs are needed, therefore, per priority species (see 1.1.1 of M 227 Quarterly Report No. 1).

Crude protein samples of local isolates of *P. cinnamomi* were assessed electrophoretically with 13 enzyme systems. The isozyme profiles thus generated were subjected to a preliminary analysis.

Electrophoresis of local isolates of *P. citricola* and *P. megasperma* was initiated.

Results

From the 13 enzyme systems, with which *P. cinnamomi* isolates were tested, 18 isozymes (zones of activity) were recognized. Preliminary analysis, i.e. comparing isolate profiles, yielded detectable variation in ca. 40% of the isozymes, and we observed more genetic variation in this species than has previously been reported (Old *et. al.* 1984, Old *et. al.* 1988 and Oudemans & Coffey 1991).

We were able to recognize as many isozymes in *P. citricola* and *P. megasperma* as were found in *P. cinnamomi*.

A further 7 weeks will be required to complete electrophoresis and document the intraspecific variation in local *Phytophthora* species.

References

Old KM, GF Moran and JC Bell. 1984. Can. J. Bot. **62** : 2016-22.

Old KM, MJ Dudzinsky and JC Bell. 1988. Aust. J. Bot. **36** : 355-60.

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

PROGRAMME FOR THE NEXT QUARTER

The third quarter is of 13 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	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 is scheduled for completion in the fourth quarter.

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

Final Comment

The above work schedule for the third quarter is a modified version of that presented in the research application to MERIWA. In the application staff training was to begin in Stage 4. This has been brought forward to Stage 3 in the work programme above, so that staff can be trained extensively over quarters 3 & 4 rather than intensively in the last. Re-evaluation of the time required to complete Stage 4, comparison of isozyme and morphological methods (formerly research application Stage 3), has resulted in it being shortened. Otherwise all other work areas are progressing in accordance with the research application programme. No obstacles have been encountered at this stage, and none are foreseen for the third quarter.