ESSENTIAL OILS & PLANT EXTRACTS

RIRDC Sub-Program 2.5

November 1998

RIRDC Publication No 98/86
FOREWORD

This year RIRDC has produced *Research in Progress, June ’98*, which contains short summaries of continuing projects as well as those that were completed during 1997-98 for all of the Corporation’s 21 program areas.

The complete report on all the programs is only available in electronic format on our website at http://www.rirdc.gov.au

The following report is a hardcopy extract covering Sub-program 2.5. It contains all entries from continuing Essential Oils and Plant Extracts research projects funded by RIRDC. This program aims to support the growth of a profitable and sustainable essential oils and natural plant extracts industry in Australia.

This report is the newest addition to our extensive catalogue of over 250 research reports, videos and CD-Roms of projects supported by RIRDC. Please contact us for the latest publications catalogue or view it on our website.

**Peter Core**  
Managing Director  
Rural Industries Research and Development Corporation
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RESEARCH IN PROGRESS

Project Title
Establishment of a genetic and biodiversity resources of Eucalyptus polybractea

RIRDC Project No: ANU-24A
Start Date: 1 July, 1996
Finish Date: 30 September, 1998
Researcher: Dr. Michael Slee
Organisation: Department of Forestry
Australian National University

Contacts:
Phone: (02) 6249 2224
Fax: (02) 6249 0746

Objectives
- The project will establish a genetic and biodiversity resource using material collected from throughout the range of the blue mallee (Eucalyptus polybractea). It will also examine in detail the taxonomy of the species.
- It will provide a reliable source of marketable seed and other material of guaranteed genetic quality and thus encourage expansion of the use of the species for oil production, for agroforestry and land rehabilitation.

Current Progress
Trees representing 11 different seed sources, (seven NSW and four Victorian), were raised in the nursery in Canberra and planted at Tallimbalong, West Wyalong, NSW in October 1997. The procedures used were those practised by G.R. Davis Pty Ltd, whose staff did the planting. Despite the drought most of the 1800 trees have grown well — survival is 92% and average height in May 1998 was 53cm.

The layout design is based on 10 tree line plots and has separated the NSW and Victorian sources. The trees representing each seed source are together and are replicated three times — thus the layout for each state is a randomised block with three replications.

Progeny of 27 NSW and 20 Victorian parent trees are included and individual parent identity has been maintained for all except one Victorian batch. The progeny form subplots.

The excellent growth means it will be possible to assess the planting for oil production in November 1998.

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Project Title
Production of betaine(s) from Australian Melaleuca spp for use in agriculture to reduce plant stress

RIRDC Project No: CSC-55A
Start Date: 1 July, 1995
Finish Date: 30 August, 1998
Researcher: Dr. Naidu Bodapati
Organisation: CSIRO Molecular Science Cunningham Lab
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ST LUCIA QLD 4067

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Objectives
- To establish the agronomic and commercial feasibility of growing Melaleuca spp containing betaine/betaine (proline) analogues by conducting field trials
- To develop lab-scale and industrial prototype methodologies for the production of betaines from Melaleuca species by June 1998.

Current Progress
Field trials were conducted at Tyagarah (NSW), Samford (near Brisbane) and two sites near Dimbulah (Qld) with M. alternifolia, M. bracteata, M. cuticularis, M. glomerata, M. lanceolata, M. pauperiflora, and M. uncinata. Of the betaine or betaine analogue accumulating species, only M. bracteata showed the ability to grow well under sub-tropical and tropical conditions of NSW and Qld. This species contained N-methyl-trans-4-hydroxy-L-proline (MHP) in leaves to about 1.8% dry wt and in stems to about 0.6-1%. Estimated potential yield of this solute varied between 200 to 300 kg/ha. Up to 95% of MHP present in plants could be simply extracted by rushing the spent biomass in water following distillation. Aqueous extract of MHP from the biomass has been purified on cation exchange column to 98% purity.

To find a high MHP yielding genotype of M. bracteata, 46 provenances have been collected from field locations. MHP content ranged from 0.1 to 2.15% on a dry weight basis. We are currently raising seedlings of the above 46 accessions along with the initially used M. bracteata to plant field trials at Tyagarah (NSW), Maryborough, and two sites near Dimbulah (QLD).
### Project Title
**Economic feasibility of native and scotch spearmint production in Tasmania & Victoria**

**RIRDC Project No:** DAV-101A  
**Start Date:** 1 July, 1995  
**Finish Date:** 30 September, 1998  
**Researcher:** Mr. Fred Bienvenu  
**Organisation:** Department of Natural Resources & Environment

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#### Objectives
- Investigate the potential of Native and Scotch Spearmint for production of high quality oil in Victoria and Tasmania by determining the effects of major production variables on yield and composition of oil produced.  
- Assess the effect of oil composition on the marketability of spearmint oils in the essential oil markets of the world.  
- Estimate cost of production and potential returns from commercial production of oil of spearmint.  
- Produce a manual for the cultivation of spearmint which includes: planting/establishment, pest and disease management, nutrition and harvest strategies to maximise yield and quality.

#### Current Progress
In 1997/98, the collaborative research project into spearmint (*Mentha cardiaca* & *M. spicata*) production in Tasmania and Victoria has produced a clearer insight to the agronomic practices required. Control of the devastating disease mint rust (*Puccinia menthae*) is of paramount concern to would-be producers. Studies in conjunction with University of Melbourne have been conducted to ascertain the details of the different races of mint rust that may attack spearmint.

The fungicide treatments performed with similar levels of control to those found in the previous season. Baycor® (bitertanol) and Folicur® (tebuconazole) have again showed a higher level of mint rust control than the world industry standard in peppermint, Tilt® (propiconazole). In previous studies into mint rust in peppermint (*Mentha piperita*), Baycor® (bitertanol) was considered to be far less effective than Tilt® (propiconazole). This helps to demonstrate the importance of not merely copying agronomic practices from allied crops without thorough local testing.

Past concerns for the longevity of a spearmint stand can now be somewhat ameliorated with improved levels of mint rust control. The data so far developed will greatly assist with the production of a "growers" manual for industry development.

### Project Title
**Adding value to essential oils and other natural food ingredients by secondary processing in dense carbon dioxide**

**RIRDC Project No:** DAV-97A  
**Start Date:** 1 January, 1995  
**Finish Date:** 30 July, 1998  
**Researcher:** Dr. Brian Imison  
**Organisation:** Australian Food Industry Science Centre

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#### Objectives
- To improve the profitability and sustainability of the Australian essential oils industry through the development and industrial application of high level technology. This will involve the use of dense CO2 secondary processing for the production of high value fractionated citrus oils and other natural food ingredients.

#### Current Progress
Pilot scale trials have been completed on the adsorption and supercritical CO2 desorption of orange oil, using a 400 g expanded column of silica gel adsorbent. A high concentration limonene fraction is directly produced and flavour fractions are recovered during the high pressure desorption steps.

This pilot scale process has been developed and scaled up, using a 2.5 kg bed of adsorbent and low and high flavour concentrations in the feed.

Aspects studied on both plants were the cycle selectivity, productivity, effect of desorbing pressure programming, fraction yields, and adsorbent regeneration and reuse.

The use of continuous countercurrent liquid/liquid supercritical extraction is currently being scaled up and re-examined, using a high concentration feed with consistent composition produced by batch blending.

Assessment of key flavour fractions by a commercial flavour company has, to date, indicated that some were of commercial interest. However, fraction yields and commercial value indicate that for orange oil, the supercritical extraction techniques studied are unlikely to give an acceptable internal rate of return on the plant capital investment. Further assessment of the latest
flavour fractions and detailed costings still need to be done to complete this project.

**Project Title**
A model of best practice in peppermint oil production – Tasmania and Victoria

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<td><strong>Researcher:</strong></td>
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<td><strong>Organisation:</strong></td>
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<td><strong>Contacts:</strong></td>
<td>Phone: (03) 6226 2619 Fax: (03) 6226 2642</td>
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<td><strong>E-mail:</strong></td>
<td><a href="mailto:Rob.Clark@agsci.edu.au">Rob.Clark@agsci.edu.au</a></td>
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**Objectives**

- To increase the commercially achievable yield of high quality peppermint oil in Tasmania and Victoria by collating existing knowledge on factors affecting the yield and quality of peppermint into a model and manual of "best practice".
- Using the model and manual of "best practice", monitor production in Tasmania and Victoria and recommend manipulation of cultural practices to maximise the yield and quality of oil.

**Current Progress**
Work in the Best Practice Project has progressed on three fronts:

1) Assessment of the benefits and costs of using elite propagation material for plantation establishment. US producers purchase plant material from specialist providers to ensure freedom from pathogenic agents. Essential Oils of Tasmania has established a tip cutting nursery project under the guidance of the research team and tissue culture techniques have been used to produce a second line of meristem material. These will be compared for yield and vigour with commercially (stolon) propagated material in field trials during the coming year.

2) Following study of the progress in commercial production during the 97/98 season, the research group has focussed on the importance of enabling producers to measure and regulate irrigation and nitrogenous fertiliser use during the summer. These two matters alone we believe account for the largest proportion of crop failures, and offer the best opportunity to lift yields in the short to medium term. There are a number of commercially available methods for determining nitrogen and water status in commercial crops and we hope to develop a pilot monitoring project on at least one commercial field during the coming season.

Another management issue requiring serious attention is the harvest itself. We have ample evidence (and the concurrence of producers) that the harvest operation may be responsible for losses of up to 30% of available oil, and propose to develop a simple 'Harvest Managers Guide' for use in 1999, to be incorporated in the crop management system described below.

3) Incorporating the problems mentioned above, and following the model of the grain production management systems such as 'TopCrop', 'MEY-Check' and others, we hope to develop a checkpoint - based management system for peppermint in southern Australia. This would provide basic management guidance for individual growers, helping them develop an intuitive understanding of mint growth and oil production, as well as establishing a framework for measuring and collating performance indicators across growers, districts and seasons.

With respect to the development of the model, a review of the available literature is complete, and a broad outline of a scheme has been devised with draft checklists prepared for three of a probable six checkpoints presently completed for discussion.

**Project Title**
Development of production and harvesting systems for Echinacea angustifolia DC

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<td><strong>Finish Date:</strong></td>
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<td><strong>Researcher:</strong></td>
<td>Mr. Nicholas Walker</td>
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<td><strong>Organisation:</strong></td>
<td>Plantalab Laboratories Pty Ltd &quot;Billenya&quot; Mail Service 852 TOOWOOMBA QLD 4352</td>
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<td><strong>Contacts:</strong></td>
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**Objectives**

- To develop profitable and sustainable production and harvesting systems for the production of *E. angustifolia* root for the Australian medicinal herb industry. Import replacement, penetration of export markets, mechanical harvesting methods and organic production techniques are major project objectives.

**Current Progress**
A system has been developed to produce high quality *E. angustifolia* seedlings ready for transplanting 3 to 4 months from sowing. This system, called “The Echo Valley Herbs Transplant Production System” or EVHTPS produces transplants that will satisfy OPAC organic production guidelines and involves; cold stratification of seed, semi-mechanised seed sowing and propagation media preparation, a number of biological control measures for insect and disease
management including the use of vesicular arbuscular mycorrhizal fungi (VAMF), an antagonistic soil fungus, *Trichoderma harzianum*, and an entomopathogenic nematode, *Steinernema feltiae*, a tray and rack system for efficient seedling transportation completes the system.

Vegetative propagation techniques have been developed with the aim of using these in the breeding of a superior synthetic variety of *E. Angustifolia*. Preliminary evaluation of superior individuals is underway.

Organic field production methods have been developed, and are continually upgraded to facilitate mechanical harvesting requirements; controlled traffic with permanent raised beds, trickle irrigation, fertigation, and organic weed control through the use of a woven plastic weed mat/composted organic matter combination. A revolutionary vehicular platform for crop manipulation is currently being developed, the design of this machine allows the operator to be suspended horizontally above the crop with his/her arms free for precise weeding of transplants, replanting of misses, or general crop inspection for insect/disease damage.

Use of a modified elevator-digger has proved superior to a tree lifting blade in harvesting undamaged root. A semi-mechanised root washer is being developed. A domestic marketing chain has been established. Further marketing opportunities will be investigated later in 1998.

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**Project Title**

**Generation of high quality Australian Skullcap products**

**RIRDC Project No:** UNC-6A  
**Start Date:** 1 June, 1997  
**Finish Date:** 30 June, 2000  
**Researcher:** Professor Ron Wills  
**Organisation:** University of Newcastle Central Coast Campus PO Box 127 OURIMBAH NSW 2258

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Fax: (02) 4348 4145  
E-mail: ftbhw@cc.newcastle.edu.au

**Objectives**  
- To generate compositional information on skullcap (*Scutellaria lateriflora*) and optimise the genetics of seed stocks, and postharvest handling and processing systems, to enable growers and processors to maximise quality in the end product and thus obtain price premiums on the world market.

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**Current Progress**

Initial studies have concentrated on developing analytical methodology using HPLC to separate and identify the key flavanoid compounds that are generally considered to be the active pharmacological constituents in *Scutellaria lateriflora*. A general method has now been established involving extracting a ground sample in a methanol-water mixture in an ultrasonic bath and separation and quantification using a reversed phase column and ultraviolet absorption detection. Nine peaks of interest are routinely obtained in the skullcap extracts and studies are in progress to positively identify the individual peaks using mass spectrometry. Trials on fresh skullcap have shown that considerable changes in flavanoid compounds occurs very soon after harvest which has implications for careful handling of harvested plants to preserve quality.

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**Project Title**

**Australian ginseng research program- crop establishment project**

**RIRDC Project No:** POP-1A  
**Start Date:** 1 June, 1996  
**Finish Date:** 30 September, 1998  
**Researcher:** Mr. Tony Sadler  
**Organisation:** c/- New Crops Program Gatton College University of Queensland 12 Coolibah Court MT CROSBY QLD 4306

**Contacts:**  
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Fax: (07) 5460 1112

**Objectives**  
The Australian Ginseng Research Program: Crop Establishment Project has five sub-projects which have the following objectives:

- To identify those areas in Australia that are suited for the production of ginseng;
- To identify those factors that contribute to  
  - loss of seed viability for both *Panax ginseng* and *Panax quinquefolius* and  
  - the time taken for seed germination;
- To reduce root dormancy of both *Panax ginseng* and *Panax quinquefolius*;
- To identify the most appropriate cultivars of *Panax ginseng* and *Panax quinquefolius* for Australian growing regions; and
- To test the feasibility of tissue culture as a means of reproducing ginseng cultivars for commercial plant propagation.

**Current Progress**

- A mathematical model has been produced and maps of Australia identifying the probable boundaries to successful ginseng cultivation are currently being generated. The accuracy of those boundaries remains dependent on the results of the chill summation root experiments.
• The final experiments to establish a method of breaking seed dormancy early have been completed. The data is currently being analysed. Two robust methods of reducing dormancy time have been established, involving temperature control and the use of an endogenous chemical. The final data analysis is expected to establish an efficient protocol for commercial use of these methods.

• Root dormancy experiments have continued to cause difficulties. However, the results of experiments currently under way appear very promising. It is expected that analysis will confirm both a chemical method of breaking root dormancy and a time-temperature relationship for dormancy.

• Contact has been made with researchers in China and Canada who appear to be in progress of developing cultivars. A visit has been arranged to view the operations in Canada in mid June.

• Field-grown material has been successfully placed in vitro and multiplication achieved through somatic embryogenesis. This has been the final step of the project in readiness for propagating cultivars when available.

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**Project Title**
Development of a commercial natural sprout inhibitor from dill and caraway for potato storage

**RIRDC Project No:** SCA-1A  
**Start Date:** 1 July, 1996  
**Finish Date:** 30 September, 1998  
**Researcher:** Dr. Lee Peterson  
**Organisation:** Specialist Crop Advice  
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NICHOLLS RIVULET  
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**Objectives**
• To develop a natural product based on carvone for commercial application in potato storage.

**Current Progress**
A review of previous cultural practices utilised for caraway and dill production for their essential oil has been completed and the adaptation of such practices for carvone production examined.

From this review it was determined that caraway would not be suitable for economic carvone production and the decision was made to concentrate on dill as a source of carvone.

First year field trials examined weed control measures through a herbicide screening trial. Only observational data was obtained due to the loss of the trial plots post flowering due to grazing from wildlife.

Second year field trials of dill were much more successfully established examining sowing date and density effects. A small trial examined a range of varieties available from Europe.

Yield and quality data has been collected from all second year trials as well as growth data and is presently being analysed.

Harvest and distillation techniques have been reviewed and a potential commercial harvest technique trialed on a small scale. These trials have produced a small quantity of dill oil which will be the subject of rectification studies examining the fractionation of carvone from the oil.

Samples from this study will enable preliminary market evaluation of an Australian produced natural potato sprout inhibitor.

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**Project Title**
Control of mint rust (Puccinia menthae) on peppermint – epidemiology and chemical control

**RIRDC Project No:** UM-16A  
**Start Date:** 1 July, 1993  
**Finish Date:** 30 September, 1998  
**Researcher:** Dr. Gerald Halloran  
**Organisation:** The University of Melbourne  
Royal Parade  
PARKVILLE VIC 3052

**Contacts:**  
Phone: (03) 9344 7128  
Fax: (03) 9344 5570

**Objectives**
• To determine and demonstrate an effective strategy for the control of peppermint rust based on minimal use of chemicals sprays.
• To identify fungicides which will effectively control P. menthae without contamination of distilled oil.
• In conjunction with (1) and (2) to conduct detailed studies of the epidemiology of mint rust eg. life cycle and the influence of temperature and humidity on its capacity for growth and reproduction.

**Current Progress**
Mint rust is caused by a rust fungus that has a complicated life cycle involving five spore types. This cycle, as it occurs in NE Vic, has been studied for 4 years now and it is evident that only two spore types occur, of which the summer spore remains viable throughout winter and carries the disease into the next season. Experiments have been undertaken examining the effects of temperature and leaf wetness on the
infection process. Optimum conditions are at least six hours of leaf wetness and temperatures of 15º - 20ºC. Below 15ºC the fungus is inhibited, and above 27ºC it is killed. Control measures should therefore begin early in the season.

There is a lot of variation within the fungus population. At least 8 races have been identified from 18 collections of rust, and there are clear differences between those races which attack peppermint and those which attack spearmint.

Of six fungicides trialed on Scotch spearmint over the past 2 years, Baycor and Foliar performed best under Victorian conditions. It is hoped that these results can also be applied to Peppermint

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**Project Title**

Generation of high quality Australian Echinacea products

**RIRDC Project No:** UNC-4A  
**Start Date:** 1 September, 1996  
**Finish Date:** 30 September, 1998  
**Researcher:** Professor Ron Wills  
**Organisation:** University of Newcastle Central Coast Campus PO Box 127 OURIMBAH NSW 2258  
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E-mail: ftbhw@cc.newcastle.edu.au

**Objectives**

- To study changes in the composition of Echinacea purpurea in different plant parts during growth, and during postharvest handling and processing, develop quality standards, and rapid quality testing procedures.
- The data will generate improved postharvest and processing technology which will be utilised by MediHerb to market high quality Australian-grown echinacea internationally.

**Current Progress**

Analytical methods using HPLC to determine the level of alkylamides and caffeoyl phenols in echinacea extracts have been established and a routine service is now available to the industry. Examination of changes in these active constituents during growth shows the major concentration of active constituents is in the roots followed by the flowers and lowest in the stems and leaves. The concentration of active constituents was relatively constant over the growing period with some decline in over-mature plants during senescence. It seems that an increase in plant size is beneficial as the concentration of active constituents is not decreased during the additional plant growth. Preliminary studies of changes in active constituents during processing to obtain a liquid extract and subsequent concentration or drying showed considerable losses of active constituents during both extraction and subsequent concentration and drying.

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**Project Title**

Yield & quality of concentrate from Boronia megastigma (Nees)

**RIRDC Project No:** UT-10A  
**Start Date:** 1 August, 1995  
**Finish Date:** 30 October, 1999  
**Researcher:** Professor Robert Menary  
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**Objectives**

- To study (on a commercial scale) the production of volatiles in vivo pre- and post-harvest and to tailor management practices to maximise volatile yield.

**Current Progress**

Significant increases in the production of floral extract, volatiles and beta-ionone occurred during post-harvest incubation of freshly harvested flowers at 12-16ºC. Oxygen is required and temperature control is crucial, flower respiration appears to be a regulating factor. Clones differ in their post-harvest biosynthetic ability, potential increases being 20% of extract concentration (% dr. wt.), 120% of total volatiles and 190% of beta-ionone. Large scale (150kg) incubations have been successful, producing similar increases and paving the way for improved post-harvest handling techniques on-farm. Optimally harvested flowers (80% open) have the potential to produce more volatiles after harvest than late harvested flowers (90% + open).

The biochemical basis for the increases in extract and volatiles after harvest include hydrolysis of glycosidically bound volatiles or de novo synthesis. The former appears to be most likely; identification of potential precursors and hydrolytic enzymes is being pursued. There is an increase in the range of volatile compounds released from standard extracts by hydrolysis with enzymes made from flower material more advanced than 75% open flowers, suggesting that hydrolysis of glycosides is a natural part of flower senescence.

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**Project Title**

A new commercial flavour product from Tasmannia lanceolata

**RIRDC Project No:** UT-11A  
**Start Date:** 1 October, 1995  
**Finish Date:** 30 September, 1998  
**Researcher:** Professor Robert Menary
Objectives

- To refine methods of specification for the new product.
- To survey the available populations of the species, and select and propagate plants showing commercial promise.
- To establish two pilot plantations of the species for development of management and harvest technologies.

Current Progress

A variety of extracts of *T. lanceolata* were sent to Toyotama and have been assessed by Dr. Ishizuka. Clones FG1, GL6, HY9 and PP1 rated well in terms of flavour and spice characteristics. For their current purposes, PP1 and TR5 were determined to be the most useful.

Principle co-ordinate analysis and cluster analysis were used to provide an insight into the chemical relatedness of plants within a localised area. A paper dealing with this work has been accepted for publication by the *Journal of Agricultural and Food Chemistry*.

Safrole concentrations in leaves have been followed throughout the season.

Chemical characterisation of the Native Pepper extract has identified over 75% of the components, including most of the major constituents. A new HPLC/MS/MS system will be commissioned by the CSL early in June. Our separatory methods will be transferred to the new system, where the further identification of unknowns will proceed.

A new method for screening clones for temperature tolerance is being investigated. The collection of 140 clones is being maintained in a nursery clone bank.

A report from the marketing company, EOT, suggests that the process of product registration has been initiated, and we are waiting for a response from FEMA.

Note: This report relates to the modified objectives as agreed in October 1996.

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**Project Title**

Determination of pesticide minimum residue limits in essential oils

**RIRDC Project No:** UT-13A

**Start Date:** 1 July, 1996

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**Project Title**

Maximising of yield and productivity of peppermint through double harvesting

**RIRDC Project No:** VMP-1A

**Start Date:** 1 October, 1996

**Finish Date:** 30 September, 1998

**Researcher:** Dr. Leo Cahill

**Organisation:** VicMint Partners Pty Ltd

**Start Date:** 87 The Crescent

**ASCOTVALE VIC 3032**
Sub-program 2.5 - Essential Oils and Plant Extracts
RIRDC Completed Projects in 1997-1998 and Research in Progress as at June 1998

Objectives
- To establish the agronomic conditions needed to maximise yield and productivity of peppermint through double harvesting.
- To examine the market reaction of double cut and blended Australian peppermint oils.

Current Progress
Peppermint oil is used as a flavouring in toothpaste, chewing gum and confectionery and approximately 5000 tonne per year is consumed worldwide of which Australia imports about 60 tonne p.a. This research project is investigating means to increase the yield of our peppermint crops through having two harvests per season. This research on double harvesting has been carried out on plots on research stations and on commercial crops. The main effects studied are the timing of the first and second harvests and use of nitrogen fertilisers. Preliminary results show that the oil from both the first and second harvests are complete mature oils of high quality. However achieving sufficient yield from the second harvest is proving difficult and requires further research.

Project Title
Developing essential oils of honeydew melon, carrot and spinach for export

RIRDC Project No: DAS-39A
Start Date: 1 July, 1994
Finish Date: 31 March, 1998
Researcher: Mrs. Shirlie Sylvia
Organisation: Department of Primary Industries and Resources SA
LOXTON Centre
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Objectives
- To develop essential oil production of honeydew melon, carrot and spinach in the Riverland with an estimated production area of 300ha valued at $0.6m, through the evaluation of varieties and development of extraction techniques suitable for the production of essential oils for Japanese and Asian export markets, with expected annual exports of 100,000 litres of essential oils valued at $1.5-2m.

Current Progress
Tall spinach varieties are high yielding, suited to machine processing, with overseas market demand for their juice flavour and aroma. Dedicated spinach processing crops are achievable if harvesters and herbicides for broadleaf weeds are developed to reduce labour costs. Short, hand harvested, spinach varieties are preferred by the fresh market. Methods to reduce high sodium in spinach juice are required through management of fertiliser, irrigation and planting time. Spinach maturing by spring, has better quality due to reduced aphid-spread Cucumber Mosaic Virus, and bolting.

Honeydew and muskmelon juice and aroma have overseas market demand. Production costs are high and fruit perishable and bulky. Processing local, fresh market reject fruit reduces costs and transport, but production is variable. Melons with tight maturities, enabling a once-over-pick are preferred and require further development. Extraction equipment to exclude skins, and methods to reduce pH in juice are being developed, as markets reject off-flavours and additives.

Overseas demand for carrot juice has declined due to surplus. If markets redevelop, processors are well placed to use the 20-30% overrun fresh market carrots for juice and aroma extraction.

The final report is now completed and under peer review to reach RIRDC by 31 June 1998.

Project Title
Development of an onion oil industry in Tasmania

RIRDC Project No: VOO-1A
Start Date: 1 February, 1994
Finish Date: 30 September, 1998
Researcher: Mr. Breven Howe
Organisation: Vecon Pty Ltd
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DEVONPORT TAS 7310

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Objectives
- To produce commercial food grade onion oil from waste onions by developing an extraction process capable of producing oil which meets the demands and specifications of international markets.

Current Progress
- Development of techniques and equipment for replicable laboratory scale distillation and/or extraction of onion samples.
- Development of a pyruvate assay for to allow measurement of the activity of the enzyme associated with aroma compound production.
- Investigation of the effects of varying fermentation time, temperature, pH, and degree of onion comminution on oil yield and quality. Optimum conditions have been determined, although the ideal time of fermentation varies with the physiological state of the onions.
- Investigation of the effects of the addition of the co-enzyme pyridoxal-5-phosphate and the growth
hormone GA, in promoting oil production and altering oil quality. The promotory effect again appears to be dependent on the physiological state of the onions.

- Investigation of the optimum storage condition and pre fermentation treatment of onions. Both, physical wounding of the onions and a period of cold treatment (vernalisation) in the weeks prior to fermentation, have been shown to promote oil yield but more work is required to fully understand these effects.

The feasibility of measuring enantiomeric ratios in commercial essential oils using chiral columns was assessed. Literature, dealing with the enantiomeric excesses (ee) encountered in natural extracts has been coordinated. The retention order of the racemic mix of the standards (-)-menthol, (+)-limonene, (-)-limonene, (±)-pinene and -ionone, (±)-terpinen-4-ol, -terpineol and racemic mix of menthone isomers were determined by comparison to the GC profiles of essential oils known to contain only one enantiomeric form of the chemicals. Different conditions of pressure, temperature and loading quantities on 20% permethylated -cyclodextrin chiral columns were trialed. Separation was achieved for all enantiomeric forms. Elution orders were confirmed for enantiomers of limonene, linalool and terpinen-4-ol. After optimisation using GC FID, the chemical identities were confirmed by GC MSD.

The separation of components of peppermint oil and lavender oil was trialed on the chiral column. Comparison with the purified chemicals available indicated that retention time of enantiomeric form is only slightly affected by the matrix in which it is introduced to the chiral column. MSD proved to be a valuable tool in identifying the presence of other components of essential oil which co elute with the enantiomer of interest.

**Project Title**
Investigation of potential new opportunities for commercial liquid CO2 extraction

**RIRDC Project No:** BRA-1A  
**Start Date:** 15-Sep-1997  
**Finish Date:** 30-Apr-1998  
**Researcher:** Dr. Lee Peterson  
**Organisation:** 9 Rodgers  
**Contacts:** Phone: (03) 6295 1897  
Fax: (03) 6295 1897

**Objectives**
- To investigate the potential of alternative methods of extraction of an Australian native for essential oil production
- To investigate the market acceptance of product(s) derived from extraction trials.

**Current Progress**
The objective of this preliminary study was to investigate the suitability of using a new liquid CO2 refinery to refine extracts of essential oils for sale in overseas markets. A sample of boronia flowers was solvent extracted using a commercial extraction plant and a good yield of oleoresin was obtained. Samples of this oleoresin has been refined using a laboratory scale liquid CO2 refinery. Various refined samples have been analysed using GC equipment. Results to date have indicated that the CO2 refined product is very clear and generally free of coloured plant compounds. The yields of volatile compounds appear promising and good yields of the ionone were achieved. Suitable refined product samples will be sent to potential overseas customers for market evaluation in the near future. If promising market opportunities are available, more work may be conducted to fine-tune the extraction and refining process to increase the yield and quality of the refined product.

Botanical Resources Australia Pty Ltd (BRA) is currently a major producer, manufacturer and exporter of pyrethrum products to world markets. New plant extract products would complement the existing manufacturing processes such as extraction and refining and will allow BRA to diversify and become a supplier or a range of botanical products.