PT216
Development of a commercial assessment to detect parasitoids of the potato moth

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FINAL REPORT

Development of a commercial assessment method to detect parasitoids of the potato moth.

Project PT216

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INDUSTRY SUMMARY

Potato moth is a serious pest of potatoes and other solanaceous crops in Australia. Several species of biological control agents (parasitoid wasps) exist nationally and, with cultural control measures, these form the basis of IPM strategies. The potato moth can be monitored easily using pheromone traps or other crop monitoring techniques. As part of this monitoring, growers need to know whether caterpillars have been attacked by parasitic wasps. The wasps will reduce the build-up of later generations of potato moth populations. However, the parasitoids develop inside their host and thus it is impossible for growers and scouts to visually check parasitism levels. Conventional means of monitoring parasitoids involve rearing larvae to adult stages: a process which is too time- consuming to be of practical use to growers in daily decision making about the crop.

This project has resulted in a rapid test being available to growers which can detect the presence (or absence) of each of the three common parasitoid wasps. The technique involves Phast-Gel electrophoresis, a biochemical method that uses enzymes specific to each species as indicators of the presence of these species. The enzymes show up as bands on an electrophoretic gel, with certain bands diagnostic for the different parasitoids.

Caterpillars or leaf samples can be sent to laboratories having suitable equipment, where the test can be done within 24 hours. The results will enable growers to make informed decisions on control of potato moth, and give confidence in IPM strategies.

TECHNICAL SUMMARY

Potato moth (*Phthorimaea operculella*) is a serious pest of potatoes and other solanaceous crops in Australia. Several species of biological control agents (parasitoid wasps) exist nationally and, with cultural control measures, these form the basis for IPM strategies. The potato moth can be monitored easily using pheromone traps or other crop monitoring techniques. However, the parasitoids develop inside their host and so are essentially invisible to growers and crop scouts. Conventional means of monitoring parasitoids involve rearing larvae to adult stages: a process which is too time consuming to be of practical use to growers in daily decision making about the crop.

This project developed a rapid test that can detect the presence (or absence) of each of the three common parasitoid wasps, *Orgilus lepidus, Apanteles subandinus* and *Copidosoma koehleri*. The technique involves PhastGel electrophoresis, a biochemical method that uses esterases specific to each species as indicators of the presence of these species within potato moth caterpillars. The banding patterns on an electrophoretic gel are diagnostic for the different parasitoids, and can be used as "fingerprints".

The technique does not detect parasitoids when the hosts weigh less than 2mg or are less than 12 days old (when reared at 23°C). This should not limit the value of the test as such small larvae are not likely to be collected in the field. If they are, they can be reared until above the critical weight.

INTRODUCTION.

Potato moth (*Phthorimaea operculella*) is one of the major insect pests of potatoes in Australia (Rothschild, 1986). In some regions such as Bundaberg and Mareeba it also is a serious pest of other solanaceous crops such as tomatoes, capsicums, eggplants and tobacco. The caterpillar stages of the potato moth can cause significant damage to the leaves but most economic losses are due to caterpillars feeding on tubers or fruit.

Biological control agents that limit populations of *P. operculella* are present in potato growing areas and, as part of an IPM strategy, have been shown to give effective control of the potato moth (Horne 1990, 1991). The biological control agents are parasitic wasps (*Orgilus lepidus, Apanteles subandinus* and *Copidosoma koehleri*) that attack the young caterpillars. The wasps develop inside the caterpillars, eventually killing their hosts before the pupal stage.

The adult wasp parasitoids, which are smaller than the potato moth, are not easily seen in crops and are not recognised by growers. Since the developing wasps are internal parasites and are not visible. Parasitised caterpillars are practically indistinguishable (externally) from non-parasitised caterpillars.

Current monitoring and sampling procedures (Horne 1993) can rapidly indicate changes in potato moth populations, but monitoring parasitoids takes approximately four weeks. This is due to the time taken to rear parasitoids through from egg or

early larval stages. A major impediment to the adoption of IPM is that growers are reluctant to rely on a biological control agent that they cannot see and do not know is actually there.

The lack of visible evidence of the parasitoids often means that growers will resort to insecticides (with visible results) that also kill the biological control agents, and so destroy any IPM strategy. Growers are then committed to continuing use of insecticides, with the attendant problems of cost, user safety and residues.

This project aimed to develop a rapid means of detecting parasitoids using PhastGel electrophoresis. Biochemical detection of parasitoids has been described for a number of insects such as wasps attacking aphids (Wool et al. 1978; Castanera et al. 1983) and caterpillars (Castrovilla and Stock, 1981). However, no such research has been done on parasitoids of the potato moth. The method relies on the principle that insects belonging to different species (or other taxonomic divisions) contain characteristic enzymes, which although having the same specificity, have different physico-chemical properties.

Wool et al. (1978) first recognised that electrophoresis could be used to detect parasitoids. Enzymes can be separated by gel electrophoresis and subsequent staining for particular allozymes can produce chromatograms ("finger prints") that are diagnostic for particular species (Powell and Walton 1989). The process has been used for detection of aphid parasitoids more than any other group, but techniques for the detection of parasitoids of Diptera and Lepidoptera have also

been described. These have been reviewed by Powell and Walton (1989). A parasitised potato moth larva can be detected since the parasitoid contributes additional bands to the particular finger print.

This project has addressed the needs of growers by providing the means to rapidly ascertain the presence (or absence) of biological control agents. By giving growers confidence that biological control agents exist in their crops we have reduced the need for insecticides and promoted IPM. It will also allow use of mass-release of parasitoids as a control option, as the effectiveness can be quickly assessed.

MATERIALS AND METHODS

This project was conducted initially at the Institute for Plant Sciences (IPS), Burnley, and then subsequently at the Institute for Horticultural Development (IHD), Knoxfield. Equipment essential for this project, a PhastSystem™ electrophoresis apparatus (Amrad Pharmacia Biotech., North Ryde, NSW) was available at IPS and has recently been purchased by IHD.

The three species of wasps and the potato moth were maintained in culture at IPS and then at IHD. Proteins, unique to each of the three parasitoid wasps, were identified by using a PhastGel electrophoretic technique.

Isozyme bands characteristic of parasitised larvae were identified and then a series of experiments was conducted to determine the earliest stage at which parasitism could be identified for each species.

The sensitivity of the test was evaluated using parasitised potato moth larvae at various ages and stages of development.

Field trials in selected Victorian commercial potato crops were used to validate the technique. These crops were monitored weekly to determine the percentage parasitism by conventional methods and at particular times during the season biochemical methods were also used. The results of the biochemical evaluation were compared with estimates of parasitism derived by conventional rearing techniques.

Estimates of the percentage of caterpillars parasitised were calculated for each outcome of a testing procedure with 12 lanes and either 2, 3 or 5 caterpillars combined and tested per lane. A 95% lower confidence limit for the percentage parasitism was also calculated.

ELECTROPHORESIS PROTOCOL

(See Appendix 1)

RESULTS

Bands diagnostic for each of the three parasitoid species were detected using Phast-gel electrophoresis (Figure 1). Initially, bands for each species were found using a range of procedures. However, it was important for commercial application that a single electrophoretic technique would simultaneously detect all three species. The methods that we have developed, which accomplishes this requirement is described in Appendix 1. The bands were initially produced using *P. operculella* pre-pupae or large larvae apparrently about to pupate. This is the stage at which the parasitoids normally kill their hosts and when the developing parasitoid larvae are at their maximum stage of development. A range of electrophoretic methods were tested, including iso-electric focussing, staining for several enzymic activities and using different gel gradients. The most suitable method for our diagnostic purpose was obtained using native 10 to 15% polyacrylamide gels and stained for esterase allozymes.

Detection of parasitism at an early stage is necessary for the system to be useful to potato growers. Experiments were conducted with each of the three parasitoid species to determine the earliest stage at which parasitism can be detected. Levels of parasitism determined using gel electrophoresis on larvae 12-15 days old (reared at 23°C) were consistent with those determined by conventional methods taking 30-35 days. However, parasitism could not be reliably detected in larvae less than 12-15 days old (figure 2). The relationship between age of larvae and weight was then examined. The ages of larvae from field collected samples are unknown, but

weights can be measured and larvae too small (and therefore presumably too young) are not used for the gel electrophoresis method. Larvae parasitised by *Orgilus lepidus* and *Apanteles subandinus* weigh less than unparasitised caterpillars, but those parasitised by *Copidosoma koehleri* weigh more (figure 3). Detection of parasitoids was shown to be reliable when larvae greater than 2mg were used (figure 4).

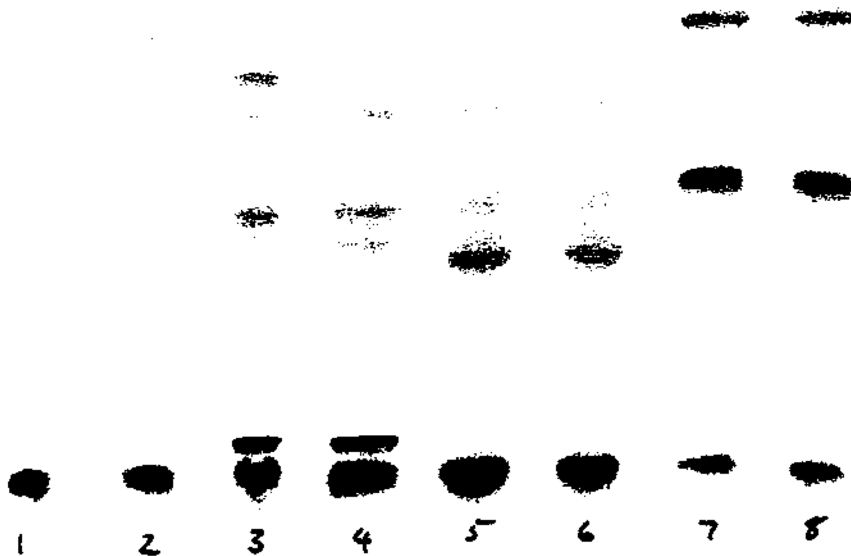
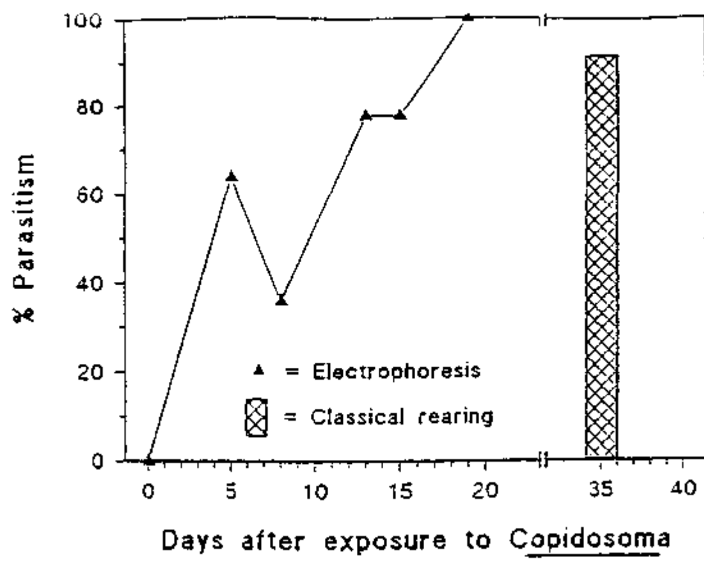
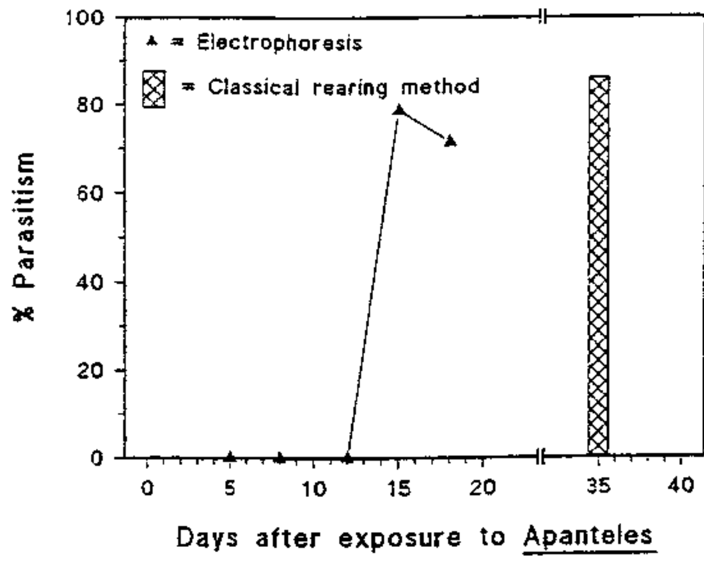


Figure 1: Banding patterns obtained by gel electrophoresis. Lanes 1 and 2; unparasitised potato moth; Lanes 3 and 4, *Copidosoma*; Lanes 5 and 6 *Orgilus lepidus*; Lanes 7 and 8, *Apanteles subandinus*.

Maximum likelihood estimates of the percentage of caterpillars parasitised are presented in Table 1. Each estimate is the most likely level of parasitism given the number of positive bands found on 2 gels (12 available lanes), when either 2, 3 or 5 caterpillars are combined and tested per lane. For example, if 8 positive laneways are recorded, the estimated level of parasitism is 19.7% (if 5 caterpillars are used per lane), 30.7% (if 3 caterpillars are used per lane) or 42.3% (if 2 caterpillars are used per lane). Table 1 also shows the lower 95% confidence limit for the level of parasitism. We can be sure that the true parasitism is at least as great as this figure.





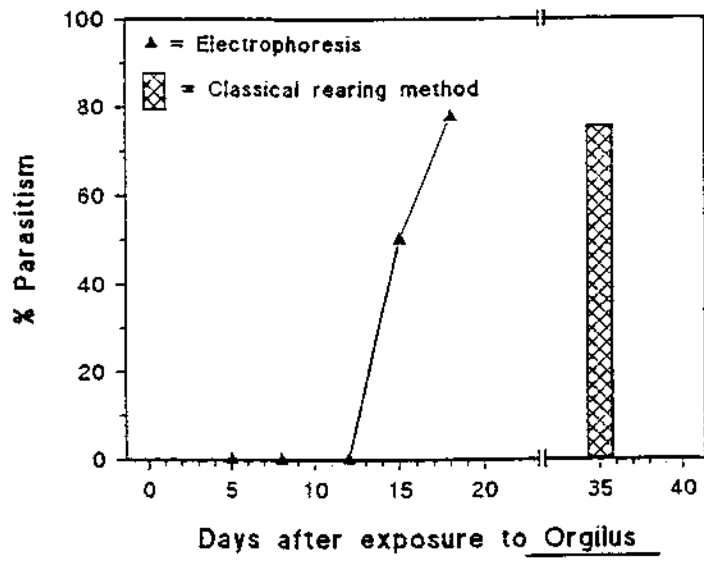


Figure 2: Detection of parasitism versus days after parasitism, using gel electrophoresis and conventional rearing methods.

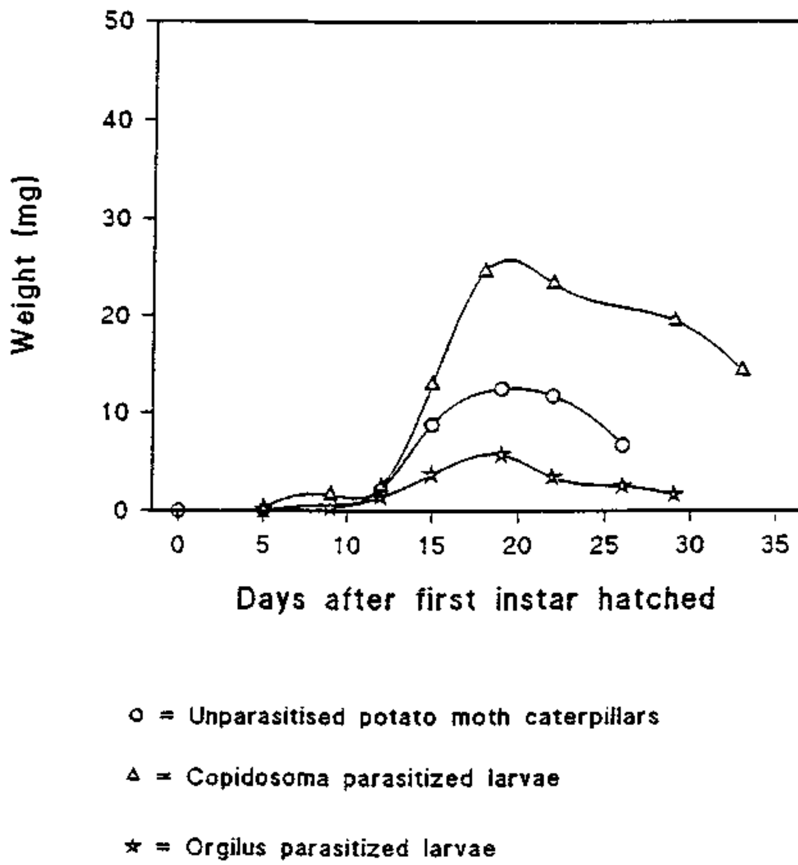


Figure 3: Weights of parasitised and non-parasitised caterpillars reared at 23°C.

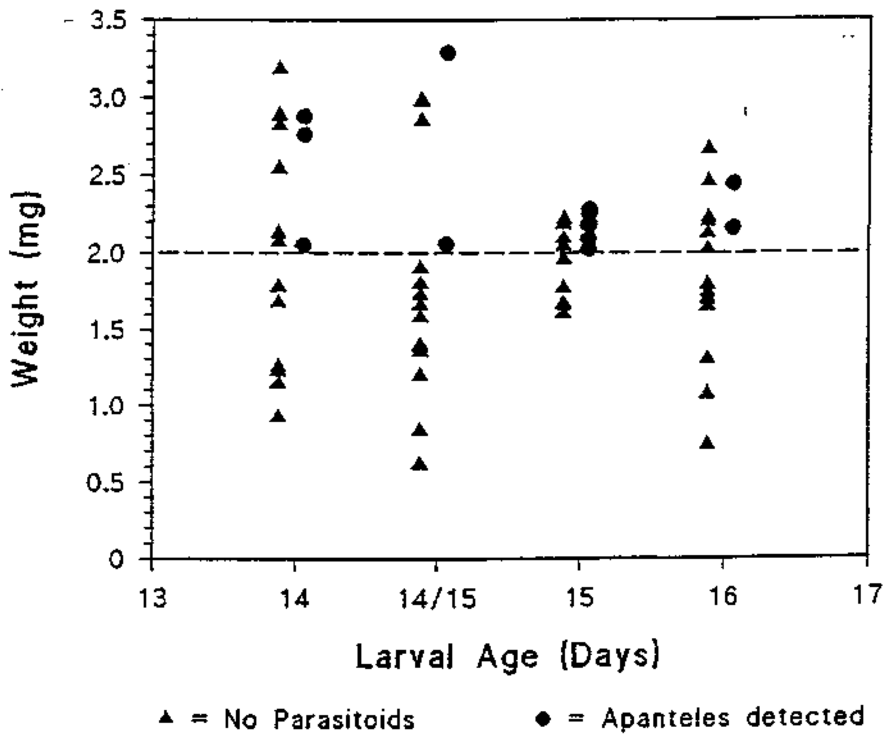


Figure 4: Detection of parasitism in larve of different ages and weights. A mixture of parasitised and non-parasitised larvae were tested.

Three caterpillars per laneway would be a reasonable choice as it gives sufficiently precise estimates at a reasonable cost. If this is the number used, then 36 caterpillars would be required for each test.

Table 1: Maximum likelihood estimates of (a) percent parasitism, and (b) lower 95% confidence limits, given the number of laneways on the gel with positive bands (minimum 0, maximum 12), and with groups of either 2, 3 or 5 caterpillars per laneway.

+ve bands	Groups of 2		Groups of 3		Groups of 5	
	a	b	a	b	a	b
0	0	0	0	0	0	0
1	4.3	.4	2.9	3.0	1.7	.4
2	8.7	2.1	5.9	1.4	3.6	.9
3	13.4	4.5	9.1	3.0	5.6	1.8
4	18.4	7.4	12.6	5.0	7.8	3.0
5	23.6	10.8	16.4	7.3	10.2	4.5
6	29.3	14.6	20.6	10.0	12.9	6.1
7	35.5	19.0	25.3	13.1	16.1	8.1
8	42.3	23.9	30.7	16.7	19.7	10.4
9	50.0	29.7	37.0	20.9	24.2	13.1
10	59.2	36.6	45.0	26.2	30.1	16.7
11	71.1	45.3	56.3	33.1	39.2	21.4
12	100	58.2	100	44.1	100	29.5

DISCUSSION

Neither potato moth nor parasite populations in crops are static. The population numbers are cyclic as successive generations develop. Typically, potato moth populations increase, and then decrease as a consequence of developing parasitoid populations. A reduction in the number of hosts consequently limits the number of parasitoids (Horne 1990). When results from tests are analysed they need to be related to these host-parasite population cycles. That is, **either (i) samples should be taken weekly and the levels of parasitoids monitored precisely or (ii) simple presence/absence of parasitoids recorded.** Regular samples need to be taken if a precise level of parasitism is needed, to know where the sample fits in the parasitoid/host cycles.

Descriptions of the entire process, with detailed instructions of field sampling techniques, biochemical equipment and methods and interpretation of results in the context of IPM can now be developed as a commercial kit for industry use. The methods are fairly simple but the capital cost of equipment is relatively high. **Laboratories that already have the equipment, or are prepared to purchase it, will be able to offer a service nationally.** Samples of caterpillars in leaves may be sent by express-post or courier to laboratories at a central location for analysis. For example, caterpillars from Bundaberg, Queensland have been successfully tested in Melbourne, Victoria using this procedure.

It is intended to offer the service to growers, nationally, through the

Diagnostics Service Group based at IHD, Knoxfield. Pilot schemes involving growers in Victoria and Queensland are planned for the 1994/95 season. However, details of the techniques are available for any laboratory wishing to be involved.

Parasitoids could not be detected in very young caterpillars. This restriction on larval age is not seen as limiting the use of the method in practice. Two of the parasitoids attack young larvae and so caterpillars should be at least one week old before sampling. This will ensure that, if parasitoids are present, then caterpillars will have been exposed at the vulnerable stage. Otherwise, levels of parasitism will be seriously underestimated (Horne 1993). Caterpillars less than 2mg are very difficult to find in the field and so samples consisting of caterpillars too small to test are very unlikely to be collected.

Maximum benefit will be obtained from this project if it is linked to other IPM work. Extension material advising growers of this tool will be distributed in conjunction with notes on other aspects of IPM in potatoes. It will be of particular use when combined with mass-rearing and release of parasitoids, and it can be used to increase growers awareness of beneficial insects, thereby generating greater confidence in adopting IPM strategies.

The results of this work will be integrated with on-going IPM projects in potatoes, funded by HRDC and Agriculture Victoria. Pilot schemes involving groups of growers in various districts, and a variety of solanaceous crops, will assess the most profitable way to use this new monitoring procedure and at the

same time promote grower awareness.

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APPENDIX 1: PROTOCOL FOR PHASTGEL ELECTROPHORESIS

For detailed information relating to use of the PhastSystem electrophoresis instrument the reader is referred to the user's manual (Amrad Pharmacia Biotech., North Ryde, NSW).

BUFFERS AND REAGENTS REQUIRED:

Extraction Buffer - 25mM MOPS/Na^+, pH 6.8, (containing 5mM EDTA and 5mM DTT).

Esterase staining buffer - 50mM phosphate/Na^+, pH 6.9.

'stop' solutiion - 5%$^v/_v$ aqueous acetic acid

gel preserving solution - 5%$^v/_v$ aqueous glycerol

PVP, insoluble (polyvinylpyrrolidone)

-napthylacetate

Fast Blue RR

PROCEDURE: (this example uses one test individual per lane on each of two gels)

- Isolate larvae to be tested i.e.: 12 test larvae, 2 unparasitised larvae (control 1), and 2 parasitised larvae (control 2).
- Ensure that larvae each weigh more than 2mg.
- Keep all samples and extracts on ice to maintain enzymic activity.

- Place each larva into a micro-centrifuge tube and add 10μl of extraction buffer to each tube. (20μl for larger larvae such as *Copidosoma* [parasitised]). Also add PVP to each tube (1:1 by weight of sample).

- Centrifuge in a microfuge at maximum speed for about 20 seconds to ensure all contents are at the bottom of the tube.

- Grind the contents of each tube thoroughly, using a small plastic pestle.

- Centrifuge for 5min at maximum speed.

- Collect 5μl of supernatant from each sample and transfer to a new tube. Before transferring, wipe the end of the pipette with a tissue to remove any lipid.

- Add to the supernatant 2μl 33% $^v/_v$ NP-40 and 0.5μl of 0.5M $CaCl_2$.

- Centrifuge for a further 5 minutes at maximum speed.

- While the samples are centrifuging, prepare the PhastSystem for either a 10-15 % or 8-25 % Native Gradient electrophoresis run.

- Typical loading of sample extract per lane is 1μl.

- When completed, place each gel into a petri dish containing 25ml of esterase staining buffer.

- Add 7.5mg of alpha-naphthyl acetate, dissolved in 750μl ethanol, to the petri dishes and agitate them gently in the dark.

- After approximately 1 minute add 12.5mg of Fast Blue RR.

- Leave the gels agitating in the dark until bands have developed to the required intensity. This usually only takes a few minutes.

- Rinse the gels with water and place into a few mls of "stop" solution to terminate the enzymic reaction.

- After 10-15 minutes replace the stop solution with gel preserving solution.

- Leave the gels for a few hours. They may then be dried down for recording and preservation.