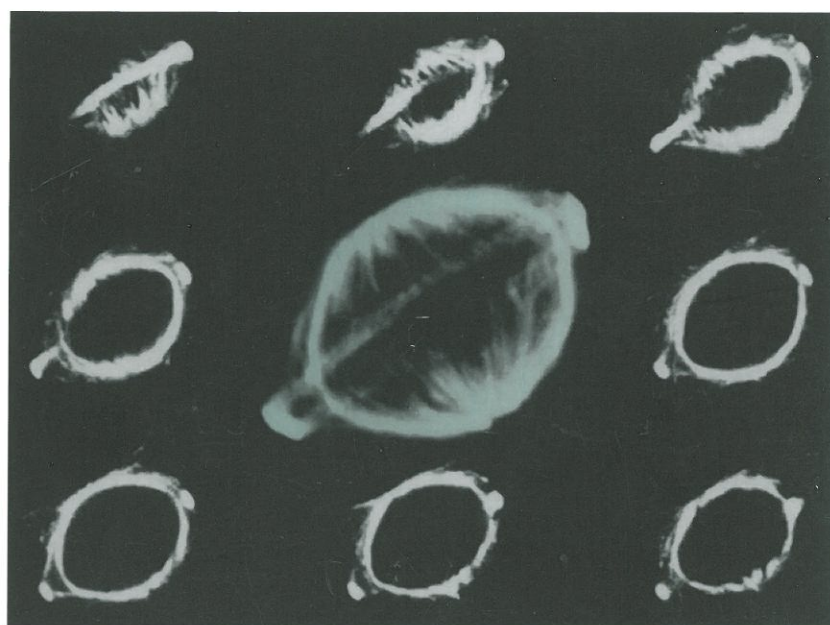


BIOLOGIC

*Research School of Biological Sciences
Australian National University*



CONFOCAL MICROSCOPY AND PLANT CELLS

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Planting an Idea

November 1992

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Welcome to a new edition of *Biologic*.

The new edition springs from recent research success in the Research School of Biological Sciences which we want to share with you.

We hope to give you something of the flavour of what it is like to engage in research at the forefront of biology.

Perhaps the most important development in our research agenda has been the establishment of a seven year Cooperative Research Centre between several groups in this Research School and in CSIRO Division of Plant Industry. Now a year old, this venture is in full swing. The previous editor of *Biologic*, and School Secretary in RSBS, Chris Buller has been appointed Executive Officer in the Centre. Chris has taken a particular interest in the educational programs of the Centre and we can expect to see these activities reported in future issues of *Biologic*. This edition has been produced by James Whitehead and Brok Glenn.

The freedom to engage in full time research in biology is a privilege available to few in our society. Although we in Australia invest heavily in education and research, only a small part of this investment finds its way to independent creative enquiry of the sort undertaken in RSBS. It is a privilege shared with relatively few institutions world-wide, such as the Max Planck Institute in Germany and the Carnegie Institute in the U.S.A. Whether privately endowed or publicly funded, the independent researchers in these laboratories are a well-spring of innovation and insights. After only 25 years, you can be proud that your investment in RSBS continues to generate "high impact science", research and scholarship which has changed the way we think about several areas of biology.

The Research School celebrates the silver jubilee of its founding in 1967 as this issue of *Biologic* is released. The School's anniversary present to itself, and to the University, is a 184 seat, \$1.7 million lecture theatre and computer

laboratory. Built as a joint venture with the Faculty of Science, it will be used for undergraduate teaching each morning during the semester, and for research seminars and symposia at other times. The lecture theatre will be named for Sir Rutherford Robertson, second Director of RSBS, who guided the expansion of Australian scientific Research in the 1960s.

The infrastructure of tertiary education and of research in universities has changed markedly since the foundation of this Research School. The Robertson Theatre symbolises, in a small way, our independent response to these changes. In the absence of grants for capital works, the construction of the lecture theatre is funded from equal contributions from syndicated R&D projects in biology undertaken by members of RSBS, from collaborative projects with the Faculties and from School funds.

We are grateful to our colleagues whose research achievements made this venture possible, and to the University's R&D company, ANUTECH which was responsible for negotiation of these programs. The Robertson Theatre is thus part of our investment in a future which may well see closer collaboration in research and teaching, and the emergence on the ANU campus of a unique research university.

Biologic is another part of our investment in the future. It is an investment which depends on you getting to know us better. Your appreciation of our ambitions and achievements will help determine how well we can do what we do. Most of all we hope *Biologic* will reach out to the next generations of students and researchers in biology who will continue and develop what we do!

Barry Osmond

A POTENT BREW

Chemical Archaeology of Kava



A seemingly unlikely collaboration between Dr Charles Hocart of the Plant Cell Biology Group in RSBS and Dr Barry Fankhauser and Mr David Buckle of the Prehistory Department in the Research School of Pacific Studies has helped determine the function of archaeologically interesting pottery.

One can often make reasonable guesses at the function of a piece from general appearances, for example, a fire-blackened exterior may indicate a cooking pot or a combination of decoration, shape and style may indicate a vessel of religious significance. Specifically, the question these researchers posed was; had these particular Pacific potsherds been used for making or drinking kava? In answering this, it was necessary to know something of the origin and chemistry of kava.

Kava is the traditional drink of the native people of Oceania and is made from the roots of *Piper methysticum*. It first came to the attention of Europeans as an intoxicant during the 1768-1771 voyage of Captain James Cook through the South Pacific. It was drunk throughout Polynesia (with the exception of New Zealand, Easter Island, Rapa, and coral atolls where the plant could not grow), in parts of the Melanesian island groups of Vanuatu, the Solomon Islands and Papua New

Guinea, and in the Micronesian islands of Pohnpei and Kosrae. Subsequently this practice was discouraged by missionaries and in some places this prohibition was very successful, especially when combined with the introduction of alcohol. More recently, kava drinking has undergone something of a revival as people have been encouraged to reassert their cultural values along with campaigns for political independence and national identity, and in fact a kava cup is featured on the national flag of the Micronesian state of Ponape.

The kava plant, *Piper methysticum*, is an upright shrub with characteristic large heart-shaped leaves and a pithy jointed stem, growing to a height of between six to twelve feet. Although it is a dioecious species only male plants are known and no fruits or seeds have ever been reliably reported. The plant is thus entirely dependant on man, both for vegetative propagation from selected stem cuttings, and for its distribution during the great migratory voyages. The obvious importance attached to the kava plant by its cultivators may lie in the general paucity of stimulants and intoxicants to be obtained from the flora of the South Pacific; not even alcohol was known. Hence, it is reasonable to speculate that the great number of cultivars which are known today, many with a particular traditional use, may have arisen as a result of careful selection for plants with useful chemical characteristics.

The drink is traditionally made using the freshly harvested rhizome or the fine root system from *Piper methysticum*. This is cut up and cleaned before being thoroughly pulverised, either by chewing or grinding, and is then steeped in water, filtered and drunk. Prepared from fresh roots, the drink has a greenish milky look and is much stronger than the grey mixture obtained from the powdered dried rhizome which is now commercially available. It has a strong characteristic but pleasant smell, in contrast to the acrid, and highly astringent taste. The descriptions of the after-effects uniformly indicate a pleasant euphoria, with ease of conversation and increased perception followed by a deep natural sleep. Kava also has properties as a local anaesthetic and consumption results in a numbing of the mouth and tongue. In addition, kava has been described as having diuretic, soporific, anti-convulsive, spasmolytic, analgesic and anti-fungal activities, hence its use in traditional medicine to treat venereal disease, gout, rheumatism, diarrhoea and asthma.

Although the reputed psychoactive properties of kava have often been the focus of the casual outside observer, kava has played a much broader, social, economic, medicinal and religious role in the traditional societies in which it has been used. Consequently, it has attracted much attention from anthropologists, botanists, chemists, pharmacologists and doctors.

Fortunately for our purposes, the physiological action of kava has prompted numerous chemical investigations dating back more than a century. The sum of this work has been the identification and synthesis of many of the constituents of kava, with attention focussed on those eliciting a pharmacological effect, namely the kava lactones. These can be divided into two groups; the major kava lactones (Fig 1) and the minor kava lactones with the former accounting for more than 96% of the whole extract. As might be expected from the anecdotal evidence regarding the use of the various cultivars of the kava plant, each cultivar may be chemically typed according to the proportions of kava lactones present. It is also interesting to note that the kava lactones are all insoluble in water, hence the importance of a thorough emulsification step in the preparation of the drink.

The kava lactones may be readily quantified and identified by using the combined technique of gas chromatography-mass spectrometry (GC/MS) which Charles normally uses in his analyses of the cytokinin group of plant growth regulators. In simple terms, the gas chromatograph can separate the components of a mixture which is then directly

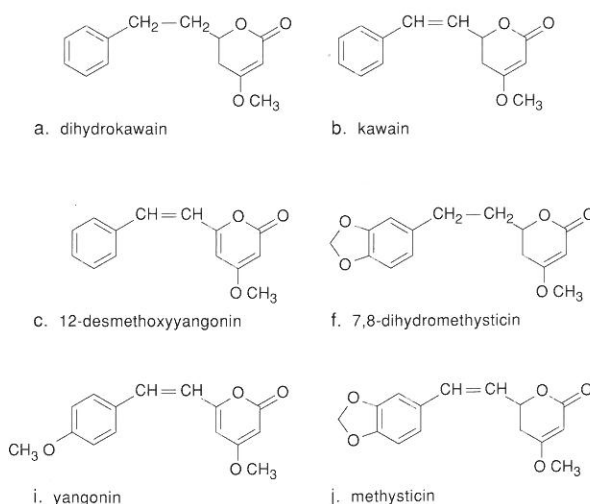


Fig 1. Chemical structures and names of the major kava lactones.

transferred into the source of the mass spectrometer where they are fragmented in a chemically characteristic way and the mass of each fragment determined. Each compound then has its own unique fingerprint or mass spectrum (Fig 2). This is a very sensitive technique and is widely used today, for example, to analyse blood and urine samples for drugs, and for environmental monitoring of industrial waste and agricultural chemicals.

One of the central elements in any kava ceremony is the kava bowl. In modern times these have typically been large round shallow wooden bowls, often with legs and characteristically decorated. These bowls are used only for kava. However, it is thought that these wooden bowls only came into general use four to five hundred years ago, for the most part replacing pottery bowls. The broken remains of such pottery are commonly found on archaeological surveys and in the absence of ethnographic records it has been necessary to functionally interpret these potsherds on the basis of their similarity to modern wooden bowls. The standard of glazing on these pots was frequently perfunctory allowing the contents to soak into the porous body of the pot. We felt that as the kava lactones are essentially insoluble in water, that they would not be readily leached out of the potsherds by the action of ground water and hence that sufficient material may still be present for us to detect. Today we are now testing this hypothesis and attempting to extract and identify this material from these remains of purported kava bowls. For the purposes of comparison, we firstly

extracted a sample of powdered dry kava roots with chloroform and identified each of the GC peaks from their mass spectra (Fig 3). The next step was to repeat this with an ancient potsherd which we extracted with chloroform and analysed by GC/MS. If kava lactones were still present in the matrix of the potsherd, then we would expect to find them in very small amounts. Therefore, in order to maximise our sensitivity we used a MS technique called selected ion monitoring in which instead of looking at every ion in the mass range of interest, only the characteristic or fingerprint ions of the compounds are monitored. In the example shown (Fig 4), we have been able to identify two kava lactones, methysticin and yanonin, confirming that this particular ancient Fijian potsherd once formed part of a kava bowl or cup.

The distribution and number of cultivars of the kava plant, together with the known movement of people, suggest that kava drinking may be quite an old practice but to date this has been no more than supposition. However, it has generally been possible to context date pottery (eg carbon dating charcoal in the same layer of an excavation), thus we can now unequivocally link kava drinking, a major aspect of the ceremonial cultures of many Pacific societies, to the archaeological record. In the future, it may be possible to extend this type of analysis to other potsherds and, by looking for other sets of telltale chemical markers (e.g. alkaloids, terpenoids), to determine their former function.



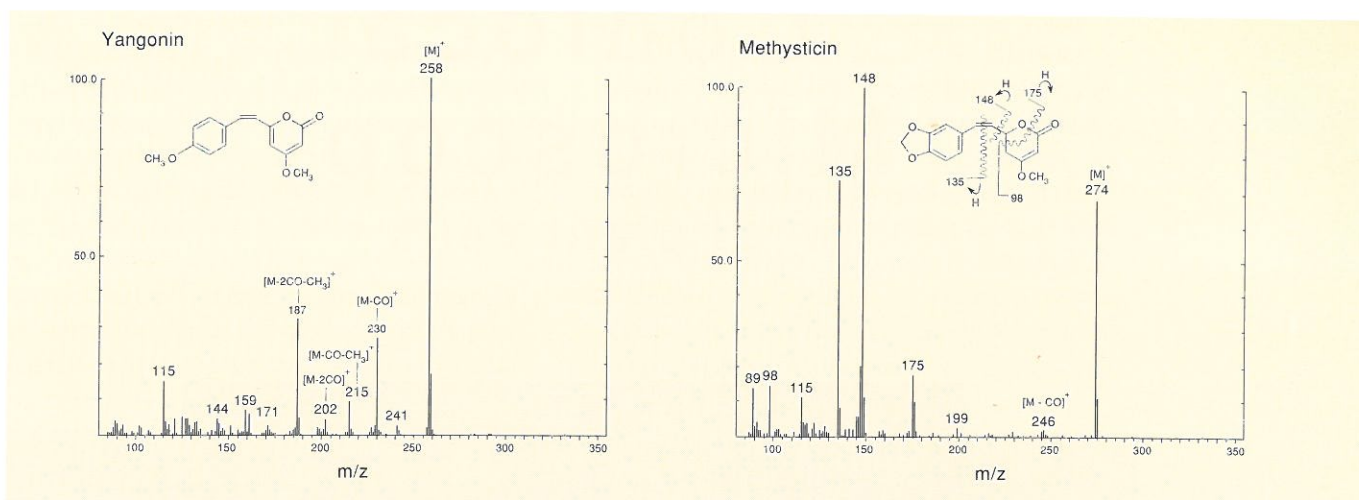


Fig 2. Mass spectra of yangonin and methysticin (electron impact, 70eV).

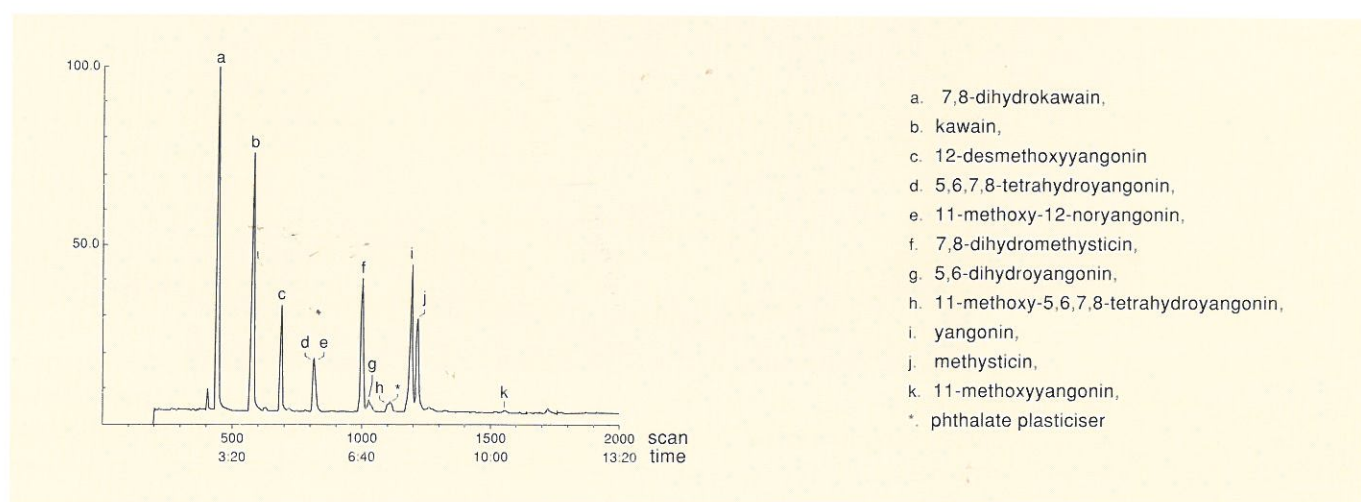


Fig 3. Total ion currents from GC/MS analysis of a chloroform extract of a kava root.

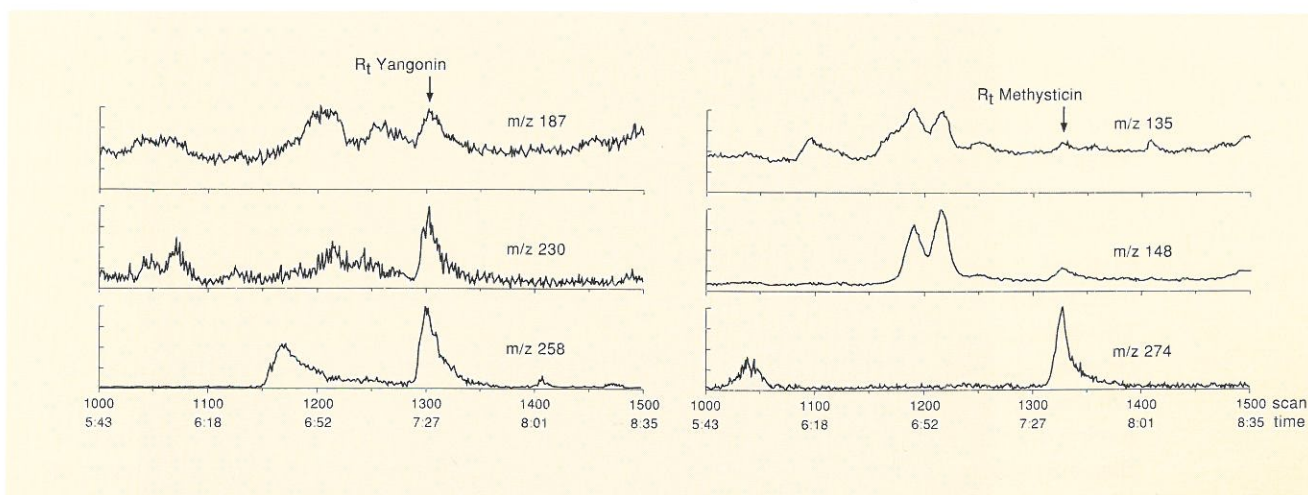


Fig 4. Ion currents observed during GC/MS of the chloroform extract of an ancient potsherd containing yangonin (m/z 258, 230, and 187) and methysticin (m/z 274, 148 and 135). Ion current maxima occurred at the retention times (R_t) of the authentic compounds.

Confocal Microscopy

of plant cells



Plant cells are closely confined by the cell wall that surrounds them. The properties of cell walls and the sites of their insertion thus determine the size, shape and expansion of all plant cells, and, in the aggregate, of the entire plant.

An understanding of factors that regulate plant cell growth and division is the focus of active research in the Plant Cell Biology Group in RSBS. Two of these factors are known to be filamentous components of the plant cytoskeleton, namely microtubules and actin microfilaments. Microtubules are responsible for the separation of chromosomes during division, and also determine cell shape by controlling the orientation of reinforcing cellulose microfibrils in the wall. Actin filaments have a major role in cytoplasmic streaming and also participate in the alignment of new cell walls.

Much of the basic information concerning the cytoskeleton has been revealed by transmission electron microscopy of fixed and sectioned material, a laborious task particularly if three dimensional reconstructions are required.

Approximately 10 years ago there was a breakthrough in the study of the plant cytoskeleton with the development, by members of the Plant Cell Biology Group, of a technique for fluorescently labelling microtubules. The technique uses antibody molecules that are specific for tubulin, the protein with which microtubules are built. Labelling with fluorescent antibodies allows

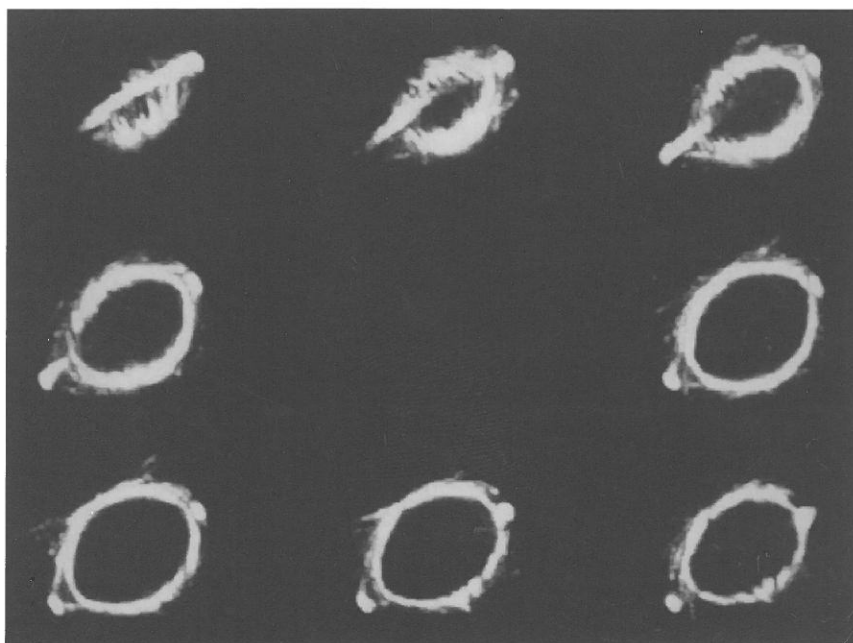
arrays of microtubules and actin filaments to be visualised in whole, fixed cells by epifluorescence microscopy. Using this method, rapid advances in understanding the plant cytoskeleton were made in laboratories throughout the world. As revolutionary and versatile as this procedure has proven to be, there are, however, limitations to the information that can be gleaned from such studies. Examination of fine detail is often hampered by background fluorescence arising out of the plane of focus, and by the limited resolution of the fluorescence light microscope. Bleaching of fluorochromes by the mercury vapour light source is also a problem.

Recently a completely new type of fluorescence microscopy that overcomes many of these problems has been developed. The technique is called confocal laser scanning microscopy. Confocal microscopes are used predominantly for fluorescence studies, but reflection and transmission images may also be collected. Light from an argon ion laser passes through an illumination aperture and objective lens and is focussed on the specimen. Light returning from the specimen passes back through the lens and is focussed on a second detection aperture which allows only the portion of the beam that is in focus to pass to the detector. The principle of the confocal microscope relies on the fact that the illumination and detection apertures are with one another. This means that stray light emanating from above or below the plane of focus is excluded. It is this

light that in a conventional microscope would result in blurring of the image. The ability to completely exclude out of focus light means that confocal microscopes can take discrete "optical sections" through the sample, even when the sample is very thick. A complete image at any focal plane is built up by scanning the laser beam across the specimen. The image is viewed on a monitor as it is being collected and noise reduced by frame averaging functions before being stored in digital form on a computer.

Spatial resolution in the x-y plane, parallel to the microscope stage, can be as much as 1.4 times greater than on a non-confocal microscope, and has been measured at approximately 0.2 μm . In addition, discrimination in the z axis is remarkable. Optical sections 0.3 μm in depth can be made, free from any information from elsewhere in optical sections from the same cell can be added together as a linear projection to give an extended depth of focus. These three dimensional reconstructions can be rotated about any angle, enabling cells to be viewed from orientations not possible in a conventional microscope. Stereo images can be compiled generating life like three dimensional views of whole cells, or of the inside of cells viewed through an optically cut surface.

All these powerful imaging capabilities were made available in RSBS through the instigation of Professor Brian Gunning and the purchase of a Biorad MRC 600 confocal laser scanning microscope. Just as was shown by the introduction of



Progressive optical sections of a cell showing microtubules that have been stained with an antibody and fluorescent label.

the antibody technology in the early 1980's, the new technology of confocal microscopy offers the potential for rapid advances in many fields of study. Within Plant Cell Biology, in the year in which the microscope has been operating, its use has led to a wealth of new insights into the cytoskeleton of plants cells and the regulation of plant cell growth and division. Indeed, demand for time on the confocal microscope is so high that users need to be prepared to work the midnight to dawn shift ! Projects for which it is being used include examination of the cytoskeleton in a range of higher and lower plants to determine how specific arrangements of microtubules are organised and how they may have evolved ; determination of the distribution of cell cycle proteins in plants and algae; and analysis of the infection of plants by pathogenic fungi.

Most of these studies have been carried out on fixed and therefore dead material, yet the elements being examined are very dynamic in nature. Until recently there has been no way in which these components could be visualised in living cells. Because plant cells are enclosed within a cell wall it is notoriously difficult to get foreign materials, like fluorescent antibodies, into the cell. Removal of the wall can damage or even kill a cell, and in the case of multicellular structures, results in the loss of tissue integrity. A resolution to this dilemma is, however, now at hand.

During a recent visit to RSBS, Professor Peter Hepler, from the University of Massachusetts, instructed members of the Plant Cell Biology Group in the art of microinjection. This technique, developed by Professor Hepler and his co-workers, allows substances to be injected into living plant cells that are surrounded by cell walls. The confocal imaging system has been set up in combination with a Zeiss inverted microscope and microinjection equipment. The aim of the work carried out by Professor Hepler and members of the Plant Cell Biology Group was to microinject specific fluorescent probes into plant cells and

examine dynamic processes in living cells as they grew and divided. The tremendous success of this work has meant that there has been a constant buzz of excitement in the Group all year !

In one study, Dr. Geoff Wasteney purified and injected fluorescently labelled tubulin into two different types of plant cells. Inside the living cells, the fluorescent tubulin was incorporated into microtubules which grew and were reorganised as the cells were being observed. Dr Wasteney has been able to record individual microtubules, 25 nm in diameter, as they grew and shortened, a process called dynamic instability. In another study, Dr Ann Cleary has shown that the injected fluorescent tubulin incorporated into microtubules predicts the plane of cell division prior to the onset of mitosis. They were also observed to form the mitotic spindle. Injection of a probe that is specific for plant actin revealed that actin is also a dynamic component of the plant cytoskeleton. Further research work by Dr Peter John and Professor Hepler have documented the role of fluorescently labelled cell cycle proteins in the initiation of cell division.

The success of these studies was contingent on the use of the confocal microscope. The injection of fluorescent probes in to the cytoplasm of cells results in quite high levels of background fluorescence from which fine elements must be resolved. In addition, fluorochromes are prone to bleaching and fluorescently labelled microtubules can be broken down by excessive irradiation. The great sensitivity of the confocal system and the additional enhancement functions allow cells to be observed as they incorporate probes without damaging or inhibition cellular processes. Not surprisingly, there has thus been intense interest in the use of the confocal microscope to study dynamic events in living cells. The results obtained so far indicate that, in the future, confocal microscopy will be a major tool for imaging cells not only in plants but in a diverse range of biological systems.



B R O A D C A S T



One of the founding objectives for RSBS, set out in the proposal to the Australian Universities Commission in 1965, states that where possible the School should exploit the special features of the Australian biota. In the continued response the School is establishing a Biodiversity database to provide answers to all kinds of questions and promises a new era and concept in cooperative research.

Computers are forever challenging us with new ways of doing science. Even by conservative estimates, thousands of institutions and perhaps millions of researchers are now served by Internet, a vast communications web that links together computers, institutions and people all around the world allowing for unprecedented sharing of information.

One development of this global connectivity is the establishment of public domain databases. Public domain databases are freely available for anyone to use, copy or contribute to via an open network and provide for the promotion of both the research and the institution. The task of overseeing the input of all the information and designing for its accessibility is that of the Bioinformatics Group within RSBS.

The local backbone for Internet is AARNET, the Australian Academic Research Network.

The existing services and information available on Internet are astounding. Access to world-wide electronic mail and electronic news groups covering hundreds of topics are just the beginning. Being connected to Internet means having the resources of literally thousands of computers at your fingertips. Recognizing the advantages of free information exchange, many computer sites now allow guest logins by users over the network. What is more they make available various data, software and services that can be freely copied or used.

In some areas of research, notably molecular biology, distribution of information over Internet has grown explosively. Genbank, EMBL, and many other molecular biology databases are publicly available to all comers. But not only can biologists access data over the network, they can also obtain, free of charge, most of the software they need to interpret it! Several aids, such as Archie, Gopher and WAIS (Wide Area Information Servers), are now available to help users navigate their way around Internet's vast resources. They make it possible to retrieve files from anywhere in the world at the touch of a button. The following examples can only hint at the incredible range of information already available:

- * On-line access to telephone directories, bibliographies and library catalogues in many parts of the world;

- * Free software - many sites maintain libraries of public domain software. The Free Software Foundation at MIT develops and distributes high-quality, free software under its GNU Project.

- * Molecular biology databases, software and bibliographies - the Australian National Genomic Information Service (ANGIS) at the University of Sydney maintains up-to-date copies of the major databases.

- * Satellite and weather data - the University of New Mexico alone makes available 90 gigabytes worth!

- * Geographic data - electronic atlases, census data and summaries such as the CIA World Databank and Factbook (maps, facts and figures about every country in the world).

- * Electronic texts - Project Gutenberg, a public domain project, produces electronic versions of

English language texts, such as Roget's Thesaurus and the Complete Works of Shakespeare.

Free exchange of information implies give as well as take and while some people will view the idea of providing information on the network as time consuming, or as reducing the initial impact of research findings. The Bioinformatics Group, comprising Molecular Evolution and Systematics and the Centre for Structure and Molecular Function, see it as a golden opportunity. The School is preparing to provide a public domain database concerning biodiversity. Already the Molecular Evolution and Systematics Group, aware of these realities, is aggressively planning to exploit Internet to promote their work. The all important task of making the data accessible and determining how the output of the Biodiversity project will be made available to other researchers is the known as the "informatics" of the project. The job of deciding what methods should be followed in building the software to store and manage the data is conducted by Drs David Green, Georg Weiller and Jack Palmer.

Work is in progress to make available the Virus Identification Data Exchange developed by Professor Adrian Gibbs - the first ever comprehensive systematic treatment of plant viruses on a world scale. This information is already in use in discrete computerised form in nearly all developing countries. Fuller availability together with the capacity to update the data holdings will ensure that this School database will remain a landmark in plant pathology research. Another component of the Biodiversity database includes a computerised bank of morphological, anatomical, cytological and geographic characters amassing more than 400 different kinds of information, for each of the 764 genera of grasses. Dr Les Watson has been working since 1970 on producing this unique resource. In its present form the database can provide full descriptions of the grasses of all countries and translate them into an appropriate language allowing for interpretation and visualisation of the data. Provision has been made to generate keys for identification purposes for any geographic or taxonomic sub-set of the database. Again the information is to be made freely available using the Public Domain Database for Networking Biodiversity.

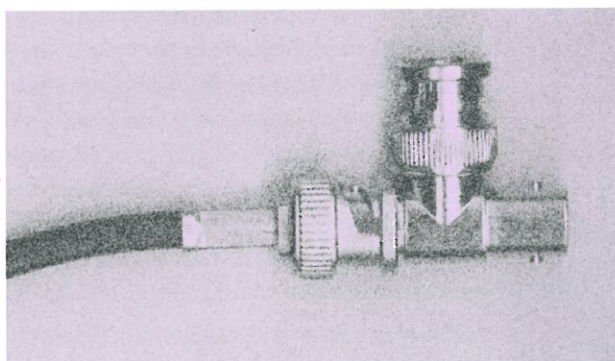
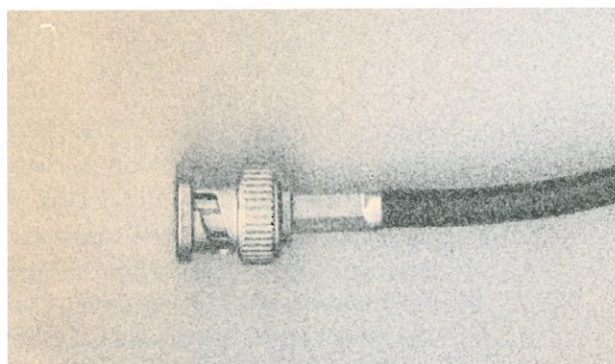
Visibility on Internet is beginning to play a role in determining the prestige and influence of both individuals and institutions. With publication

delays often running into years, researchers are increasingly turning to Internet to distribute their results quickly. Furthermore the sheer number of journals means that published work is often missed by other researchers. Electronic collections of papers and references provide a way to "advertise" both your past work and new publications. Also, technical innovations these days often require sophisticated software. If you want people to adopt your methods, then you need to provide them with your software. The fastest and simplest way to do this is to make it freely available on Internet.

Internet provides many opportunities for institutions to promote themselves. Sometimes a single unique "product" can draw attention to a site. James Cook University, for instance, is rapidly becoming known for its daily weather satellite images. Many sites have become famous as the coordinating centres of major network projects, such as those mentioned above. As a premiere biological research institution RSBS is well poised to take a lead in setting up a (non molecular) Biology Data Base that would allow the School to deliver our research on a national and international basis. For example, we plan to offer a Complex Systems Data Base, develop the work of Professor Dennis Carr and provide a Eucalypt database again with imaging information, or set-up a billboard for world-wide biology conferences. At the same time other individuals would be able to address RSBS and see the many features of the School. In addition to School research publications this could include access to PhD abstracts, RSBS Annual Reports, Robertson Symposium proceedings and issues of Biologic.

At present ANU is poorly represented on Internet. Despite being at the forefront in many fields of research this University provides the network with little that is unique. Apart from molecular biology, relatively little biological information is available on Internet. The contribution of a Biodiversity database allows for many opportunities while highlighting Australian biota and the School satisfies a founding objective: the requirement to fulfil a national role.

For the past twenty five years the School has served the Australian biological community and firm plans are in place to provide a continued and wider availability of the best biological research using the computer facilities of the University. The consequence of this planning will provide for meaningful interaction queries and interpretations of the database.



Charting the evolution of success

At first there seems little to link the record charts - those listings you pick up in record stores to learn what others are buying - with biological research.

Dr John Hancock, newly arrived in the Molecular Evolution and Systematics Group at RSBS thinks otherwise. Since his school years his two strongest interests have been science and analysing the record charts. There is, he says, a strong similarity between the kind of evolution research he now does, the analysis of the patterns of gene evolution and the processes underlying them, and analyses of record charts. The main objective is to take a group of individual "observations", be they gene sequences or individual charts, and to try to understand what patterns emerge and what processes have been involved in generating them.

There is much more to weekly "Top Of The Pops" than who happens to be No.1 this week. One striking aspect of the Australian charts compared to the English charts is the sustained success of one or two "golden oldies". For example, Neil Diamond, who recently visited Australia, has seen his "Hot August Night" LP in the Australian national chart for more than 160 weeks, while elsewhere in the world it stopped selling in the 1977. But a much more interesting aspect of chart analysis is looking for the new trends in Pop music. This is not always as easy as it may seem - the most important LP's are not always the biggest hits. The current success here and around the World of Nirvana, who played at ANU recently, certainly signals a return to the old fashioned values of guitar music, but who would have picked My Bloody Valentine's first LP as the landmark it has undoubtedly become.

Because of such difficulties in defining critically important records, Dr Hancock and his colleague Neil Rawlings in Cambridge, UK, have confined themselves to plotting the rises and falls of successful artists, and trying to trace back from their successes and failures the underlying trends in the music scene. For example, using a system

they have developed to link chart success directly to record sales, they have been able to assess which artists have been most successful in the British charts since 1971 (they are currently working their way through the sixties, publishing their results in their quarterly magazine, "Chartwatch"). Their analysis shows great success for artists producing dance-based music from 1983, when Michael Jackson's "Thriller" was the dominant record in the British charts, to 1990, when the New Kids On The Block were in full swing. Since then, though, they detect a much more directionless music scene - Bryan Adams was the most successful artist last year, mostly due to his massive hit "(Everything I Do) I Do It For You", rather than to any real enthusiasm for his music among the music-buying public. Perhaps more indicative was the success of REM's "Out Of Time" LP, which they see as a strong indicator of the revival of intelligent, guitar-based music.

While analyses of the Australian charts are still developing, there are strong indications of a difference between the musical tastes of the Australian and the British audiences. Like the Americans, the Australians have a taste for R'n'B-based artists, while the Brits have tended to prefer



dance-oriented artists in recent years. Indeed many of the most well-established Australian artists, like Jimmy Barnes, John Farnham and Diesel, are virtually unknown outside Australia. Arguably, this can be seen as encouraging - at least Australia has its own taste and the confidence to back it, something that may have contributed to making Australia the third biggest exporter of music, after the USA and the UK.

So what has this got to do with evolution? Dr Hancock's particular interest is in the way simple DNA sequences - DNA sequences that show a lot of internal repetition - influence the evolution of genes. This has involved him in a considerable amount of computer analysis that has fed and fed off his chart analysis, making him more prone to use statistical methods to look at DNA sequences than he would otherwise have been. The result has been models he has proposed to explain the evolution of ribosomal RNAs - component molecules of every cell that are now widely used in building trees of evolutionary relationships between species - and of a protein factor, TBF, that is intimately involved in the initiation of gene transcription.

At the RSBS Dr Hancock is expanding two new interests. One is the role of simple DNA sequences in the evolution of genes controlling pathways in development. Many genes that have been shown to be important in the development of the fruit fly *Drosophila melanogaster* also contain simple DNA sequences. Such regions are thought to be generated by a process known as slippage, and it is possible that the generation of such regions can lead to the evolution of new functions within a molecule that can add to its original functions. A second interest is the role of simple DNA sequences and slippage in the evolution of whole genomes (the total DNA content of an organism) - something he hopes to be able to link into the growing efforts to characterise a wide range of genomes, including the human.

"Chartwatch" magazine can be obtained by contacting John at the Molecular Evolution and Systematics Group, RSBS. The magazine is obtainable by post only. Prices are \$4 for a single issue, \$15 for a year's subscription (Surface Mail from the UK) and \$19 for an Air Mail subscription.

Frequent travellers between RSBS and the CSIRO Division of Plant Industry are no doubt aware of the seemingly sheep-worn path which exists in the various lawns intervening these two dynamos of plant science research in Australia.

The formation of the Cooperative Research Centre (CRC) for Plant Science in the second half of 1991 will see a need for a major upgrading of this path over the next 7 years as the bi-directional flow of foot traffic and ideas inexorably increases.



Planting an Idea

- the CRC for Plant Science -

The Plant Science Centre (which it is more conveniently called) was one of the first CRCs to be established under the federal governments CRC program (the brainchild of a past director of RSBS - Ralph Slatyer) and represents a legitimising of the many de facto interactions that have existed between the two institutions over the years. A new bedfellow in the name of Biocem (pronounced biosem) Pacific P/L completes the trinity of partners in the Centre.

Most inmates of RSBS are no doubt aware of the Biocem lab and the Plant Science Centre lab on the 3rd level but may not realise that a similar Plant Science Centre lab has been established in Plant Industry. These labs and a number of established labs are now host to a total of 32 Post Docs, PhD students, and research assistants who are directly supported by the Centre (see figure). Moreover the Centre also employs an Executive Officer, Administrative Officer and an Education Officer (yours truly).

Who are all these people and what activities do they do? I have tried to simplify the answer to this question by providing you with a lineage (figure) come pedigree commencing with the originators of the Centre - the current co-directors Prof Brian Gunning and Dr Jim Peacock, and Biocem's Dr Eric Hutner. As you can see Centre research is divided into four programs titled: Plant Growth and Performance, Plant Development, Plant Disease Mechanisms and Prevention, and Arabidopsis. Each program has a number of projects that loosely sit under these titles and it is here that the true co-operative nature of the research being undertaken can be seen. To date 2 research fellows, 10 post doctoral fellows and 9 research support personnel have been appointed to projects and there are more to come. Furthermore there are 7 PhD students undertaking research directly aligned with the Centres research interests and who have gained Graduate Assistantship awards or other ANU scholarships from the Centres Education Program.

While this may give you an idea of the size of this enterprise, it does not convey any of the excitement inherent in the research projects being undertaken nor the degree to which collaboration is taking place, so let me give you just one example: Improving the transpiration efficiency of wheat and oilseeds. This project stems from co-operative research that has taken place for a number of years now between members of the Plant Environmental Biology Group (RSBS) and Crop Adaptation (PI) which has identified carbon isotope discrimination as a potentially useful indicator of genotypic differences in transpiration efficiency (ie the amount of dry matter produced per unit water used by a plant) in a range of species. Under water-limited conditions, any improvement in transpiration efficiency should lead to greater and more stable yields. It is the word "potentially" that this CRC supported project aims at evaluating and moreover converting into "reality". The envisaged outcome is that within 3 years, Australian wheat and oilseed breeders will have available a rapid and accurate method of screening for transpiration efficiency within segregating populations thereby improving yield and soil sustainability. Longer term goals include the incorporation of greater transpiration efficiency (via backcrossing) into germplasm offered to Australian breeders.

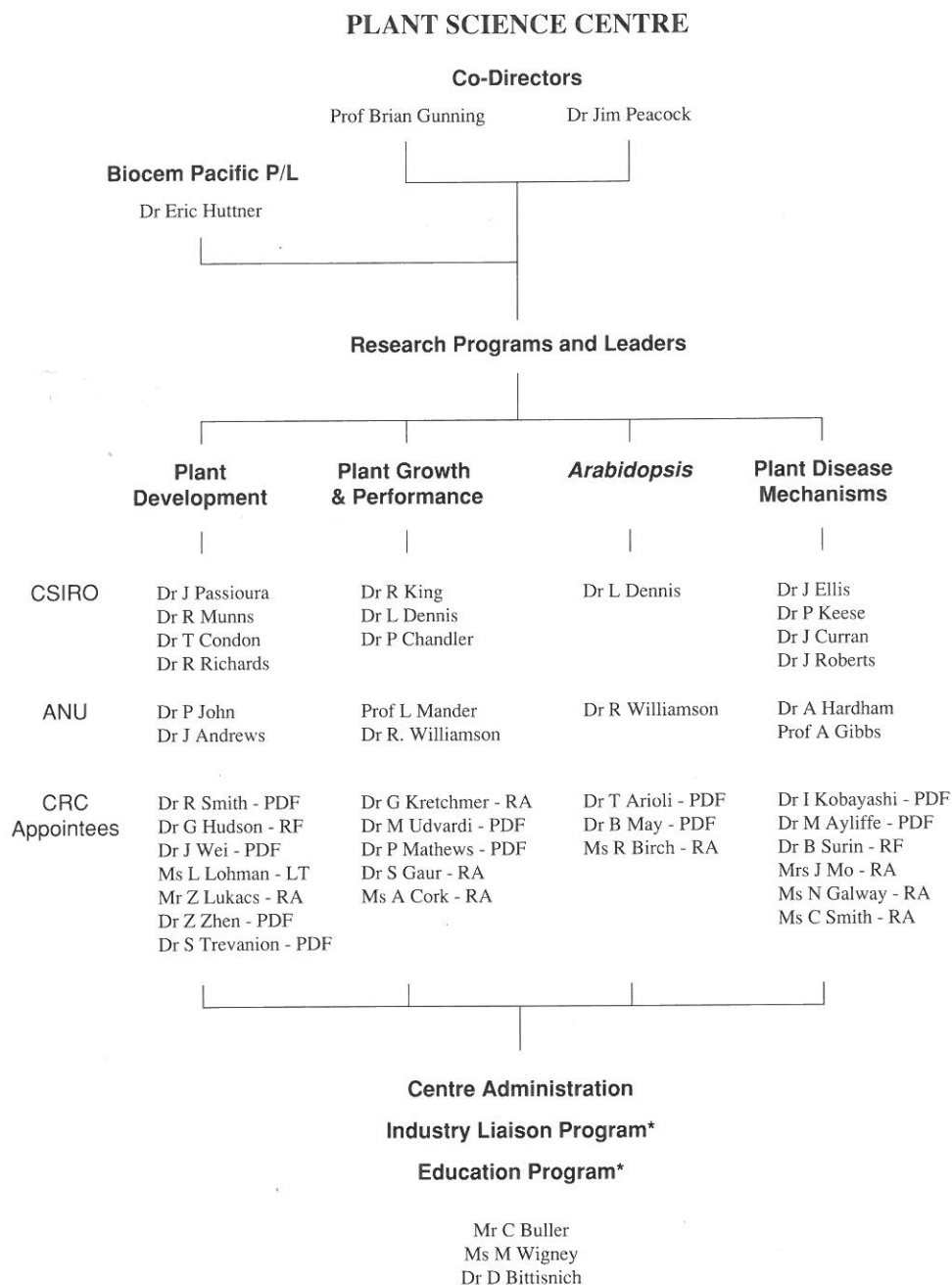
Although the course of Plant Science Centre research appears set, it is important to point out that new projects and ideas are continuously being considered. For example the Centre currently has a submission for supplementary funding to the CRC Secretariat aimed at establishing a barley transformation sub-program. The sub-program aims to use genetic engineering coupled with traditional plant breeding to improve barley malting quality, disease resistance and germplasm stock. The value of barley crops to growers is about \$600 M but this has been declining over recent years due to the relatively poor performance of local varieties. Even marginal improvement in varieties via the barley transformation sub-program could lead to higher returns to farmers, brewers and maltsters, and breeders.

You may note that apart from the research programs, the Centre maintains an Education Program and an Industry Liaison Program. The latter is concerned essentially with fostering bilateral associations, and the bipartisan benefits which may flow, between the Centres research programs and relevant industry interests - the lack of commercialisation of public sector generated ideas is a continuing bugbear of both science and commerce in Australia and this Program aims at forging and maintaining this link in relation to the Centre's research output.

The Education Program also attempts to foster associations but on a more grass roots (sic) level. Its aims are to encourage enquiry and research into plant science in students ranging from primary to tertiary education via a number of sub-programs including: a scholarship scheme - which provides Honours and PhD awards to students undertaking study in an area of Centre interest; a schools science education centre - "The Green Machine" - especially aimed at promoting plant science both regionally and nationally (the education centre is currently being established on the ANU campus in association with CSIRO Education Programs and the ACT Schools Authority); and the setting up in 1993 of a Master of Science course in Plant Molecular Biology and Biotechnology - this course, which is to be taught by staff from both the ANU and CSIRO Plant Industry, services both university and industry demands by providing both a structured research course for graduate students and workshops (eg the Introduction to Plant Genetic Engineering workshop which some of you may have seen advertised) for non-degree students.

This brief overview of the Plant Science Centre has hopefully provided you with an idea and feeling for some of the activities which are being undertaken and which are proposed. Born essentially from casual associations of RSBS and Plant Industry interests, the Plant Science Centre now provides a mechanism for strengthening and extending these collaborations (not to mention money) and planting this ideal further afield.

Talking about planting - we will have to see if Buildings and Grounds can do something (sic) with that rut-like track between RSBS and PI, its bound to become much worse. Anyone interested in establishing a turf hoof damage research project? Better still, I am sure there would be a good trade in a fast-food concession out the front of Physics.



RESEARCH SCHOLARSHIPS

FOURTH YEAR (HONOURS) SCHOLARSHIPS IN BIOLOGICAL SCIENCES



The Research School of Biological Sciences is offering three scholarships for suitably qualified students wishing to undertake their fourth (honours) year at the Australian National University in 1993.

Scholarships are for one year and are valued at \$6000 plus limited travel expenses (where applicable).

Applicants should be completing, or have recently completed, the third year of a relevant Bachelor's degree course with majors in the fields relevant to the biological sciences.

CLOSING DATE FOR APPLICATIONS: 30 NOVEMBER 1992.

Application forms and further information may be obtained from:

The School Secretary
Research School of Biological Sciences
GPO Box 475
Canberra ACT 2601
ph (06) 249 4138