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RIRDC Completed Projects in 2011–12 and Research in Progress at June 2012

Tea Tree Oil

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Foreword

RIRDC produces summaries of completed and continuing projects for each financial year. Our intention is to:

- Provide stakeholders with early access to the results of ongoing and completed work to inform their decisions, and
- Inform researchers of results to shape research direction.

Tea Tree Oil *RIRDC Completed Projects 2011–12 and Research in Progress at June 2012* contains short summaries of projects funded by the Program. The Tea Tree Oil Program aims to support the continued development of a profitable and environmentally sustainable Australian Tea Tree Oil Industry that has established international leadership in marketing, value adding, product reliability and production.

The research objectives of the Tea Tree Oil Program are to:

1. Enhance production systems to maintain the competitiveness of Australian growers
2. Identify regulatory regimes and market barriers, and enhance the ability of industry to meet safety standards
3. Demonstrate proof of concept/efficacy for innovative applications of tea tree oil
4. Foster communication that increases understanding and thereby encourages greater use of tea tree oil.

This report is an addition to RIRDC's diverse range of over 2000 research publications most of which are available for viewing, free downloading or purchasing online at www.rirdc.gov.au. Purchases can also be made by phoning 1300 634 313.

Craig Burns
Managing Director
Rural Industries Research and Development Corporation

Contents

Completed Projects 2011–2012

Enhancing production systems to maintain the competitiveness of Australian growers

Demonstrating proof of concept/efficacy for innovative applications of tea tree oil

Research in Progress at June 2012

Enhancing production systems to maintain the competitiveness of Australian growers

Demonstrating proof of concept/efficacy for innovative applications of tea tree oil

Other supported activities

Collaborative Programs

PRJ-006878 Minor use of chemicals (National Weeds and Productivity Research Program)

PRJ-007524 Discovery of genetic resistance markers to Myrtle rust in Myrtaceae (Essential Oils and Plant Extracts Program – Myrtle Rust response)

Scholarships

PRJ-007367 The genetics of oil yield in *Melaleuca alternifolia* and *Eucalyptus loxophleba*

New Projects

PRJ-008456 Using tea tree residues & legumes to develop a low-emission tea tree industry (Clean Energy Futures – Action on the Ground Project)

Completed Projects - Enhancing production systems to maintain the competitiveness of Australian growers

PRJ-004221 Tea tree oil communication project

Start Date:	05/06/2009
Finish Date:	16/05/2012
Researcher:	Tony Larkman
Organisation:	Tony Larkman
Email:	tlarkman@attia.org.au
Objectives	This project is designed to put into place some tools that will, over many years into the future, provide a basis for development of the tea tree oil industry. It will do this by: Developing, fostering and integrating a Quality Assurance (QA) system which ensures that purchasers of Australian tea tree oil that the quality of the product is demonstrably controlled from the paddock to the point of export. This will include collation and dissemination of generic safety data to help ensure that tea tree oil is handled and stored appropriately from point of manufacture to point of consumption. Creating and implementing a web site that has substantial interest not only for stake holders in the industry but also for casual users of tea tree oil by enabling quick, efficient and useful communication of the uses, results of research and other communication which will increase understanding and greater use of Australian tea tree oil. This will include a database of existing uses of tea tree oil worldwide. Creating a categorised dossier of existing tea tree oil research that will be available to researchers and anyone involved in tea tree oil from paddock to end-user. Abstracts and/or summaries of existing research will also be developed and made available to facilitate information gathering.
Background	Legislation regarding the safety of chemical substances is developing globally. This legislation has the potential to severely impact on the pure Australian tea tree oil industry, 90% of which is exported, unless all sectors of the supply chain are educated and prepared to manage the safe production and supply of pure Australian tea tree oil. This project aims to provide the tea tree industry with communication capability well into the future through a website, a Quality Assurance & compliance system and a database of literature on tea tree oil research. These tools assist all sectors of the tea tree oil supply chain to manage any future threats through a better understanding of the production, storage, transport, safety, effectiveness and uses of pure Australian tea tree oil.
Research	A website, a Quality Assurance system and a database of research on tea tree oil were developed.
Outcomes	The website has been successful with traffic increasing by more than 250%. Eighty percent of tea tree growers/producers are certified as Quality Assured representing nearly 90% of Australian production of tea tree oil. Quality Assured oil is now in demand from exporters while some international purchasers are investigating integrating it into their own systems. The literature database, listing more than 1,000 documents from 1904 to today, is available on the ATTIA website (www.attia.org.au)
Implications	The combination of a Quality Assured product and an agile and responsive set of communication tools will help to ensure that pure Australian tea tree oil continues to enjoy its reputation both as a premium commodity as well as a clean, green, safe and effective product.

Completed Projects - Enhancing production systems to maintain the competitiveness of Australian growers

PRJ-005771 Improving the sustainability of plant protection in tea tree oil production systems

Start Date:	15/10/2010
Finish Date:	31/05/2012
Researcher:	Peter Entwistle
Organisation:	North East Agricultural Services
Email:	peter.entwistle@gmail.com
Objectives	To enhance production systems and to maintain the competitiveness of Australian Tea Tree producers by: Identifying more effective chemical control options for Pyrgo beetles in Tea Tree. Determining any risk of chemical residue in Tea Tree Oil from these control options. Obtaining permits from the APVMA for the selected effective insecticides Identifying safe and effective selective broadleaf herbicides for use in Tea Tree. Identifying safe and effective post harvest pre-emergent herbicides for use in Tea Tree. Identifying safe and effective in crop directed spray herbicide options for use in Tea Tree. Obtaining permits from the APVMA for the selected effective herbicides. To determine the suitability of emergency fungicides for Myrtle Rust control in Tea Tree.
Background	Australian tea tree plantations are facing increased impacts on production from insect pests and weeds. Pyrgo beetles are becoming difficult to control and the chemistry that is available to producers is very limited and is becoming less effective. The control of weeds in plantations has previously been investigated by RIRDC project DAN-74A but further work is required as weed problems have evolved in the industry over time.The use of pre-emergents, broadleaf selective herbicides and, directed sprays needs to be investigated. Myrtle rust is possibly a significant future threat to the viability of tea tree plantations in Australia. The safe use of fungicides for its control needs to be determined.
Research	The use of Integrated Pest Management in other Australian industries was investigated to find other chemistry that may be used to effectively control Pyrgo beetles in tea tree. A bioassay was used to determine which insecticides should be taken to field trial. Field trials were subsequently carried out. A range of herbicide field trials were carried out to determine what options for pre-emergent, selective and directed sprays were available for tea tree growers.
Outcomes	Abamectin and indoxocarb for control of Pyrgo beetles have been permitted for use in Australian tea tree production by the APVMA. Further submissions for the use of simazine and metolachlor have been made to the APVMA to be used as post-harvest pre-emergent herbicides in tea tree. A permit for linuron to specifically control Cuphea carthagenensis, an inceasingly significant weed in the industry, has also been applied for. No further suitable broadleaf selective herbicides have been determined at this stage.
Implications	The control of Pyrgo beetles in the Australian tea tree industry will now be more effective with the use of abamectin and indoxocarb. The overall use of insecticides is now likely to decline with the ongoing use of this products that promote effective Integrated Pest Management in tea tree. The herbicide options that are currently being applied for will give tea tree producers a more targeted

and effective range of weed control tools. The implementation of both insect pest and weed control options will allow tea tree producers in Australia to remain both profitable and sustainable in the future.

Completed Projects - Demonstrating proof of concept/efficacy for innovative applications of tea tree oil

PRJ-000459 Tea tree oil to prevent staphylococcal infections in dialysis patients

Start Date:	15/10/2010
Finish Date:	31/05/2012
Researcher:	Professor T V Riley
Organisation:	The University of Western Australia
Email:	Thomas.Riley@uwa.edu.au
Objectives	To demonstrate that tea tree oil (TTO) products were efficacious in the prevention of staphylococcal infections associated with dialysis catheters To demonstrate that TTO products were a suitable alternative to existing products used to prevent staphylococcal infections with dialysis catheters To provide clinical data on the efficacy and safety of TTO products
Background	The end-stage renal disease (ESRD) patient population in the world has grown dramatically with a estimated global prevalence of 280 cases per million. ESRD is now linked with congestive heart failure and diabetes as these are the leading causes of ESRD. In Australia at the end of 2009 there were 18,243 (834 per million) people receiving renal replacement therapy, of which 10,341 were receiving dialysis treatment. The usual treatment for peritoneal dialysis patients is to receive nasal mupirocin therapy to clear any carriage of *Staphylococcus aureus.* After such treatment staphylococcal carriage rates and exit-site infections are reduced but rates of peritonitis are not.
Research	A multicentre double-blind, randomized clinical trial with two arms (one mupirocin and the other TTO) was designed with nasal application of the medication. A total of 100 renal patients undergoing dialysis were to be enrolled for a treatment period of 6 months. This designed had to be abandoned as only 5 peritoneal dialysis patients were enrolled over a 12 month period. All received TTO.
Outcomes	TTO did have some efficacy against staphylococcal infections as none of the five patients had *S. aureus* peritonitis. However two had exit-site infections and both had carried the organism in their nose. One patient of these had not been compliant with medication. As there was no mupirocin arm, no comparisons can be made with the usual treatment. During the trial no patient reported any side effects of the TTO treatment, so this trial can be added to the growing data on the safety of TTO products.
Implications	TTO shows promise as a topical antibacterial for application to catheter exit-sites, but if TTO is to break into the healthcare market there needs to be a concerted effort to provide the necessary evidence. Products such as nasal gel and body wash need not be confined to this one application but are useful in many medical settings. In this age of increasing medical costs and decreasing government funding to hospitals and healthcare facilities an opportunity exists for cheaper alternatives to antibiotics to establish themselves in these settings.
Publications	To date there are no publications from this work.

Research in Progress - Enhancing production systems to maintain the competitiveness of Australian growers

PRJ-003689 Highly improved tea tree varieties to maximise profit

Start Date:	27/07/2009
Finish Date:	30/04/2014
Researcher:	Trevor Olesen
Organisation:	The Department of Primary Industries, an office of the Department of Trade and Investment, Regional Infrastructure and Services (NSW)
Email:	trevor.olesen@dpi.nsw.gov.au
Objectives	To release highly improved seed and clones to maximize the production of high quality oil and plantation profitability. Progressive genetic improvement is achieved through: the on-going development of established seed orchards (Clonal seed orchard est. 1994 [CSO1], clonal seed orchard est. 2006 [CSO2], second generation seedling seed orchard est. 2001 [SSO2], second generation partial seedling seed orchard est. 2001 [SSO2p]) using a recurrent breeding strategy. the establishment of new orchards (third generation seedling seed orchard est. 2010 [SSO3] and third generation partial seedling seed orchard est. 2010 [SSO3p]) to ensure the long-term advancement of genetic gain. the establishment of new progeny, yield and clonal trials to provide data for orchard culling and selections for cloning and to quantify yield gains from orchard progeny and elite clones. investigating the insect resistance of specific genotypes, with a view to reducing insecticide use. investigating the frost tolerance and survivability of specific genotypes, with a view to reducing losses to climatic extremes.
Current Progress	The project has continually released seed to industry since 1997. Seed sales total 12kg (enough to plant over 1700 ha). Released seed is from the best provenances, together with improved seed from both seedling and clonal seed orchards. Over 1.1kg of seed from the clonal orchard has been sold since 2004 when yield gains of 69% (averaged over 5 harvests) were confirmed for this seedlot. Demand for improved seed increased significantly during 2008 and still remains relatively high. To supply the demand, seed from the second-generation seedling seed orchards was released. Seed sales from these orchards now exceed 4.1kg. Sales for 2012 total 250g. During 2010, 8 trials were established: 2 orchards to further improve yields; 3 yield trials to quantify gains and 3 progeny trials to provide data to cull the orchards. Assessment of these trials indicated excellent survival rates (>98%), good growth, no recorded frost damage, minimal pyrgo damage and no Myrtle rust. In April 2012, the progeny and yield trials in north Queensland were assessed for growth and trees harvested to determine the oil yield for 6 different seed sources. The progeny and yield trials located in northern NSW will be assessed for growth and oil yields later this year. A germination by temperature trial was undertaken to study rates of germination and seedling growth at 5, 10, 15, 20, 25, 30, 35 and 400C. Results suggest that 30 and 25 degrees are about optimal for germination and seedling vigour respectively. The tree breeding database program (TTDAT) continues to be developed for the tea tree breeding project. The database management

program will better ensure the security of project data while enhancing the capacity for long-term data storage and retrieval.

Research in Progress - Enhancing production systems to maintain the competitiveness of Australian growers

PRJ-005801 Genetic markers for yield improvement in tea tree

Start Date:	12/05/2011
Finish Date:	30/05/2012
Researcher:	William Foley
Organisation:	Australian National University
Email:	william.foley@anu.edu.au
Objectives	To identify single nucleotide polymorphisms (SNPs) in DNA from candidate genes of *Melaleuca alternifolia*. To genotype 400 samples of *Melaleuca alternifolia* DNA to identify which individuals have particular patterns of SNPs To perform a formal association analysis to identify which SNPs are associated with high foliar concentrations of desirable essential oils To design and validate genotyping assays to monitor these SNPs in breeding populations of *M. alternifolia*.
Current Progress	The work has progressed well but there will be a delay in completion. All 400 samples that were collected from a single population have had the DNA extracted, and the ten candidate genes have been amplified in all individuals. The amplicons were purified, shredded given an individual barcode using a protocol that we designed. This allows massive cost savings over conventional methods but is extremely laborious. All samples are awaiting sequencing on the Illumina Hi-Seq at the Australian National University, which is scheduled for the next fortnight. This part of the work is close to schedule, but the phenotype data (oil yield and profile) has to be repeated because of misunderstanding with the team at Wollongbar. Unfortunately, the plantation was harvested in October 2011 and so we must wait until the plants have re-grown sufficiently for re-sampling. We will inspect the plants in the first week of May and expect to re-sample in August. Thus the final associations between oil yield and SNPs in the candidate genes will not be completed before end of September at the earliest and we expect to be able to report in October 2012.

Research in Progress - Demonstrating proof of concept/efficacy for innovative applications of tea tree oil

PRJ-005131 Anti-tumour mechanisms of action and prophylactic activity of tea tree oil

Start Date:	30/06/2009
Finish Date:	07/03/2015
Researcher:	Manfred Beilharz
Organisation:	University of Western Australia
Email:	sara.rooney@uwa.edu.au
Objectives	Examine the efficacy of various poloxamer gels of Tea Tree Oil (TTO) and TTO components and other appropriate pharmaceutical formulations of TTO in terms of: (i) skin penetrative capacity and efficacy in non-tumour bearing mice and (ii) anti-tumour efficacy in a range of tumour bearing mice. (iii) safety and any side effects in mice These studies are designed to optimise the efficacy of TTO in the current subcutaneous tumour models. Identify the mechanism by which TTO induces local immune activation by measuring the: (i) activation of DCs in vitro following TTO treatment and the possible interaction of TTO components with toll-like receptors (TLRs) (ii) relationship between immune activation and tumour clearance by anti-tumour effector T cells. This will enhance the likelihood of describing a unique mechanism underlying our current phenomenology. Examine the in vivo preventative and therapeutic efficacy of TTO formulations in a SSUV induced murine skin cancer model to: (i) prevent/ protect against the development of skin carcinogenesis e.g. actinic keratoses (AK) (precancerous lesions)/skin cancers by treating “high risk” SSUV exposed mice with TTO formulations (ii) prevent/protect against skin carcinogenesis by concominantly exposing mice to SSUV and TTO formulations (iii) investigate the efficacy of TTO formulations to inhibit and possibly regress the growth of SSUV induced skin carcinomas This de nova cancer induction will further delineate the underlying mechanisms of immune activation and will identify TTO’s ability to prevent and treat skin cancer.
Current Progress	Animal ethics to establish the murine LK2 UV-induced squamous carcinoma model in our laboratory and to conduct UV experiments in mice at Telethon have been approved. The LK-2 cell inoculum and tumour growth rate has been trialled, with treatments ongoing. It appears that topical TTO/DMSO similarly affects established LK-2 tumours as seen in AE17 tumours. The topical TTO clinical trial for the treatment of AKs is currently under review with NHMRC Project grants and the Raine Research Foundation. Prophylaxis experiments assessing topical TTO treatment in melanoma and LK-2 tumours are underway. Preliminary results show prophylaxis of LK-2 tumour growth is not affected by the current topical treatment regime. Future experiments will examine varied tumour inoculums and will attempt a cycling TTO treatment regime. Preliminary experiments using 2% TTO/DMSO and 2% TTO in PBS/tween for intratumoral injections are very encouraging, but further repeats are necessary to examine any enhancement in tumour regression or time taken to reach endpoint

compared with the topical 10% TTO/DMSO regime. It appears that T effector cells do not play a part in tumour clearance, as their depletion did not affect TTO induced tumour regression. In vitro DC studies have been recommenced and are underway. Lastly, our paper "Topically applied *Melaleuca alternifolia* (tea tree) oil causes direct anti-cancer cytotoxicity in subcutaneous tumour bearing mice" has been accepted for publication in the Journal of Dermatological Science.

Research in Progress - Demonstrating proof of concept/efficacy for innovative applications of tea tree oil

PRJ-006245 In vitro activity and clinical efficacy of tea tree oil products against acne

Start Date:	30/07/2011
Finish Date:	26/07/2013
Researcher:	Kate Hammer
Organisation:	University of Western Australia
Email:	katherine.hammer@uwa.edu.au
Objectives	The broad aim of this research is to investigate the anti-acne potential of tea tree oil products. The specific objectives of this project are to: Determine the activity of commercially available products against the bacteria implicated in acne by broth dilution and time-kill methods Compare the antibacterial activity of products to that of tea tree oil Identify the formulations with the best spectrum of antibacterial activity for the treatment of acne Evaluate the clinical efficacy of the most promising tea tree oil product in a pilot study using human volunteers
Current Progress	The in vitro testing of tea tree oil and tea tree oil products has been completed. The minimum inhibitory concentrations (MICs) of non-formulated tea tree oil for 10 isolates of Propionibacterium acnes ranged from 0.25 - 1% with 90% of strains inhibited by 1%. For the Tea Tree Medicated Gel for Acne MICs ranged from 0.3 - 1.2% product, which corresponds to a tea tree oil range of 0.062 - 0.25%. The Tea Tree Face Wash also showed inhibitory activity with MICs of less than 0.25% product. Given that the currently available Tea Tree Medicated Gel has good in vitro activity it has been selected for the pilot study evaluating efficacy for treating mild to moderate facial acne in human subjects. In preparation for the pilot study a draft protocol has been developed using guidelines provided by the Therapeutic Goods Administration (The Australian Clinical Trial Handbook) and in consultation with two Doctors; a dermatologist and registrar. This, in addition to other trial documents such as the patient information and consent form, is due to be finalised by the end of June and submitted for approval to the Human Research Ethics Committee.

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RIRDC is a partnership between government and industry to invest in R&D for more productive and sustainable rural industries. We invest in new and emerging rural industries, a suite of established rural industries and national rural issues.

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