J. W. Gottstein Memorial Trust Fund

The National Educational Trust of the Australian Forest Products Industries

INVESTIGATING NATURAL PLANT RESISTANCE AS A MANAGEMENT TOOL AGAINST BROWSING HERBIVORES

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2004 GOTTSTEIN FELLOWSHIP REPORT

JOSEPH WILLIAM GOTTSTEIN MEMORIAL TRUST FUND

The Joseph William Gottstein Memorial Trust Fund was established in 1971 as a national educational Trust for the benefit of Australia's forest products industries. The purpose of the fund is *"to create opportunities for selected persons to acquire knowledge which will promote the interests of Australian industries which use forest products for the production of sawn timber, plywood, composite wood, pulp and paper and similar derived products."*

Bill Gottstein was an outstanding forest products research scientist working with the Division of Forest Products of the Commonwealth Scientific Industrial Research Organization (CSIRO) when tragically he was killed in 1971 photographing a tree-felling operation in New Guinea. He was held in such high esteem by the industry that he had assisted for many years that substantial financial support to establish an Educational Trust Fund to perpetuate his name was promptly forthcoming.

The Trust's major forms of activity are,

1. Fellowships and Awards - each year applications are invited from eligible candidates to submit a study programme in an area considered of benefit to the Australian forestry and forest industries. Study tours undertaken by Fellows have usually been to overseas countries but several have been within Australia. Fellows are obliged to submit reports on completion of their programme. These are then distributed to industry if appropriate. Skill Advancement Awards recognise the potential of persons working in the industry to improve their work skills and so advance their career prospects. It takes the form of a monetary grant.

2. Seminars - the information gained by Fellows is often best disseminated by seminars as well as through the written reports.

3. Wood Science Courses - at approximately two yearly intervals the Trust organises a week-long intensive course in wood science for executives and consultants in the Australian forest industries.

4. Study Tours - industry group study tours are arranged periodically and have been well supported.

Further information may be obtained by writing to,

The Secretary,
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Dr Julianne O'Reilly-Wapstra graduated BSc (Hons) in Zoology from The University of Tasmania in 1994. She has worked as a Scientific Officer for the Parks and Wildlife Service in Tasmania, as a Wildlife Technician in the USA, and as a Research Assistant for the Cooperative Research Centre for Temperate Hardwood Forestry and the CRC for Sustainable Production Forestry before completing in 2004 her PhD thesis titled "The phenotypic and genetic basis of browsing resistance of *Eucalyptus globulus* to marsupial herbivores" at The University of Tasmania. Currently she is a Postdoctoral Research Fellow for the CRC for Sustainable Production Forestry and the School of Plant Science at The University of Tasmania. In her current position as Project Leader of the Vertebrate Browsing Group in the Resource Protection Program of the CRC-SPF she is primarily focused on management strategies to reduce the amount of browsing damage incurred in eucalypt plantations. Dr O'Reilly-Wapstra received the Commonwealth Queens Trust Award for Young Australians in 1997, and the Commonwealth Department of Agriculture, Fisheries and Forestry Science and Innovation Award for Young People in 2002.

Executive Summary

Browsing of seedlings and trees by pest species in forestry plantations is an economic problem in eucalypt and pine plantation establishment and management throughout Australia. An exciting area of research in Australian forestry is the use of natural plant resistance as a means of reducing the amount of damage herbivores cause in plantations. Research into natural plant resistance to herbivores is being conducted at the Macaulay Land Use Research Institute in Aberdeen, Scotland, by Dr. Glenn Iason and his colleagues. The plant herbivore group at the Macaulay Institute is the largest group of researchers in Europe studying the interactions and consequences of herbivores in heterogeneous landscapes. In April 2004 I traveled to the Macaulay Institute to conduct a research project in collaboration with Dr Iason titled 'Investigating natural plant resistance as a management tool against browsing herbivores". Scots pine (*Pinus sylvestris*) is a native species to Scotland that is browsed significantly by vertebrate and invertebrate herbivores including the large black slug (*Arion ater*). Needles from Scots pine are rich in terpenes, a plant secondary metabolite, which act as an anti-herbivore defence mechanism. The concentration of terpenes between individual trees varies significantly and is maternally inherited. The specific aims of my research project were to investigate the relationship between Scots pine genotype, herbivory by the slug, and plant terpene concentration. My project provided insight into plant resistance in another forestry system and how this is utilised by researchers to better their understanding of the ecological processes between a tree species of interest and the animal pests that feed on it. This knowledge is used directly by researchers in Scotland in implementing management strategies when establishing Scots pine plantations and when conserving remnant Scots pine native forests. The knowledge, skills and collaborative links that I established during my stay at the Macaulay Institute will benefit future research in the management of browsing pest species in Australian forestry.

Background and Significance of the Study Trip

Browsing of seedlings and trees by pest species in forestry plantations is an economic problem in eucalypt and pine plantation establishment and management throughout Australia. In 1990 the cost of browsing damage in plantations in Tasmania alone was estimated at $2.8million/annum by Forestry Tasmania, based on estimated replanting rates of 40% (Coleman et al. 1997). Browsing by vertebrate and invertebrate herbivores can affect tree form, reduce growth rates and can lead to tree death. For example, mammal browsing damage on *Eucalyptus nitens* seedlings significantly reduced net growth rate at five plantations (Bulinski & McArthur, 1999), and individual eucalypt trees or entire plantations in juvenile phases may be totally defoliated by the autumn gum moth (Neumann & Collett, 1997). Some of the major insect pest species in eucalypt plantations include the autumn gum moth (*Mnesampela privata*), the leaf beetles, *Chrysophtharta agricola* and *Chrysophtharta bimaculata,* the scarab beetles (*Heteronyx* spp) and the African black beetle (*Heteronychus arator*). The mammalian herbivores responsible for damage in plantations are the common brushtail possum (*Trichosurus vulpecula*), Bennetts wallaby (*Macropus rufogriseus*), red-bellied pademelon (*Thylogale billardierii*) and the European rabbit (*Oryctolagus cuniculus*).

At present damage control largely relies on reducing the localised abundance of target species by lethal control, using poisoning and shooting of mammals, and insect pesticides. The lethal control of native mammals and the widespread use of pesticides in the environment are currently politically and socially controversial issues, and alternatives to these control methods are being investigated. An exciting area of research in Australian forestry is the use of natural plant resistance as a means of reducing the amount of damage herbivores cause in plantations.

It has been well documented in many plant species that individuals within a population can vary greatly in their resistance to herbivores, and that plant secondary metabolites play a role in this variation in resistance. If characteristics contributing to resistance can be identified, and demonstrated to have a genetic basis, they may then be selected for in breeding programs to exploit natural resistance to herbivory. To date, this field of research in Australia, on plantation species and associated pests, is still quite limited, however very promising results have been documented. My PhD research identified genetic variation in browsing resistance of *Eucalyptus globulus* to browsing mammals (O'Reilly-Wapstra et al 2002) and Jones et al (2002) also identified genetic variation in resistance of *E. globulus* to the autumn gum moth and found that cuticular waxes were the plant trait conferring this resistance.

Study trip aims

Research into natural plant resistance to herbivores is being conducted at the Macaulay Research Institute in Aberdeen, Scotland, by Dr. Glenn Iason and his colleagues. The plant herbivore group at the Institute is the largest group of researchers in Europe studying the interactions and consequences of herbivores in heterogeneous landscapes. Scots pine (*Pinus sylvestris*) is a native species that is browsed significantly by vertebrate and invertebrate herbivores including the large black slug (*Arion ater*). Needles from Scots pine are rich in

terpenes, plant secondary metabolites, which act as an anti-herbivore defence mechanism (Langenheim 1994), and the concentration of terpenes between individual trees varies significantly and is maternally inherited (Kinloch et al. 1986). Eucalypt species are also rich in terpenes and consequently the defensive chemical profile of Scots pine and eucalypts may be similar.

I aimed to travel to Scotland and for three months to conduct collaborative work with Dr. Iason, from the Macaulay Institute, to investigate natural plant resistance in his system and return to Australia to apply new insight, knowledge and techniques to our systems. The specific aims of this project were to investigate the relationship between *P. sylvestris* genotype, herbivory by *A. ater* and plant terpene concentration.

The Macaulay Land Use Research Institute

The Macaulay Land Use Research Institute was established in 1987 and currently employs 270 people. The Institute is a multidisciplinary-based research institute employing soil, plant and animal scientists, geographers, socio-economists and IT specialists. The research carried out is an integrated study of the physical, environmental, and social consequences of land use, which is undertaken at various levels of physical, chemical, and biological organisation. The research is aimed at meeting the needs of land managers and those formulating and implementing land use policy in the UK and Europe.

The Macaulay Land Use Research Institute, Aberdeen, Scotland, U.K.

The Scots Pine System

Scots pine (*Pinus sylvestris)* is an important tree species in Scotland for its use in plantations and restoration programs. Scots pine grows naturally throughout Europe, ranging from Scotland in the west, Siberia in the east, Scandinavia in the north and the Mediterranean in the south. Scots pine is considered a keystone species in Scottish woodland ecosystems, however, the current natural distribution of this species is patchy where remnant populations cover only 1% of the former range. Consequently this species is the focus of restoration and regeneration programs. Additionally, Scots pine is also considered an important economic plantation species and is the only native conifer grown for commercial purposes in the United Kingdom. The tree can grow 36 m in height and 1.5 m around the girth and can live for up to 300 years. The timber is known as "redwood" or "red deal". It is a reasonably strong, low-density timber that has as its greatest appeal the attractive yellow / red coloured finish of the wood. Its use in building includes roof timbers, stairs, doorways skirting, and furniture. It is also used for telegraph poles, fences and paper pulp. In the past it was also used to make ships, ship masts and water wheels.

Native Scots pine woodland at Balmoral field site, Scotland

Scots pine plantation, Braemar, Scotland

There are numerous vertebrate and invertebrate herbivores that feed on Scots pine seedlings and saplings. These include the generalist mollusc, the large black slug (*Arion ater*), native mice, voles, hares and deer. Browsing by these herbivores on Scots pine is detrimental to regeneration programs and commercial plantations. In northern Scotland, growth rates of Scots pine are slow (see photo below of 40 year old Scots pine trees) due to the climatic conditions. Browsing by herbivores is an added pressure that affects growth rates and survival leading to large economic losses. Scots pine contains a group of plant secondary metabolites called terpenes which have been shown in many plant species to act in defence against herbivores. Eucalypts are also rich in terpenes and consequently the defensive chemical profile of Scots pine and eucalypts may be similar.

Native Woodlands Field Day

During my stay in Aberdeen I participated in a field day workshop that was designed to discuss the management of small Scots pine plantations to ensure gains by the growers while maintaining biodiversity that is lacking but of high value in Scottish woodlands. This field trip allowed me to view plantations and natural Scots pine stands to see first hand the issues (e.g. deer browsing) associated with maximizing the output from plantations. Due to historical intensive land use in the United Kingdom, plantations often cover the due role of commercial output and while maintaining biodiversity values in woodlands of a native species. Deer browsing is a major problem in plantation establishment and management (see photo of deer fence below) and at present fencing and culling appear to be the only successful management strategies, however, these methods are expensive. This scenario

mirrors the mammal browsing problem we are faced with here in the Australian forestry industry.

Intensive land use in Scotland. Heather habitat for grouse, Scots pine woodland and grazing habitat for cattle.

Slow growth rates; forty year old Scots pine plantation, Braemar, Scotland.

A deer fence showing deer exclusion on the left side and the effects of deer browsing on the right side, Braemar, Scotland

Research experiments and results

I conducted two research experiments with the herbivore research group at the Macaulay Institute. The current work conducted by this group is varied but centers around understanding the relationships between different Scots pine genotypes, Scots pine defensive chemistry (terpenes) and the herbivores that feed on Scots pine.

Research by the herbivore group at the Macaulay Institute.
This photo shows deer exclusion plots at the Balmoral Field Site. This trial examined the effects of deer browsing on Scots pine seedling recruitment and survival.

Considerable research had been conducted on the mammalian herbivores in the Scots pine system, however, little was known about the effects of browsing by the generalist herbivore, the large black slug (*Arion ater*). Consequently my research project focused on investigating the relationship between Scots pine genotype, herbivory by *A. ater* and Scots pine terpene concentration.

Arion ater is a common herbivore in grasslands and woodlands in Scotland. When mature the slug can reach 10-20 cm in length. This species has been introduced into Australia on several occasions and small populations exist in South Australia, New South Wales and Victoria. Large populations, however, have failed to establish and this species does not appear to be 'successful' in establishing in Australia.

The large black slug (Arion ater).

Experiment 1

The first trial I conducted at the Macaulay Institute investigated browsing of two week old Scots pine seedlings by *A. ater* in a captive feeding trial. Slugs were caught for the trial in nearby Scots pine forest. Seedlings were grown from seed, collected from 6 known mothers and consequently, the genotype of all seed was known. The 6 mothers differed in their defensive terpene profiles; two mothers were high in terpene concentration, two were intermediate in terpene concentration and two were relatively low in terpene concentration. Fourteen individual slugs were used in the feeding trial. Each slug was allocated randomly to 14 separate plastic tub (49 x 40 x 45 cm) enclosures which contained soil for planting the seedlings. Only one slug was allocated to each enclosure to remove any effects of mutual interference with other individuals. A copper mesh wooden frame was placed around the top of each enclosure to keep slugs inside the enclosures. A strip of shade cloth (5 x 50 cm) was placed at the two ends of each enclosure to provide 2 possible refuges for each slug. Three hundred and thirty six seedlings were used in the feeding trial, 56 from each of the six mothers. Twenty four seedlings, four from each six mothers were planted in a 6 x 4 grid design in each of the 14 enclosures. Prior to the seedlings being planted in the tubs, one needle was cut from each seedling for terpene analysis.

Two week old Scots pine seedling used in experiment 1.

Arion ater, *shade cloth shelter and two planted Scots pine seedlings in experiment 1.*

Experiment 2

In the second trial I investigated the effects of three different terpene concentrations (high, medium and low) on the intake of a cereal diet by *A. ater*. In this trial I used two different terpenes that are quantitatively dominant in Scots pine needles, 3-carene and alpha pinene. Thirty six slugs were used in this feeding trial and kept in individual containers. The feeding trial was a paired choice feeding trial, where each slug was offered a choice of two diets at the one time; one control diet (a cereal diet with no added terpene) and the other an experimental diet (a cereal diet laced with either low, medium or high concentration of either the 3-carene or the alpha pinene terpene).

The results of these trials are still being analysed for publication and consequently only a preliminary description of the results will be discussed. In experiment one, *A. ater* readily consumed the two week old seedlings and would consistently eat the seedling at the soil level. This resulted in seedling death and consequently, the effects of slug browsing on Scots pine seedlings is significant. There was large variability in the number of seedlings that an individual slug would consume in one night. The maximum number of seedlings consumed in one night by an individual slug was 11. There appeared to be no selective feeding by slugs among seedlings from the 6 different genotypes. The terpene profiles of each individual seedling presented in the trial are yet to be analysed, but it does not appear that selection of seedlings by slugs was determined by terpene concentration.
In experiment two, there was considerable variation in the amount of cereal diet that was eaten by slugs. Slugs ate significantly less of the cereal diets that contained high concentrations of the terpene diet compared to the control diet (not laced with terpene) that was offered at the same time. There was no significant difference in the amount of diet eaten when slugs had a choice of the control and the low terpene diet, or the control and a medium terpene diet. This result is consistent for the two terpenes tested, 3-carene and alpha pinene.

These results suggest that Scots pine trees with high constitutive levels of terpenes will be resistant to browsing by the generalist herbivore, the large black slug, while trees with intermediate or low levels of terpenes will be susceptible to browsing.

Outcomes of the Study Trip

There are several significant outcomes of this study trip. The research group with which I was associated with at the Macaulay Institute is the largest group of researchers in Europe studying the interactions and consequences of herbivores in heterogeneous landscapes. Collaborative links between forestry researchers at the Macaulay Institute and my research group have been consolidated, and the intellectual and professional benefits of collaborating with this research group are second to none. The benefits, to Australian forestry research, of interacting with such a group will continue for as long as the collaborative links are maintained.

While in Aberdeen, I gained considerable knowledge of a different forestry system where browsing by herbivores is also a significant problem for plantation establishment and native forest regeneration. The research conducted focused on understanding the range in natural plant resistance (terpene concentration) of Scots pine and how this may be utilised in natural resistance against herbivores. Eucalypts are also rich in terpenes and consequently, an understanding of the role of terpenes as defences in Scots pine is beneficial to our understanding of the role of terpenes as defences in eucalypts. The research I am conducting in Tasmania aims to utilise natural plant resistance as a means of reducing the browsing damage occurring in plantations. The political and social climate is placing considerable pressure on the forestry industries to implement alternative, non-lethal strategies when managing browsing damage in plantations. The knowledge, skills (e.g. in chemical analysis, experimental design and statistics) and insight that I have gained during my stay in Aberdeen will greatly benefit future research into these alternatives.

While at the Macaulay Institute I presented a seminar on the current status of our research into natural plant resistance. Here in Australia, the outcomes of my study trip will be presented in seminars and the scientific findings from my research project will be published in an international refereed journal.

Acknowledgement

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