Underground structures and mycorrhizal associations of *Solanum centrale* (the Australian bush tomato)

Angela Dennett

Faculty of Agriculture, Food and Natural Resources

University of Sydney

Supervisors:

Prof. Lester Burgess

Dr Peter McGee

Dr Maarten Ryder

SIDERE MENS EADEM MUTATO

DESERT KNOWLEDGE CRC

Acknowledgements

Contributions to this project have been made by so many people from across Australia they are almost too numerous to acknowledge. Funding contributions towards this project were graciously received from the Desert Knowledge CRC.

Personal thanks firstly to my main supervisor, Professor Lester Burgess, for guiding me through field observations; laboratory analysis and thesis assembly; and for being so willing to help even when it has taken up his personal time. Likewise, thank you to supervisors Dr Peter McGee of Sydney University and Dr Maarten Ryder of CSIRO Sustainable Ecosystems for giving this project direction and guidance in their specific areas of expertise. The assistance of all my supervisors has not only resulted in the production of this thesis but has inspired me to begin a career as a research scientist.

Thanks also to Yvonne Latham, Marie O'Hanlon and Bruce Hawke for their extensive and invaluable contributions in both field and laboratory work. Furthermore, field work in Alice Springs could not have been conducted without the resources and advice generously contributed by the staff of the Alice Springs Desert Park and Horticultural department at Charles Darwin University in Alice Springs. Special thanks to Ruth Brown, Tim Collins, Jayde Kudrenko, Wayne Tregear, and Michael "the budding horticulturist" for providing tools and digging in the dirt in less than ideal conditions. Thanks to Max Emery of Pwerte Arntarntarenhe and Neville Bonney of Wallowa (the Palmer site) for allowing sampling on their properties and offering advice and assistance. Similarly, the permission to sample on Napperby Station and at Owen Springs Detention Centre (Hatt Rd) was gratefully received.

Thank you to the technical staff and students in the Agronomy, Plant Pathology and Mycorrhiza Laboratories - Jarka Geisler, Ivan Desailly, Tim Knight, Suneetha Medis and Leonie Whiffen for guiding me through the diverse glasshouse and laboratory work; and to Dr Bob Caldwell for assisting me with the phosphorus analysis. Much appreciated assistance was also provided by Anne Simpson and Emine Korkmaz of the Electron Microscopy Unit in the many attempts to obtain root sections. I am also grateful for the information provided by David Symon of the South Australian Botanical Gardens, Tony Bean of the Queensland Herbarium and Brendan Lepschi of the National Herbarium - Australia's experts on native *Solanum* spp..

Finally, I am indebted to my family for their encouragement and support throughout this project. Your love and concern has been a massive help and a great example, supporting me in my faith which got me through this year. Thank you.

Underground structures and mycorrhizal associations of *Solanum centrale* (the Australian bush tomato)

Contents

Literature review

Word count – 9294
References – 121 (69 from 1999 to 2006)
Tables – 5
Figures – 10

Thesis

Word count – 8355
References – 76 (38 from 1999 to 2006)
Tables – 7
Figures – 17

Abstract

Solanum centrale (Solanaceae) is an Australian indigenous food plant with potential uses as a desert crop species. This project investigated the little understood underground structures and mycorrhizal associations of *S. centrale* to assist the domestication process. Eight naturally colonised and cultivated sites in South and Central Australia were observed and samples of lateral and fine (secondary) roots were taken for anatomical study and to determine resprouting ability in the glasshouse. In addition, a glasshouse trial spanning 10 weeks was conducted to determine the response of *S. centrale* seedlings to mycorrhizal fungi and phosphorus nutrition at zero P, low P (0.15 g) or high P (0.3 g), applied as soluble phosphate.

S. centrale was found to form clonal communities connected by underground lateral roots. Shoots were produced at irregular intervals on lateral roots and responded extremely rapidly and favourably to simulated disturbance in the glasshouse. Tap roots were occasionally found with a shoot and lateral roots often turned downwards to grow vertically. Fine roots were sparsely located on laterals and were old and brittle at the driest sites. Despite this, mycorrhizas were observed in the fine roots at all field sites.

In the glasshouse experiment, the effect of mycorrhizal fungi on root colonisation and plant growth and development varied depending on phosphorus application. Mycorrhizal fungi colonised 48% of root length in inoculated zero P plants but only 2% of roots at low P and no roots at high P. Inoculation with mycorrhizal fungi had no effect on dry weight, root length and plant height of low or high P plants, but significantly increased growth at the zero P level ($P<0.05$). However inoculation increased the average root phosphorus content by 42%, decreased root to shoot ratio by 92% and decreased root biomass by 41% at all P levels, despite negligible colonisation at low or high P. This suggests that the presence of mycorrhizal fungi in the rhizosphere influences plant morphology. Furthermore, the absence of mycorrhizal associations under high P suggests that no benefit is conferred to the host in high nutrient environments. This may prevent an unnecessary drain of photosynthate from the plant when P is not limiting.

The positive response of *S. centrale* to mycorrhizal inoculation, and its ability to rapidly resprout from roots, highlights its suitability to an arid environment. This work demonstrates the potential of *S. centrale* as a cultivated desert food plant.

Additional keywords

Aboriginal food; arid-zone flora; root-sprouting; vegetative reproduction; phosphorus response; plant hormones.

Part 1 - Literature review

Solanum centrale (bush tomato) – root system adaptations to aridity and importance in the Native Food Industry

Angela Dennett
Faculty of Agriculture, Food and Natural Resources
University of Sydney

Supervisors:
Prof. Lester Burgess
Dr Peter McGee
Dr Maarten Ryder

Contents

List of Figures

List of Tables

Author's note:

Considering its role as a staple food, the literature on *Solanum centrale* is very thin indeed – searches of appropriate online databases typically reveal less than five papers which mention the species in the title or abstract. As such, much of this review involves short segments of information gleaned from literature on a wide range of topics, from taxonomy to agronomy to natural history to sociodemographics. In several areas (particularly in reference to root systems and the Native Food Industry), many authors did not appear to have a thorough knowledge of the previous research in related areas. Thus this review is expected to be of benefit not only in the understanding of the root systems of *Solanum centrale* but characteristics of arid zone flora and the place of Australia's desert plants in global food production.

I. INTRODUCTION

For thousands of years, indigenous Australians living in the vast arid interior have relied on Australia's edible flora and fauna for sustenance. The term 'native foods' encompasses animals including kangaroos, emus and witchetty grubs, and plants together with a few macrofungi (such as *Laccocephalum myllitae*) (Latz, 1999). This review is primarily concerned with edible plant species that have commercial potential. Many of these species possess unique characteristics which have been poorly researched and documented, despite their importance to Aboriginal communities and potential role in the fledgling native food industry. In recent years several desert plant species, including *Solanum centrale* (the desert raisin), have been proposed as subjects for research which will assist development of these species for food production. In particular, the below-ground features of many desert species are poorly understood despite their importance in survival and reproduction though drought, frost and fire, and in obtaining the scarce water and mineral nutrients of the arid region. This review consolidates the scattered information on *S. centrale* and contains a consideration of the root morphology and mycorrhizal associations in plant species with either similar taxonomy or a similar natural environment to the desert raisin.

II. AUSTRALIAN NATIVE FOOD PLANTS

(1) Introduction

Since the arrival of humans in Australia, plants have been used for food, medicine, tools, shelter and firewood (Everard *et al*., 2002). The diversity of edible species in Australia has been slowly refined to a list of palatable species which were eaten by Aboriginal groups for thousands of years prior to European colonisation. Today, "native plant foods" are 'edible indigenous plant components, harvested from the wild or farmed' (Walker, 1996). These edible plant products can be grouped into fruits, seeds, roots, galls, herbs/vegetables and nectars, sweet secretions and gums (Everard *et al*., 2002).

(2) Aboriginal uses

Indigenous Australians relied entirely upon native plants and animals for food until European settlement. Tribes living near the coast generally had a greater variety and number of plant and animal species available to them including fish and crustaceans. The use of plants varied with the abundance of animal food sources (Wilby, 2006).

The Aboriginal plant foods consumed in the greatest abundance in Central Australia varied with the region and the season. Almost all species are only available for part of the year and thus the indigenous diet varied considerably between seasons. Some plants form underground storage structures such as yams and bush potato which can be harvested in dry periods. Furthermore, the uses of food species depended greatly on their storage ability and complexity of preparation. This was usually to remove toxins in either juices or seeds (Brand, 1989).

Thorough knowledge is essential for correct selection, collection, preparation and preservation of food plants. This is of particular importance in the Solanaceae family where plants of similar appearance may be poisonous or require special processing before consumption (Hiddins, 2001).The depth of Aboriginal knowledge on the collection, preparation and preservation of species of *Solanum* and other bushfoods has been acknowledged by European explorers and scientists for well over a hundred years (Gilmore, 1934). For example, explorer David Carnegie reports finding 'several wooden sticks on which were skewered dried fruits' whilst exploring Central Australia in the 19th Century, which he (almost certainly incorrectly) identified as the exotic *Solanum sodomeum* (Apple of Sodom) (Carnegie, 1898).

Although not cultivated in the European sense, Aboriginal groups cared for the land in ways which increased its productivity. In arid Australia, regular firing was used to encourage new growth, stimulate germination of many species such as Hakeas and Eucalypts, release nutrients from dead and decaying material and manage animals (Gott, 2005). There are also reports of the diversion of waterways after rains to encourage growth of key food species, such as the desert raisin (Isaacs, 1987). In NSW, Quandongs were cross-pollinated by Aboriginal groups to obtain favourable genotypes (Gilmore, 1934).

Today, native plants still have an important role for many indigenous peoples. Wild harvesting for personal consumption, income or enjoyment is practiced in some communities. This is a cultural as well as functional activity, serving to educate the younger generation about the tribal language, ways of the land and Aboriginal Dreamtime traditions.

In some places it has become a social event not just for women but whole families, promoting exercise and health within the community (Cribb *et al*., 2005). In Central Australia several native species are used in the diet; these include members of *Solanum*, particularly *S. centrale*, *S. ellipticum* and *S. cleistogamum* (Turner-Neale, 1994).

Thus the role of native foods in Australia is far from purely economic. For many Aboriginal communities, particularly in Central Australia, native foods have great social and cultural significance.

(3) The Australian native food industry

(*a*) History of the native food industry

Trade in native foods had its origins amongst indigenous tribes long before the arrival of Europeans (Carnegie, 1898). Since the arrival of the First Fleet the use of native plants has decreased, partially due to losses of cultural knowledge, killing or displacement of tribal groups and introduction of high yielding exotic plant and animal species.

The first native food to be developed into its own industry was the macadamia nut. The potential of macadamias was largely ignored in Australia and the industry began with the introduction of the plants to Hawaii in the 1800s (Courtney, 2003). Australia is now the worlds biggest producer with gross production valued at $101.5m over the 2003-2004 financial year (Australian Bureau of Statistics, 2005). Macadamia nuts are the most economically successful of the native food plants and are no longer considered part of the "Native Food Industry".

The modern native food industry can be traced back to the activities of seed collectors in the 1960s and 1970s (Miers, 2004). Native foods began as novelty items associated with Aussie outback stereotypes (such as Paul Hogan and the Bush Tucker Man), gimmicky terminology and a strong association with the 'outback' to obtain media and international attention (Cherikoff, 2000). Some interest and investment was generated in the 1980s and early 1990s by nutritionists and pioneering farmers looking for ecologically sustainable farming strategies (Brand, 1989; Phelps, 1995).

In 1996, a conference was held by the Australian Native Bushfood Industry Committee (ANBIC). It is clear from the sudden increase in published literature that the conference generated considerable momentum and enthusiasm within the scattered

participants of the industry. A business and marketing paper was produced by Econsult (Australia) (1996) for the conference, which contained an industry profile and recommendations. The industry was valued at $13,683,060 in the 1994/95 financial year. Interviews with 25 industry participants revealed an average anticipated growth of 74% over three to five years. If this growth were sustained, the industry would reach its growth target of $100m in three years.

Since that time, research has begun with funding from several leading organisations in Australia (including RIRDC and CSIRO) into production, processing and value-adding for native plant products. Business partnerships involving Aboriginal communities were developed to obtain wild harvested produce and some farmers made small investments in domestic production (Bunt *et al*., 2004). Existing technology was utilised in production and processing. For example, a modified wheat header has been used to harvest bush tomatoes (Hele, 2001).

Such developments over the last 10 years have led to small but significant growth in the industry. Excluding macadamia nuts, the native food industry was estimated to be worth between $10m and $12m in 1995/1996 (Graham & Hart, 1997), $14m in 1997 (Stynes, 1997), $10m and $16m in 2000 (Cherikoff, 2000), $16m in 2002 (Department of Natural Resources and Environment, 2002) and between $15m and $20m in 2004 (Miers, 2004) (Fig. 1). This is significant considering there were “no Australian indigenous foods already on the table” in the late 1980s (Brand, 1989). However it is far below the industry aim of $100m by 2000 after the ANBIC conference. Because of the complexity of supply it is impossible to obtain reliable estimates for total industry value (Robins, 2004).

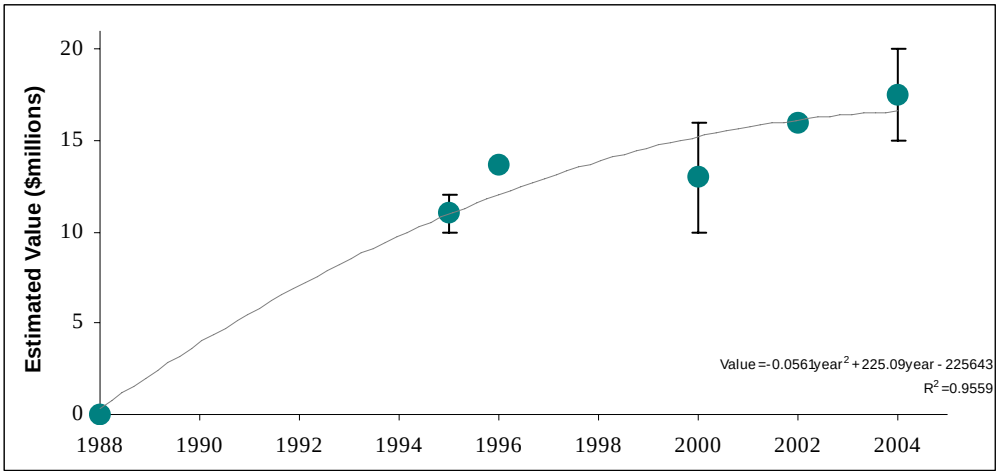


Fig. 1 Trend in estimated value of the native food industry. Error bars indicate upper and lower bounds of estimates for that year. See text for sources.

(*b*) The native food industry today

The native food industry is still very small relative to other agricultural industries. It is not unusual for industry participants to be involved in multiple sectors including nurseries, cultivation or wild harvest, processing and marketing (Robins, 2004). A list of key species of either current or potential commercial value is given in Table .

Returns for suppliers are low compared to returns to processors and end users. Few suppliers derive all of their income from producing or harvesting native foods (Stynes, 1997). The value to farmers and wild harvesters of commercial native food products for 2003 was estimated at $3.3m (Foster, Jahan & Smith, 2005). This is a large increase from the estimated $645 000 generated in income directly from native foods for growers, nurseries and harvesters in 1994/95 (Econsult (Australia), 1996).

Table 1 Species marketed in the Native Food Industry. Source: (Isaacs, 1987; Robins, 2004; Stynes, 1997)

Common name	**Botanical name**	**Uses**
Aniseed Myrtle	*Backhousia anisata*	Leaves dried and milled
Bush tomato	*Solanum centrale*	Fruit eaten whole, dried or ground
Davidson's plum	*Podocarpus elatius*	Whole, deseeded halves or frozen puree
Kakadu plum	*Terminalia ferdinandiana*	Whole or dried fruit
Lemon aspen	*Acronychia acidula*	Whole fruit
Lemon myrtle	*Backhousia citriodora*	Whole fresh leaves on stem, leaves dried and milled
Native citrus	*Citrus glauca, Citrus australasica* and other *Citrus spp.*	Whole
Mountain pepper	*Tasmannia lanceolata*	Dried and milled leaf or dried berries
Muntries	*Kunzea pomifera*	Consumed as whole or dried fruit or made into jams and chutneys
Quandong	*Santalum acuminatum*	Fruit
Native mint	*Prostanthera rotundifolia*	Dried and milled leaves
Riberry	*Syzygium luehmannii*	Whole fruit, seeded fruit
Wattle seed	*Acacia spp.*	Whole seed, roasted and milled
Wild lime	*Citrus spp*.	Fruit

The primary problems in the Australian native food industry relate to supply of products. Supply varies with the species, with some oversupplied and some undersupplied for current demand (Graham & Hart, 1997). For example, Robins (2004) reports that the quandong, wattle seed, lemon myrtle and native citrus are in over supply, whereas the native pepper, riberries and desert raisin are undersupplied. Furthermore, in species where wild harvest takes place (such as the desert raisin) the supply can be irregular both through the year and from season to season. The inability to appropriately supply the market reveals a lack of information amongst industry players (Atech Group & Total Earth Care, 1999) and creates problems when attempting to supply bulk buyers such as restaurants and the export market. Irregularity of supply and value-adding of products has not been sufficient to launch an export campaign.

Many native food species are strongly associated with Aboriginal communities. Currently seven of the 13 key species utilised by the native food industry are mainly sourced from wild harvesting (Robins, 2004). Different Aboriginal groups have different views on the right to cultivate, harvest and sell native food species. Adding to this conflict is the issue of ownership of intellectual property and plant variety rights for Aboriginal groups. This issue is largely unresolved (Bunt *et al*., 2004; Econsult (Australia), 1996).

(*c*) Growing bushfoods

The target species for the native food industry are currently grown throughout Australia. They are sourced from a variety of climates, soils and ecosystems ranging from the wet forests of southeast Australia (native pepper) to tropical north east Queensland (Davidson's plum) to the arid interior (bush tomatoes) and the Kimberly region (Kakadu plum) (Robins, 2004). In many cases the cultivation and harvest of native foods is the greatest constraint to the industry, even more so than creating demand and awareness. Little is known about the agronomy of many species compared to exotic horticultural crops (Miers, 2004). This is partially because several are still being domesticated, and also because the climatic and soil conditions in naturally productive parts of Australia are quite different to the rest of the world. In particular, Australia's deserts possess a great variety of food species which far exceeds the naturally productive food species in regions such as the Sahara and Sinai Deserts.

Given the current under-utilisation of Australia's arid region for horticultural production, comparatively little is known about the best ways to grow native foods in the region. Many techniques used in temperate and tropical regions for agricultural production such as crop rotation and traditional cultivation are not always applicable in the desert. The climatic extremes, unreliable rainfall and soils which are often saline, have a high pH and low organic matter content create specific challenges for growth (Miers, 2004). Techniques which have been trialed on a variety of species in Central Australia include drip irrigation, wind breaks, fertilisers, mounding, artificial shade, windbreaks, mulching and water harvesting, with varied results (Miers, 2004; Ryder & Latham, 2005).

(*d*) Industry goals

The native food industry faces many challenges. These include regularity of supply, food safety (Hegarty, Hegarty & Wills, 2001), appropriate names, labeling and packaging (Econsult (Australia), 1996), harvesting food in isolated parts of the country (considering the high cost of freight) (Miers, 2004) and obtaining vigourous varieties which produce quality products (Graham & Hart, 1997). Of these food safety is a small but perhaps the most urgent problem, considering that there are currently no industry standards for levels of chemicals in any products (apart from macadamias). Natural toxins are present in many species of native plant, such as protease inhibitors in raw acacia seeds and solasodine in the seeds of *Solanum coactiliferum* (Hegarty *et al*., 2001). Furthermore based on the name 'bushfoods', consumers may incorrectly assume the product they consume has been wild harvested and as such has not been treated with pesticides or herbicides.

As the native food industry grows, more farmers and agribusinesses will take part. Few participants in the industry derive their entire income from native foods (Stynes, 1997). This trend may or may not continue, depending on the returns from native species facilitated by increased demand in the marketplace. The variety of species allows growers to choose a species suitable to their area (taking into account climate, soils, pathogens and access to markets) and which fits in with their current enterprises (considering crop rotations and existing capital such as harvesting equipment and irrigation infrastructure).

As the current challenges in the industry are overcome more attention can be paid to marketing. Restaurants and commercial catering outlets such as QANTAS are prime targets for the industry, servicing both the need for public education of native foods and creating a steady demand for products. The industry also has the potential to exploit the 'clean, green' image of growing native plants (Phelps, 1995).

(4) Importance of native foods

(*a*) Indigenous community development

Outside the towns the population of Australia's arid regions is very sparse, comprised mostly of small Aboriginal communities. According to Guenther *et al*. (2005), approximately 33 000 Aboriginal people live in the desert – one-fifth of the desert population. This group is highly dispersed over the region with 72% living in groups of 50 people of less. The population is also highly mobile – based on data from the Australian Bureau of Statistics (2002), Guenther *et al*. (2005) determined that 39% of communities in South Australia, 48% of communities in Western Australia and 21% of communities in the Northern Territory experienced an increase of population of at least 50% at some stage in the course of a year.

There are fewer employment and educational opportunities in the desert than in other remote parts of the country (Miers, 2004). Conventional agriculture and horticulture cannot support the current population of Central Australia because very few exotic animal and crop species are suited to the conditions. The tourism, pastoral and mining industries create some opportunities but these are limited by geography and demand (Guenther *et al*., 2005).

Many of the adaptations of indigenous groups to life in the desert were behavioral and social, such as high residential mobility, broad foraging and intimate knowledge of the environment (Smith, 2006). This contrasts with the European strategy of relying on technology to overcome the challenges of Australia's arid region. Conflicting social and political models between Aboriginals and Europeans have led to a loss of traditional knowledge, skills and social structures which are ideal for life in the desert. Much of this knowledge is based around obtaining food. There is considerable potential to develop

knowledge and skills into a viable source of income which stems from a more traditional lifestyle (Miers, 2004).

The unique sociodemographics, politics, economics and environment of the arid region make it an ideal location to promote the harvest of native foods for income amongst Indigenous communities. Much of the arid region is under the control of traditional indigenous inhabitants and as such has the advantages of large areas of undeveloped land and Aboriginal knowledge of local species. Growing native foods is a way of maintaining the strong connections between Aboriginal peoples and their country and allowing the social processes which shape these connections to influence their lifestyle (Guenther *et al*., 2005). Therefore native foods have significant potential as a source of income for Aboriginal communities and are a unique opportunity for communities who wish to stay closely associated with their traditional lands.

Some desert communities are already experimenting with native food plots, such as Koonibba in South Australia (Grosser, 2003). Species such as the desert raisin can either be grown in market gardens on outstations or produce can be collected from wild stands (Miers, 2004). Wild harvest of plants is traditionally performed by Aboriginal women (Everard *et al*., 2002).

There are several other benefits of growing native foods in the arid region. Agricultural activities are incredibly restricted by the climatic extremes. Existing enterprises (almost entirely limited to very extensive grazing) mean the current returns from a “life off the land” in Central Australia very subject to erratic beef and wool prices (Phelps, 1997). Also, desert plantings have fewer problems with pathogens due to the climatic extremes, which naturally restrict many pathogenic fungi and bacteria. Such benefits may attract non-indigenous land holders to producing edible desert plants.

(*b*) Sustainability and conservation

With the focus on sustainable farming by governments and research agencies, there is considerable potential for planting productive native species on arable land. Most native foods can be grown with relative ease without acidity-causing fertilisers and require less water than exotic species when grown in their natural range. Therefore even before the financial returns are as high as alternative agricultural uses, farmers may adopt native plant species because of their environmental benefits.

(*c*) Global food security

Use of the deserts for food production is an issue of importance not only for the people who live there. Global trends in population growth and environmental changes (such as global warming) create specific challenges to agriculture over the next 50 years. Estimates of the carrying capacity of Earth vary between 2.5 billion people (on an American diet consuming 30-40% more calories than required) and 40 billion (if using all the flat land to produce food, even though much of it is unsuitable) (Botkin & Keller, 2003). Furthermore, even though the specific effects are unproven global warming will undoubtedly create some problems for agriculture around the globe (International Panel on Climate Change, 2001). Therefore in the future the use of arid and other currently non-arable regions for food production will become increasingly important.

Considering this, Australia has a potentially vital role in global food security in the coming years. Approximately 3.5 million km^2 of the continent is considered arid (Smith, 2006) (Fig. 2). Finding ways of using these areas to provide food, resources and medicine is becoming of increased importance. Research into plant (and animal) species which thrive in our arid interior must continue to not only provide a source of wealth to desert communities but also as a potential contribution to the global food supply.

III. *SOLANUM SPP*. IN CENTRAL AUSTRALIA

(1) Taxonomy

The genus *Solanum* is the largest genus in the family Solanaceae. It is one of approximately 90 genera in the family with over 2600 species. *Solanum spp*. are found worldwide, preferring tropical and warm temperate areas (Symon, 1981). Several crops are found in this genus, including potatoes (*Solanum tuberosum*) and eggplant or aubergine (*S. melongena*). Weeds of agricultural importance include deadly nightshade (*S. nigrum*) and kangaroo apples (*S. aviculare* and *S. laciniatum*). Several other crop species are found within the family Solanaceae including capsicum (*Capsicum annuum*) and tomato (*Lycoperscicon esculentum*, also classified as *Solanum lycoperscicon*).

There are 23 Solanaceous genera found in Australia, of which six are endemic (Purdie *et al*., 1982). Within the genus *Solanum*, Australia's approximately 100 species are overwhelmingly endemic (Symon, 1982). Of these species, 24 can be found in the arid zone (Fig. 2, Table 2).

(2) General Description

Most Australian *Solanum spp*. are small shrubs with short lived shoots which develop from adventitious buds on the roots (Symon, 1981). Arid zone species include 18 herbaceous perennials and just six woody shrubs (Symon, 1982). Some individuals may have hairs or prickles on leaves or stems, depending on both the species and its environment. Species in the subgenus *Leptostemonum* (including *S. centrale*) are poor competitors in later successional stages and respond positively to fire and mechanical disturbances such as road works (Bean, 2004).

Table 2 Native *Solanum spp*. found in Australia's arid zone. After Isaacs (1987); Latz (1999); Symon (1982).

Section	Group	Species	Edible
Graciliflorum	-	*chenopodinum*	
		ferocissimum	
Melongena	Andromonoecious	*chippendalei*	Y
		diversiflorum	Y
Oliganthes	Small, hard fruited	*ashbyae*	
		eardleyae	
		gilesii	
		lasiophyllum	
		lachnophyllum	
		petrophillum	Y
		quadriloculatum	
	Small, yellow fruited	*centrale*	Y
		coactiliferum	
		esuriale	Y
		hesperium	
		lacunarium	
		oligacanthum	
		orbiculatum	
		plicatile	
		sturtianum	
		terraneum	
	Small, green fruited	*cleistogamum*	Y
		ellipticum	Y
		horridum	
	Total species	24	

While Aboriginal knowledge of the use of these plants for food is quite detailed, few scientific studies have been performed on the agronomic and human health properties of edible species. Several species of *Solanum* contain the poisonous alkaloid solanine (Smith & Smith, 1999) and are either inedible or require substantial pre-treatment before consumption. Different species were prepared in different ways by Aboriginal communities, including removal of seeds, rubbing in the dirt and cooking (Women from Laramba (Napperby) community, 2003). The most commonly consumed species are *S. centrale*, *S. cleistogamum* and *S. ellipticum* (Turner-Neale, 1994). Of these species *S. centrale* has the greatest commercial use and has been identified as a primary focus for research by the Desert Knowledge Cooperative Research Centre.

Solanum spp. were commonly used by Aboriginal communities as a staple food in arid Australia. This is partly due to their abundance in a variety of seasonal

conditions their storage potential (Peterson, 1979). Although agriculture as known by Europeans was not practiced as such, fire was used extensively to manipulate the abundance and vigour of *Solanum spp*. (Isaacs, 1987). *S. centrale* seed was scattered near campfires on burnt areas (Smith & Smith, 1999) and productive patches have been serviced by damming water courses after heavy rain (Isaacs, 1987). The distribution and abundance of arid zone *Solanum spp*. was further manipulated by anthropochory, as Aboriginals gathered and transported fruits between campsites. Although the viability of seeds in faeces is uncertain, the inedible seeds of species such as *S. chippendalei* were thrown away and thus could produce new plants (Symon, 1979).

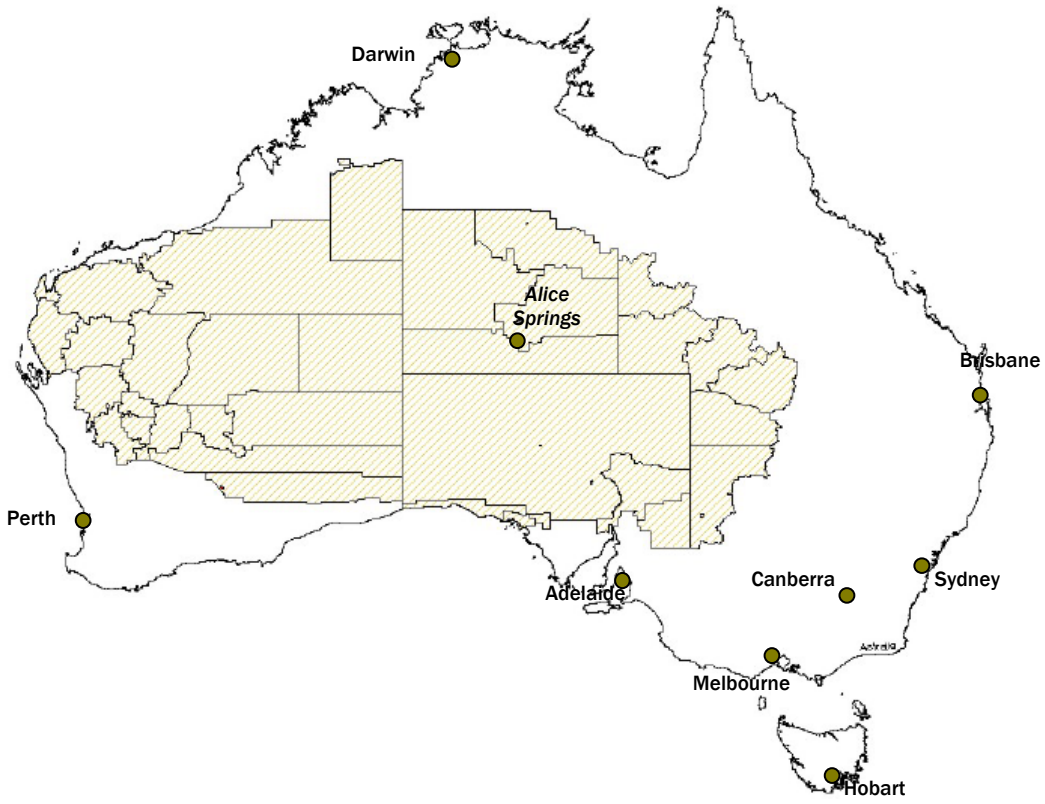


Fig. 2 Australia's arid region. After Guenther *et al*. (2005), based on data from the Australian Bureau of Statistics (2000)

(3) *Solanum centrale*

Despite the fact that this species is considered a staple food, next to no research has been published. Almost every reference to this species in the literature is as part of larger studies or surveys which were completed predominantly over the past two decades.

(*a*) Taxonomy

Solanum centrale J. M. Black was identified in early literature as *S. nemophilum* F. v. Mueller (Cleland & Johnston, 1939). Black (1934) originally described the species from a specimen found on Macdonald Downs Station in 1932. He correctly identified the Aboriginal name as ‘akitjura’, commenting that they are eaten “dry or mashed up in water”. Because of the large number of species in the Solanaceae family and the Solanum genus, the taxonomy is split into several sub-groupings within standard nomenclature (Table 3). Some Aboriginal names for *S. centrale* are given in Table 4.

Table 3 Taxonomy of *Solanum centrale*

Family	**Solanaceae**
Subfamily	*Solanoideae*
Tribe	*Solaneae*
Genus	*Solanum*
Subgenus	*Leptostemonum*
Section	*Oliganthes*
Group*	*S. macoori*
Species	*centrale*

*= The ‘group’ was assigned by Whalen (1984) and is not a formal taxonomic reference but a name which classifies similar species.

Table 4 Aboriginal names for *Solanum centrale*

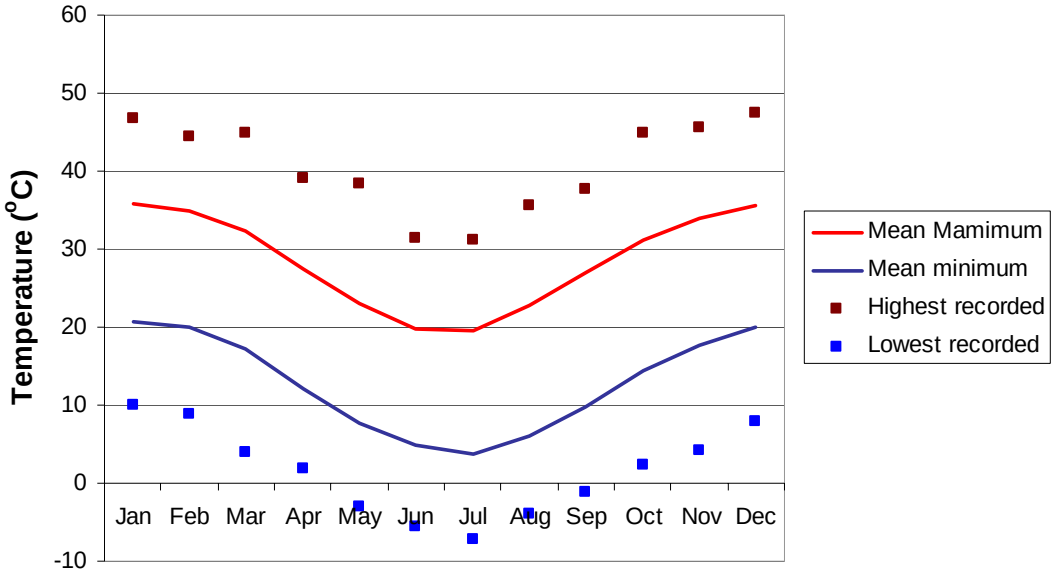
People group	**Names for *S. centrale***	**Source**
Alyawarra	akatjira or akatyerr	(Smith & Smith, 1999)
Anangu	kampurarpa or kati.kati	(Smith & Smith, 1999)
Anmatyerr	akatyerr or katyerr	(Women from Laramba (Napperby) community, 2003)
Arrernte	akatyerre, katyerre	(Smith & Smith, 1999), (Latz, 1999)
Kaytetye	arlkerre	(Latz, 1999)
Pintupi	kampurarrpa or kanytjilyi	(Latz, 1999)
Pitjantjatjara	akudjura or akatjira	(Smith & Smith, 1999)
Warlpiri	yakajirri	(Smith & Smith, 1999)

(*b*) Distribution

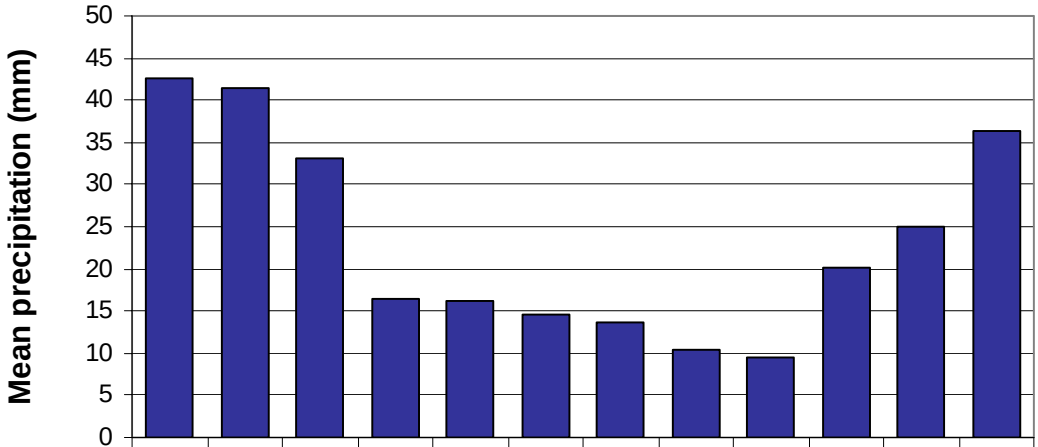
S. centrale is found on the sandy plains and dunes of arid Australia (Fig. 3). Within its distribution the rainfall is highly variable but typically less than 250mm per year (Fig. 4). Winter night temperatures often produce frosts, and summer temperatures can reach over 45^{o}C (Bureau of Meteorology, 2006). The species prefers light to medium textured soils away from escarpments and gibber plains. These are generally the spinifex sand plains, dune fields and adjacent mulga areas (Latz, 1999).

Fig. 3 Distribution of *S. centrale*. After (Purdie, Symon & Haegi, 1982)

A



B



	Jan	Feb	Mar	Apr	May	Jun	Jul	Aug	Sep	Oct	Nov	Dec
Maximum	313.5	235.8	362.9	117.1	108.7	81.8	244.8	158	89.7	115.5	138.8	288.2
Mean	42.7	41.4	33.1	16.4	16.1	14.6	13.6	10.4	9.4	20.1	25.1	36.3
Minimum	0	0	0	0	0	0	0	0	0	0	0	0

Fig. 4 Temperatures and rainfall patterns for Alice Springs, as measured at the Post Office. (A) Mean daily maximum and mean daily minimum temperatures and the highest and lowest temperatures recorded. (B) Mean monthly precipitation, with data table which indicates the maximum and minimum recorded monthly precipitation. Both graphs illustrate the high climatic variability and extremes of temperature and precipitation which can be experienced in the desert. After BOM (2006).

(*c*) Description

(i) Habit and ecology

Solanum centrale is a herbaceous perennial shrub, 15cm to 45cm high. There is a large amount of phenotypic variability within the species and Aboriginals identify a difference between dune and interdune forms (M. Ryder, personal communication). Patches of 15 to 50 plants were found by Miers (2004) on Pantharrpilenhe in a survey of native foods, and he noted larger stands can be found. The species thrives on disturbed sites such as grader tracks and sites with a regular firing – in fact the general vigour of the community appears to be stimulated by fire (Symon, 1981).

(ii) Vegetative growth and Inflorescence

All parts are pubescent with an often rusty tomentum of stellate hairs. Leaves are ovate-oblong and pale green (Symon, 1981). The species possesses several features which are conducive to survival in the arid zone, including dense hairs on leaves and stems and often spines which protect from consumption by marsupials (Symon, 1986) (Fig. 5).

The inflorescence is a cyme or pseudo-raceme containing typical Solanum flowers, with a purple or mauve corolla and yellow attenuate-oblong anthers. Symon (1981) describes the style as approximately 8mm long with a bent stigma; however short, erect styles have been observed on some flowers (M. Ryder, personal communication). Short styles apparently occur in *Solanum* sect. *Oliganthes* (Whalen & Costich, 1986).

It is possible this species is andromonoecious (has both bisexual and male/ female-sterile flowers on the same plant), or exhibits varying degrees of andromonoecy depending on the environmental conditions. All members of the *S. macoorai* group (to which *S. centrale* belongs) are apparently andromonoecious (Whalen, 1984). The ratio of hermaphroditic to female-sterile flowers in andromonoecious *Solanum spp*. depends on genotype and possibly environmental conditions (Whalen & Costich, 1986). These authors list sect. *Oliganthes* as self-compatible however several species in the *Leptostemonum* are self-incompatible, which is considered the ancestral condition (Whalen & Anderson, 1981).

Fig. 5 Drawing of *Solanum centrale* shoots, flower and fruit. Drawn from a pot grown plant which was collected at Musgrave Park Station, northern SA. Scale: x 2/3. After Symon (1981).

(iii) Fruit set

Fruiting occurs during warmer months of the year whenever the plant receives sufficient rain. Pollination is usually by hover flies or native bees (Symon, 1981). The fruit are small berries 1-2cm diameter which ripen from green to yellow then naturally dry on the bush (Symon, 1979) (Fig. 6). Fruit mature over two to three months and are available from November to the start of winter. Miers (2004) found an average of 8-10 fruit per plant in his survey, which was conducted in a year of low rainfall and low winter temperatures. An average of 54 seeds (ranging from 17 to 94) were found in *S. centrale* fruits (Symon, 1987).

Older plants produce less flowers and fruit (Miers, 2004). Therefore fire is an essential part not only for production of food but the reproduction of the species in natural systems. The fruit are dispersed by mammals and birds, including emus (Symon, 1979).

Fig. 6 Dried desert raisin fruits. After Cribb, Latham & Ryder (2005)

(iv) Root systems

The root systems of Australia's *Solanum spp*. are poorly understood. Unlike its relative the potato (*S. tuberosum*), no tuberous-rooted Solanums have been described (Symon, 1982). Several authors note that *S. centrale* propagates clonally by structures often called suckers or rhizomes (Anderson & Symon, 1989; Hele, 2001; Maconochie, 1982; Symon, 1981). Clonal communities can occupy a large area, particularly in disturbed sites such as grader tracks, roadsides and after fire (Miers, 2004). Peter Latz, quoted in Symon (1981) noted that the underground structures of many Central Australian *Solanum spp*. allow rapid regeneration and fruit set within four months of a fire in favourable conditions.

The longevity of aerial stems of *S. centrale* is unknown. Severe frosts will kill shoots of *S. centrale* in the winter months. It appears that over time the shoots will lose vigour and after a few years following a disturbance there may be no shoots remaining (Bean, 2004; Latz, 1999).

(*d*) Uses

(i) Indigenous uses

Historically *S. centrale* was one of the staple foods for Aboriginals living in Central Australia, including in drought periods (Latz, 1999). Fruits dry on the bush and are available from July/August to the following Autumn, or can be stored for long periods. Traditionally they are eaten fresh, dried on sticks or ground and made into balls or a cake (Low, 1988). The whole dried fruit can be eaten, unlike many other *Solanum spp*. (such as *S. chippendalei*) which require the removal of the bitter and poisonous seeds and/or juices (Smith & Smith, 1999).

(iv) Industry uses

The native food industry markets *S. centrale* as the 'bush tomato'. The name of the species is a critical part of marketing and must be consistent across the industry (Econsult (Australia), 1996). However, because several species of *Solanum* were consumed in Central Australia, the name 'bush tomato' is confusing to locals as it could apply to any of the species. Therefore in this review, *S. centrale* is referred to as the desert raisin, its local common name.

Demand for desert raisins has risen over the past decade. In 1995/96, approximately five tonnes of raw produce were used by processors (Graham & Hart, 1998). Recent estimates state as much as 8-10 tonnes are traded annually (Robins & Ryder, 2004). In 1997 there were approximately 12 000 commercial plantings of *S. centrale* (Graham & Hart, 1997).

The native food industry is promoting the use of 'akudjura' (the ground form) as a spice, in foods containing normal tomatoes (such as pasta, casseroles, salad dressings, soups, pizza and quiches), as an additive to cheeses and as a key component in chutneys, sauces and salsa. The taste is described as piquant, mildly hot and somewhat similar to a sun-dried tomato (Smith & Smith, 1999). Unprocessed dried fruits have limited appeal in the current Australian market because of this intense taste.

According to an analysis by Brand-Miller, James & Maggiore (1993), *Solanum centrale* is relatively high in vitamin C, with 19mg in 100g of fresh fruit on average or 17mg in 100g of dried fruit. The energy content of the dried fruit is relatively high with an estimated 1174kJ per 100g of edible material: this is comparable to pitted dates which have approximately 1200kJ per 100g. Fresh fruit is given as 570kJ per 100g on average, with an average water content of 61.9%, however the energy contents corresponded to the water content of the sample (higher water content reduced the energy value). Because fruit dries on the bush it is difficult to obtain objective measurements of both fresh and dried fruit. The average nutritional value of dried fruit is given in Table 5. In an earlier study of only one sample, 606 kJ/100g of fruit with a water content of 61.2% was observed (Brand *et al*., 1983).

The species has also been identified as a possible nursery plant because of its attractive foliage (Miers, 2004). However before cultivars can be released considerable breeding is required to produce lines without spines and a low potential to become a weed in irrigated gardens due to clonal reproduction.

Table 5 Average constituents in 100g of dried *S. centrale* fruits. After (Brand Miller, James & Maggiore, 1993).

Water g	**Energy kJ**	**Protein g**	**Fat g**	**Carbohydrate g**	**Dietary Fibre g**	**Ash g**	**Thiamin mg**	**Vitamin C mg**
12.5	1174	8.5	3.8	67.3	23.4	5.0	0.85	17

(*e*) Growth and Production

(i) Germination

In the wild, desert raisins germinate from seed produced on plants which develop following fire and/or sufficient rain. These seeds have extremely low germination (typically less than 5%) (Ahmed 06). It is uncertain whether this low germination is due to environmental conditions, genetics or dormancy factors. In native stands this strategy preserves the seed bank and assists species survival in the

erratic Central Australian conditions, but it is also one of the main obstacles to commercial cultivation (Johnson & Ahmed, 2003).

Recent experiments have achieved up to 95% ± 2.9 germination, however this varied greatly depending on the batch of seed (Ahmed *et al*., 2006). Miers (2004) obtained 68-78% germination in 15-30 days using fire. Similarly, Smith & Smith (1999) have had no definite results from use of smoke or heat on a barbeque. All these experiments indicate a combination of scarification, soaking, leaching and smoke will probably achieve the highest possible germination.

In experiments on kangaroo apples (*Solanum aviculare* and *S. laciniatum*), fluctuating temperatures and light provided the highest germination percentage, suggesting that seed germinates best when exposed to conditions on the soil surface (Porter & Clark, 1979). The same two species also had greater than 80% and 90% germination respectively when treated with Gibberellic acid. In another study, *S. laciniatum* germinated best when soaked in Gibberellic acid for six days or KNO_3 for nine days (Sudiatso & Wilson, 1974). Therefore the effect of light, temperature fluctuations and Gibberellic acid on *S. centrale* should also be investigated.

(ii) Agronomy

For a potential arid zone crop very little is known about the behavior of the species under cultivation. It appears desert raisins require warm, dry conditions and lighter textured, well drained soils (Hele, 2001) (Fig. 7). In a series of trials documented by Ryder and Latham (2005), desert raisins only became well established after the first season in the warmer, drier sites. The failure of the crop at one site (Lyrup in SA, near the Murray River on the NSW boarder) was attributed to an unspecified root disease. This is similar to observations of commercial cultivators who have noted damping-off in some crops (Hele, 2001; Miers, 2004). Under cultivation the best yields are generally obtained once the crop becomes established as a perennial, although suggestions have been made of growing this crop as an annual to allow complete mechanical harvesting (Hele, 2001). Estimates of fruit production from a mature crop range between 0.7t/ha to approximately 4t/ha (Graham & Hart, 1998; Hele, 2001; Robins & Ryder, 2004).

To facilitate the warm, dry conditions required by bush tomatoes, use of mounds to increase temperature and facilitate drainage have proven successful (Ryder & Latham, 2005). Further research into the best growing conditions are essential for commercial cultivators.

Fig. 7 Cultivated *Solanum centrale* shrub. After (Cribb, Latham & Ryder, 2005)

IV. ROOT SYSTEMS

(1) Introduction

The root system is comprised of the set of ‘descending axies which grow in the opposite direction from the stem’ (Jackson, 1900) plus any other underground parts of the plant. This includes primary and secondary roots, storage organs and plant reproductive or perennating structures. The primary role of root systems is to obtain water and nutrients in addition to providing stability and in some cases organs for regrowth and reproduction.

The root system is closely associated with the soil flora and fauna. The concentration of microbes in the rhizosphere is far greater than the bulk soil. This has been attributed to the priming effect of root exudates (Kuzyakov, 2002). The soil biota can be both detrimental and beneficial to plant health and often the stability and diversity within the soil ecosystem will determine the stability and diversity of plant communities (Klironomos, 2002).

The below ground structures of a plant are designed to support growth in a certain set of environmental conditions. Soil profiles vary in texture, pH, water-holding capacity, salinity, aeration and nutrient availability and exist under varied rainfall, sunlight and temperatures. The sizes and shape of root systems tend to vary predictably along climatic gradients, coinciding with the amount, variability and seasonality of rainfall (Schenk & Jackson, 2002). Furthermore, factors within plant microenvironments such as competition and microbes further influence an individual plant’s root morphology. These influences combine with genetic differences between plant species to form the diverse physiology of root systems observed in natural ecosystems.

(2) Anatomy and morphology of root systems in arid regions

(*a*) Introduction

The root systems of plants adapted to arid regions possess a range of unique features conducive to survival and reproduction in hot, dry conditions and generally poor soils. Because of the lack of crop species sourced from the arid zone and difficulties in sampling, very little literature has been published on the anatomy and physiology of arid zone root systems.

Plants possess two strategies to overcome seasons of extreme conditions – tolerance or avoidance. The desert ephemerals are the most extreme example of avoidance, completing their lifecycle in a few months following favourable conditions. On the other extreme some species tolerate hot dry conditions using a combination of anatomical and physiological features, such as thick cuticle and water-storing parenchyma (Knox *et al*., 2003). Many species use a combination of strategies including 'ephemeral roots' which respond to rainfall such as the desert succulent *Ferocactus acanthodes* (Nobel, 2002); or 'ephemeral shoots' which die back in unfavourable conditions such as the deciduous desert bush *Calligonum comosum* of North Africa (Kassas & Girgis, 1970). Some species can change their lifecycle in response to arid conditions – for example the perennial *Zilla spinosa* may grow like an annual; *Acacia tortilis* has a maximum height anywhere between 1m and 7m (Kassas & Girgis, 1970).

(*b*) Acquisition of resources

(i) Water

Water acquisition is one of the greatest challenges to arid zone species. Some root systems respond extremely rapidly to water: for example, *Agave desertii* produced a new root 6mm long only five hours after watering (Nobel, 1988). Such a rapid response presumably occurs from already existing cells in root primordia (Nobel, 2002). Other roots seek permanent water – models based on literature reviewed by Schenk & Jackson (2002) found that trees and shrubs tend to have rooting depths greater than 2m in water limiting environments. For semi-shrubs (such as the desert *Solanum spp*.) root systems tended to be shallower and wider in arid than humid climates (Schenk & Jackson, 2002).

Other plants have sophisticated methods of water preservation. At a site receiving 250-300mm annual rainfall, *Artemisia tridentata* was found to redistribute water downwards through the root system (Ryel *et al*., 2004). This slowed the immediate uptake of water from the surface layers however created uniformity of soil water, preserving moisture at depth for later use.

Most plants will not produce roots in the top few centimeters of soil to avoid not just high temperatures but high diurnal fluctuations. The soil temperature in the top few centimetres of an unshaded soil surface can exceed 70^{o}C (Nobel, 2002).

(ii) Nutrients

Plants growing in nutrient-poor soils have a number of adaptive responses to increase the supply of nutrients. These include increased proportion of roots to shoots, altered metabolism, forming mycorrhizal or rhizobial associations and altered root morphology (Miller, 2005). For example in certain nutrient conditions the Proteaceae form cluster or proteoid roots (Lamont, 2003) (Fig. 8). Some members of the Cyperaceae form carrot-shapped dauciform roots (Shane, Dixon & Lambers, 2005). However Winkworth (1966) noted that the soils of Australia's spinifex sandplains were only deficient in nitrogen, phosphorus and possibly sulfur for the growth of oats and sorghum.

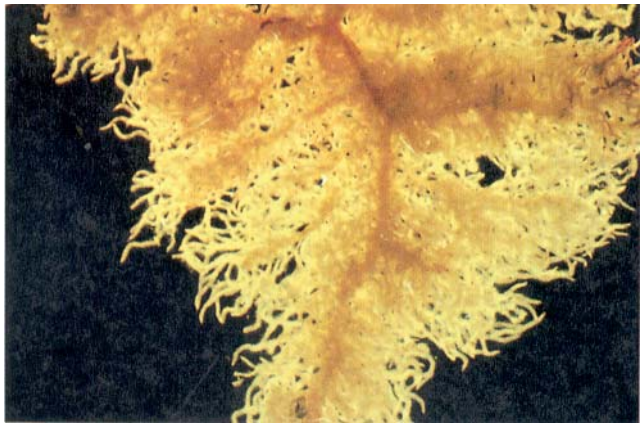


Fig. 8 Proteoid roots of *Banksia sp*. The high density of root tissue around each main branch is caused by proliferation of lateral rootlets. After Knox *et al*. (2003)

(*c*) Reproduction and survival

Perennating root systems are an effective strategy for plants to survive fire and drought and exhibit a fast response to favourable conditions. According to Maconochie (1982), reproduction from adventitious buds on the root systems has been observed in 18 of the 77 plant families found in Central Australia and 87% of these species are found in the sand dune/sand plain system. The author notes that on heavier soils regeneration from seed is adequate to maintain plant populations.

Many authors note that little mention is made of the root sprouting phenomenon on herbarium specimens or in other literature (Nord, Christensen & Plummer, 1969; Symon, 1981). Nevertheless the ability to form clonal communities is well noted amongst herbarium curators (A. R. Bean, personal communication; B. Lepschi, personal communication; D. E. Symon, personal communication). According to Symon (1981), most of the shrubby Australian *Solanum spp*. exhibit this form of survival and reproduction, forming extensive underground root systems which connect plants in a community.

Often underground reproductive structures are loosely referred to as rhizomes in the literature without evidence their anatomy has been properly documented. A rhizome is officially defined as:

> *"the rootstock or dorsiventral stem, of root-like appearance, prostrate on or underground, sending off rootlets, the apex progressively sending up stems or leaves."* (Jackson, 1900)

By not adhering to the accepted definition the term rhizome creates confusion in the literature between casual observations and detailed root morphology studies.

Winkworth (1966) notes that many of the plant species in spinifex communities are either ephemeral or perennate from rhizomes or roots, such as *Rulingia loxophylla* (Sterculiaceae). *Eremophila sturtii* (Myoporaceae) thrives in the semi-arid regions of Australia. It also has the ability to shoot from lateral roots 15-25cm below the surface once severed from the main plant and when soil erodes or is removed (Wiedemann & Kelly, 2001). Several *Atriplex spp*. (Chenopodiaceae) form clonal communities connected by woody lateral roots 15-30cm below the surface (Nord *et al*., 1969). A single *Atriplex gardneri* plant in loose soil was found to have 226 buds (of which 20 had emerged) along a total of 16.5m of lateral roots. The authors noted that this phenomenon was observed in irrigated nursery crops, dryland plantings and in native stands.

The ability to sprout from roots is well described for woody forest species such as *Populus tremuloides* and *Sassafras albidum* and in temperate climates is apparently more common in trees and biennials than shrubs (Klimesova & Klimes, 2003). Growth from adventitious buds on roots is typical of some plant families (e.g. Podostemataceae) or genera including *Euphorbia* (Euphorbiaceae) and *Rorippa* (Brassicaceae) (Klimesova & Martinkova, 2004). This ability to reproduce from underground laterals may confer weedy characteristics to the species, particularly if it is competitive in temperate cropping regions (North American Plant Protection Organisation, 2003a; North American Plant Protection Organisation, 2003b; Symon, 1981; Wiedemann & Kelly, 2001). This is exacerbated by the fact that root sprouting is often triggered by disturbance (including ploughing and fire) and high nutrient levels (Klimesova & Martinkova, 2004).

Several endangered species also possess the ability to sprout from roots. *Elliottia racemosa* (Ericaceae) or the Georgia Plume is an endangered shrub or small tree from Georgia in the USA. Agriculture, fire, deer browsing and logging have contributed to the clonal spread of this species with very low seed set (Godt & Hamrick, 1999). The rare *Eucalyptus curtisii* can also form clonal communities of several hundred square metres not from roots but from an underground lignotuber which expands radially and eventually decays in the centre (Smith, Hughes & Wardell-Johnson, 2003).

Several species from the monocotyledonous family Restionaceae also exhibit underground laterals (Meney, Pate & Dixon, 1990). This contrasts with reports than the phenomenon is unknown in monocolyledons (Klimesova & Martinkova, 2004). The root sprouting species have thicker and deeper rhizomes and a higher concentration of carbohydrates in the rhizomes than species from the same family which survive predominantly by reseeding following fire (Pate, Meney & Dixon, 1991). High fire frequency tends to favour species which can resprout after a disturbance. Long intervals between firing decrease vigour and cause more intense and deadly fires (Bell, 2001).

Several species in the genus *Solanum* have underground reproductive structures - the most recognised are undoubtedly the tubers of the potato (*S. tuberosum*). *S. carolinense* and *S. elaeagnifolium* both propagate clonally from underground laterals (Department of Agriculture/Weights and Measures, 2006; North American Plant Protection Organisation, 2003b) (Fig. 9). Thus the phenomenon observed in arid *Solanum spp*. has been documented in both species found in the same environment and species which are taxonomically related.

Fig. 9 Clonal reproduction from lateral roots of *Solanum elaeagnifolium.* After Department of Agriculture/Weights and Measures (2006).

V. MYCORRHIZAL ASSOCIATIONS

(1) Introduction

Mycorrhizal associations are highly complex, mutualistic relationships between vascular plants and certain members of the fungal kingdom. The relationship is generally specialised, specific and symbiotic between hosts and fungi and confers benefits at both the plant and ecosystem level (Peterson, Massicotte & Melville, 2004).

Several types of mycorrhizal associations are observed in natural systems. The most common are Arbuscular Mycorrhizas (AM, also referred to as Vesicular-Arbuscular Mycorrhizae), Ectomycorrhizas (and the related ectendo-, arbutoid and monotropid associations), Ericoid Mycorrhizas and Orchid Mycorrhizas. Mycorrhizas occur in about 80% of land plants (Brundrett *et al*., 1996).

(2) Arbuscular Mycorrhizas

(*a*) Description

Arbuscular Mycorrhizas (AM) are found in more than 75% of plant species in habitats from the tundra to the tropics (Peterson *et al*., 2004; Schussler, Schwarzott & Walker, 2001). Fungus species that form AM are all from the newly reclassified phylum Glomeromycota (Schussler *et al*., 2001). AM are characterised by the presence of arbuscules (highly branched haustoria which invaginate plasma membranes of cortical cells of the root – Fig. 10), intraradical hyphae in plant roots (intercellular or intracellular) and extraradical mycelium with spores (Peterson *et al*., 2004). Arbuscules are presumably the site for nutrient exchange however coils may also be a site of nutrient transfer, having as large a surface area as arbuscules

(Jakobsen, Smith & Smith, 2002). Although not always present, many AM fungi possess terminal or intercalary lipid-filled bodies termed ‘vesicles’ either within roots or on the extraradical hyphae (Peterson *et al*., 2004).

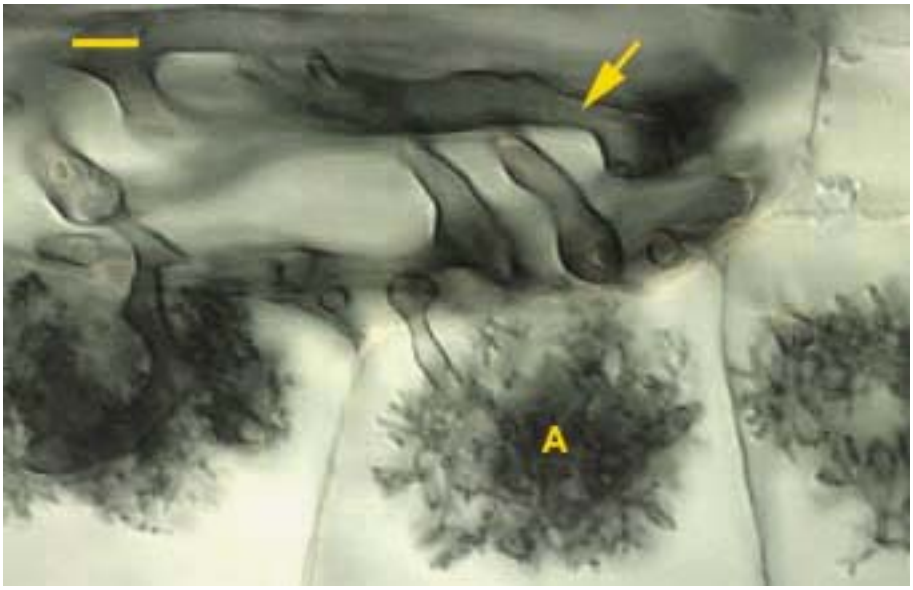


Fig. 10 Mycorrhizas in the inner cortex of a root of *Asarum canadense*, showing arbuscules (A) and hyphae (arrow). In this species arbuscules only form in the cortical cells adjacent to the endodermis. Bar = 10μm. After(1999).

Generally, AM fungi show little host specificity with plants. However fungi require more specific soil conditions than the host, in particular being more susceptible to soil pH (Brundrett *et al*., 1996). Also in some experiments a preference for fungal species has been observed (Kubota, McGonigle & Hyakumachi, 2005). The specificity of host-fungal relationships is still being investigated.

Two morphological forms of AM have been described: Paris- and Arum-type. Paris-type morphology is characterized by hyphal coils which grow intracellularly. The Arum-type consists of intercellular hyphae which grow longitudinally through the root and produce arbuscules on typically right-angled branches. These forms can co-exist on the same root system as in tomato and cucumber (Kubota *et al*., 2005) and the same fungal species can form either morphology in different host species (Brundrett *et al*., 1996).

Colonisation begins when root exudates from young and growing roots are recognized by an AM fungus, resulting in hyphae growing towards the root. Appressoria form at the join between adjacent epidermal cells and hyphae then develop an intercellular and intracellular network within the outer cortex. Arbuscules then form within cortical cells and vesicles may also develop to accumulate storage products as hyphae elongate through the root. Vesicles remain viable after arbuscules senesce. After a few weeks the first arbuscules start to senesce and cross walls develop in the older hyphae sealing off senescent parts from remaining viable hyphae (Brundrett *et al*., 1996).

The soil hyphal network can remain intact even after the host plant is removed, thus remaining useful for subsequent plants of the same or other species (Brundrett *et al*., 1996). The perennial mycelium is of considerable importance in native and minimum tillage cropping systems where the soil hyphal networks are not destroyed by cultivation each season. Glomalean fungi produce networks of thinner 'absorbative' hyphae supported by thicker 'runner' hyphae used for distribution of nutrients. Runner hyphae range from 5μm to 20μm thick, whilst absorbative hyphae are generally less than 2μm in diameter (Brundrett *et al.*, 1996). Up to 3m of hyphae may grow from each centimeter of root (Knox *et al*., 2003), and tens of metres of hyphae can grow in a gram of soil (e.g. Tisdall & Oades, 1979, Rillig, Wright & Eviner, 2002).

Some families are considered to be non-mycorrhizal such as the Proteaceae, Brassicaceae and Juncaceae (Tester, Smith & Smith, 1987). However new observations are regularly recorded – for example mycorrhizal associations have recently been found in *Hakea verrucosa* (Proteaceae) (Boulet & Lambers, 2005). Pattinson & McGee (2004) found mycorrhizas in *Banksia aricifolia* (Proteaceae) however this association did not benefit the plant. O'Connor, Smith & Smith (2001) postulate that the absence of colonisation is not sufficient evidence to conclude a species is non-mycorrhizal. Colonisation or formation of arbuscules may be ephemeral, highly dependant on environmental conditions such as rainfall, or only make up a small percentage of total root length. Also, spore numbers vary between seasons and years, partially due to extremes of wet and dry conditions in soil (Uhlmann *et al*., 2006).

Dependence on mycorrhizas differs between species and cultivars (Ryan & Graham, 2002). Plants exist somewhere along a continuum between absolute reliance on AM (obligate) to never associated (non-mycorrhizal). In a given set of environmental conditions for a species, the mycorrhizal dependency and marginal benefit of associations depends largely upon plant root anatomy, physiology and phenology. Species with a large root surface area, particularly extensive fibrous root systems, are less likely to depend on mycorrhizas for nutrition. Similarly, long-lived roots, which often have lignified or suberised layers, are generally better at withstanding pathogen attack and desiccation but are less able to absorb nutrients and may depend more heavily upon mycorrhizal associations (Brundrett, 1999). Furthermore the mycorrhizal dependency of plants differs between annuals and perennials. In observations by Collier, Yarnes & Herman (2003) in the Chihuahuan Desert, perennials had a much greater level of colonisation within the roots - 70% in perennials compared to 6% in annuals.

(*b*) Advantages and disadvantages of arbuscular mycorrhizas

Arbuscular mycorrhizas have an impact on both plants and ecosystems. The most prominent benefit is increased uptake of mineral nutrients (particularly phosphorus) by the plant. This is achieved through increased surface area of absorptive surfaces which are in contact with the soil and access by hyphae into smaller pores (Handreck, 1997). Furthermore, acquisition of nutrients via hyphae is more efficient in terms of carbon compared to the equivalent length of root (Jakobsen *et al*., 2002). At the ecosystem level, AM may alter the species composition and rate of succession in communities (Read, 1991).

In conditions of low water availability, AM can also increase the water uptake of the plant. Hyphae (diameter of 2-5μm) access smaller micropores in soil than do root hairs (10-20μm). Several additional mechanisms which help alleviate drought stress have been reported in mycorrhizal plants including maintaining gas exchange at lower leaf water potential and release of antioxidant enzymes to combat oxidants in drought-stressed plants (Ruiz-Lozano, 2003). In a study on *Glomus intraradices* the external mycelium slightly improved soil structure and increased absolute permanent

wilting point of a soil to about -4MPa, meaning slightly more water was available for plant growth (Augé *et al*., 2001). The reasons for increased plant response to dry conditions are complex. In a study on tomatoes, Subramanian, Santhanakrishnan & Balasubramanian (2006) concluded that the enhanced resistance to drought stress was due to the enhanced nutrient uptake in the mycorrhizal plants as opposed to non-mycorrhizal plants. Mycorrhizas had an increasing marginal benefit on height and fruit production with decreasing irrigation.

Many studies have shown enhanced disease resistance by mycorrhizal plants, including Solanaceous species such as tomatoes (Cordier *et al*., 1998; Fritz *et al*., 2006; García-Garrido & Ocampo, 1988; García-Garrido & Ocampo, 1989). In the case of *Phytophthora parasitica*, this is due to the induction of systemic defenses such as cell wall thickening around intercellular pathogen hyphae and callose-rich encasements where pathogens are entering cortical cells. Sections of root infected with mycorrhizal hyphae have a further localized resistance in the form of phenolics and cell wall appositions (Cordier *et al*., 1998). AM also contribute indirectly to plant defense by increasing plant vigour (García-Garrido & Ocampo, 1989). This increased tolerance of stressful conditions such as drought, salinity, high temperatures, poor soils and pH extremes. The overall effects of AM on disease are relatively minor (Ryan & Graham, 2002).

AM provide benefits to natural ecosystems. Fungal mycelia improve soil structure and thus air and water relations as they grow in and around soil particles. They have also been implicated in sequestration of heavy metals, more rapid succession on disturbed sites (Peterson *et al*., 2004) and in increasing plant species diversity (van der Heijden, 2002).

Because AM fungi are obligate biotrophs the plant is essential for their survival, even when there is no benefit to the plant. Plants provide photosynthate which sustains fungal growth (Jakobsen *et al*., 2002). Because many plants are able to support a wide variety of mycorrhizal fungi, they increase the fungal diversity in soil.

The net benefit of AM associations may change over time. If the mycorrhizal association does not increase carbon fixation by more than the organic energy removed the fungus has a detrimental effect on the plant. This may happen in low light situations and where soil nutrients are in adequate supply (Jakobsen *et al*., 2002; Ryan & Graham, 2002). Whether or not a plant can prevent fungal invasion of its roots differs between species (Jakobsen *et al*., 2002).

(*c*) Arid zone mycorrhizal associations

There is relatively little research on mycorrhizal associations in deserts. Australia's arid zone is characterized by high temperatures, low rainfall and slightly alkaline sandy soils which are very low in organic matter and nutrients – conditions which seem likely for a beneficial mycorrhizal association. The presence of AM in arid regions may be associated with increased nutrient storage given the rapid growth response required by plants when conditions become favourable.

Mycorrhizas are found throughout Australia's deserts. O'Connor, Smith & Smith (2001) found 73% or 38 out of 52 species from three sites in the Simpson desert were mycorrhizal. This survey included eight species from four families which were considered largely non-mycorrhizal. In a global study comparing the distribution of mycorrhizal fungi between deserts and temperate grasslands, Treseder & Cross (2006) found a similar number of plant species and a similar percentage of the root lengths were colonized by mycorrhizas in both regions.

Several studies have indicated that a high proportion of species in sandy soils are mycorrhizal e.g. Jacobson (1997); O'Connor *et al*. (2001); Uhlmann *et al*. (2006). Tarafdar & Praveen-Kumar (1996) found that mycorrhizal inoculation of fields in an arid environment improved shoot biomass and uptake of some nutrients in a tree, grass and crop species (*Prosopis juliflora*, *Cenchrus ciliaris* and *Vigna aconitifolia* respectively). In addition to providing plants with the nutrients they need in conditions with sporadic rainfall and poor soil nutrition, AM fungi play a major role in soil stabilisation (Rillig *et al*., 2002).

Several desert species exhibit unusual AM morphologies. In a glasshouse study of the saltbush *Atriplex nummularia* (Chenopodiaceae) arbuscules were absent but the plants still exhibited a significant growth response to AM (Plenchette & Duponnois, 2005). A weak colonisation without arbuscules was observed by Pattinson & McGee (2004) on *Banksia ericifolia*. Similarly, O'Connor *et al*. (2001) found internal hyphae and coils but not arbuscules or vesicles in *Solanum ellipticum*, but internal hyphae and arbuscules and sometimes coils and vesicles in *Nicotiana velutina* - another Solanaceous desert species.

(*d*) Mycorrhizal Associations in the *Solanum spp*.

The family Solanaceae contains many mycorrhizal members. Tomato plants are weakly mycorrhizal and exhibit a weak response to phosphorus fertiliser (Bolan, 1991). Disease suppression of the soil borne pathogens *Phytophthora parasitica, Erwinia carotovora* and *Pseudomonas syringae* by the presence of mycorrhizal associations in tomato has been reported by several authors (Cordier *et al*., 1998; García-Garrido & Ocampo, 1988; García-Garrido & Ocampo, 1989). Suppression of the symptoms of the foliar fungal pathogen *Alternaria* was also observed in tomato (Fritz *et al*., 2006).

The percentage of tomato roots colonized by the fungus *Glomus mosseae* was given as 56% and 57% by García-Garrido & Ocampo (1988) and García-Garrido & Ocampo (1989) respectively, and up to 58% by Cordier *et al*. (1998). Similar results were observed for intracellular hyphae of *G. intraradices* (Smith, Smith & Jakobsen, 2004). Only 19% colonisation was observed on tomato plants grown in field-collected soil by Kubota, McGonigle & Hyakumachi (2005). Between 45 and 50% was observed on inoculated tomatoes after a month by Subramanian *et al*. (2006), however control plants which received maximum irrigation always had 5% less colonisation.

Although the mycorrhizal status of *Solanum centrale* has not been published, hyphae and coils (but not arbuscules or vesicles) were observed in the roots of *S. ellipticum*, another edible desert Solanum, with less than 30% colonisation (O'Connor *et al*., 2001). *S. nigrum* and *S. surattense* are both considered mycorrhizal, and the family Solanaceae is considered usually mycorrhizal in arid environments (Trappe, 1981). However, *Solanum* is a weakly mycorrhizal genus (Boerner, 1992) and early successional plants are generally non-mycorrhizal (Pankow, Boller & Wiemken, 1991). Thus it is difficult to speculate on the mycorrhizal status of *S. centrale*.

VI. CONCLUSION

There is considerable scope to expand on the patchy research on *Solanum centrale* – indeed, most Australian desert species. This research project will contribute substantial information in a variety of areas, including anatomy and morphology of underground parts, vegetative reproduction, storage products, fertiliser and mycorrhizal response, plus smaller observations which will add to the published information in areas from germination to flowering. Specifically, this project will investigate three hypothesies. Firstly, that *S. centrale* forms clonal communities connected by underground structures; secondly that Rhizomes connect shoots of *S. centrale* and finally that Arbuscular mycorrhiza improve plant growth and development.

This information has application in both botany and agronomy and will assist individuals and groups looking to grow the desert raisin for income. Very little research has been conducted on the behavior of desert foods under cultivation (Bunt *et al*., 2004; Miers, 2004). By providing background knowledge on the desert raisin this project will assist Indigenous Communities in creating a lifestyle more closely associated with their traditional lands. Thus this project has not only scientific but also social and economic implications. It is part of a strategy within the Australian native food industry to incorporate traditional knowledge and practices with scientific investigation to develop a sustainable industry.

VII. REFERENCES

AHMED, A. K., JOHNSON, K. A., BURCHETT, M. D. & KENNY, B. J. (2006). The effects of heat, smoke, leaching, scarification, temperature and NaCl salinity on the germination of Solanum centrale (the Australian bush tomato). *Seed Science and Technology* **34,** 33-45.

ANDERSON, G. J. & SYMON, D. E. (1989). Functional dioecy and andromonoecy in Solanum. *Evolution* **43,** 204-219.

ATECH GROUP & TOTAL EARTH CARE. (1999). Improving access to bushfood production and marketing information. Rural Industries Research and Development Corporation, Canberra.

AUGÉ, R. M., STODOLA, A. J. W., TIMS, J. E. & SAXTON, A. M. (2001). Moisture retention properties of a mycorrhizal soil. *Plant and Soil* **230,** 87-97.

AUSTRALIAN BUREAU OF STATISTICS. (2000). Australian Standard Geographical Classification (ASGC), 2000, Canberra.

AUSTRALIAN BUREAU OF STATISTICS. (2002). Housing and infrastructure in Aboriginal and Torres Strait Islander Communities - 2001, Canberra.

AUSTRALIAN BUREAU OF STATISTICS. (2005). 2003-04 Value of agricultural commodities produced, Australia, Canberra.

BEAN, A. R. (2004). The taxonomy and ecology of Solanum subg. Leptostemonum (Dunal) Bitter (Solanaceae) in Queensland and far north-eastern New South Wales, Australia. *Austrobaileya* **6,** 639-816.

BELL, D. T. (2001). Ecological response syndromes in the flora of southwestern Western Australia: fire resprouters versus reseeders. *The Botanical Review* **67,** 417-440.

BLACK, J. M. (1934). Additions to the flora of South Australia. *Transactions and Proceedings of the Royal Society of South Australia* **58,** 168-186.

BOERNER, R. E. J. (1992). Plant life span and response to inoculation with vesicular-arbuscular mycorrhizal fungi II. Species from weakly mycotrophic genera. *Mycorrhiza* **1,** 163-167.

BOLAN, N. S. (1991). A Critical-Review on the Role of Mycorrhizal Fungi in the Uptake of Phosphorus by Plants. *Plant and Soil* **134,** 189-207.

BOTKIN, D. B. & KELLER, E. A. (2003). *Environmental science : earth as a living planet*, 4th edition. Wiley, New York.

BOULET, F. M. & LAMBERS, H. (2005). Characterisation of arbuscular mycorrhizal fungi colonisation in cluster roots of Hakea verrucosa F. Muell (Proteaceae), and its effect on growth and nutrient acquisition in ultramafic soil. *Plant and Soil* **269,** 357-367.

BRAND, J. C. (1989). Australian Indigenous Foods - the Commercial Potential. *Food Australia* **41,** 600-602.

BRAND, J. C., RAE, C., MCDONNELL, J., LEE, A., CHERIKOFF, V. & TRUSWELL, A. S. (1983). The nutritional composition of Australian Aboriginal bushfoods: 1. *Food Technology in Australia* **35,** 293-298.

BRAND MILLER, J., JAMES, K. W. & MAGGIORE, P. M. A. (1993). *Tables of composition of Australian Aboriginal foods*. Aboriginal Studies Press, Canberra, ACT.

BRUNDRETT, M. (1999). Section 3: Arbucular Mycorrhizas. In *Mycorrhizas in Forestry and Agriculture*. CSIRO Forestry and Forestry Products, Adelaide.

BRUNDRETT, M., BOUGHER, N., DELL, B., GROVE, T. & MALAJCZUK, N. (1996). *Working with Mycorrhizas in Forestry and Agriculture*. Australian Centre for International Agricultural Research, Canberra.

BUNT, C., TAYLOR, C., LEAKEY, R., VIEIRA, T. & OLDHAM, S. (2004). Proceedings - Indigenous Bush Tucker Industry Summit. Cairns Region Economic Development Corporation, Cairns, Queensland.

BUREAU OF METEOROLOGY. (2006). Climate averages for Alice Springs Post Office, Available from <http://www.bom.gov.au/climate/averages/tables/cw_015540.shtml>.
CARNEGIE, D. W. (1898). *Spinifex and Sand: a narrative of five years' pioneering and exploration in Western Australia.* C. Arthur Pearson, London.
CHERIKOFF, V. (2000). Marketing the Australian native food industry. Rural Industries Research and Development Corporation, Canberra.
CLELAND, J. B. & JOHNSTON, T. H. (1939). Aboriginal names and uses of plants at the Granites, Central Australia. *Transactions and Proceedings of the Royal Society of South Australia* **63,** 22-26.
COLLIER, S. C., YARNES, C. T. & HERMAN, P. R. (2003). Mycorrhizal dependency of Chihuahuan Desert plants is influenced by life history strategy and root morphology. *Journal of Arid Environments* **55,** 223-229.
CORDIER, C., POZO, M. J., BAREA, J. M., GIANINAZZI, S. & GIANINAZZI-PEARSON, V. (1998). Cell defense responses associated with localised and systemic resistance to Phytophthora parasitica induced in tomato by an arbuscular mycorrhizal fungus. *Molecular Plant-Microbe Interactions* **10,** 1017-1028.
COURTNEY, P. (2003). Macadamias: Australia's bushfood success, vol. 2006. Landline, 24/8/2003.
CRIBB, J., LATHAM, Y. & RYDER, M. (2005). Desert Delicious: Indigenous Australian foods for the global palate. *A Journal of the Built and Natural Environment* **16**.
DEPARTMENT OF AGRICULTURE/WEIGHTS AND MEASURES. (2006). White Horsenettle. In *Pests under eradication*. County of San Bernardino, San Bernardino, USA.
DEPARTMENT OF NATURAL RESOURCES AND ENVIRONMENT. (2002). Native food. In *Agriculture Notes*, vol. 2006 (ed. Department of Natural Resources and Environment). Farm Diversification Information Service, Bendigo, VIC.
ECONSULT (AUSTRALIA). (1996). Business and Marketing Paper. In *Culture of the Land; Cuisine of the People* (ed. Australian Native Bushfood Industry Committee). Rural Industries Research and Development Corporation, Canberra.
EVERARD, P., EVERARD, M., MURIKA, TJAMPU, T., KANYTJI, PUMANI, S. & MILATJARI. (2002). *Punu: Yankunytjatjara Plant Use*, 2 edition. IAD Press, Alice Springs.
FOSTER, M., JAHAN, N. & SMITH, P. (2005). Emerging animal and plant industries - their value to Australia. Rural Industries Research and Development Corporation, Canberra.
FRITZ, M., JAKOBSEN, I., LYNGKJAER, M. F., THORNDAL-CHRISTENSEN, H. & PONS-KUHNEMANN, J. (2006). Arbuscular mycorrhiza reduces susceptiility of tomato to Alternaria solani. *Mycorrhiza***,** Published online.
GARCÍA-GARRIDO, J. M. & OCAMPO, J. A. (1988). Interaction between Glomus mosseae and Erwinia carotovora and its effects on the growth of tomato plants. *New Phytologist* **110,** 551-555.
GARCÍA-GARRIDO, J. M. & OCAMPO, J. A. (1989). Effect of VA mycorrhizal infection of tomato on damage caused by Pseudomonas syringae. *Soil Biology and Biochemistry* **21,** 165-167.
GILMORE, M. C. (1934). *Old days: old ways*. Angus and Robertson, Sydney.
GODT, M. J. W. & HAMRICK, J. L. (1999). Population genetic analysis of Elliottia racemosa (Ericaceae), a rare Georgia shrub. *Molecular Ecology* **8,** 75-82.
GOTT, B. (2005). Aboriginal fire management in south-eastern Australia: aims and frequency. *Journal of Biogeography* **32,** 1203-1208.
GRAHAM, C. & HART, D. (1997). Prospects for the Australian native food industry. Rural Industries Research and Development Corporation, Canberra.
GRAHAM, C. & HART, D. (1998). Bushfoods. In *The new rural industries: a handbook for farmers and investors* (ed. K. Hyde). Rural Industries Reseasrch and Development Corporation, Canberra.

GROSSER, S. (2003). Bush Tucker Fruit Salad. In *SA Country Hour*. ABC, South Australia. Aired 23rd March, 2003.
GUENTHER, J., YOUNG, M., BOYLE, A., SCHABER, E. & RICHARDSON, J. (2005). Growing the desert: regional and educational profiles of the Australian desert and its Indigenous peoples - Stage 1 Report. National Centre for Vocational Education Research, Adelaide.
HANDRECK, K. A. (1997). Phosphorus requirements of Australian native plants. *Australian Journal of Soil Research* **35,** 241-289.
HEGARTY, M. P., HEGARTY, E. E. & WILLS, R. B. H. (2001). Food safety of Australian plant bushfoods. Rural Industries Research and Development Corporation, Canberra.
HELE, A. (2001). Bush Tomato/Desert Raisin Production. In *Fact Sheets*, vol. No. 8/03. Primary Industries and Resources SA, Adelaide.
HIDDINS, L. (2001). *Bush tucker field guide*. Penguin, Ringwood, Vic.
INTERNATIONAL PANEL ON CLIMATE CHANGE. (2001). *Climate change 2001: the scientific basis*. Cambridge University Press, Cambridge.
ISAACS, J. (1987). *Bush food : Aboriginal food and herbal medicine*. Weldons, McMahons Point, N.S.W.
JACKSON, B. D. (1900). *Glossary of botanic terms*. Duckworth & Co., London.
JAKOBSEN, I., SMITH, S. E. & SMITH, F. A. (2002). Function and diversity of Arbuscular Mycorrhizae in carbon and mineral nutrition. In *Mycorrhizal Ecology*, vol. 157. *Ecological Studies* (ed. M. G. A. van der Heijden and I. Sanders), pp. 75-92. Springer-Verlag Berlin Heidelberg, Berlin.
JOHNSON, K. A. & AHMED, A. K. (2003). Investigations into in vitro manipulation of Solanum centrale. *Acta Horticulturae* **616,** 169-175.
KASSAS, M. & GIRGIS, W. A. (1970). Habitat and plant communities in the Egyptian Desert: VII. geographical facies of plant communities. *Journal of Ecology* **58,** 335-350.
KLIMESOVA, J. & KLIMES, L. (2003). Resprouting of herbs in disturbed habitats: is it adequately described by Bellingham-Sparrow's model? *Oikos* **103,** 225-229.
KLIMESOVA, J. & MARTINKOVA, J. (2004). Intermediate growth forms as a model for the study of plant clonality functioning: an example with root sprouters. *Evolutionary Ecology* **18,** 669-681.
KLIRONOMOS, J. N. (2002). Feedback with soil biota contributes to plant rarity and invasiveness in communities. *Nature* **417,** 67-70.
KNOX, B., LAIDGES, P., EVANS, B. & SAINT, R. (2003). *Biology*, 3rd edition. McGraw-Hill, Sydney.
KUBOTA, M., MCGONIGLE, T. P. & HYAKUMACHI, M. (2005). Co-occurrence of Arum- and Paris-type morphologies of arbuscular mycorrhizae in cucumber and tomato. *Mycorrhiza* **15,** 73-77.
KUZYAKOV, Y. (2002). Review: Factors affecting rhizosphere priming effects. *Journal of Plant Nutrition and Soil Science - Zeitschrift Fur Pflanzenernahrung Und Bodenkunde* **165,** 382-396.
LAMONT, B. B. (2003). Structure, ecology and physiology of root clusters - a review. *Plant and Soil* **248,** 1-19.
LATZ, P. K. (1999). *Pocket bushtucker*. IAD Press, Alice Springs, N.T.
LOW, T. (1988). *Wild food plants of Australia*. Angus & Robertson, Sydney.
MACONOCHIE, J. R. (1982). Regeneration of arid zone plants: a floristic survey. In *Evolution of the flora and fauna of arid Australia* (ed. W. R. Barker and P. J. M. Greenslade), pp. 141-144. Peacock Publications, Frewville, SA.
MENEY, K. A., PATE, J. S. & DIXON, K. W. (1990). Comparative Morphology, Anatomy, Phenology and Reproductive Biology of Alexgeorgea Spp. (Restionaceae) from South-Western Western Australia Australia. *Australian Journal of Botany* **38,** 523-542.

MIERS, G. (2004). Cultivation and sustainable wild harvest of bushfoods by Aboriginal communities in Central Australia. Rural Industries Research and Development Corporation, Canberra.
MILLER, R. M. (2005). The nonmycorrhizal root - a strategy for survival in nutrient-impoverished soils. *New Phytologist* **165,** 655-658.
NOBEL, P. S. (1988). *Environmental biology of agaves and cacti*. Cambridge University Press, New York.
NOBEL, P. S. (2002). Ecophysiology of roots of desert plants, with special emphasis on Agaves and Cacti. In *Plant roots : the hidden half* (ed. Y. Waisel, A. Eshel and U. Kafkafi). M. Dekker, New York.
NORD, E. C., CHRISTENSEN, D. R. & PLUMMER, A. P. (1969). Atriplex species [or Taxa] that spread by root sprouts, stem layers and by seed. *Ecology* **50,** 324-326.
NORTH AMERICAN PLANT PROTECTION ORGANISATION. (2003a). Euphorbia esula L. complex. In *NAPPO Pest Fact Sheets*, vol. 2006. NAPPO.
NORTH AMERICAN PLANT PROTECTION ORGANISATION. (2003b). Solanum carolinense L. In *NAPPO Pest Fact Sheets*, vol. 2006. NAPPO.
O'CONNOR, P. J., SMITH, S. E. & SMITH, F. A. (2001). Arbuscular mycorrhizal associations in the southern Simpson Desert. *Australian Journal of Botany* **49,** 493-499.
PANKOW, W., BOLLER, T. & WIEMKEN, A. (1991). The significance of mycorrhizas for protective ecosystems. *Experientia* **47,** 391-394.
PATE, J. S., MENEY, K. A. & DIXON, K. W. (1991). Contrasting growth and morphological characteristics of fire-sensitive (obligate seeder) and fire-resistant (resprouter) species of Restionaceae (S. Hemisphere Restiads) from south-western Western Australia. *Australian Journal of Botany* **39,** 505-25.
PATTINSON, G. S. & MCGEE, P. A. (2004). Influence of colonisation by an arbuscular mycorrhizal fungus on the growth of seedlings of Banksia aricifolia (Proteaceae). *Mycorrhiza* **14,** 119-125.
PETERSON, L. R., MASSICOTTE, H. B. & MELVILLE, L. H. (2004). *Mycorrhizas: Anatomy and Cell Biology*. NRC Research Press, Ottawa, Canada.
PETERSON, N. (1979). Aboriginal uses of Australian Solanaceae. In *The Biology and taxonomy of the Solanaceae*, vol. 7. *Linnean Society Symposium Series* (ed. J. G. Hawkes, R. N. Lester and A. D. Skelding), pp. 171-189. Academic Press, London.
PHELPS, D. (1995). Australia's Bush Food Industry - a potential for sustained economic and ecological development in the rangelands. *Range Management Newsletter* **95,** 5-6.
PHELPS, D. G. (1997). Bush food industry in Western Queensland. Rural Industries Research and Development Corporation, Canberra.
PLENCHETTE, C. & DUPONNOIS, R. (2005). Growth response of the saltbush Atriplex nummularia L. to inoculation with the arbuscular mycorrhizal fungus Glomus intraradices. *Journal of Arid Environments* **61,** 535-540.
PORTER, N. G. & CLARK, S. M. (1979). Effect of temperature and light on the germination of seed of Solanum aviculare and S. laciniatum. *New Zealand Journal of Experimental Agriculture* **7,** 307-310.
PURDIE, R. W., SYMON, D. E. & HAEGI, L. (1982). Solanaceae. In *Flora of Australia*, vol. 29 (ed. R. Robertson, B. G. Briggs, H. Eichler, L. Pedley, J. H. Ross, D. E. Symon, P. G. Wilson, A. McCusker and A. S. George). Australian Government Publishing Service, Canberra.
READ, D. J. (1991). Mycorrhizas in ecosystems. *Experientia* **47,** 376-391.
RILLIG, M. C., WRIGHT, S. F. & EVINER, V. T. (2002). The role of arbuscular mycorrhizal fungi and glomalin in soil aggregation: comparing effects of five plant species. *Plant and Soil* **238,** 325-333.

ROBINS, J. (2004). Native Foods - Overview. In *The New Crop Industries Handbook*, vol. Publication No 04/125 (ed. S. Salvin, M. Bourke and T. Byrne), pp. 338-345. Rural Industries Research and Development Corporation, Canberra.

ROBINS, J. & RYDER, M. (2004). Native Foods - Bush Tomato. In *The New Crop Industries Handbook*, vol. Publication No 04/125 (ed. S. Salvin, M. Bourke and T. Byrne), pp. 346-352. Rural Industries Research and Development Corporation, Canberra.

RUIZ-LOZANO, J. M. (2003). Arbuscular mycorrhizal symbiosis and alleviation of osmotic stress. New perspectives for molecular studies. *Mycorrhiza* **13,** 309-317.

RYAN, M. H. & GRAHAM, J. H. (2002). Is there a role for arbuscular mycorrhizal fungi in production agriculture? *Plant and Soil* **244,** 263-271.

RYDER, M. & LATHAM, Y. (2005). Cultivation of native food plants in south-eastern Australia. Rural Industries Research and Development Corporation, Canberra.

RYEL, R. J., LEFFLER, A. J., PEEK, M. S., IVANS, C. Y. & CALDWELL, M. M. (2004). Water conservation in Artemisia tridentata through redistribution of precipitation. *Oecologia* **141,** 335-345.

SCHENK, H. J. & JACKSON, R. B. (2002). Rooting depths, lateral root spreads and below-ground/above-ground allometries of plants in water-limited ecosystems. *Journal of Ecology* **90,** 480-494.

SCHUSSLER, A., SCHWARZOTT, D. & WALKER, C. (2001). A new fungal phylum, the Glomeromycota: phylogeny and evolution. *Mycological Research* **105,** 1413-1421.

SHANE, M. W., DIXON, K. W. & LAMBERS, H. (2005). The occurrence of dauciform roots amongst Western Australian reeds, rushes and sedges, and the impact of phosphorus supply on dauciform-root development in Schoenus unispiculatus (Cyperaceae). *New Phytologist* **165,** 887-898.

SMITH, K. & SMITH, I. (1999). *Grow your own bushfoods*. New Holland, Frenchs Forest, N.S.W.

SMITH, M. (2006). Australian deserts, deserts past - the archaeology and environmental history of the Australian deserts. In *Year book Australia, 2006* (ed. D. Trewin). Australian Bureau of Statistics.

SMITH, S., HUGHES, J. & WARDELL-JOHNSON, G. (2003). High population differentiation and extensive clonality in a rare mallee eucalypt: Eucalyptus curtisii - Conservation genetics of a rare mallee eucalypt. *Conservation Genetics* **4,** 289-300.

SMITH, S. E., SMITH, F. A. & JAKOBSEN, I. (2004). Functional diversity in arbuscular mycorrhizal (AM) symbioses: the contribution of the mycorrhizal P uptake pathway is not correlated with mycorrhizal responses in growth or total P uptake. *New Phytologist* **162,** 511-524.

STYNES, B. (1997). Opportunities for contributing to the development of Aboriginal food plants. *Tropical Grasslands* **31,** 311-314.

SUBRAMANIAN, K. S., SANTHANAKRISHNAN, P. & BALASUBRAMANIAN, P. (2006). Responses of field grown tomato plants to arbuscular mycorrhizal fungal colonization under varying intensities of drought stress. *Scientia Horticulturae* **107,** 245-253.

SUDIATSO, I. S. & WILSON, D. R. (1974). Seed germiantion of Solanum laciniatum Ait. *New Zealand Journal of Agricultural Research* **17,** 455-458.

SYMON, D. (1986). A survey of Solanum prickles and marsupial herbivory in Australia. *Annals of the Missouri Botanical Garden* **73,** 745-754.

SYMON, D. E. (1979). Fruit Diversity and Dispersal in Solanum in Australia. *Journal of the Adelaide Botanic Gardens* **1,** 321-332.

SYMON, D. E. (1981). A revision of the genus Solanum in Australia. *Journal of the Adelaide Botanic Gardens* **4,** 1-367.

SYMON, D. E. (1982). Solanum (Solanaceae) in Arid Australia. In *Evolution of the Flora and Fauna of Arid Australia* (ed. W. R. Barker and P. J. M. Greenslade), pp. 335-339. Peacock Publications, Frewville, SA.

Part 2 – Research paper

Underground structures and mycorrhizal associations of *Solanum centrale* (the Australian bush tomato)

Angela Dennett
Faculty of Agriculture, Food and Natural Resources
University of Sydney

Supervisors:
Prof. Lester Burgess
Dr Peter McGee
Dr Maarten Ryder

Contents

List of tables

List of figures

Introduction

The unique flora of Australia's arid interior must adapted to extreme climate, poor nutrition and regular fire. Plants posses a number of mechanisms which allow them to survive this desert environment, including avoiding drought by rapidly completing their life cycle, forming thick cuticle or water-storing parenchyma, or the development of perennating storage organs which can rapidly resprout given favourable conditions (Maconochie 1982; Knox *et al.* 2003). Among these desert species are food plants which have supported the Indigenous tribes of Central Australia for thousands of years (Everard *et al.* 2002).

Solanum centrale J. M. Black, known as the bush tomato or desert raisin, is an Australian desert food plant in the Solanaceae family (Table 1). This family contains many important crop species including potatoes (*S. tuberosum*), tomatoes (*Lycoperscicon esculentum*) and eggplant (*S. melongena*). *S. centrale* is a perennial subshrub up to 45cm in height which thrives on disturbed sites throughout Central Australia (Symon 1981). The shoots are well adapted to desert life, possessing a dense coving of stellate hairs to reduce water loss and often spines to protect from herbivory (Symon 1981; Symon 1986). *S. centrale* berries are produced within 4 months of rain and dry naturally on the bush (Isaacs 1987). Because they produced fruit for most of the year and could be found in many habitats, this species was a staple food source for Aboriginal people (Symon 1981; Latz 1999).

Table 1 Taxonomy of *Solanum centrale* J. M. Black.

Taxonomic rank	Taxon
Family	Solanaceae
Subfamily	*Solanoideae*
Tribe	*Solaneae*
Genus	*Solanum*
Subgenus	*Leptostemonum*
Section	*Oliganthes*
Group[A]	*S. macoori*
Species	*S. centrale*

[A]= The 'group' was assigned by Whalen (1984) and is not a formal taxonomic rank but a name which classifies similar species.

Little is known about the underground parts of *S. centrale*. However it has been previously reported that this species is clonal and can form communities of 50 plants or more in communities which stretch tens of metres (Miers 2004). The underground connections between shoots in these clonal communities have been called both suckers and rhizomes by various authors (Symon 1981; Maconochie 1982; Anderson and Symon 1989; Hele 2001) but without mention of their anatomy, morphology or physiology. Rhizomes are generally defined as underground diatropic shoots which form rootlets along the axis and progressively send up stems or leaves from the apex (Jackson 1900; Markarov and Golovko 1995; Knox *et al.* 2003). By this definition, a rhizome must possess stem anatomy and form both roots and shoots along its length.

The underground parts of various *Solanum* spp. from around the world include rhizomes, tubers and lateral roots. The root system of *S. tuberosum* is well documented, including its ability to store starch and resprout from perennating underground tubers (terminal swellings of lateral rhizomes) (Niemira *et al.* 1995). However no tuberous-rooted *Solanum* spp. have been described in Australia (Symon 1982). Several weedy *Solanum* spp. native to North America are able to produce shoots from perennating lateral roots, including *S. carolinense* and *S. elaeagnifolium* (Lemerle and Leys 1991; Miyazaki and Ito 2004; Onen *et al.* 2006). Symon (1981) reports that most of the shrubby Australian *Solanum* spp. also have this ability, noting that a root sprouting habit is rarely indicated on herbarium specimens. It is possible that the underground parts of *S. centrale* act as both a carbohydrate store and organs of vegetative reproduction – ideal features for perennial desert species.

Like the macromorphology of underground parts, the ability of *S. centrale* (and all desert species) to acquire minerals and water from fine roots must be well adapted to the extreme and unpredictable desert conditions. Mycorrhizal associations are one well-documented method of increasing mineral acquisition in both agricultural and native ecosystems. Mycorrhizas are symbiotic plant-fungi relationships with fungi from the phylum Glomeromycota (Schussler *et al.* 2001). The most common type are arbuscular mycorrhizas, where the fungal hyphae grow intercellularly or intracellularly in the root cortex and form arbuscules for nutrient exchange with the host (Peterson *et al.* 2004). Mycorrhizas are known to improve plant water and mineral acquisition, particularly

phosphorous. This is achieved by fungal hyphae which increase exploration of the soil for the host which in turn provides the photosynthates required for fungal growth (Handreck 1997; Jakobsen *et al.* 2002). Mycorrhizas can also alter plant morphology such as the root to shoot ratio (McArthur and Knowles 1993) and provide protection against disease (Cordier *et al.* 1998; Fusconi *et al.* 1999; Fritz *et al.* 2006).

Although arbuscular mycorrhizas have been reported in several desert species around the world, there is little information in the literature. A survey of 52 species in the Simpson Desert of Australia found mycorrhizas in the roots of 73% of those species, including *Solanum ellipticum*, a close relative of *S. centrale* (O'Connor *et al.* 2001). Several arid and semi-arid Australian native plants are considered non mycorrhizal, including members of the Proteaceae family (Tester *et al.* 1987). However new observations are regularly recorded – for example, Boulet and Lambers (2005) found mycorrhizas in the roots of *Hakea verrucosa*. Members of the Solanaceae family are considered to be usually mycorrhizal in arid environments (Trappe 1981).

Because of its obvious potential as a crop species, the native food industry is developing a strategy to domesticate *S. centrale* (Collins 2002). This involves combining traditional knowledge and practices, little of which is published, with scientific investigation to develop a sustainable industry and improve the livelihoods of Aboriginal people. This project will address the need for further research as part of this strategy and will contribute information on both the basic structure and function of this species and infer how it may behave under cultivation. Furthermore, this project provides insight into the nature of desert root systems with particular reference to the role of mycorrhizal associations.

The aim of this project is to document the anatomy and possible function of the below ground structures and mycorrhizal associations of *Solanum centrale*. From this broad aim, three hypotheses have been proposed - firstly, that *S. centrale* forms clonal communities connected by underground structures; secondly that rhizomes connect shoots of *S. centrale* and finally that arbuscular mycorrhizas occur in the fine roots and improve plant growth and development.

Materials and Methods

Field specimens

The nature of the underground parts of *S. centrale* was studied at six sites. These included both cultivated and naturally colonised sites which were visited in either summer 2005, winter 2006, or in both seasons.

December 2005

Three disturbed sites in the Northern Territory which were naturally colonised by *S. centrale* were visited in the summer of 2005. These were Pwerte Arntarntarenhe (24°20’12”S, 133°42’31”E), a wild stand on Napperby Station (22°32' S, 132°46' E) and a wild stand along Hatt Rd (23°48’58”S, 133°48’43”E). Pwerte Arntarntarenhe and HattRd were extremely dry, whereas Napperby had experienced a storm the night before sampling and was wet to approximately 10cm. Fires had occurred within the past 2 years at Napperby and HattRd. Further site descriptions are given in Appendix 1.

From each site approximately 1 m^2 of underground connections and fine roots were excavated. Samples of underground laterals were immediately wrapped in wet paper and fine roots were immediately preserved in 50% ethanol. All samples were stored at room temperature, except laterals which were stored at 10°C for up to 5 days. In addition to laterals and fine roots, one large plant from Napperby with many lateral roots was heavily pruned, removed from the ground and stored in the same manner as the laterals.

July 2006

Six colonies of *S. centrale* were studied at five locations. The HattRd site in the Northern Territory was revisited and a new 1 m^2 section of the same colony was excavated. Root samples were also taken from a colony of cultivated (but unfertilised) plants at Palmer in South Australia (34º52´8.90”S, 139º08´44.85”E) and a wild site at Aileron (22°38’00”S, 133°20’49”E). Two types of lateral root samples from these sites were taken – one wrapped in dry paper, the other in wet paper and sealed in a plastic bag. Fine roots from these three sites were immediately preserved in 50% ethanol.

Fine root samples were also taken from three further sites - two separate cultivated locations on Pwerte Arntarntarenhe about 50 m from the wild stand visited in December 2005, and in the cultivated bushfoods garden at the Alice Springs Desert Park (23°42'16.18", 133°50'22.93"). These two sites are regularly irrigated and fertilised.

Observation and analysis of lateral roots samples

Glasshouse resprouting and growth

Samples of underground laterals were planted in the glasshouse on return from the field. Laterals from December 2005 (Pwerte Arntarntarenhe and Hatt Rd) and the large plant from Napperby were sown into 1 x 0.4 m rectangular tubs filled with Amgrow® Pot n' Peat for Indoor Plants. Fragments from 10 cm to 20 cm long collected July 2006 (wrapped in both wet and dry paper) were planted in 25 cm pots in a mixture of approximately 3:2:2:2 seed raising mix: fine sand: course sand: Narrabri soil.

All plants were under lights from approximately 6am to 6pm and the temperature was maintained at 26°C or greater. Field material was watered to field capacity daily for the first month for material collected December 2005 but only for the first seven days for material collected July 2006. After this, watering was reduced to two or three times a week for both samples. Tubs were fertilised with approximately 1 g Hortico All Purpose Compound Fertiliser with trace elements® after 46 days then with Osmocote Exact Standard® (N:P:S = 15:4:7.5) after 6 months.

Seven seedlings were donated by the Alice Springs Desert Park. These were transplanted into Darlington potting mix in 25 cm pots in December 2005 and treated as laterals. To observe the ability of the plant to resprout following removal of the shoots (common by fire, herbivory or mechanical disturbance), and to support observations from field collected material, seedlings and resprouted laterals were pruned and disturbed in several ways. Two plants were pruned right back to their base, leaving a ball of woody stems 5-10 cm in diameter, and one additional plant had just the dead branches removed. One harshly pruned and the lightly pruned (dead branches only) plant were watered immediately. The other harshly pruned plant was left for a month without water.

Chemical analysis

Hand sections were stained for the presence of fats and oils, starch, lignin, reducing sugars and cellulose using the methods described in Berlyn and Miksche (1976). These are summarised in Table 2.

Table 2 Staining methods for determining carbon storage products in root material.

Stain	Recipe	Method
Fats and oils	0.5 g Sudan III in 100 mL water was stirred overnight then Filtered through Whatman no. 54 filter paper	Stain dropped onto specimen then observed after 20 min.
Starch	1 g KI and 1 g I_2 dissolved in 100mL water	Stain dropped onto specimen.
Reducing sugars	Solution A: 80 g $CuSO_4.5H_2O$ in 1L water Solution B: 346 g Sodium Potassium Tartrate and 100 g NaOH in 1 L water	Sample was finely chopped and mixed with equal volumes of A and B. Solution was then boiled and allowed to cool.
Lignin	0.1 g Phloroglucinol crystals and 16 mL conc. HCl in 100 mL of 95% ethanol	Stain dropped onto specimen.
Cellulose	Solution A: Starch stain as above Solution B: 75% H_2SO_4	Specimen was placed on a slide and stained with solution A then covered with a coverslip. While observing under a microscope, 1 drop solution B was added to side of coverslip. Location of further colour change indicated cellulose.

The Phosphorus content of lateral roots was measured by taking between 0.13 g and 0.16 g of plant material which had been dried in an oven at 60^{o}C. Samples were digested by boiling the sample in 9 mL of 69% nitric acid (AnalaR®) until the mixture weighed approximately 1 g then making the mixture up to 25 mL in nano-pure water. The solution was further diluted by taking 2.5 mL and making it up to 50 mL in nano-pure water then

a subsample was analysed using the FOSS TECATOR FIAstar^{tm} 5000. Detailed methods used by the instrument are provided by FOSS (2000). This instrument performs a spectrophotometric analysis for ortho-phosphate based on the reaction between Ammonium Molybdate and the sample. Colour was measured by the instrument at 720 nm and ortho-phosphate concentration was determined by the standard curve given in Appendix 1, Fig. A1.

Field mycorrhizal associations

Fine roots were preserved immediately after collection in the field in 50% ethanol. Samples were rehydrated by diluting the ethanol in four stages over 2 hours. Roots were then cleared and stained based on the method of Vierheilig *et al*. (2005). Roots were cleared in 10% KOH in a 65^{o}C waterbath for 90 to 120 min followed by rinsing in acidified water (10% vinegar in distilled water). Samples were stained in a solution containing 5% black ink in vinegar by heating in the 65^{o}C waterbath for 30 min. These samples were subsequently destained in acidified water for at least 30 minutes at room temperature then rinsed in distilled water and placed into lactoglycerol (50% lactic acid, 50% glycerol).

Percentage colonisation by mycorrhizal fungi was estimated according to the method of McGonigle *et al*. (1990). Roots were mounted on slides parallel to the long axis and observed microscopically. The slide was moved a fixed distance each time to give at least 100 different views for at least seven rows of roots. The presence of hyphae, vesicles and arbuscules was noted at the point where the vertical cross-hair intersected the root.

Effect of mycorrhizas on phosphorus response

The effect of mycorrhizas on plant growth was assessed in a replicated glasshouse experiment.

Germination

The seed used in the mycorrhizal and germination experiments was extracted from fruit collected from Napperby Station. Batches of 50 unwashed large seeds were soaked in 500 mg/L Gibberellic acid for 24 hours. Seeds which sank in the Gibberellic Acid solution were air dried then batches of 50 seeds were placed onto a bed of sterilized coarse river sand and ash in sealed plastic containers. The surface was sprayed with water then the sealed containers were placed in a germination cabinet at $9^{o}C/24^{o}C$ light/dark for 12 hours each. After 25 days of no germination, seeds were re-sprayed then placed back into the cabinet until the first seeds germinated, after which containers were removed. Rate of germination was subsequently recorded daily.

Mycorrhizal inoculum

Three mycorrhizal fungi were used: *Glomus intraradices* (NBR 8.7) isolated from ACRI, Narrabri; *Glomus mosseae* (NBR 4.1) from ACRI Narrabri; and *Glomus coronatum* (BUR 11) from a roadside at Burren Junction. Fungi were cultured on leek roots in either an autoclaved coarse/fine sand mix or autoclaved Narrabri soil/river sand mix. Pots were inoculated by placing a 1-2 cm ball of unwashed chopped leek roots approximately 3cm below the seedling. Non-mycorrhizal pots received about the same amount of Narrabri soil which was attached to leek roots in an equivalent position.

Glasshouse conditions

Plants were grown in sterilized 10 cm pots with drainage holes covered by paper towel and then filled with a mix of washed and autoclaved river sand containing 2:1 fine:coarse sand. Seeds which had germinated in the previous 3 days were transplanted at about 1 cm depth. Seedlings which died shortly after transplantation were replaced.

The experiment was conducted in the glasshouse at 26^{o}C and given artificial light from 6am to 6pm. The experimental design was blocked to account for possible gradients in light intensity.

Nutrients were provided in the form of a slightly modified Hoaglands solution (Hoagland and Arnon 1938) containing one of three levels of phosphorus (P) (Table 3). Pots were given equal amounts of nutrient solution once a week throughout the experiment, plus watered once a week for four weeks then twice a week thereafter.

All plants were harvested 71 days after the initial transplanting. Shoot height and number of leaves were recorded before soil was gently rinsed from the roots and, once dry, roots and shoots were weighed separately. Root subsamples ranging in weight from 0.004 g to 1.035 g (depending on the total weight) were placed into 50% ethanol before the shoots and the remainder of the roots were dried in a 70^{o}C oven. Desiccated roots and shoots were weighed separately.

Table 3 Modified Hoaglands solution given to each pot once a week during the mycorrhizal trial.

Nutrient	Concentration
KNO_3	1.011 g/L
$Ca(NO_3)_2.4H_2O$	0.7085 g/L
$MgSO_4.7H_2O$	0.4930 g/L
H_3BO_3	5.101 mg/L
$ZnCl_2.7H_2O$	7.1885 mg/L
$MnCl_2.H_2O$	4.225 mg/L
$CuSO_4.5H_2O$	0.6242 mg/L
$NH_4Mo_7O_{24}$	0.6179 mg/L
KH_2PO_4	
High P	2.042 g/L
Low P	1.021 g/L
Zero P	0 g/L

Mycorrhizal colonisation

Roots were cleared and stained according to Brundrett *et al*. (1994). Clearing occurred in 10% KOH for 6 days after which roots were stained in Trypan Blue by heating until boiling point on a hotplate for a few minutes. Stained samples were rinsed in acidified water then stored in lactoglycerol (1:1:1 water: glycerol: lactic acid).

Root length was estimated according to Brundrett *et al*. (1994). Subsamples were placed over a 1cm grid and the number of times a root crossed a grid line was counted under the dissecting microscope. Length in centimetres was estimated by the formula:

$$Length = \frac{\left(Intsersections \times \frac{11}{14} \right)}{SubsampleWeight} \times TotalWeight$$

Colonisation was estimated as described in 'Field mycorrhizal associations'; however percentage colonisation was based on approximately 50 random views.

Phosphorus analysis

The Phosphorus content of both roots as shoots was measured using the method described in 'Chemical analysis'. Subsamples of approximately 0.1 to 0.15 g of plant material (or all available material if required) were analysed. Whole plant phosphorus was calculated by adding the phosphorus contents of roots and shoots and dividing by whole plant weight.

Sample analysis was conducted over two days. Average absorbance (particularly for higher values) was greater on the second day than the first and thus reported concentrations on the second day are expected to be higher than the first. The second day contained all root samples and only a few shoot samples as well as seven repeat shoot samples measured on the first day to estimate this effect. Shoot samples measured only on the second day (pots 84 to 105) were adjusted according to this effect, estimated as:

$$EquivalentDayOneResult = -0.0003 DayTwoResult^2 + 1.4235 DayTwoResult$$

This equation was forced to pass through the origin and had a high regression sum of squares (R^2=0.9979). Root samples were not adjusted.

Statistical analysis

Several plants died late in the experiment and thus treatments were unequally replicated. Variables analysed were plant biomass (root, shoot and whole plant); height; Phosphorus content (roots, shoots and whole plant average); dry weight (root, shoot and whole plant); root to shoot ratio (dry weights); leaves per mm height; root length; root length to weight ratio; plant height to shoot dry weight ratio and the number of leaves (as measured 4 days before harvest and at harvest). All stated analyses were performed in GenStat ver.8.1 with a 5% level of significance and treatments were compared using the 5% least significant difference (lsd).

Continuous variables were analysed using Residual Maximum Likelihood (REML), usually with a log transformation. Number of leaves were analysed using log-linear modelling. Details of transformations and error structures for individual variates are given in Appendix 2. Treatment averages for all log-transformed variables are reported as geometric means.

Two root phosphorus readings (from samples 44 and 45) had suspected contamination and were excluded. Sample 82 was excluded from whole plant phosphorus analysis due to suspected incorrect dilution. Root sample 102 had a negative phosphorus reading and thus was removed prior to log-transformation.

Results

Root macromorphology

Field observations

Clonal communities of *S. centrale* shoots were observed at all field sites. These patches were greater than 50m in diameter at Hatt Rd and Napperby. Each excavated shoot was connected to at least one other shoot nearby by a woody lateral root 2-10 mm in diameter which grew predominantly horizontally at 5-15 cm depth (Fig. 1). In all locations at least one lateral root was found to bend downwards until the end was growing vertically down into the soil. At Pwerte Arntarntarenhe one vertical root travelled to 70 cm depth then abruptly stopped and grew horizontally back into the soil profile.

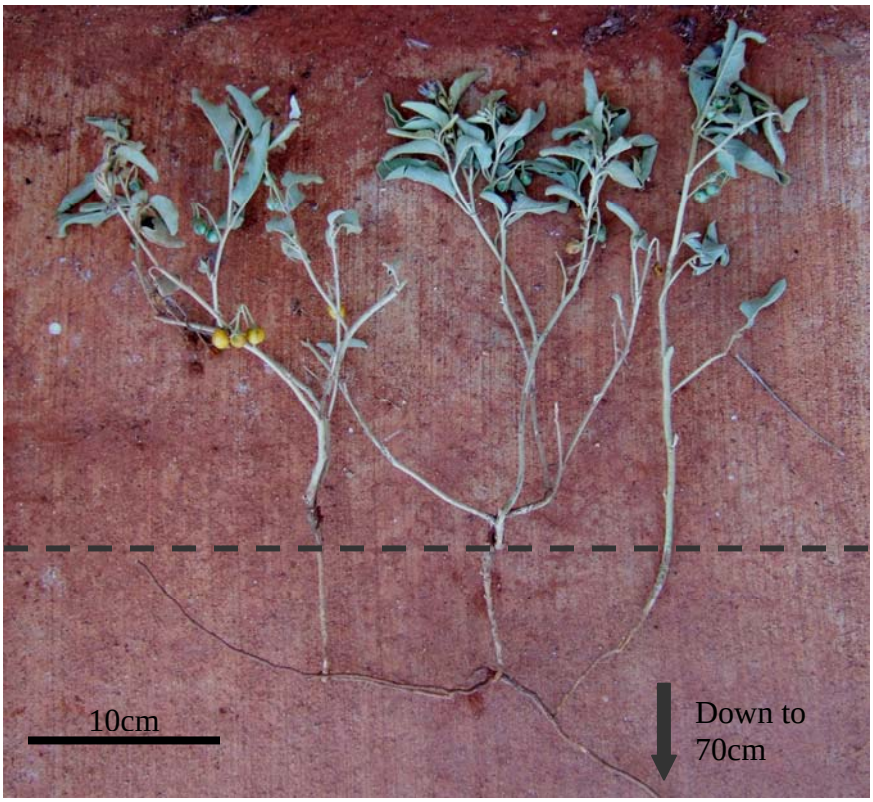


Fig. 1. A small portion of excavated shoots and roots of *Solanum centrale* from Pwerte Arntarntarenhe. Dotted line indicates original soil surface.

Six types of junction in the woody lateral and vertical roots were observed:

1. Shoot with several laterals emerging from the root system in multiple directions and at least one thick vertical (tap) root, possibly originating from seed (Fig. 2a);
2. Shoot with only two laterals in a straight line, plus one thick vertical root (Fig. 2b);
3. Shoot growing from a straight lateral with no vertical root (a 'T junction') (Fig. 2c);
4. Lateral which bends to vertical and grows down into the soil profile (Fig. 2d);
5. Branching in a lateral, either off to the side or downwards (Fig. 2e); or
6. Abrupt change in direction of a lateral or vertical, probably due to a rock or other impenetrable soil obstacle (Fig. 2f).

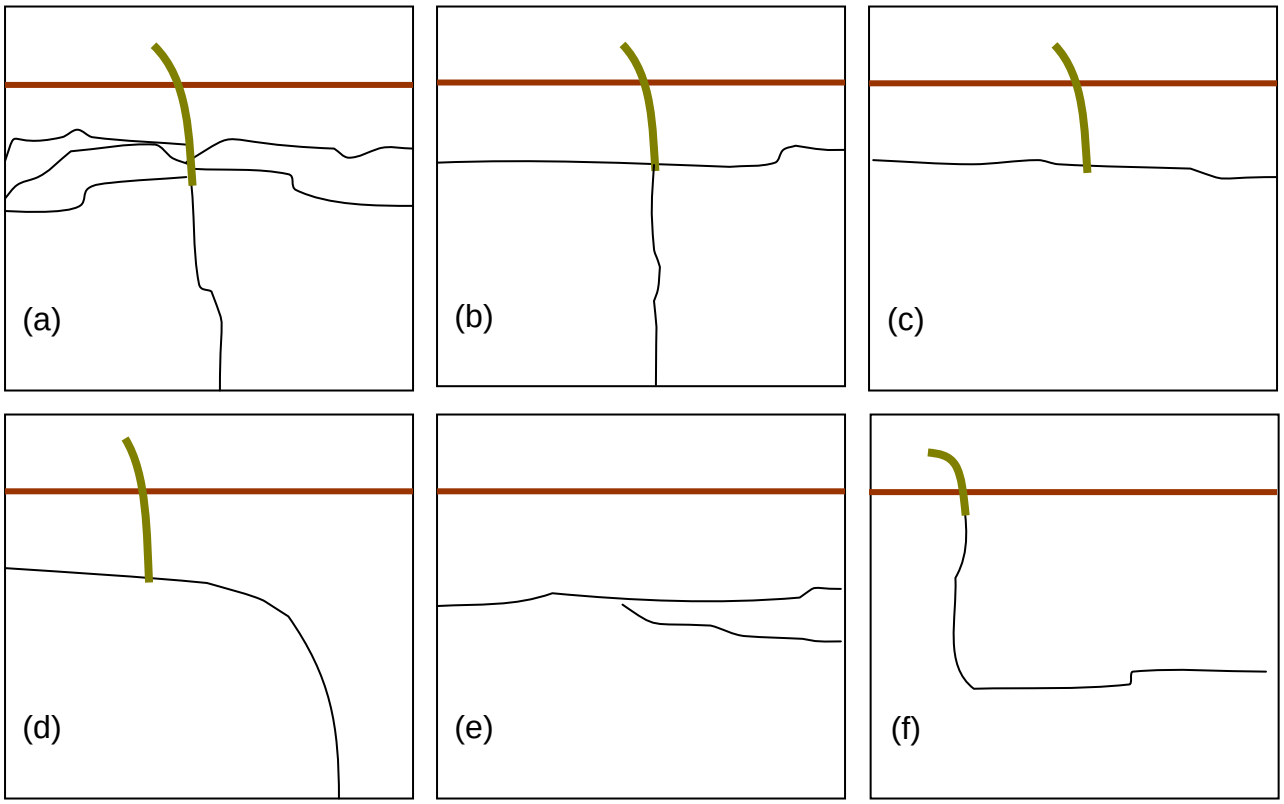


Fig. 2. The six types of root macromorphology observed in the field: (a) Multiple laterals emerging from a taprooted seed plant; (b) New shoot with tap root; (c) New shoot as T-junction; (d) Diving lateral; (e) Branching lateral; (f) Suspected soil obstacle

The connections within an excavated cultivated *S. centrale* community from Palmer are illustrated in Fig. 3. The original plant had produced several woody laterals from the thick taproot between 5 and 15 cm below the surface. This plant exhibited many fine secondary roots growing both on the taproot and as long fine roots which branched into feeder rootlets.

In just three years, this plant produced a colony about 4 m in diameter despite rainfall and irrigation amounting to only about 400 mm/annum. New shoots tended to emerge in clusters, suggesting bursts of resprouting from a continuously expanding root zone. The irregular distances from neighbouring shoots suggest laterals continue to grow underground without shoot emergence until sprouting is triggered.

The number of shoots in a colony appeared to increase with rainfall/irrigation. The low rainfall HattRd and Pwerte Arntarntarenhe wild sites exhibited fewer shoots per square metre than Palmer (an unfertilised but higher rainfall site). The highest shoot density was observed at the Desert Park and Pwerte Arntarntarenhe bushfood gardens, where the canopy was closed due to the high number of shoots and large healthy plants. However it is uncertain whether all shoots in a colony are interconnected and therefore whether the entire colony is comprised of clones.

Fine (secondary) roots were sparsely located along these laterals and were extremely brittle and fine, particularly at the drier HattRd and the naturally colonised Pwerte Arntarntarenhe sites. A greater frequency of fine roots was observed at Palmer and in the cultivated garden bed on Pwerte Arntarntarenhe than at the wild sites. In addition, more fine roots were observed on the tap root of the parent plant than any daughter shoots, including daughter shoots from which a tap root emerged. It is presumed these fine roots are ephemeral and can develop rapidly following rainfall.

Resprouting ability in the glasshouse

All fragments of laterals which had been wrapped in damp paper resprouted after being replanted and watered, even after being out of the ground for a week or more. Laterals from Palmer resprouted whilst still wrapped in damp paper over 2 weeks after excavation. Once replanted, shoot growth was very rapid: flowers were formed within 12 weeks from replanting. Healthy shoots were produced from fragments as small as 10 cm

in length. Laterals from Palmer which were excavated from dry soil then wrapped in dry paper were also able to resprout over three weeks after originally being excavated once planted and watered. Laterals from all sites produced one or more shoots in a single flush after replanting. Generally after sprouts were established new shoots did not emerge for several months.

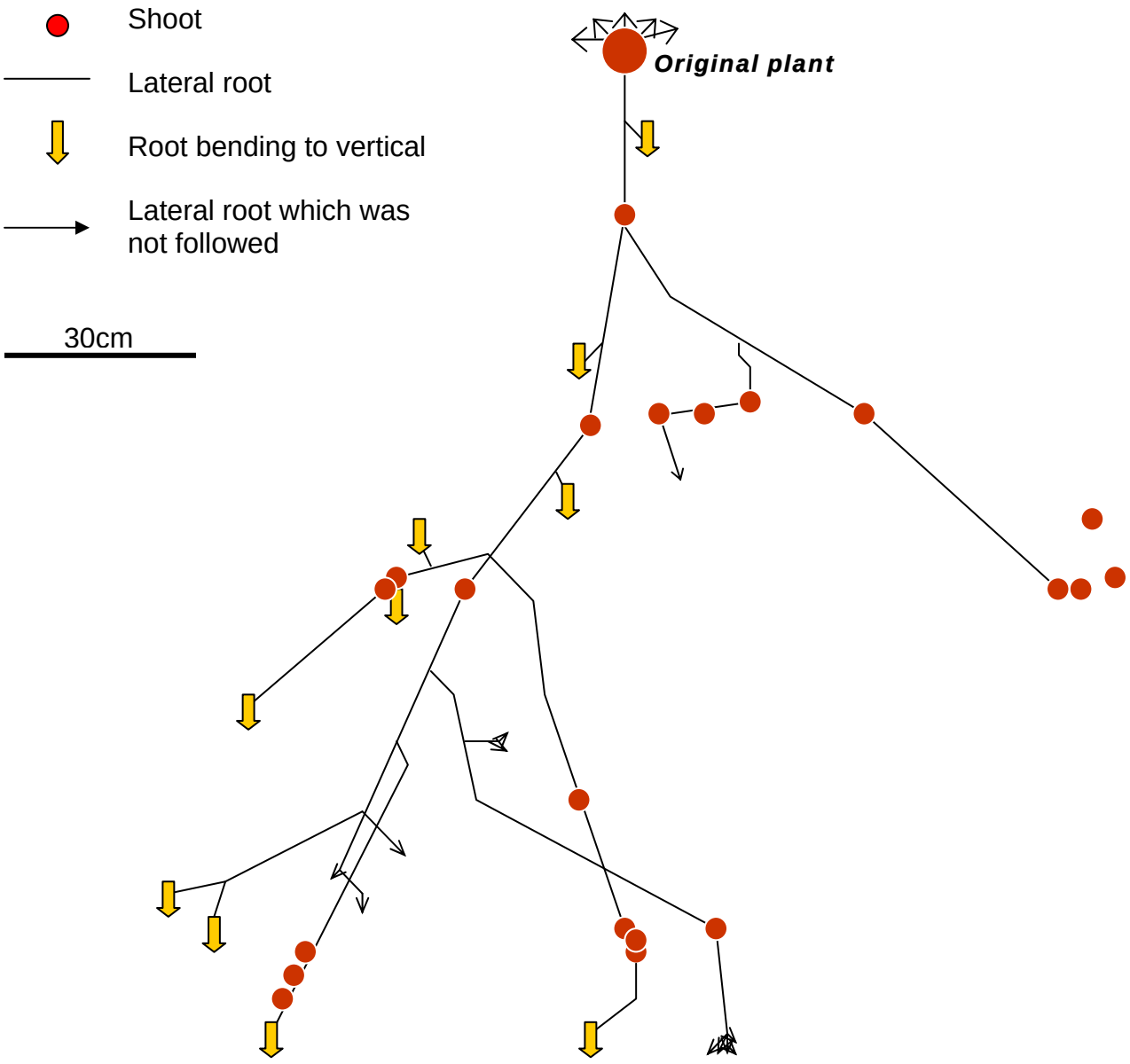


Fig. 3. Cultivated community of *S. centrale* clones from Palmer in South Australia. The original seed plant from which the colony was established is shown at the top of the diagram.

Seedling vigour declined after about 6 months, even when watered and fertilised. In general, shoots had a very high rate of leaf turnover and after the initial growth burst branches produced fewer and fewer new leaves and eventually dried out. In contrast to field observations, branches elongated to the point where they could not support themselves and bent over, then again grew towards the light. This suggests the low light intensity of the glasshouse relative to Central Australia was causing shoot elongation.

Seedlings responded extremely favourably to pruning, with new shoots emerging from stems which had been cut back to almost ground level. Similar to root sprouting, new growth from shoots was stimulated by water, with one seedling remaining dormant for 2 months whilst water was withheld. Pruned plants grew rapidly and flowers were produced on new growth within a month.

Anatomy

Thick laterals and vertical roots were found to possess polyarch root anatomy with secondary growth similar to that found in taproots or woody stems (Fig. 4). Growth rings are visible within this secondary growth for laterals from Palmer but are less clear in laterals from Hatt Rd and Pwerte Arntarntarenhe. Fine roots possessed typical dicotyledonous polyarch root anatomy with a much smaller vascular region than observed in the laterals. Stems, whether above or below the soil surface, possessed dicotyledonous stem anatomy with secondary thickening and a clearly defined vascular region (Fig. 5). Pith is present in the centre of the stem, unlike in lateral roots.

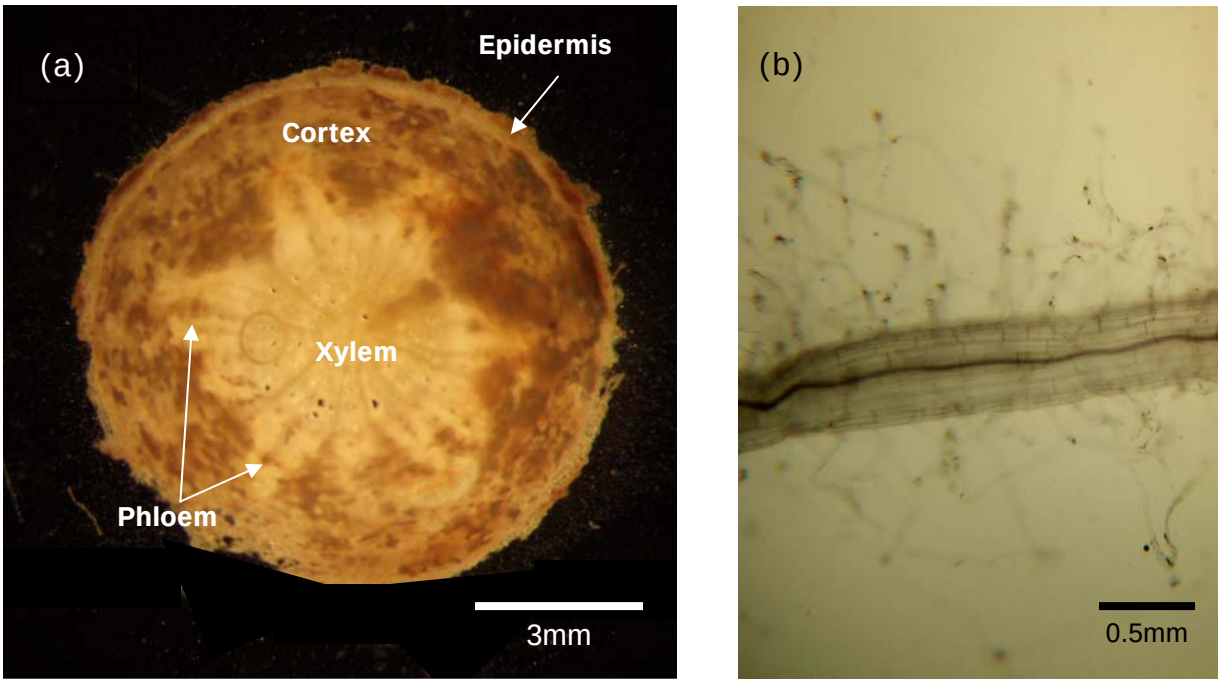


Fig. 4. *S. centrale* roots from Palmer, a cultivated site in South Australia: (a) Cross section of lateral root; (b) Root hairs on secondary (fine) root.

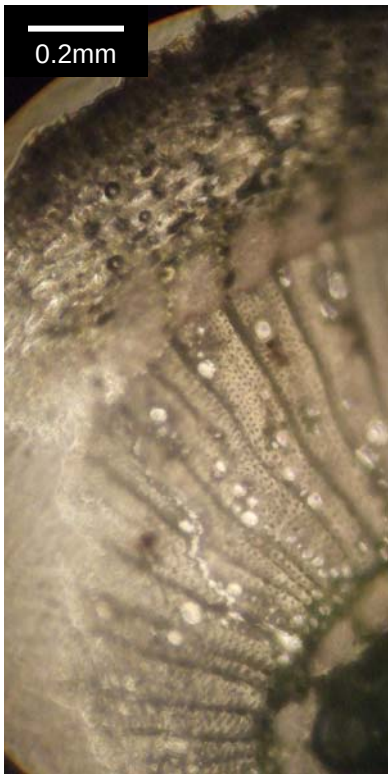


Fig. 5. Cross section of stem emerging from a lateral root from Palmer, South Australia. Section was taken from just below the soil surface.

Root storage products

Staining revealed large numbers of starch granules in the root cortical cells, including as part of parenchymatous inclusions into the vascular region. Starch granules were also observed in the cortex of secondary roots (Fig. 6).

Staining also revealed large amounts of lignin in the stele present as secondary thickening of xylem vessels. No fats or oils were observed.

The phosphorus content of one seed sample and samples of lateral roots collected from an unfertilized but cultivated site (Palmer) and three wild sites are given in Table 4. Seed collected from Napperby had a high concentration of phosphorus relative to that measured in lateral roots from the same location.

Table 4 Phosphorus concentration of lateral roots from Palmer and three wild sites in Central Australia and the seed phosphorus content in fruits collected at Napperby.
Seed was collected at Napperby on the same day as laterals.

Site	Phosphorous concentration (%)
Palmer	0.12
Aileron	0.04
HattRd	0.05
Napperby	0.07
Seed	0.15

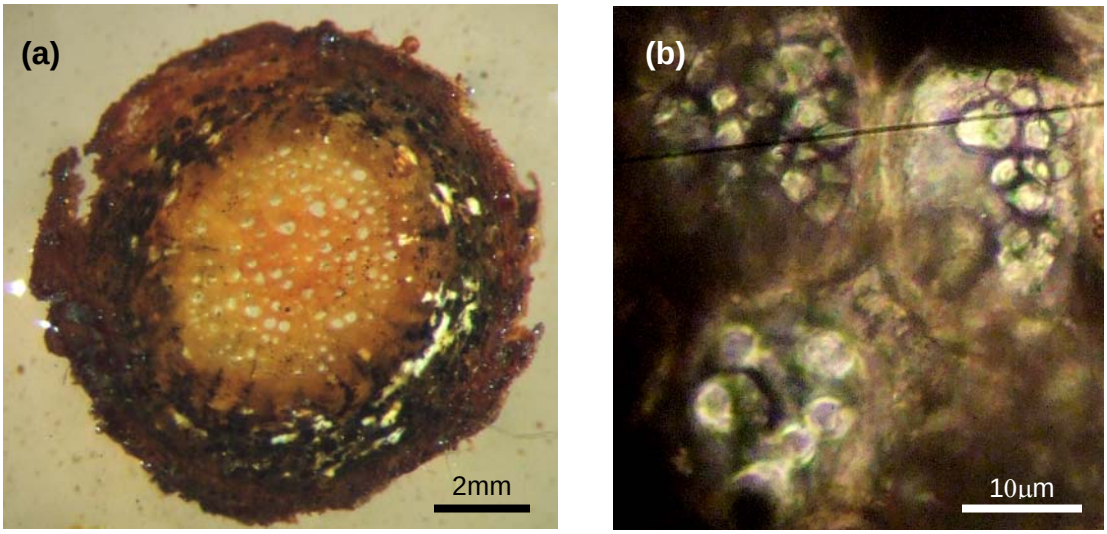


Fig. 6. Starch in *S. centrale* laterals from Palmer, a cultivated site in South Australia: (a) Starch distribution in a lateral root stained in 2% iodine; (b) Unstained starch granules in root parenchyma.

Field mycorrhizal colonisation

Hyphal coils, arbuscules and vesicles were observed in roots from all sites, although arbuscules were rare in older roots (Fig. 7). Old roots were sampled to measure percentage colonisation because the fine roots were so sparse and the sampling sites so dry. Highest colonisation was observed at the sites with the freshest roots – the cultivated and irrigated sites on Pwerte Arntarntarenhe (Table 5).

Table 5 Average percentage of root length colonised by mycorrhizal fungi from two natural stands (Palmer and HattRd) and two cultivated stands (Pwerte Arntarntarenhe) of *S. centrale*.

Site	Colonisation (%)
Palmer	40.0
Pwerte Arntarntarenhe	
Low fert.	51.0
High fert.	63.6
Hatt Rd	16.3

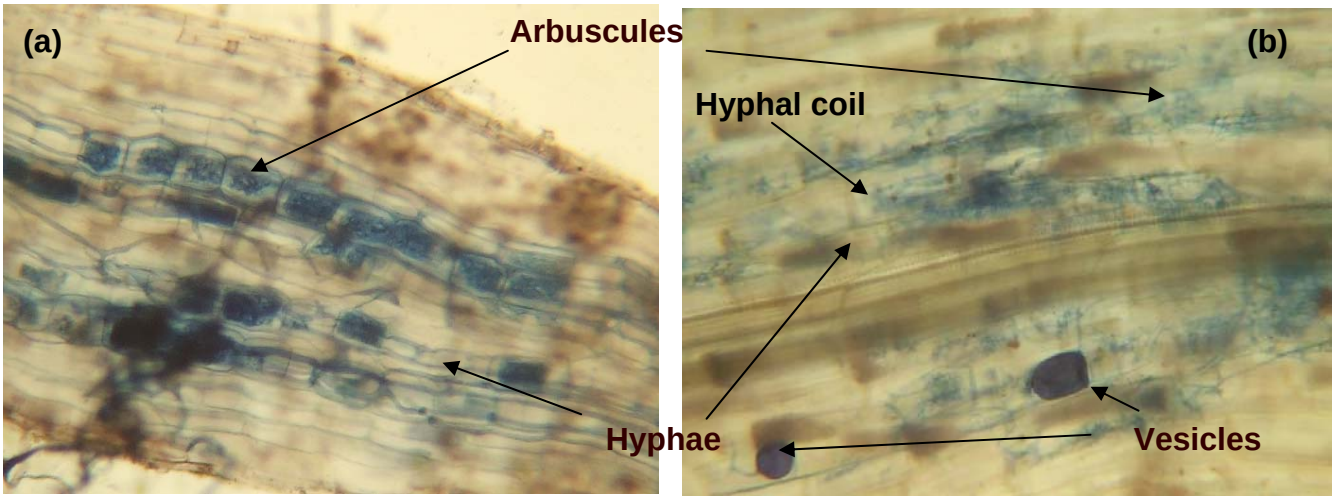


Fig. 7. Mycorrhizal associations in the roots of *S. centrale* from the cultivated Palmer site in South Australia: (a) Younger root; (b) Older root, with visible hyphal coils and vesicles.

Effect of mycorrhizas in the glasshouse

For most measures of plant growth and development a significant interaction was observed between mycorrhizas and phosphorus nutrition at the 5% level, where mycorrhizas had a greater effect at zero P than at low or high P. This was clear from visual inspection of sample pots for each treatment, where zero P, uninoculated pots were clearly stunted (Fig. 8). For most variables, no significant difference was detected between low and high P treatments, or with mycorrhizal inoculation at those high nutrition levels (Table 6). Where there was no interaction between mycorrhizas and phosphorus nutrition, the phosphorus level was highly significant ($P<0.001$ for all variables except shoot biomass, where $P=0.030$, and number of leaves where $P=0.001$). Results for individual variables are reported below.

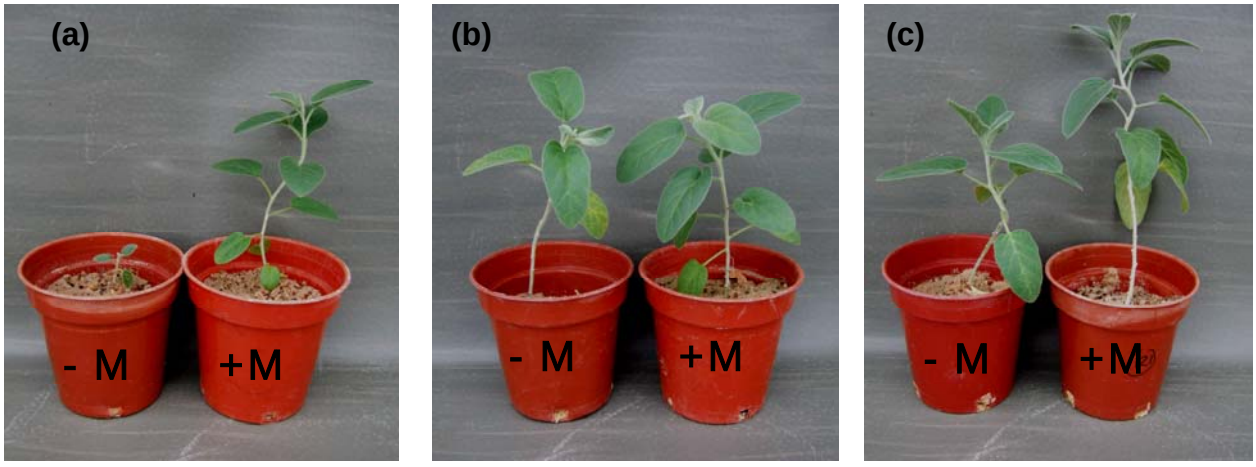


Fig. 8. Effect of inoculated versus non-inoculated treatments on the growth of *S. centrale* at three levels of applied phosphorus: (a) Zero P; (b) Low P; (c) High P. +M = inoculated; -M = uninoculated.

Table 6 Probability of the null hypothesis that there is no effect of mycorrhizal inoculation, phosphorus or blocks for all tested variables.

Variate	Interaction	Mycorrhizas	Phosphorus	Block
Biomass				
- whole plant	0.185	0.004	<0.001	0.554
- shoots	0.695	0.882	0.030	<0.001
- roots	0.408	0.012	<0.001	0.932
Dry weight				
- roots	<0.001	<0.001	<0.001	<0.001
- shoots	<0.001	<0.001	<0.001	<0.001
- whole plant	<0.001	<0.001	<0.001	<0.001
Height	<0.001	<0.001	<0.001	<0.001
% Phosphorus				
- shoots	0.664	0.957	<0.001	0.232
- roots	0.197	0.030	<0.001	<0.001
- whole plant	<0.001	0.002	<0.001	<0.001
Root:shoot dry weight	0.117	<0.001	<0.001	0.003
Root length	<0.001	<0.001	<0.001	<0.001
Root length:weight	0.001	0.348	<0.001	0.042
Leaves per mm height	<0.001	<0.001	<0.001	0.125
Height:shootDW ratio	<0.001	<0.001	<0.001	<0.001
Number of leaves				
1 week before harvest	0.002	<0.001	0.001	0.425
harvest date	<0.001	<0.001	<0.001	0.010

Colonisation

No mycorrhizas were observed in the roots of inoculated high P plants and the roots of only one inoculated low P plant (pot 26) exhibited any colonisation (estimated at just 2% of root length). Inoculated plants at zero P exhibited 47.7% colonisation. This excludes one zero P, inoculated plant in which no colonisation was observed. These roots had abundant root hairs, similar to uninoculated roots.

Plant Biomass

No interaction was observed between inoculation and phosphorus treatments for root, shoot or whole plant biomass (Table 6). Inoculation decreased biomass in both roots and the overall plant but not shoots (Fig. 9). Inoculated plants had an estimated root biomass 0.71 times that of uninoculated plants (95% CI: 0.53, 0.95).

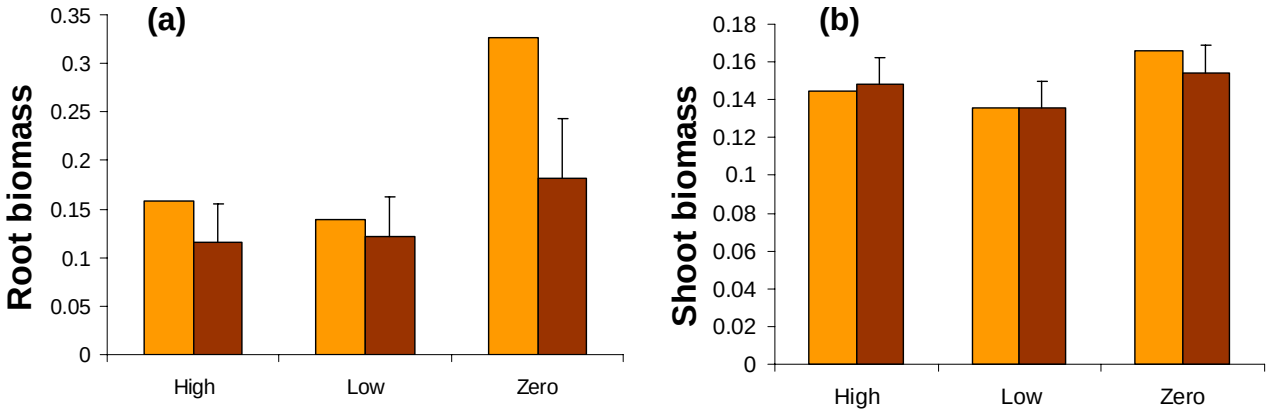


Fig. 9. Mean biomass at three levels of phosphorus nutrition in inoculated and uninoculated pots for (a) Roots; (b) Shoots. Error bars indicate the 5% lsd for differences between inoculated and uninoculated treatments at each level of phosphorus.

Dry weight

A significant interaction was observed between inoculation and P level for shoot, root and whole plant dry weight ($P<0.001$). The uninoculated zero P treatment produced a lower dry weight ($P<0.05$) than all other treatments for all three variables (Fig. 10). No statistical difference was detected between inoculated zero P plants and either low or high P due to the high variability. However inoculation appears to reduce the mean root dry weight at low P ($P=0.236$) and high P ($P=0.116$) without a concurrent decrease in mean shoot dry weight.

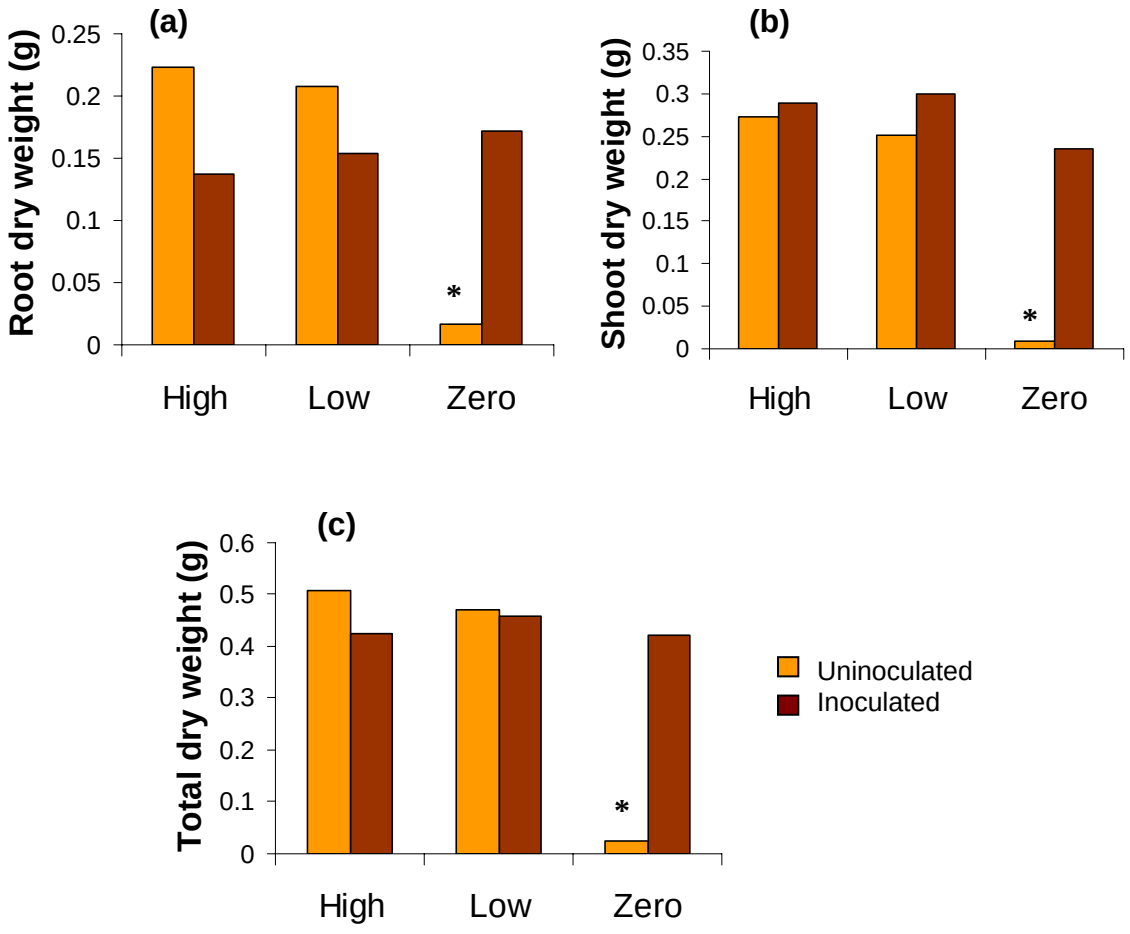


Fig. 10. Dry weight at three levels of phosphorus nutrition in inoculated and uninoculated pots for (a) Roots; (b) Shoots; (c) Whole plant. * indicates the treatment is significantly different to all other treatments at the 5% level.

Height

No difference was detected between inoculated or uninoculated, low or high P plants and inoculated, zero P plants (Fig. 11). Uninoculated Zero P plants were, on average, 13.3% of the height of plants in other treatments.

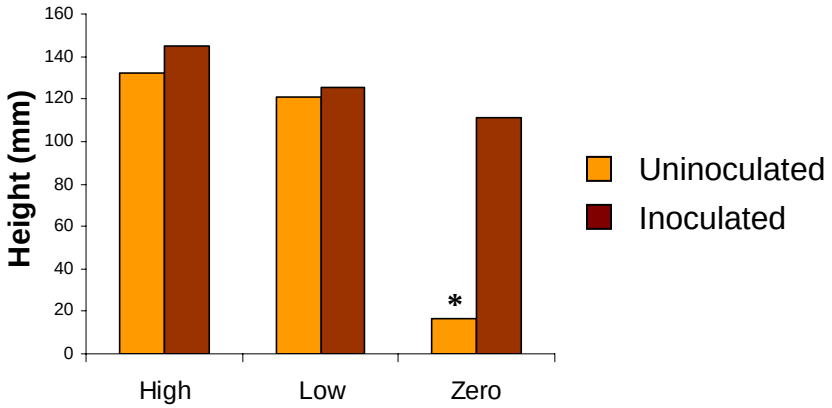


Fig. 11. Plant height at three levels of phosphorus nutrition in inoculated and uninoculated pots.
* indicates the treatment is significantly different to all other treatments at the 5% level.

Phosphorus content of roots and shoots

No interaction was detected between phosphorus nutrition and inoculation for root or shoot phosphorus percentage however when average whole plant phosphorus was determined the interaction became significant ($P<0.001$).

Root phosphorus was on average 1.42 times greater in inoculated plants than uninoculated plants ($P=0.030$, 95% CI: 1.16, 1.73). This is despite the negligible mycorrhizal colonisation in inoculated low or high P treatments.

As expected, both root and shoot phosphorus content was greater under low and high P than zero P (Table 7). Zero P shoots had a phosphorus percentage of 0.34% less than low P (95% CI: 0.26%, 0.42%) and 0.35% less than under high P (95% CI: 0.27%, 0.44%). Indeed, no difference in phosphorus content was detected between low and high P for either roots or shoots. This indicates the plant is in the zone of luxury consumption, even at low P (12.5% Hoaglands) nutrition.

Note that total phosphorus provided during the trial (if all P added from beakers remained in the pot) was approximately 0.3 g phosphorus for the high nutrient and 0.15 g phosphorus for the low P. A typical plant with 0.6% phosphorus weighed approximately 0.5 g DW and thus would contain 0.03 g of phosphorus - about 10% of what was provided for the high P treatment. Average seed phosphorus content was 0.15%, equating to just 2 μg of phosphorus per seed (Table 4).

Table 7 Phosphorous content of roots, shoots and whole plant average at three levels of phosphorus nutrition for inoculated and uninoculated pots.
* indicates a significant difference between inoculated and uninoculated pots at that level of phosphorus for that variate.

		High P	Low P	Zero P
Whole plant P (%)	Inoculated	0.57	0.60	0.14*
	Uninoculated	0.55	0.55	0.07*
Root P (%)	Inoculated	0.80*	0.71	0.10*
	Uninoculated	0.62*	0.58	0.05*
Shoot P (%)	Inoculated	0.50	0.49	0.18
	Uninoculated	0.53	0.50	0.14

Leaves per mm height

Like most variates, number of leaves per mm height exhibited an interaction between phosphorus nutrition and mycorrhizas ($P<0.001$). The non-mycorrhizal, zero P treatment had between 2.30 times and 2.85 times more leaves per mm than other treatments (Fig. 12). This reflects the stunting caused by lack of phosphorus, where the plant preferentially allocates phosphorus to leaf rather than vertical stem growth when under limiting conditions.

There was no significant difference in other treatments at the 5% level.

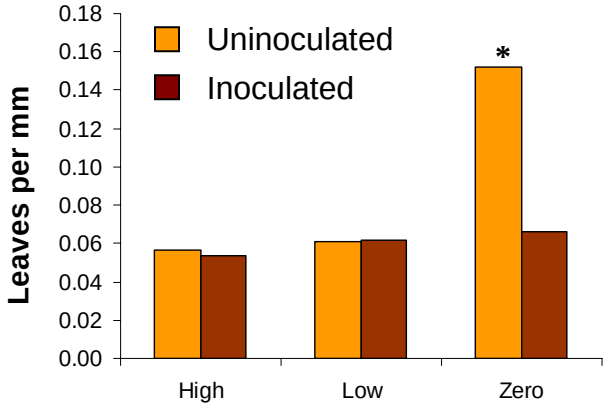


Fig. 12. Leaves per mm height at three levels of phosphorus nutrition in inoculated and uninoculated pots. * indicates the treatment is significantly different to all other treatments at the 5% level.

Root to shoot ratio

Inoculation approximately halved the root to shoot ratio: specifically, inoculated plants had an average root to shoot ratio of 53.7% of that of uninoculated plants ($P<0.001$, 95% CI: 0.333, 0.81). Phosphorus also altered the root to shoot ratio, with zero P treatments 1.87 times higher than the high P and 1.80 times higher than the low P treatment (Fig. 13).

Interestingly, mycorrhizas were not found to interact with the level of Phosphorus nutrition for this variable. This suggests that mycorrhizas still influenced root to shoot ratio at low and high P whereas in most other variables mycorrhizas had no effect at high nutrition. Similar to root phosphorus content, this effect is occurring despite insignificant colonisation at low or high nutrition.

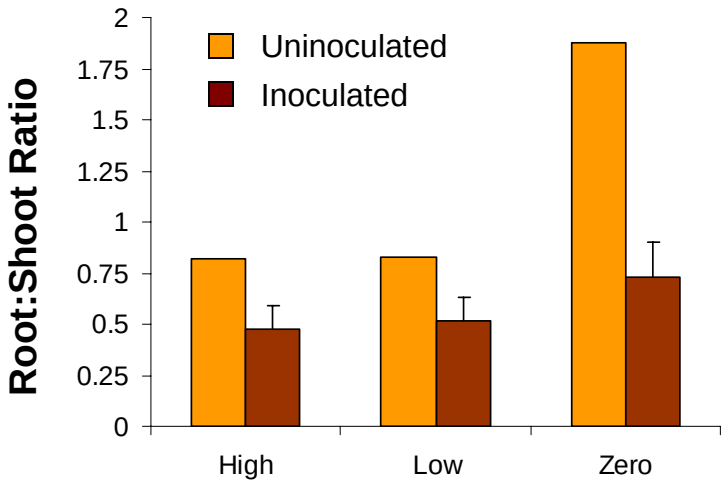


Fig. 13. Root to shoot ratio at three levels of phosphorus nutrition in inoculated and uninoculated pots. Error bars indicate the 5% lsd for comparison between inoculated and uninoculated pots at each level of Phosphorus.

Root length

A significant interaction was clearly evident for root length between inoculation and phosphorus nutrition ($P<0.001$, Fig. 14). No difference in root length was detected between inoculated or uninoculated, low or high P plants and inoculated, zero P plants. The roots of zero P, uninoculated plants were, on average, 0.149 times shorter than other treatments (average 95% CI: 0.147, 0.187).

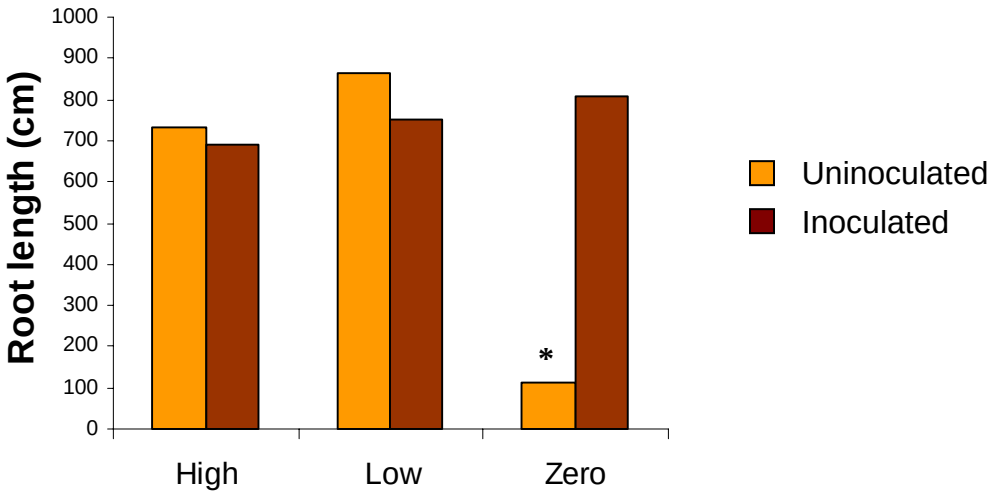


Fig. 14. Root length at three levels of phosphorus nutrition in inoculated and uninoculated pots.
* indicates that treatment is significantly different to all other treatments at the 5% level.

Root length to weight ratio

An interaction was observed between inoculation and phosphorus nutrition on the root length to root weight ratio (P=0.001, Fig. 15). This ratio for the zero P, uninoculated plants was, on average, 3.79 times greater (at the 5% level) than all other treatments. Furthermore, zero P, inoculated plants had a significantly greater root length:weight ratio than both the high P treatments and the inoculated low P treatment has a 0.051 probability of being significantly higher than the high phosphorus, uninoculated treatment. Specifically, inoculated zero P plants were on average 1.57 times greater than the high phosphorus treatments and the low P, inoculated plants were 1.24 times greater than the high phosphorus, uninoculated plants. This suggests that poor phosphorus nutrition increases the root length to weight ratio: in other words, zero P pots had longer and thinner roots than those from inoculated pots. The difference between inoculated low P and uninoculated high P treatments suggests inoculation may increase root length to weight ratio when phosphorus is not limited, however the difference caused by inoculation at high and low P was insufficient to make a firm conclusion.

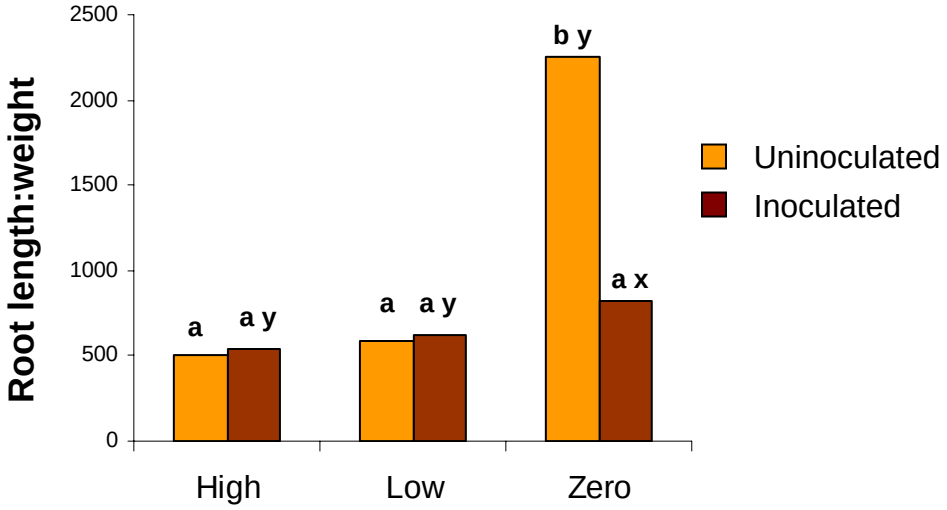


Fig. 15. Root length to weight ratio at three levels of phosphorus nutrition in inoculated and uninoculated pots. Treatments marked with a are different to b and x different to y at the 5% level.

Height to shoot dry weight ratio

A highly significant interaction was observed for height to shoot DW ratio, again due to the extremely different result in the zero P, uninoculated treatment ($P<0.001$). This treatment had an average ratio of 4.05 times greater than the ratios of all other treatments (Fig. 16). In other words, plants under severe phosphorus deprivation allocated proportionally more resources into leaves rather than vertical stem growth. This confirms the result for number of leaves per mm height.

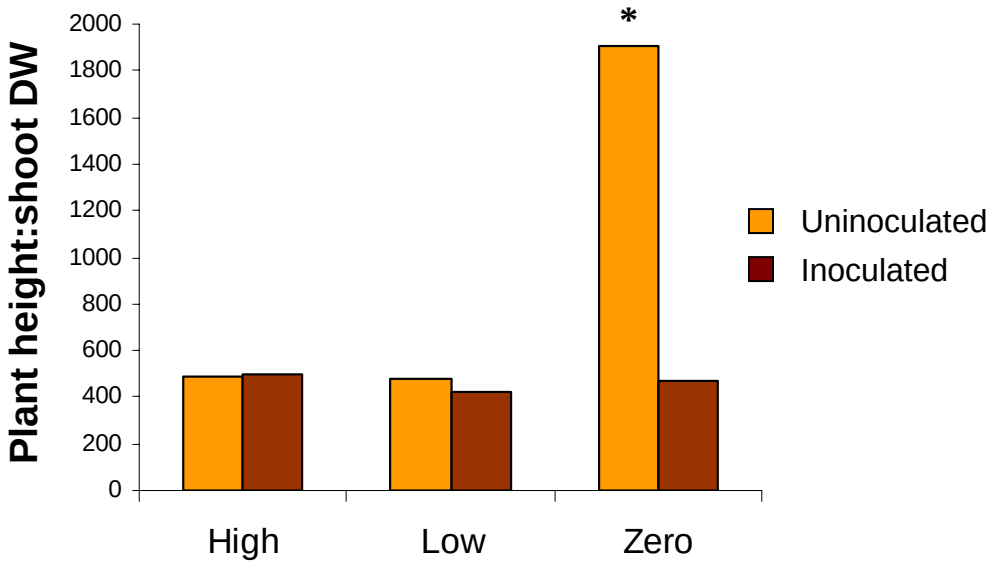


Fig. 16. Plant height to shoot dry weight ratio for three levels of phosphorus nutrition in inoculated and uninoculated pots. * indicates that treatment is significantly different to all other treatments at the 5% level.

Number of leaves

The number of leaves was again subject to an interaction between inoculation and phosphorus nutrition for both dates (P=0.001 for day 67, P<0.001 for day 71). The number of leaves increased between the two measurement dates for all treatments except uninoculated, zero P plants. Inoculation appeared to cause plants to retain their leaves, although this effect was only statistically significant at the zero P level for both days and the low P level on the first day (Fig. 17).

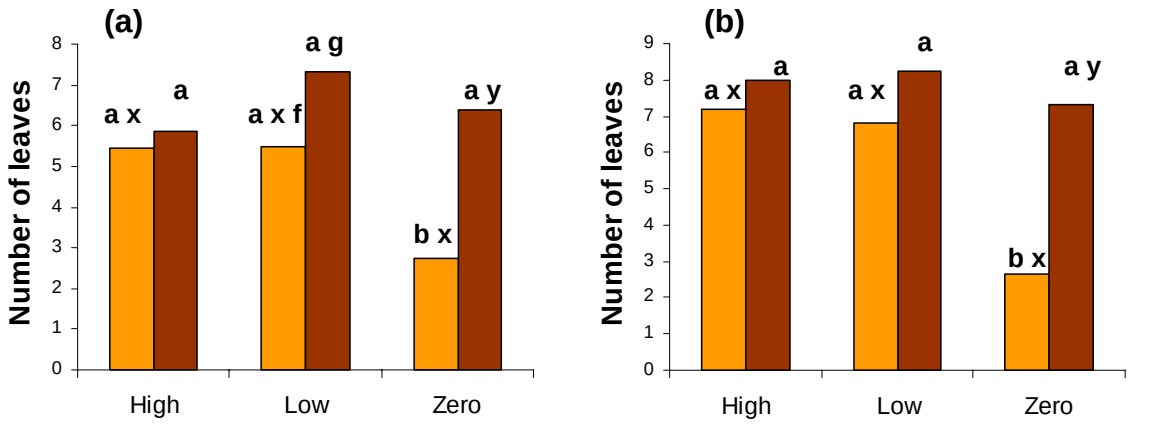


Fig. 17. The number of leaves measured on two harvest dates at three levels of phosphorus nutrition in inoculated and uninoculated pots: (a) On day 67 from transplanting; (b) On harvest day (day 71). Letters designate treatments which are significantly different at the 5% level, with the difference between letters a and b, x and y, and f and g being declared significant.

Discussion

Root macromorphology and anatomy

The field studies of the underground structures of *Solanum centrale* confirm the hypothesis that this species forms clonal communities. Such communities were observed in both natural and cultivated conditions. Furthermore the underground lateral connections were found to possess root rather than stem anatomy and therefore the hypothesis that the underground connections are rhizomatous is rejected. Authors of recent botanical literature make a distinction between root-sprouters and rhizomatous plants (Bell *et al.* 1996; Klimesova and Martinkova 2004; Guerrero-Campo *et al.* 2006) whereas older literature was not always as definitive e.g. (Symon 1981; Maconochie 1982).

Having said this, the lateral roots of *S. centrale* still form shoots and fine roots along its axis, a common feature of rhizomes (Jackson 1900). They also store large amounts of starch (Berry and Jackson 1976) and retain meristematic cells which allow rapid shoot formation (Guerrero-Campo *et al.* 2006). Thus the function of lateral roots is similar to rhizomes in that they are underground organs of vegetative reproduction and store non-structural carbohydrates. This is similar to truly rhizomatous native species which resprout following fire such as native *Alexgeorgea* spp. (Pate *et al.* 1991), *Patersonia* spp. (Rudall 1984) and members of the Restionaceae (Meney *et al.* 1990). Furthermore, in *S. centrale* the carbohydrate reserves presumably enable the plant to resprout from roots not only whilst connected to the plant but also from root fragments as was observed in the glasshouse.

Colonies appear to require disturbance to maintain vigour. In the glasshouse, unpruned seedlings which were given adequate nutrition and water were slow growing and produced less flowers over time compared to plants which were pruned. Given how rapidly flowers form following resprouting from either roots or shoots, it appears the greatest period of productivity occurs shortly after a disturbance (Klimesova and Martinkova 2004). This appears to be a characteristic of native *Solanum* spp. as many (if not all) can be classified as pioneer species (Bean 2004). *S. centrale* was observed growing vigourously along the verges of recently graded roads in Central Australia during field sampling. A preference of native

desert *Solanum* spp. for disturbed sites was noted by Symon (1981), who comments that "One wonders how they fared before road making was common." Indeed, based on the results of this experiment it appears regular firing and fruit collection by indigenous groups and grazing by desert marsupials and emus would have provided sufficient disturbance to maintain frequently visited colonies before European settlement.

The ability to resprout from roots is well documented by Cuda *et al.*(2002) and the North American Plant Protection Organisation (2003) for *Solanum elaeagnifolium* and *S. carolinense*. The description of the root system of *S. carolinense* by Miyazaki and Ito (2004) is almost identical to that observed for *S. centrale.* The seed plant in an *S. carolinense* colony produced horizontal roots 5-10mm in diameter. Like the roots of *S. centrale*, these laterals grow parallel to the surface and produce shoots at intervals or bend to vertical some distance from the seed plant.

The six types of underground junctions described in Fig. 2 form under different conditions. Most colonies in natural conditions would begin from a single seed. These seed plants were readily identified by the root system, with multiple laterals emerging from the rootstock as it turned into a thick taproot. A T-junction (shoot without a taproot) presumably forms when a shoot emerges rapidly on a lateral in favourable conditions (Maconochie 1982). Vertical roots were found occasionally at a junction with the shoot or where a lateral root abruptly turns to grow vertically down. This is most likely to search for water at depth (Davies and Bacon 2003). Laterals may begin to grow vertically as the number of shoots in a colony increases but no taproots are formed at the point where the root sprouts. The reason a tap root is rarely formed at the junction with a shoot is uncertain.

Shoots of *S. centrale* were often found in clusters where two or more had emerged quite close together from a lateral root. Based on this observation it is speculated that high soil moisture triggers several shoots to emerge at once, followed by fruit production and root expansion until the next significant rain or irrigation event. Furthermore, observations indicate that resprouting from roots was hindered by the presence of mature shoots. This may be a hormonal suppression or a direct effect of depletion of starch reserves following a burst of resprouting. This corresponds to observations of *S. carolinense*, where no new shoots were formed once existing shoots reached a certain maturity (Miyazaki *et al.* 2005).

S. carolinense, S. elaeagnifolium and other root-sprouters such as *Knautia arvensis* and *Centaurea scabiosa* are vigourous weeds in cropping regions (Symon 1981; Klimesova and Martinkova 2004). Colonies are very difficult to eradicate as the viability of roots is not affected by cultivation and most herbicides. In fact, cultivation stimulates sprouting from root fragments (Klimesova and Martinkova 2004; Urakawa and Koide 2004). The difficulty in eradicating colonies or preventing colony expansion has been noted in *S. centrale* (Tim Collins, pers. comm.; Maarten Ryder, pers. comm..). This will create challenges when using *S. centrale* as a crop - it will be difficult, for example, to rotate the field with other crop species, or even to sow new varieties, because of the difficulty in totally removing the *S. centrale* colony.

Conversely, its ability to resprout from both roots and shoots then rapidly produce fruit may make a colony of *S. centrale* highly productive. There is considerable potential to use root fragments rather than seed to establish crops, given how low germination is currently a major hindrance to commercial production (Stefanski 1998; Ahmed *et al.* 2006). Slashing or burning shoots followed by regular irrigation can induce fruit production within weeks. Alternatively, mechanical harvesting of entire shoots would trigger the same resprouting if followed by rain/irrigation and may prove highly feasible if fruit can be easily separated from shoot material. Mechanical harvesting has been trialed in South Australia using a modified cereal header with some success (Hele 2001).

Although it is clear that shoots form from lateral roots, there was great difficulty in sectioning and staining plant material for anatomical study. The lignified stele and soft, starch-filled cortex proved almost impossible to section using standard microtomy. Both fresh and reconstituted laterals were fixed and embedded in resin however both broke apart as the knife sliced the block. More advanced techniques are required to enable a detailed study of the root anatomy, including the possible use of freeze drying and harder resin. Other techniques such as MicroCT (Pierret *et al.* 2003), briefly trialed to give a three-dimensional visualization of laterals, may prove particularly useful in determination of the structure of adventitious buds which were noted but not examined in this study.

Mycorrhizal associations

Field colonisation

The hypothesis that mycorrhizal fungi naturally colonise the fine roots of wild plants was confirmed for both fertilised and unfertilised sites. Because young roots could not be obtained from all sites, accurate estimation of root length colonisation was impossible (Tester *et al.* 1987). The low level of colonisation in *S. centrale* at naturally colonised sites may be due to the scarcity of roots, low density of propagules in soil or dormancy in the fungus induced under drying conditions. Few arid zone mycorrhizal fungi have been examined, and their biology in situ studied in only a few situations (McGee and Warcup 1986; O'Connor *et al.* 2001). However the percentage of root length colonised in relatively young roots from Palmer and Pwerte Arntarntarenhe was certainly higher than the less than 15% root length colonisation reported by Boerner (1992) for field collections of *Solanum* spp. and less than 30% for *S. ellipticum* from the Simpson Desert, a closely related desert food species (O'Connor *et al.* 2001).

Affect of mycorrhizas on growth

In the glasshouse experiment, inoculation had a significant effect on plant growth and development at zero P but little effect when phosphorus was provided in excess of requirements (low or high P). This supports the hypothesis that mycorrhizas can improve the growth of *S. centrale*.

The absence of colonisation in the inoculated high P treatment and negligible colonisation in the inoculated low P treatment in the glasshouse contrasts with the 63.6% root colonisation measured for the highly fertilised Pwerte Arntarntarenhe field site. Evidence in the literature on the effect of phosphorus on root colonisation is conflicting. For example, decreasing colonisation with increasing inorganic phosphorus nutrition was also observed by Olsen *et al.* (1999) in capsicum (*Capsicum annuum*) but not tomato (*Lycopersicon esculentum*), whereas the opposite was found by Schroeder and Janos (2005), with colonisation decreasing for tomato but not chili (*Capsicum annuum*). Such contrasting results illustrate the complexity of the interaction between mycorrhizas, their host and soil phosphorus.

The lack of colonisation in high P roots suggests that either *S. centrale* can reject colonisation or that the fungi themselves are suppressed by high levels of soluble inorganic phosphorus. Hyphal growth of some mycorrhizal fungi is inhibited by high soil phosphorus (Abbott *et al.* 1984) and high phosphorus concentration in roots apparently regulates colonisation (Menge *et al.* 1978). However, colonisation has still been observed under excessively high soil P (Stewart *et al.* 2005). A brief observation on the outside of one high P *S. centrale* root suggested fungal hyphae are being aborted by the plant during penetration, as has been observed in a non-mycorrhizal tomato mutant (Poulsen *et al.* 2005). In either case, the absence of colonisation at high P prevents unnecessary draining of photosynthate by the fungus when the association does not increase photosynthate production (McArthur and Knowles 1993; Olsen *et al.* 1999).

Absence of mycorrhizas in experimental plants in the glasshouse but not in the field is a common phenomenon (Peter McGee, pers. comm.). One possible explanation is that despite being fertilised, most phosphorus in the field was inaccessible compared to the soluble inorganic phosphate provided in this experiment. Alternatively, mycorrhizas may be providing field plants with other benefits including increased water uptake (Augé 2001) and reducing the effect of pathogens (Newsham *et al.* 1995). Increased water uptake is unlikely to be of benefit here as fertilised sites were also irrigated.

Improvement of growth at zero P is at least partially a nutrition effect: inoculated plants given zero phosphorus had approximately twice the phosphorus content of uninoculated, zero P plants. Uninoculated plants at the zero P level were clearly limited by lack of available phosphorus, exhibiting typical phosphorus deficiency symptoms including stunting and purple discolouration (Reuter and Robinson 1997). Measures of plant growth including root and shoot dry weight, shoot height and root length were only improved at zero P when mycorrhizas increased the phosphorus available to nutrient-stressed plants. Future experiments testing the affect of mycorrhizas on *S. centrale* should choose phosphorus concentrations that would reveal the trend in mycorrhizal response as phosphorus becomes less and less limiting. The low and high P treatments here provided the plants with phosphorus in excess of what could be utilised for growth and were thus too high to reveal such a trend. The phosphorus content of roots and shoots from the zero P treatment indicate the plants are adapted to low P conditions (Pattinson and McGee 2004).

In the zero P treatment all phosphorus found in plant tissues must have been sourced from the seed, paper towel in the bottom of pots and growing medium, particularly the small amount of Narrabri soil in each pot. Plants in inoculated pots could also source phosphorus from the leek root inoculum although the rate of breakdown is expected to be fairly small in the sterilised growing-medium over the 10 week experiment. The ability of mycorrhizal fungi to scavenge for phosphorus from soil is well documented (Bolan 1991).

No difference was observed between low and high P plants, regardless of inoculation, for dry weight, shoot biomass, root length, height, leaves per mm height, height to shoot dry weight ratio and root length to weight ratio. This suggests that even at just 12.5% of Hoaglands solution (the low P treatment), phosphorus is still not limiting for growth. Furthermore, apart from root length to weight ratio the treatment means for all variables listed above exhibited no statistical difference between the inoculated, zero P treatment and plants at both low and high P. This is despite the fact that the phosphorus concentration in high and low P plants was approximately four times that of inoculated, zero P plants (Table 7). Therefore the presence of mycorrhizas was able to compensate for the lack of readily available phosphorus at the zero P level, a phenomenon also observed by Olsen *et al*. (1999) in tomato (*Lycopersicon esculentum*) and by McArthur and Knowles (1993) in potato (*Solanum tuberosum*).

Increased nutrition is unlikely to be the sole reason for altered plant morphology and growth (Smith and Read 1997). This is evidenced by plant features which are altered by inoculation even which phosphorus is provided in excess of requirements (McArthur and Knowles 1993). In this experiment, such features included root phosphorus content (but not shoot phosphorus content), root to shoot ratio, root biomass (but not shoot biomass), and the number of leaves. Interestingly, these features were all altered by inoculation in the absence of visible colonisation. Furthermore, plant morphological characters were altered by inoculation, but not absolute weights or sizes of parts except when phosphorus was limiting. For example, inoculated plants tended to retain more leaves than uninoculated plants (Augé 2001). It is known that the relative size of carbon sinks such as the root to shoot ratio are regulated by hormones – substances which can also be influenced by mycorrhizal fungi (Ludwig-Muller 2001). Thus morphological changes induced by mycorrhizal associations are not necessarily caused by increased nutrition alone and mycorrhizal fungi may be having a

hormonal effect on *S. centrale* (Torelli *et al.* 2000). These results are in agreement with Niemira *et al*. (Niemira *et al.* 1995), who also speculated that mycorrhizal fungi in the rhizosphere were having a hormonal effect on *S. tuberosum* despite observing little or no mycorrhizal colonisation and no change in shoot phosphorus content between inoculated and uninoculated plants. Inoculation is known to influence the concentration of several plant hormones in the host plant, including Abscisic Acid, Gibberellic Acid, Jasmonic Acid and cytokinins (Allen *et al.* 1982; Shaul-Keinan *et al.* 2002; Strack *et al.* 2003). Furthermore, mycorrhizal fungi apparently produce hormones and hormone-like substances (Barea and Azconaguilar 1982; Esch *et al.* 1994).

Root phosphorus content was greater in inoculated plants under both zero and high P nutrition. This indicates that the measured increase in phosphorus content in inoculated plants was not caused by colonisation alone. There are several possible explanations for this. Mycorrhizal fungi can reportedly increase the solubility of phosphorus compounds, such as those found in the small amount of Narrabri soil in experimental pots (Bolan 1991). Several authors note that the effect of mycorrhizas on plant growth is greater when poorly soluble phosphorus sources are used compared to soluble sources such as superphosphate (Strullu *et al.* 1981). Therefore it is possible mycorrhizal fungi are somehow increasing the availability of soil phosphorus from these sources without colonising the roots. This may involve simply transporting phosphorus from elsewhere in the soil towards the roots where fungal hyphae are concentrated, presumably feeding off carbon sources in root exudates and the leek root inoculum. If hyphae exude some of this phosphorus close to the roots, uptake would be increased. This explanation is particularly feasible considering nutrients were only provided once a week and fungal hyphae may provide a temporal store of phosphorus, releasing phosphorus mid-week after plants were watered (Strullu *et al.* 1981).

Another possible explanation is that fungal hyphae rich in phosphorus are present on the outside of roots and thus digested and analysed as part of the roots. This may also explain why inoculation increased root phosphorus but not shoot phosphorus content. Alternatively, if mycorrhizal fungi are having a hormonal effect it is possible they have increased root but not shoot phosphorus storage in *S. centrale* and thus caused a greater increase in root phosphorus content compared to the effect of inoculation on shoots. It is not surprising that

this storage is occurring even in uninoculated roots considering the root sprouting ability of the species.

Whether plant morphological changes are induced by the plant or by the fungus is uncertain. However there are several benefits of such morphological changes induced by the presence of mycorrhizal fungi. For example, mycorrhizas may have decreased the root to shoot ratio because the fungal hyphae partially replaced the function of the roots in scavenging for soil phosphorus (Smith and Read 1997). The fact that the root to shoot ratio differed at all levels of nutrition indicates that the benefits of this scavenging are somehow still transferred to plants even when they are not colonised but at high P nutrition. This is because no negative effects on growth were observed by the alteration of root to shoot ratio in the presence of mycorrhizal fungi. Furthermore, phosphorus deprivation increased the root to shoot ratio because the plant allocated more resources into obtaining this vital mineral from the soil (Smith and Read 1997).

Another benefit of mycorrhizas which is particularly important for desert species is increasing plant water absorption in the field (Augé 2001). In this experiment, plants were given large amounts of water compared to what they receive in their natural habitat. This may have influenced the level of mycorrhizal colonisation (Collier *et al.* 2003).

Problems in visualization of fungal hyphae were experienced when staining for mycorrhizas. Roots of glasshouse-grown plants appeared to be more alkaline than expected and thus hyphae and arbuscules did not take up the stain inside the roots. It is possible that more colonisation was present at low and high P nutrition however vesicles were not formed and thus colonisation was not clearly visible. The method used for roots collected in the field from July 2006 would be more appropriate for *S. centrale* as the acidified rinsing and use of ink caused roots to take up stain throughout the root cortex.

The overall effects of inoculation at low and high P, coupled with the observation of mycorrhizal fungi in the roots of fertilised field plants, suggest that in fertilised cropping situations, the benefit of increased phosphorus uptake in mycorrhizal *S. centrale* may be insignificant for production. However, other benefits in the field, such as increased water acquisition (Augé 2001) or disease suppression (Fusconi *et al.* 1999), may cause plants to still form symbiotic mycorrhizal associations (Niemira *et al.* 1995).

Conclusion

The underground macromorphology, anatomy and mycorrhizal associations of *Solanum centrale* indicate this species is well adapted to life in the desert. Its underground perennating organ with ephemeral roots, starch storage and ease of resprouting when disturbed allow rapid re-growth with the onset of suitable conditions in the arid environment. Resprouting from roots also confers a great competitive advantage over species which depend on seeds for reproduction in frequently disturbed habitats. Underground organs and attached mycorrhizal fungi enable immediate access to water and minerals, resulting in a rapid response. However in relation to its use as a crop, balancing the weedy potential with productivity characteristics in cultivated *S. centrale* will require further study.

The fine secondary roots were found to support healthy mycorrhizal associations but were surprisingly sparsely distributed along lateral roots. Mycorrhizal associations were found to increase growth, increase phosphorus uptake and alter plant morphology of seedlings in the glasshouse however root length colonisation differed between field and glasshouse root samples.

Domestication of this species demands an industry wide approach. The knowledge and resources of Aboriginal landholders and elders must be combined with modern scientific research methods and the support of the Native Food Industry to produce a suitable product for the market. However based on the results of this project it is clear the bush tomato has great potential as both a desert food species and as an income source for desert communities.

SYMON, D. E. (1987). Placentation Patterns and Seed Numbers in Solanum (Solanaceae) Fruits. *Journal of the Adelaide Botanic Gardens* **10,** 179-200.
TARAFDAR, J. C. & PRAVEEN-KUMAR. (1996). The role of vesicular arbuscular mycorrhizal fungi on crop, tree and grasses grown in an arid environment. *Journal of Arid Environments* **34,** 197-203.
TESTER, M., SMITH, S. E. & SMITH, F. A. (1987). The phenomenon of "nonmycorrhizal" plants. *Canadian Journal of Botany* **65,** 419-431.
TISDALL, J. M. & OADES, J. M. (1979). Stabilization of Soil Aggregates by the Root Systems of Ryegrass. *Australian Journal of Soil Research* **17,** 429-441.
TRAPPE, J. M. (1981). Mycorrhizae and productivity of arid and semiarid rangelands. In *Advances in food producing systems for arid and semi-arid lands* (ed. J. T. Manassah and E. J. Briskey), pp. 581-599. Academic Press, New York.
TRESEDER, K. K. & CROSS, A. (2006). Global distributions of arbuscular mycorrhizal fungi. *Ecosystems* **9,** 305-316.
TURNER-NEALE, M.-M. (1994). *Arrernte foods : foods from Central Australia = Nhenhe-areye anwerne-arle arlkweme*. IAD Press, Alice Springs, N.T.
UHLMANN, E., GORKE, C., PETERSEN, A. & OBERWINKLER, F. (2006). Arbuscular mycorrhizae from arid parts of Namibia. *Journal of Arid Environments* **64,** 221-237.
VAN DER HEIJDEN, M. G. A. (2002). Arbuscular mycorrhizal fungi as a determinant of plant diversity: in search of underlying mechanisms and general principals. In *Mycorrhizal Ecology*, vol. 157. *Ecological Studies* (ed. M. G. A. van der Heijden and I. Sanders), pp. 243-265. Springer-Verlag Berlin Heidelberg, Berlin.
WALKER, D. J. (1996). Culture of the Land: Cuisine of the People. *Food Australia* **48,** 314-315.
WHALEN, M. D. (1984). Conspectus of Species Group in Solanum Subgenus Leptostemonum. *Gentes Herbarum* **12,** 179-282.
WHALEN, M. D. & ANDERSON, G. J. (1981). Distribution of gametophytic self-incompatibility and infrageneric classification in Solanum. *Taxon* **30,** 761-767.
WHALEN, M. D. & COSTICH, D. E. (1986). Andromonoecy in Solanum. In *Solanaceae - biology and systematics* (ed. W. G. D'Arcy), pp. 284-302. Columbia University Press, New York.
WIEDEMANN, H. T. & KELLY, P. J. (2001). Turpentine (Eremophila sturtii) control by mechanical uprooting. *Rangeland Journal* **23,** 173-181.
WILBY, S. (2006). Kakadu and Arnhem Land. In *Best of Australia*. Australian Geographic, Australia.
WINKWORTH, R. E. (1966). The composition of several arid spinifex grasslands in central Australia in relation to rainfall, soil water relations, and nutrients. *Australian Journal of Botany* **15,** 107-130.
WOMEN FROM LARAMBA (NAPPERBY) COMMUNITY. (2003). *Anmatyerr ayey arnang-akert = Anmatyerr plant stories*. IAD Press, Alice Springs, N.T.

References

Abbott LK, Robson AD, Deboer G (1984) The Effect of Phosphorus on the Formation of Hyphae in Soil by the Vesicular Arbuscular Mycorrhizal Fungus, *Glomus Fasciculatum. New Phytologist* **97**, 437-446.

Ahmed AK, Johnson KA, Burchett MD, Kenny BJ (2006) The effects of heat, smoke, leaching, scarification, temperature and NaCl salinity on the germination of *Solanum centrale* (the Australian bush tomato). *Seed Science and Technology* **34**, 33-45.

Allen MF, Moore TS, Christensen M (1982) Phytohormone Changes in Bouteloua Gracilis Infected by Vesicular Arbuscular Mycorrhizae 2. Altered Levels of Gibberellin-Like Substances and Abscisic Acid in the Host Plant. *Canadian Journal of Botany-Revue Canadienne De Botanique* **60**, 468-471.

Anderson GJ, Symon DE (1989) Functional dioecy and andromonoecy in *Solanum*. *Evolution* **43**, 204-219.

Augé RM (2001) Water relations, drought and vesicular-arbuscular mycorrhizal symbiosis. *Mycorrhiza* **11**, 3-42.

Barea JM, Azconaguilar C (1982) Production of Plant Growth Regulating Substances by the Vesicular-Arbuscular Mycorrhizal Fungus *Glomus Mosseae. Applied and Environmental Microbiology* **43**, 810-813.

Bean AR (2004) The taxonomy and ecology of *Solanum* subg. Leptostemonum (Dunal) Bitter (Solanaceae) in Queensland and far north-eastern New South Wales, Australia. *Austrobaileya* **6**, 639-816.

Bell TL, Pate JS, Dixon KW (1996) Relationships between fire response, morphology, root anatomy and starch distribution in South-West Australian Epacridaceae. *Annals of Botany* **77**, 357-364.

Berlyn GP, Miksche JP (1976) 'Botanical Microtechnique and Cytochemistry.' (Iowa State University Press: Ames.)

Berry MI, Jackson BP (1976) European Mandrake *Mandragora officinarum* and *Mandragora autumnalis* - the Structure of the Rhizome and Root. *Planta Medica* **30**, 281-290.

Boerner REJ (1992) Plant life span and response to inoculation with vesicular-arbuscular mycorrhizal fungi II. Species from weakly mycotrophic genera. *Mycorrhiza* **1**, 163-167.

Bolan NS (1991) A Critical-Review on the Role of Mycorrhizal Fungi in the Uptake of Phosphorus by Plants. *Plant and Soil* **134**, 189-207.

Boulet FM, Lambers H (2005) Characterisation of arbuscular mycorrhizal fungi colonisation in cluster roots of Hakea verrucosa F. Muell (Proteaceae), and its effect on growth and nutrient acquisition in ultramafic soil. *Plant and Soil* **269**, 357-367.

Brundrett M, Melville LH, Peterson LR (1994) 'Practical methods in mycorrhizal research.' (Mycologue Publications: Ontario.)

Collier SC, Yarnes CT, Herman PR (2003) Mycorrhizal dependency of Chihuahuan Desert plants is influenced by life history strategy and root morphology. *Journal of Arid Environments* **55**, 223-229.

Collins C (2002) A study into the domestication of *Solanum centrale*, Australian bush tomato. University of Adelaide.

Cordier C, Pozo MJ, Barea JM, Gianinazzi S, Gianinazzi-Pearson V (1998) Cell defense responses associated with localised and systemic resistance to *Phytophthora parasitica* induced in tomato by an arbuscular mycorrhizal fungus. *Molecular Plant-Microbe Interactions* **10**, 1017-1028.

Cuda JP, Parker PE, Coon BR, Vasquez FE, Harrison JM (2002) Evaluation of exotic *Solanum* spp. (Solanales : Solanaceae) in Florida as host plants for the leaf beetles *Leptinotarsa defecta* and *L. texana* (Coleoptera : Chrysomelidae). *Florida Entomologist* **85**, 599-610.

Davies WJ, Bacon MA (2003) Adaptation of roots to drought. In 'Root Ecology'. (Eds H de Kroon and EJW Visser). (Springer-Verlag: Berlin.)

Esch H, Hundeshagen B, Schneiderpoetsch H, Bothe H (1994) Demonstration of Abscisic Acid in Spores and Hyphae of the Arbuscular-Mycorrhizal Fungus *Glomus* and in the N-2-Fixing Cyanobacterium *Anabaena Variabilis*. *Plant Science* **99**, 9-16.

Everard P, Everard M, Murika, Tjampu T, Kanytji, Pumani S, Milatjari (2002) 'Punu: Yankunytjatjara Plant Use.' (IAD Press: Alice Springs.)

FOSS (2000) 'Determination of ortho-phosphate in water by FIAstar 5000.'

Fritz M, Jakobsen I, Lyngkjaer MF, Thordal-Christensen H, Pons-Kuhnemann J (2006) Arbuscular mycorrhiza reduces susceptibility of tomato to Alternaria solani. *Mycorrhiza* **16**, 413-419.

Fusconi A, Gnavi E, Trotta A, Berta G (1999) Apical meristems of tomato roots and their modifications induced by arbuscular mycorrhizal and soilborne pathogenic fungi. *New Phytologist* **142**, 505-516.

Guerrero-Campo J, Palacio S, Perez-Rontome C, Montserrat-Marti G (2006) Effect of stem morphology on root-sprouting and shoot-rooting abilities in 123 plant species from eroded lands in North-east Spain. *Annals of Botany* **98**, 439-447.

Handreck KA (1997) Phosphorus requirements of Australian native plants. *Australian Journal of Soil Research* **35**, 241-289.

Hele A (2001) 'Bush Tomato/Desert Raisin Production.' Available at: http://www.pir.sa.gov.au/byteserve/agriculture/agfactsheets/vegetation/bushtoms.pdf, 6 November 2006. AgDex No. 8/03.

Hoagland DR, Arnon DI (1938) The water culture method for growing plants without soil. *California Agricultural Experimental Station* **347**, 1-39.

Isaacs J (1987) 'Bush food : Aboriginal food and herbal medicine.' (Weldons: McMahons Point, N.S.W.)

Jackson BD (1900) 'Glossary of botanic terms.' (Duckworth & Co.: London.)

Jakobsen I, Smith SE, Smith FA (2002) Function and diversity of Arbuscular Mycorrhizae in carbon and mineral nutrition. In 'Mycorrhizal Ecology'. (Eds MGA van der Heijden and I Sanders) pp. 75-92. (Springer-Verlag Berlin Heidelberg: Berlin.)

Klimesova J, Martinkova J (2004) Intermediate growth forms as a model for the study of plant clonality functioning: an example with root sprouters. *Evolutionary Ecology* **18**, 669-681.

Knox B, Laidges P, Evans B, Saint R (2003) 'Biology.' (McGraw-Hill: Sydney.)

Latz PK (1999) 'Pocket bushtucker.' (IAD Press: Alice Springs, N.T.)

Lemerle D, Leys AR (1991) Control of Silverleaf nightshade (*Solanum elaeagnifolium*) increases the grain yield of wheat. *Australian Journal of Experimental Agriculture* **31**, 233-236.

Ludwig-Muller J (2001) Hormonal balance in plants during colonisation by mycorrhizal fungi. In 'Arbuscular mycorrhizas: physiology and function'. (Eds Y Kapulnik and DD D.). (Klewer: Boston.)

Maconochie JR (1982) Regeneration of arid zone plants: a floristic survey. In 'Evolution of the flora and fauna of arid Australia'. (Eds WR Barker and PJM Greenslade) pp. 141-144. (Peacock Publications: Frewville, SA.)

Markarov AM, Golovko TK (1995) Growth orientation of underground shoots of perennial herbaceous plants: 1. Decapitation of an underground shoot and photoperiod length do not affect rhizome and stolon growth. *Fiziologiya Rastenii* (Moscow) **42**, 526-532.

McArthur DAJ, Knowles NR (1993) Influence of vesicular-arbuscular mycorrhizal fungi on the response of potato to phosphorus deficiency. *Plant Physiology* **101**, 147-160.

McGee PA, Warcup JH (1986) 'Role of mycorrhizas in the regeneration of arid zone plants.' Biennial Report of the Waite Agricultural Research Institute 1984-85, Adelaide.

McGonigle TP, H. MM, G. ED, Fairchild GL, Swan JA (1990) A new method which gives an objective measure of colonization of roots by vesicular-arbuscular mycorrhizal fungi. *New Phytologist* **115**, 495-501.

Meney KA, Pate JS, Dixon KW (1990) Comparative Morphology, Anatomy, Phenology and Reproductive Biology of *Alexgeorgea* Spp. (Restionaceae) from South-Western Western Australia Australia. *Australian Journal of Botany* **38**, 523-542.

Menge JA, Steirle D, Bagyaraj DJ, Johnson ELV, Leonard RT (1978) Phosphorus Concentrations in Plants Responsible for Inhibition of Mycorrhizal Infection. *New Phytologist* **80**, 575-578.

Miers G (2004) 'Cultivation and sustainable wild harvest of bushfoods by Aboriginal communities in Central Australia.' Rural Industries Research and Development Corporation, Web-only Publication No W03/124, Canberra. Available at: file://H:%5CMy%20stuff%5CBush%20Tomatoes%5CReferences%5CRIRDC_Abo IndustryDevelopment.pdf

Miyazaki K, Ito M (2004) Root system structure and shoot arrangement of 1-year-old Solanum carolinense L. *Weed Biology and Management* **4**, 122-125.

Miyazaki K, Ito M, Urakawa S (2005) Seasonal pattern of shoot emergence and its endogenous control in horsenettle (*Solanum carolinense* L.). *Weed Biology and Management* **5**, 14-18.

Newsham KK, Fitter AH, Watkinson AR (1995) Root pathogenic and arbuscular mycorrhizal fungi determine fecundity of asymptomatic plants in the field. *Journal of Ecology* **82**, 805-814.

Niemira BA, Safir GR, Hammerschmidt R, Bird GW (1995) Production of prenuclear minitubers of potato with peat-based arbuscular mycorrhizal fungal inoculum. *Agronomy Journal* **87**, 942-946.

North American Plant Protection Organisation (2003) '*Solanum carolinense* L.' Available at: http://www.nappo.org/PRA-sheets/Solanumcarolinense.pdf, 17th May 2006.

O'Connor PJ, Smith SE, Smith FA (2001) Arbuscular mycorrhizal associations in the southern Simpson Desert. *Australian Journal of Botany* **49**, 493-499.

Olsen JK, Schaefer JT, Edwards DG, Hunter MN, Galea VJ, Muller LM (1999) Effects of mycorrhizae, established from an existing intact hyphal network, on the growth response of capsicum (*Capsicum annuum* L.) and tomato (*Lycopersicon esculentum* Mill.) to five rates of applied phosphorus. *Australian Journal of Agricultural Research* **50**, 223-37.

Onen H, Ito M, Imaizumi T (2006) Horsenettle (*Solanum carolinense* L.) plants emerged at different times after corn (*Zea mays* L.) planting. *Weed Biology and Management* **6**, 55-58.

Pate JS, Meney KA, Dixon KW (1991) Contrasting growth and morphological characteristics of fire-sensitive (obligate seeder) and fire-resistant (resprouter) species of Restionaceae (S. Hemisphere Restiads) from south-western Western Australia. *Australian Journal of Botany* **39**, 505-25.

Pattinson GS, McGee PA (2004) Influence of colonisation by an arbuscular mycorrhizal fungus on the growth of seedlings of *Banksia aricifolia* (Proteaceae). *Mycorrhiza* **14**, 119-125.

Peterson LR, Massicotte HB, Melville LH (2004) 'Mycorrhizas: Anatomy and Cell Biology.' (NRC Research Press: Ottawa, Canada.)

Pierret A, Kirby M, Moran C (2003) Simultaneous X-ray imaging of plant root growth and water uptake in thin-slab systems. *Plant and Soil* **255**, 361-373.

Poulsen KH, Nagy R, Gao L-L, Smith SE, Bucher M, Smith FA, Jakobsen I (2005) Physiological and molecular evidence for Pi uptake via the symbiotic pathway in a reduced mycorrhizal colonization mutant in tomato associated with a compatible fungus. *New Phytologist* **168**, 445-454.

Reuter DJ, Robinson JB (1997) 'Plant analysis: an interpretation manual.' (CSIRO Publishing: Melbourne.)

Rudall P (1984) Taxonomic and evolutionary implications of rhizome structure and secondary thickening in Iridaceae. *Botanical Gazette* **145**, 524-534.

Schroeder MS, Janos DP (2005) Plant growth, phosphorus nutrition, and root morphological responses to arbuscular mycorrhizas, phosphorus fertilization, and intraspecific density. *Mycorrhiza* **15**, 203-216.

Schussler A, Schwarzott D, Walker C (2001) A new fungal phylum, the Glomeromycota: phylogeny and evolution. *Mycological Research* **105**, 1413-1421.

Shaul-Keinan O, Gadkar V, et al. (2002) Hormone concentrations in tobacco roots change during arbuscular mycorrhizal colonization with *Glomus intraradices. New Phytologist* **154**, 501-507.

Smith SE, Read DJ (1997) 'Mycorrhizal symbiosis.' (Academic Press: London.)

Stefanski A (1998) Manipulating seed dormancy in bush tomato (*Solanum centrale*). University of Sydney.

Stewart LI, Hamel C, Hogue R, Moutoglis P (2005) Response of strawberry to inoculation with arbuscular mycorrhizal fungi under very high soil phosphorus conditions. *Mycorrhiza* **15**, 612-619.

Strack D, Fester T, Hause B, Schliemann W, Walter MH (2003) Arbuscular mycorrhiza: Biological, chemical, and molecular aspects. *Journal of Chemical Ecology* **29**, 1955-1979.

Strullu DG, Gourret JP, Garrec JP, Fourcy A (1981) Ultrastructure and Electron-Probe Microanalysis of the Metachromatic Vacuolar Granules Occurring in Taxus-Mycorrhizas. *New Phytologist* **87**, 537-545.

Symon DE (1981) A revision of the genus *Solanum* in Australia. *Journal of the Adelaide Botanic Gardens* **4**, 1-367.

Symon DE (1982) Solanum (Solanaceae) in Arid Australia. In 'Evolution of the Flora and Fauna of Arid Australia'. (Eds WR Barker and PJM Greenslade) pp. 335-339. (Peacock Publications: Frewville, SA.)

Symon DE (1986) A Survey of Solanum Prickles and Marsupial Herbivory in Australia. *Annals of the Missouri Botanical Garden* **73**, 745-754.

Tester M, Smith SE, Smith FA (1987) The phenomenon of "nonmycorrhizal" plants. *Canadian Journal of Botany* **65**, 419-431.

Torelli A, Trotta A, Acerbi L, Arcidiacono G, Berta G, Branca C (2000) IAA and ZR content in leek (*Allium porrum* L.), as influenced by P nutrition and arbuscular mycorrhizae, in relation to plant development. *Plant and Soil* **226**, 29-35.

Trappe JM (1981) Mycorrhizae and productivity of arid and semiarid rangelands. In 'Advances in food producing systems for arid and semi-arid lands'. (Eds JT Manassah and EJ Briskey) pp. 581-599. (Academic Press: New York.)

Urakawa S, Koide I (2004) Horsenettle (*Solanum carolinense* L.) diffusion through rotatry tilling in invaded corn fields. *Grassland Science* **50**, 194-200.

Vierheilig H, Schiweiger P, Brundrett M (2005) An overview of methods for the detection and observation of arbuscular mycorrhizal fungi in roots. *Physiologia Plantarum* **125**, 393-404.

Whalen MD (1984) Conspectus of Species Group in *Solanum* Subgenus *Leptostemonum. Gentes Herbarum* **12**, 179-282.

Appendix 1

Supporting details for methods

Table A1 Description of field sites visited in December 2005 and July 2006

Name	Location	GPS	Elevation	Topography	Vegetation	Disturbance	General description
Arntarntarenhe - wild	~120km SSW of Alice Spings, NT	24°20’12.19”S 133°42’31.89”E	435m	Flat	Cleared Acacia scrub	Rotary hoed 7 years ago	
Arntarntarenhe - cultivated	~120km SSW of Alice Spings, NT	24°20’12”S 133°42’31”E	435m	Flat	Cultivated crop plants e.g. cotton, vegetables	Rotary hoed	
Napperby	~250km NW of Alice Springs, NT	22° 32' S 132° 46' E		Flat	Open Acacia scrub	Fire in past 2 years	
Hatt Rd	~10km S of Alice Springs, NT	23°48’58”S 133°48’43”E	538m	Gentle sand dune	Dunal Shrubs	Fire in past 5 years	Little vegetation, very dry
Palmer	Adelaide Hills, SA	34º52´8.90”S 139º08´44.85”E		Rolling hills	Cultivated crops e.g. grapes, citrus; weeds e.g. Salvation Jane, Heliotrope	None since sowing	
Aileron	~200km NNW Alice Springs, NT	22°38’00.4” S 133°20’48.5”		Flat	Mallee, Grasses	Deposition of sediment	Higher rainfall area
Alice Springs Desert Park	Alice Springs, NT	23°42’16.18” 133°50’22.93”			Bushfood garden	None since sowing	Lateral roots have spread from garden bed over 10m

Table A2 Soil analysis of sampling locations

Site	pH	Electrical Conductivity (µS/cm)	Organic matter	Carbonates	Colour (dry) (wet)	Texture	Approximate % clay	Comments
Arntarntarenhe – wild	7.01	196.9	None detected	None detected	2.5YR 4,6 10R 3,4	Sandy clay loam	20-30%	Claypan to 1m then calcrete
Napperby	6.91	185	present	None detected	10R 3,4 2.5YR 3,4	Sandy clay loam	20-30%	Claypan to 1m then calcrete
Palmer	6.91	223	present	None detected	7.5YR 4,4 5YR 3,3	Sandy loam	10-20%	Extremely hydrophobic
Arntarntarenhe – cultivated	6.94	218	present	None detected	10R 3,6 10R 3,4	Fine sandy clay loam	30-35%	High in nutrients and OM from regular fertiliser
Hatt Rd	6.94	145	None detected	None detected	2.5 YR 4,6 2.5 YR 3,6	Loam fine sandy	25%	
Aileron	6.95	143.4	present	None detected	2.5YR 4,8 10R 3,4	Fine sandy clay loam	30-35%	

Table A3 Components of the soil field test kit

Test	Method
pH	pH/EC meter
Electrical conductivity	pH/EC meter
Organic Matter	Several drops of H_2O_2 were placed on dry soil. Slow fizzle indicated presence of organic matter
Carbonates	Several drops of HCl were placed on dry soil. Bubbling indicated presence of carbonates.

Appendix 2

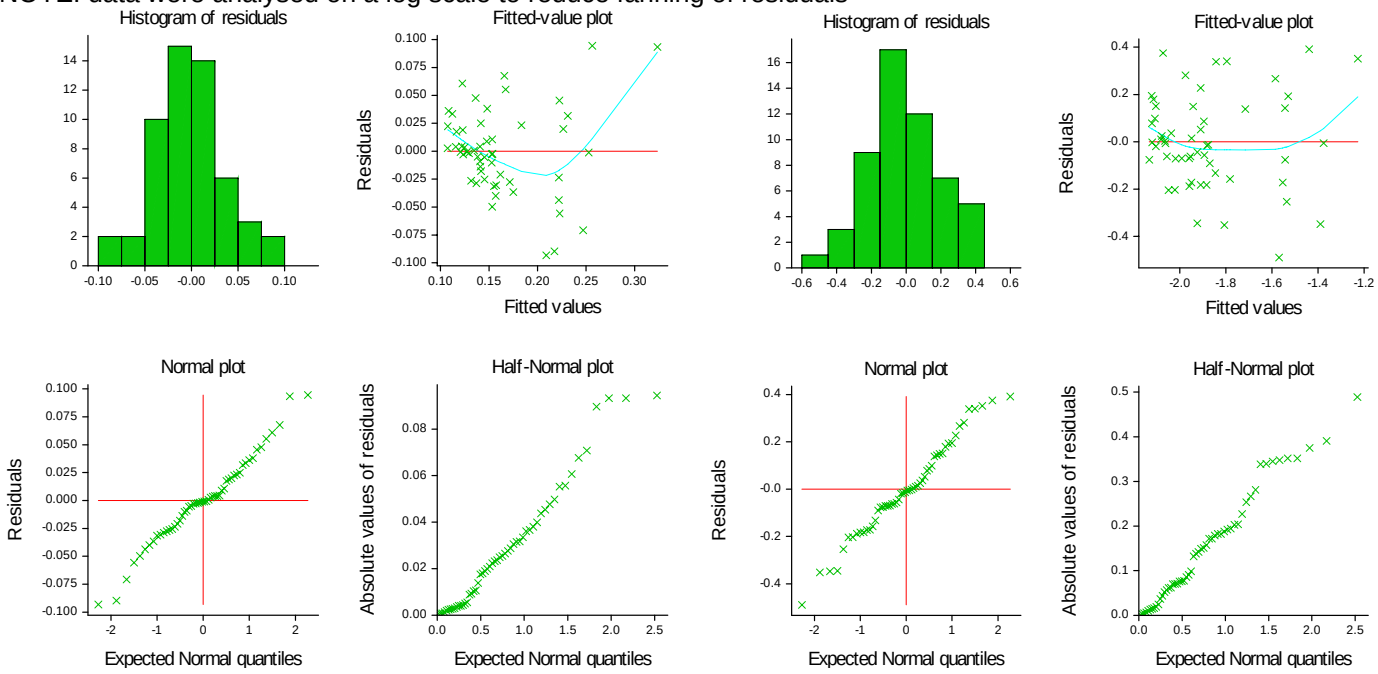
Statistical analysis

Appendix 2 - Contents

1. BIOMASS

a) Whole plant

NOTE: data were analysed on a log scale to reduce fanning of residuals




```
740  VCOMPONENTS [FIXED=Block+Mycorrhiza*Phosphorous; FACTORIAL=9]
RANDOM=Block.Treatment_Code;\
 741   INITIAL=1; CONSTRAINTS=positive
 742  REML [PRINT=model,components,means,deviance,waldTests; parameterization=sigmas;\
 743  PSE=alldifferences; MVINCLUDE=*;\
 744   METHOD=AI; MAXCYCLE=2000] log(Biomass_whole_plant)
```

REML variance components analysis

Response variate:	LOG(Biomass_whole_plant)
Fixed model:	Constant + Block + Mycorrhiza + Phosphorous + Mycorrhiza.Phosphorous
Random model:	Block.Treatment_Code
Number of units:	54 (18 units excluded due to zero weights or missing values)

Block.Treatment_Code used as residual term

Sparse algorithm with AI optimisation

Residual variance model

Term	Factor	Model(order)	Parameter	Estimate	s.e.
Block.Treatment_Code	Identity	Sigma2	0.0560	0.01301	

Deviance: -2*Log-Likelihood

Deviance	d.f.
-43.24	36

Note: deviance omits constants which depend on fixed model fitted.

Wald tests for fixed effects

Sequentially adding terms to fixed model

Fixed term	Wald statistic	d.f.	Wald/d.f.	chi pr
Block	9.74	11	0.89	0.554
Mycorrhiza	8.17	1	8.17	0.004
Phosphorous	27.59	2	13.79	<0.001
Mycorrhiza.Phosphorous	3.37	2	1.69	0.185

Dropping individual terms from full fixed model

Fixed term	Wald statistic	d.f.	Wald/d.f.	chi pr
Mycorrhiza.Phosphorous	3.37	2	1.69	0.185
Block	6.58	11	0.60	0.832

Message: chi-square distribution for Wald tests is an asymptotic approximation (i.e. for large samples) and underestimates the probabilities in other cases.

Table of predicted means for Constant

-1.879 Standard error: 0.0343

Table of predicted means for Block

Block	1	2	3	4	5	6	7	8
	-1.981	-1.624	-1.835	-1.938	-1.926	-1.899	-1.772	-1.965

Block	9	10	11	12
	-1.950	-1.939	-1.932	-1.786

Standard errors of differences between pairs

Block 1	1	*						
Block 2	2	0.207	*					
Block 3	3	0.162	0.209	*				
Block 4	4	0.162	0.209	0.170	*			
Block 5	5	0.151	0.203	0.162	0.162	*		
Block 6	6	0.151	0.206	0.162	0.162	0.151	*	
Block 7	7	0.152	0.203	0.160	0.160	0.152	0.152	*
Block 8	8	0.160	0.214	0.173	0.171	0.162	0.162	0.163
Block 9	9	0.152	0.203	0.160	0.160	0.152	0.152	0.150
Block 10	10	0.144	0.199	0.155	0.155	0.144	0.144	0.144

Block 11	11	0.152	0.203	0.160	0.160	0.152	0.152	0.150
Block 12	12	0.160	0.214	0.171	0.173	0.162	0.162	0.163
		1	2	3	4	5	6	7

Block 8	8	*				
Block 9	9	0.163	*			
Block 10	10	0.155	0.144	*		
Block 11	11	0.163	0.150	0.144	*	
Block 12	12	0.170	0.163	0.155	0.163	*
		8	9	10	11	12

Standard errors of differences

Average: 0.1655
Maximum: 0.2137
Minimum: 0.1439

Average variance of differences: 0.02776

Table of predicted means for Mycorrhiza

Mycorrhiza	N	Y
	-1.791	-1.967

Standard errors of differences between pairs

Mycorrhiza N	1	*	
Mycorrhiza Y	2	0.067	*
		1	2

Table of predicted means for Phosphorous

Phosphorous	h	l	o
	-1.953	-2.031	-1.652

Standard errors of differences between pairs

Phosphorous h	1	*		
Phosphorous l	2	0.087	*	
Phosphorous o	3	0.084	0.079	*
		1	2	3

Standard errors of differences

Average: 0.08339
Maximum: 0.08736
Minimum: 0.07909

Average variance of differences: 0.006966

Table of predicted means for Mycorrhiza.Phosphorous

Phosphorous	h	l	o
Mycorrhiza			
N	-1.889	-2.002	-1.482
Y	-2.017	-2.061	-1.822

Standard errors of differences between pairs

Mycorrhiza N.Phosphorous h	1	*				
Mycorrhiza N.Phosphorous l	2	0.111	*			
Mycorrhiza N.Phosphorous o	3	0.106	0.109	*		
Mycorrhiza Y.Phosphorous h	4	0.128	0.130	0.127	*	
Mycorrhiza Y.Phosphorous l	5	0.113	0.116	0.109	0.135	*
Mycorrhiza Y.Phosphorous o	6	0.111	0.115	0.109	0.130	0.116
		1	2	3	4	5

Mycorrhiza Y.Phosphorous o	6	*
		6

Standard errors of differences

Average:	0.1175
Maximum:	0.1351
Minimum:	0.1056

Average variance of differences: 0.01390

Standard error of differences for same level of factor:

	Mycorrhiza	Phosphorous
Average:	0.1177	0.1175
Maximum:	0.1351	0.1275
Minimum:	0.1056	0.1095

Average variance of differences:

0.01398	0.01387

b) Shoots

NOTE: original data were multiplied by 1000 to increase the precision of statistical tests

With correlated error structure

```
454  VCOMPONENTS [FIXED=Block+Mycorrhiza*Phosphorous; FACTORIAL=9]
RANDOM=Block.Treatment_Code;\
 455   INITIAL=1; CONSTRAINTS=positive
 456  VSTRUCTURE [TERMS=Block.Treatment_Code; FORMATION=direct]
MODEL=identity,diagonal;\
 457   ORDER=*,*; FACTOR=Block,Treatment_Code
 458  REML [PRINT=model,components,means,deviance,waldTests; parameterization=sigmas;\
 459  PSE=alldifferences; MVINCLUDE=*;\
 460   METHOD=AI; MAXCYCLE=2000] newbiomassShoots
```

REML variance components analysis

Response variate:	newbiomassShoots
Fixed model:	Constant + Block + Mycorrhiza + Phosphorous + Mycorrhiza.Phosphorous
Random model:	Block.Treatment_Code
Number of units:	54 (18 units excluded due to zero weights or missing values)

Residual term has been added to model

Sparse algorithm with AI optimisation

Covariance structures defined for random model

Covariance structures defined within terms:

Term	Factor	Model	Order	No. rows
Block.Treatment_Code	Block	Identity	0	12
	Treatment_Code			
		Diagonal	6	6

Estimated parameters for covariance models

Random term(s)	Factor	Model(order)	Parameter	Estimate	s.e.
Block.Treatment_Code					
	Block	Identity	-	-	-
	Treatment_Code	Diagonal	d_1	2344.	1093.
			d_2	458.7	274.9
			d_3	157.4	139.1
			d_4	165.8	172.7
			d_5	965.5	521.2
			d_6	205.5	165.6

Residual variance model

Term	Factor	Model(order)	Parameter	Estimate	s.e.
Residual		Identity	Sigma2	1.000	aliased

Deviance: -2*Log-Likelihood

Deviance	d.f.
295.64	31

Note: deviance omits constants which depend on fixed model fitted.

Wald tests for fixed effects

Sequentially adding terms to fixed model

Fixed term	Wald statistic	d.f.	Wald/d.f.	chi pr
Block	39.83	11	3.62	<0.001
Mycorrhiza	0.02	1	0.02	0.882
Phosphorous	6.99	2	3.50	0.030
Mycorrhiza.Phosphorous	0.73	2	0.36	0.695

Dropping individual terms from full fixed model

Fixed term	Wald statistic	d.f.	Wald/d.f.	chi pr
Mycorrhiza.Phosphorous	0.73	2	0.36	0.695
Block	43.27	11	3.93	<0.001

Message: chi-square distribution for Wald tests is an asymptotic approximation (i.e. for large samples) and underestimates the probabilities in other cases.

Table of predicted means for Constant

147.3 Standard error: 3.58

Table of predicted means for Block

Block	1	2	3	4	5	6	7	8
	120.9	189.9	137.4	148.8	139.0	149.8	151.1	119.3

Block	9	10	11	12
	146.6	140.3	149.9	175.2

Standard errors of differences between pairs

Block 1	1	*						
Block 2	2	17.0	*					
Block 3	3	12.4	16.8	*				
Block 4	4	12.0	16.6	12.4	*			
Block 5	5	11.7	16.7	12.7	12.3	*		
Block 6	6	10.7	16.1	11.6	11.2	11.0	*	
Block 7	7	11.8	16.5	12.2	11.9	12.1	11.0	*
Block 8	8	11.4	17.1	12.6	12.2	12.0	10.9	12.0

Block 9	9	11.8	16.5	12.2	11.9	12.1	11.0	11.6
Block 10	10	10.6	16.1	11.5	11.2	10.9	9.9	10.9
Block 11	11	11.8	16.5	12.2	11.9	12.1	11.0	11.6
Block 12	12	11.7	17.3	12.8	12.6	12.2	11.2	12.3
		1	2	3	4	5	6	7

Block 8	8	*				
Block 9	9	12.0	*			
Block 10	10	10.9	10.9	*		
Block 11	11	12.0	11.6	10.9	*	
Block 12	12	11.9	12.3	11.1	12.3	*
		8	9	10	11	12

Standard errors of differences

Average: 12.52
Maximum: 17.31
Minimum: 9.865

Average variance of differences: 160.5

Table of predicted means for Mycorrhiza

Mycorrhiza	N	Y
	148.7	146.0

Standard errors of differences between pairs

Mycorrhiza N	1	*	
Mycorrhiza Y	2	7.1	*
		1	2

Table of predicted means for Phosphorous

Phosphorous	h	l	o
	146.2	135.8	160.0

Standard errors of differences between pairs

Phosphorous h	1	*		
Phosphorous l	2	7.0	*	
Phosphorous o	3	8.9	10.0	*
		1	2	3

Standard errors of differences

Average: 8.632
Maximum: 10.01
Minimum: 6.953

Average variance of differences: 76.11

Table of predicted means for Mycorrhiza.Phosphorous

Phosphorous	h	l	o
Mycorrhiza			
N	144.4	135.9	165.7
Y	148.1	135.7	154.3

Standard errors of differences between pairs

Mycorrhiza N.Phosphorous h	1	*				
Mycorrhiza N.Phosphorous l	2	11.2	*			
Mycorrhiza N.Phosphorous o	3	15.2	18.0	*		
Mycorrhiza Y.Phosphorous h	4	7.2	12.0	15.8	*	
Mycorrhiza Y.Phosphorous l	5	6.7	11.7	15.5	8.2	*
Mycorrhiza Y.Phosphorous o	6	8.3	12.7	16.4	9.3	8.9
		1	2	3	4	5

Mycorrhiza Y.Phosphorous o	6	*
		6

Standard errors of differences

Average:	11.82
Maximum:	18.01
Minimum:	6.723

Average variance of differences: 152.2

Standard error of differences for same level of factor:

	Mycorrhiza	Phosphorous
Average:	11.82	11.75
Maximum:	18.01	16.37
Minimum:	8.246	7.220

Average variance of differences:

152.7 152.1

Without correlated error structure:

```
464  VCOMPONENTS [FIXED=Block+Mycorrhiza*Phosphorous; FACTORIAL=9]
RANDOM=Block.Treatment_Code;\
 465   INITIAL=1; CONSTRAINTS=positive
 466  REML [PRINT=model,components,deviance,waldTests; MVINCLUDE=*; METHOD=AI;
MAXCYCLE=2000]\
 467   newbiomassShoots
```

REML variance components analysis

Response variate: newbiomassShoots
Fixed model: Constant + Block + Mycorrhiza + Phosphorous + Mycorrhiza.Phosphorous
Random model: Block.Treatment_Code
Number of units: 54 (18 units excluded due to zero weights or missing values)

Block.Treatment_Code used as residual term

Sparse algorithm with AI optimisation

Residual variance model

Term	Factor	Model(order)	Parameter	Estimate	s.e.
Block.Treatment_Code	Identity	Sigma2	806.0	187.4	

Deviance: -2*Log-Likelihood

Deviance	d.f.
311.05	36

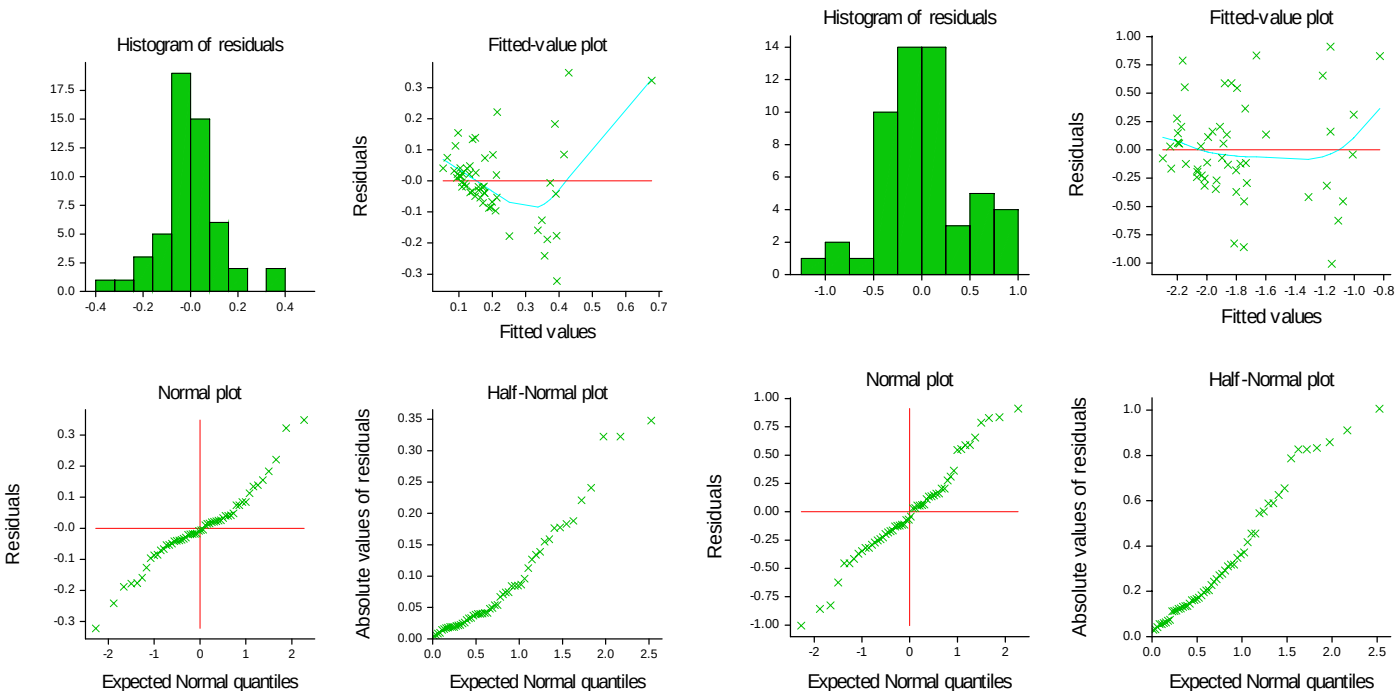
Note: deviance omits constants which depend on fixed model fitted.

Deviance change between models:
311.05 – 295.64 = 15.41
df = 5

P=0.009

c) Roots

NOTE: data were analysed on a log scale to reduce fanning of residuals




```
759  VCOMPONENTS [FIXED=Block+Mycorrhiza*Phosphorous; FACTORIAL=9]
RANDOM=Block.Treatment_Code;\
 760   INITIAL=1; CONSTRAINTS=positive
 761  REML [PRINT=model,components,means,deviance,waldTests; PSE=alldifferences;
MVINCLUDE=*;\
 762   METHOD=AI; MAXCYCLE=2000] log(Biomass_roots)
```

REML variance components analysis

Response variate:	LOG(Biomass_roots)
Fixed model:	Constant + Block + Mycorrhiza + Phosphorous + Mycorrhiza.Phosphorous
Random model:	Block.Treatment_Code
Number of units:	54 (18 units excluded due to zero weights or missing values)

Block.Treatment_Code used as residual term

Sparse algorithm with AI optimisation

Residual variance model

Term	Factor	Model(order)	Parameter	Estimate	s.e.
Block.Treatment_Code	Identity	Sigma2	0.257	0.0597	

Deviance: -2*Log-Likelihood

Deviance	d.f.
13.13	36

Note: deviance omits constants which depend on fixed model fitted.

Wald tests for fixed effects

Sequentially adding terms to fixed model

Fixed term	Wald statistic	d.f.	Wald/d.f.	chi pr
Block	4.98	11	0.45	0.932
Mycorrhiza	6.26	1	6.26	0.012
Phosphorous	18.16	2	9.08	<0.001
Mycorrhiza.Phosphorous	1.79	2	0.90	0.408

Dropping individual terms from full fixed model

Fixed term	Wald statistic	d.f.	Wald/d.f.	chi pr
Mycorrhiza.Phosphorous	1.79	2	0.90	0.408
Block	2.19	11	0.20	0.998

Message: chi-square distribution for Wald tests is an asymptotic approximation (i.e. for large samples) and underestimates the probabilities in other cases.

Table of predicted means for Constant

-1.817 Standard error: 0.0734

Table of predicted means for Block

Block	1	2	3	4	5	6	7	8
	-1.860	-1.525	-1.701	-1.775	-1.859	-1.906	-1.709	-1.851

Block	9	10	11	12
	-2.009	-1.912	-1.884	-1.807

Standard errors of differences between pairs

Block 1	1	*						
Block 2	2	0.443	*					
Block 3	3	0.348	0.447	*				
Block 4	4	0.348	0.447	0.364	*			
Block 5	5	0.324	0.435	0.347	0.347	*		
Block 6	6	0.324	0.441	0.347	0.347	0.324	*	
Block 7	7	0.326	0.434	0.342	0.342	0.325	0.325	*
Block 8	8	0.342	0.458	0.371	0.366	0.346	0.346	0.348
Block 9	9	0.326	0.434	0.342	0.342	0.325	0.325	0.320
Block 10	10	0.309	0.426	0.332	0.332	0.308	0.308	0.309
Block 11	11	0.326	0.434	0.342	0.342	0.325	0.325	0.320
Block 12	12	0.342	0.458	0.366	0.371	0.346	0.346	0.348
		1	2	3	4	5	6	7

Block 8	8	*				
Block 9	9	0.348	*			
Block 10	10	0.331	0.309	*		
Block 11	11	0.348	0.320	0.309	*	
Block 12	12	0.364	0.348	0.331	0.348	*
		8	9	10	11	12

Standard errors of differences

Average:	0.3544
Maximum:	0.4577
Minimum:	0.3082

Average variance of differences: 0.1274

Table of predicted means for Mycorrhiza

Mycorrhiza	N	Y
	-1.645	-1.988

Standard errors of differences between pairs

Mycorrhiza N	1	*	
Mycorrhiza Y	2	0.144	*
		1	2

Table of predicted means for Phosphorous

Phosphorous	h	l	o
	-1.998	-2.039	-1.412

Standard errors of differences between pairs

Phosphorous h	1	*		
Phosphorous l	2	0.187	*	
Phosphorous o	3	0.179	0.169	*
		1	2	3

Standard errors of differences

Average:	0.1786
Maximum:	0.1871
Minimum:	0.1694

Average variance of differences: 0.03196

Table of predicted means for Mycorrhiza.Phosphorous

Phosphorous	h	l	o
Mycorrhiza			
N	-1.843	-1.972	-1.119
Y	-2.153	-2.106	-1.706

Standard errors of differences between pairs

Mycorrhiza N.Phosphorous h	1	*				
Mycorrhiza N.Phosphorous l	2	0.237	*			
Mycorrhiza N.Phosphorous o	3	0.226	0.235	*		
Mycorrhiza Y.Phosphorous h	4	0.273	0.278	0.271	*	
Mycorrhiza Y.Phosphorous l	5	0.242	0.248	0.233	0.289	*
Mycorrhiza Y.Phosphorous o	6	0.237	0.246	0.235	0.278	0.248
		1	2	3	4	5

Mycorrhiza Y.Phosphorous o	6	*
		6

Standard errors of differences

Average:	0.2517
Maximum:	0.2894
Minimum:	0.2263

Average variance of differences: 0.06376

Standard error of differences for same level of factor:

	Mycorrhiza	Phosphorous
Average:	0.2522	0.2518
Maximum:	0.2894	0.2732
Minimum:	0.2263	0.2345

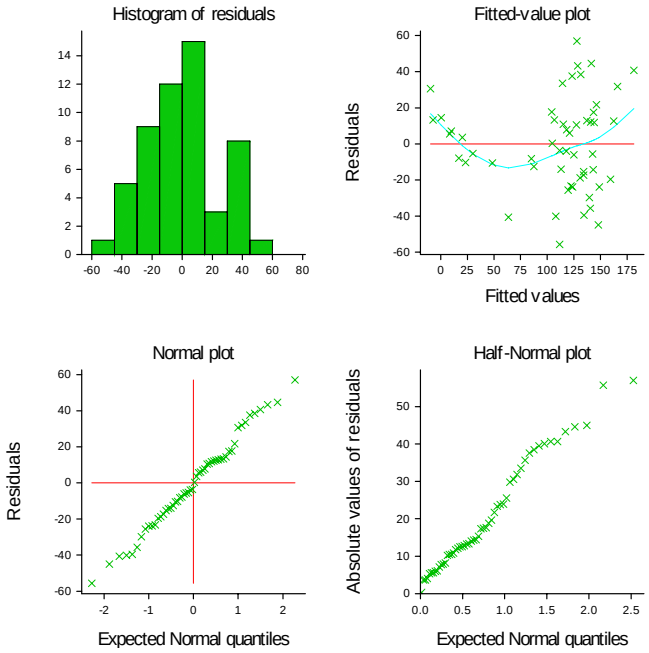
Average variance of differences:

0.06414	0.06366

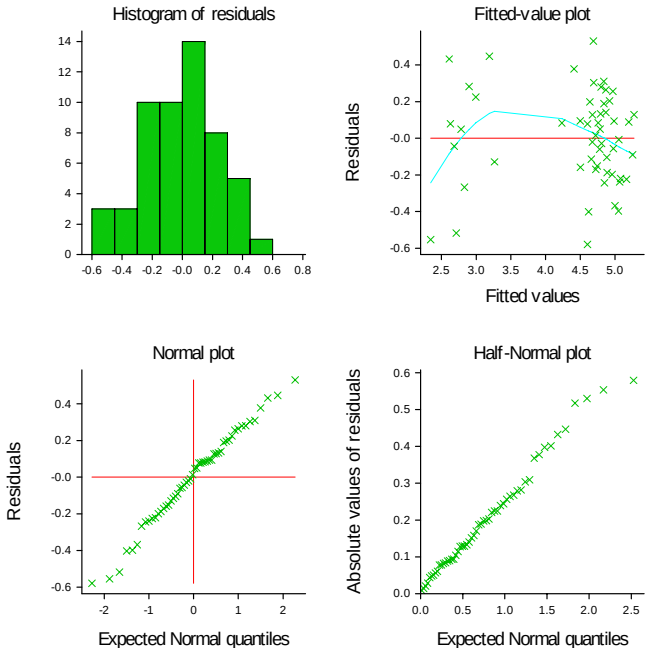
2. HEIGHT

Data were analysed on a log scale:

Original data



Log(height)



Analysis of log(height):

```
496  VCOMPONENTS [FIXED=Block+Mycorrhiza*Phosphorous; FACTORIAL=9]
RANDOM=Block.Treatment_Code;\
 497   INITIAL=1; CONSTRAINTS=positive
 498  REML [PRINT=model,components,means,deviance,waldTests; PSE=alldifferences;
MVINCLUDE=*;\
 499   METHOD=AI; MAXCYCLE=2000] log(Height)
```

REML variance components analysis

Response variate:	LOG(Height)
Fixed model:	Constant + Block + Mycorrhiza + Phosphorous + Mycorrhiza.Phosphorous
Random model:	Block.Treatment_Code
Number of units:	54 (18 units excluded due to zero weights or missing values)

Block.Treatment_Code used as residual term

Sparse algorithm with AI optimisation

Residual variance model

Term	Factor	Model(order)	Parameter	Estimate	s.e.
Block.Treatment_Code	Identity	Sigma2	0.0932	0.02167	

Deviance: -2*Log-Likelihood

Deviance	d.f.
-24.37	36

Note: deviance omits constants which depend on fixed model fitted.

Wald tests for fixed effects

Sequentially adding terms to fixed model

Fixed term	Wald statistic	d.f.	Wald/d.f.	chi pr
Block	45.42	11	4.13	<0.001
Mycorrhiza	72.13	1	72.13	<0.001
Phosphorous	187.28	2	93.64	<0.001
Mycorrhiza.Phosphorous	104.93	2	52.47	<0.001

Dropping individual terms from full fixed model

Fixed term	Wald statistic	d.f.	Wald/d.f.	chi pr
Mycorrhiza.Phosphorous	104.93	2	52.47	<0.001
Block	27.49	11	2.50	0.004

Message: chi-square distribution for Wald tests is an asymptotic approximation (i.e. for large samples) and underestimates the probabilities in other cases.

Table of predicted means for Constant

4.504 Standard error: 0.0442

Table of predicted means for Block

Block	1	2	3	4	5	6	7	8
	4.579	4.947	4.312	4.295	4.515	4.594	4.873	4.028

Block	9	10	11	12
	4.676	4.397	4.467	4.365

Standard errors of differences between pairs

Block 1	1	*					
Block 2	2	0.267	*				
Block 3	3	0.210	0.269	*			
Block 4	4	0.210	0.269	0.219	*		
Block 5	5	0.195	0.262	0.209	0.209	*	
Block 6	6	0.195	0.266	0.209	0.209	0.195	*

Block 7	7	0.196	0.262	0.206	0.206	0.196	0.196	*
Block 8	8	0.206	0.276	0.223	0.220	0.209	0.208	0.210
Block 9	9	0.196	0.262	0.206	0.206	0.196	0.196	0.193
Block 10	10	0.186	0.257	0.200	0.200	0.186	0.186	0.186
Block 11	11	0.196	0.262	0.206	0.206	0.196	0.196	0.193
Block 12	12	0.206	0.276	0.220	0.223	0.209	0.208	0.210
		1	2	3	4	5	6	7

Block 8	8	*				
Block 9	9	0.210	*			
Block 10	10	0.199	0.186	*		
Block 11	11	0.210	0.193	0.186	*	
Block 12	12	0.219	0.210	0.199	0.210	*
		8	9	10	11	12

Standard errors of differences

Average:	0.2135
Maximum:	0.2757
Minimum:	0.1857

Average variance of differences: 0.04623

Table of predicted means for Mycorrhiza

Mycorrhiza	N	Y
	4.168	4.840

Standard errors of differences between pairs

Mycorrhiza N	1	*	
Mycorrhiza Y	2	0.087	*
		1	2

Table of predicted means for Phosphorous

Phosphorous	h	l	o
	4.931	4.814	3.767

Standard errors of differences between pairs

Phosphorous h	1	*		
Phosphorous l	2	0.113	*	
Phosphorous o	3	0.108	0.102	*
		1	2	3

Standard errors of differences

Average:	0.1076

Maximum: 0.1127
Minimum: 0.1021

Average variance of differences: 0.01160

Table of predicted means for Mycorrhiza.Phosphorous

Phosphorous	h	l	o
Mycorrhiza			
N	4.886	4.797	2.822
Y	4.977	4.831	4.711

Standard errors of differences between pairs

Mycorrhiza N.Phosphorous h	1	*				
Mycorrhiza N.Phosphorous l	2	0.143	*			
Mycorrhiza N.Phosphorous o	3	0.136	0.141	*		
Mycorrhiza Y.Phosphorous h	4	0.165	0.167	0.163	*	
Mycorrhiza Y.Phosphorous l	5	0.146	0.149	0.141	0.174	*
Mycorrhiza Y.Phosphorous o	6	0.143	0.148	0.141	0.167	0.149
		1	2	3	4	5

Mycorrhiza Y.Phosphorous o	6	*
		6

Standard errors of differences

Average: 0.1517
Maximum: 0.1743
Minimum: 0.1363

Average variance of differences: 0.02314

Standard error of differences for same level of factor:

	Mycorrhiza	Phosphorous
Average:	0.1519	0.1517
Maximum:	0.1743	0.1646
Minimum:	0.1363	0.1413

Average variance of differences:

0.02328	0.02310

3. PHOSPHORUS CONCENTRATION

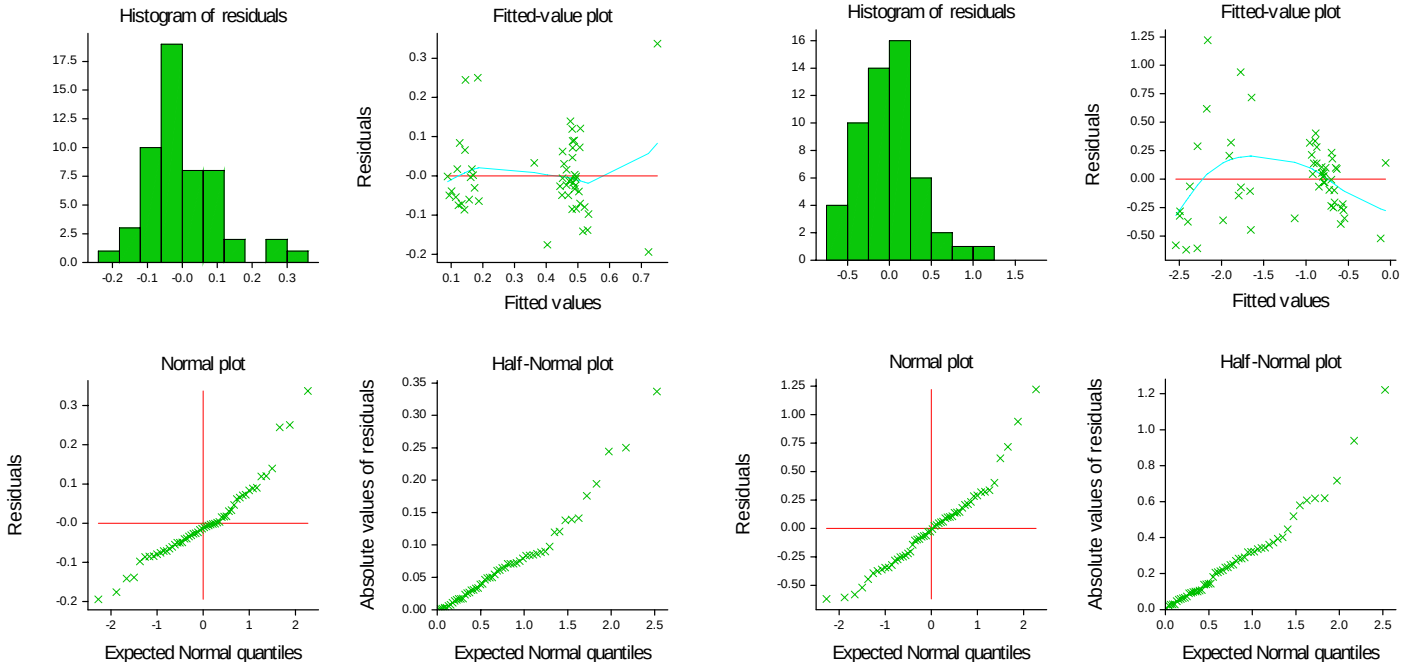
a) Shoots (%)

Several shoot measurements were adjusted as the analysis was conducted on a different day (and thus with a different standard curve and under different conditions – see materials and method)
Log transformation *increased* inequality of variance and thus no transformation was applied. Note that correlated error structure did not significantly improve the model:

Change in deviance = -94.55 - (-102.86)
= 8.31 on 36-31=5 df

P = 0.140

Original data **log(ShootPhosphorus)**




```
      368  VCOMPONENTS [FIXED=Block+Mycorrhiza*Phosphorous; FACTORIAL=9]
RANDOM=Block.Treatment_Code;\
 369   INITIAL=1; CONSTRAINTS=positive
 370  REML [PRINT=model,components,means,deviance,waldTests; PSE=alldifferences;
MVINCLUDE=*;\
 371   METHOD=AI; MAXCYCLE=2000] phos_shoots%_alt
```

REML variance components analysis

Response variate:	phos_shoots%_alt
Fixed model:	Constant + Block + Mycorrhiza + Phosphorous + Mycorrhiza.Phosphorous
Random model:	Block.Treatment_Code
Number of units:	54 (18 units excluded due to zero weights or missing values)

Block.Treatment_Code used as residual term

Sparse algorithm with AI optimisation

Residual variance model

Term	Factor	Model(order)	Parameter	Estimate	s.e.
Block.Treatment_Code	Identity	Sigma2	0.0140	0.00325	

Deviance: -2*Log-Likelihood

Deviance	d.f.
-94.55	36

Note: deviance omits constants which depend on fixed model fitted.

Wald tests for fixed effects

Sequentially adding terms to fixed model

Fixed term	Wald statistic	d.f.	Wald/d.f.	chi pr
Block	14.01	11	1.27	0.232
Mycorrhiza	0.00	1	0.00	0.957
Phosphorous	105.11	2	52.55	<0.001
Mycorrhiza.Phosphorous	0.82	2	0.41	0.664

Dropping individual terms from full fixed model

Fixed term	Wald statistic	d.f.	Wald/d.f.	chi pr
Mycorrhiza.Phosphorous	0.82	2	0.41	0.664
Block	15.71	11	1.43	0.152

Message: chi-square distribution for Wald tests is an asymptotic approximation (i.e. for large samples) and underestimates the probabilities in other cases.

Table of predicted means for Constant

0.3909 Standard error: 0.01714

Table of predicted means for Block

Block	1	2	3	4	5	6	7	8
	0.3778	0.3841	0.3448	0.3925	0.3737	0.3830	0.3651	0.6128

Block	9	10	11	12
	0.3699	0.3957	0.3522	0.3397

Standard errors of differences between pairs

Block 1	1	*

Block 2	2	0.1033	*					
Block 3	3	0.0812	0.1043	*				
Block 4	4	0.0812	0.1043	0.0848	*			
Block 5	5	0.0756	0.1016	0.0810	0.0810	*		
Block 6	6	0.0756	0.1030	0.0810	0.0810	0.0755	*	
Block 7	7	0.0760	0.1013	0.0798	0.0798	0.0759	0.0758	*
Block 8	8	0.0798	0.1068	0.0865	0.0853	0.0808	0.0808	0.0813
Block 9	9	0.0760	0.1013	0.0798	0.0798	0.0759	0.0758	0.0748
Block 10	10	0.0720	0.0995	0.0774	0.0774	0.0719	0.0719	0.0722
Block 11	11	0.0760	0.1013	0.0798	0.0798	0.0759	0.0758	0.0748
Block 12	12	0.0798	0.1068	0.0853	0.0865	0.0808	0.0808	0.0813
		1	2	3	4	5	6	7

Block 8	8	*				
Block 9	9	0.0813	*			
Block 10	10	0.0773	0.0722	*		
Block 11	11	0.0813	0.0748	0.0722	*	
Block 12	12	0.0848	0.0813	0.0773	0.0813	*
		8	9	10	11	12

Standard errors of differences

Average:	0.08271
Maximum:	0.1068
Minimum:	0.07193

Average variance of differences: 0.006937

Table of predicted means for Mycorrhiza

Mycorrhiza	N	Y
	0.3906	0.3913

Standard errors of differences between pairs

Mycorrhiza N	1	*	
Mycorrhiza Y	2	0.0336	*
		1	2

Table of predicted means for Phosphorous

Phosphorous	h	l	o
	0.5150	0.4965	0.1613

Standard errors of differences between pairs

Phosphorous h	1	*		
Phosphorous l	2	0.0437	*	
Phosphorous o	3	0.0419	0.0395	*

	1	2	3

Standard errors of differences

Average:	0.04168
Maximum:	0.04367
Minimum:	0.03953

Average variance of differences: 0.001740

Table of predicted means for Mycorrhiza.Phosphorous

Phosphorous Mycorrhiza	h	l	o
N	0.5288	0.5027	0.1403
Y	0.5012	0.4902	0.1823

Standard errors of differences between pairs

Mycorrhiza N.Phosphorous h	1	*				
Mycorrhiza N.Phosphorous l	2	0.0554	*			
Mycorrhiza N.Phosphorous o	3	0.0528	0.0547	*		
Mycorrhiza Y.Phosphorous h	4	0.0637	0.0649	0.0633	*	
Mycorrhiza Y.Phosphorous l	5	0.0566	0.0578	0.0544	0.0675	*
Mycorrhiza Y.Phosphorous o	6	0.0554	0.0574	0.0547	0.0649	0.0578
		1	2	3	4	5

Mycorrhiza Y.Phosphorous o	6	*
		6

Standard errors of differences

Average:	0.05875
Maximum:	0.06752
Minimum:	0.05280

Average variance of differences: 0.003472

Standard error of differences for same level of factor:

	Mycorrhiza	Phosphorous
Average:	0.05884	0.05876
Maximum:	0.06752	0.06374
Minimum:	0.05280	0.05473

Average variance of differences:

0.003492	0.003466

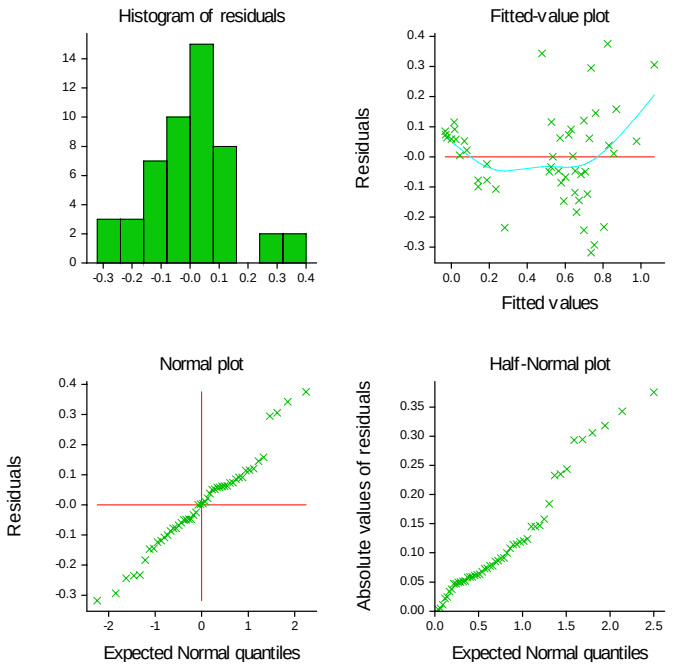
b) Roots (%)

Prior to analysis a slight negative value (calculated based on the standard curve for P content) and two outlier values resulting from suspected contamination were removed. Data were log-transformed prior to analysis. Also note that one value was already missing. Addition of a correlated error structure did not significantly improve the analysis.

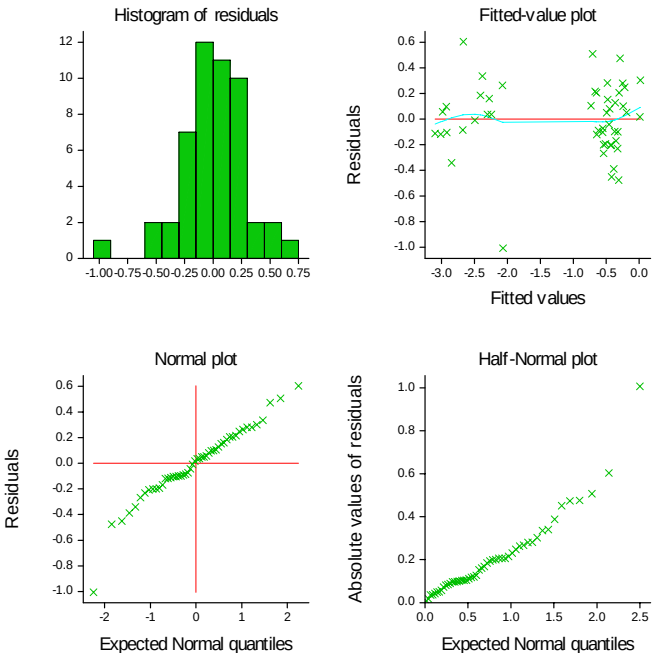
Change in deviance = -14.01 - -20.34
= 6.33 on 32-27 = 5 df

P = 0.275

Original data



log(PhosphorusRoots%)




```
447  VCOMPONENTS [FIXED=Block+Mycorrhiza*Phosphorous; FACTORIAL=9]
RANDOM=Block.Treatment_Code
 448  REML [PRINT=model,components,means,deviance,waldTests; PSE=alldifferences;
MVINCLUDE=*;\
 449   METHOD=AI; MAXCYCLE=2000] log(phosphorous_root%)
```

REML variance components analysis

Response variate:	LOG(phosphorous_root%)
Fixed model:	Constant + Block + Mycorrhiza + Phosphorous + Mycorrhiza.Phosphorous
Random model:	Block.Treatment_Code
Number of units:	50 (22 units excluded due to zero weights or missing values)

Block.Treatment_Code used as residual term

Sparse algorithm with AI optimisation

Residual variance model

Term	Factor	Model(order)	Parameter	Estimate	s.e.
Block.Treatment_Code	Identity	Sigma2	0.114	0.0279	

Deviance: -2*Log-Likelihood

Deviance	d.f.
-14.01	32

Note: deviance omits constants which depend on fixed model fitted.

Wald tests for fixed effects

Sequentially adding terms to fixed model

Fixed term	Wald statistic	d.f.	Wald/d.f.	chi pr
Block	47.86	11	4.35	<0.001
Mycorrhiza	4.69	1	4.69	0.030
Phosphorous	419.12	2	209.56	<0.001
Mycorrhiza.Phosphorous	3.25	2	1.63	0.197

Dropping individual terms from full fixed model

Fixed term	Wald statistic	d.f.	Wald/d.f.	chi pr
Mycorrhiza.Phosphorous	3.25	2	1.63	0.197
Block	7.34	11	0.67	0.771

Message: chi-square distribution for Wald tests is an asymptotic approximation (i.e. for large samples) and underestimates the probabilities in other cases.

Table of predicted means for Constant

-1.131 Standard error: 0.0517

Table of predicted means for Block

Block	1	2	3	4	5	6	7	8
	-0.904	-1.154	-1.146	-1.087	-1.239	-1.133	-1.323	-0.896

Block	9	10	11	12
	-1.210	-1.105	-1.073	-1.298

Standard errors of differences between pairs

Block 1	1	*					
Block 2	2	0.296	*				
Block 3	3	0.232	0.298	*			
Block 4	4	0.295	0.347	0.298	*		
Block 5	5	0.216	0.291	0.231	0.294	*	
Block 6	6	0.216	0.296	0.232	0.290	0.216	*

Block 7	7	0.217	0.290	0.228	0.289	0.216	0.217	*
Block 8	8	0.228	0.306	0.247	0.305	0.230	0.231	0.232
Block 9	9	0.217	0.290	0.228	0.289	0.216	0.217	0.213
Block 10	10	0.216	0.296	0.232	0.290	0.216	0.213	0.217
Block 11	11	0.217	0.290	0.228	0.289	0.216	0.217	0.213
Block 12	12	0.250	0.329	0.267	0.321	0.254	0.250	0.256
		1	2	3	4	5	6	7

Block 8	8	*					
Block 9	9	0.232	*				
Block 10	10	0.231	0.217	*			
Block 11	11	0.232	0.213	0.217	*		
Block 12	12	0.264	0.256	0.250	0.256	*	
		8	9	10	11	12	

Standard errors of differences

Average:	0.2514
Maximum:	0.3474
Minimum:	0.2131

Average variance of differences: 0.06453

Table of predicted means for Mycorrhiza

Mycorrhiza	N	Y
	-1.306	-0.956

Standard errors of differences between pairs

Mycorrhiza N	1	*	
Mycorrhiza Y	2	0.099	*
		1	2

Table of predicted means for Phosphorous

Phosphorous	h	l	o
	-0.347	-0.444	-2.601

Standard errors of differences between pairs

Phosphorous h	1	*		
Phosphorous l	2	0.125	*	
Phosphorous o	3	0.129	0.121	*
		1	2	3

Standard errors of differences

Average:	0.1249

Maximum: 0.1290
Minimum: 0.1206

Average variance of differences: 0.01560

Table of predicted means for Mycorrhiza.Phosphorous

Phosphorous	h	l	o
Mycorrhiza			
N	-0.476	-0.538	-2.903
Y	-0.218	-0.349	-2.300

Standard errors of differences between pairs

Mycorrhiza N.Phosphorous h	1	*				
Mycorrhiza N.Phosphorous l	2	0.159	*			
Mycorrhiza N.Phosphorous o	3	0.169	0.172	*		
Mycorrhiza Y.Phosphorous h	4	0.183	0.185	0.197	*	
Mycorrhiza Y.Phosphorous l	5	0.162	0.167	0.171	0.195	*
Mycorrhiza Y.Phosphorous o	6	0.165	0.168	0.175	0.189	0.173
		1	2	3	4	5

Mycorrhiza Y.Phosphorous o	6	*
		6

Standard errors of differences

Average: 0.1753
Maximum: 0.1975
Minimum: 0.1587

Average variance of differences: 0.03086

Standard error of differences for same level of factor:

	Mycorrhiza	Phosphorous
Average:	0.1761	0.1749
Maximum:	0.1950	0.1830
Minimum:	0.1587	0.1665

Average variance of differences:

0.03116	0.03064

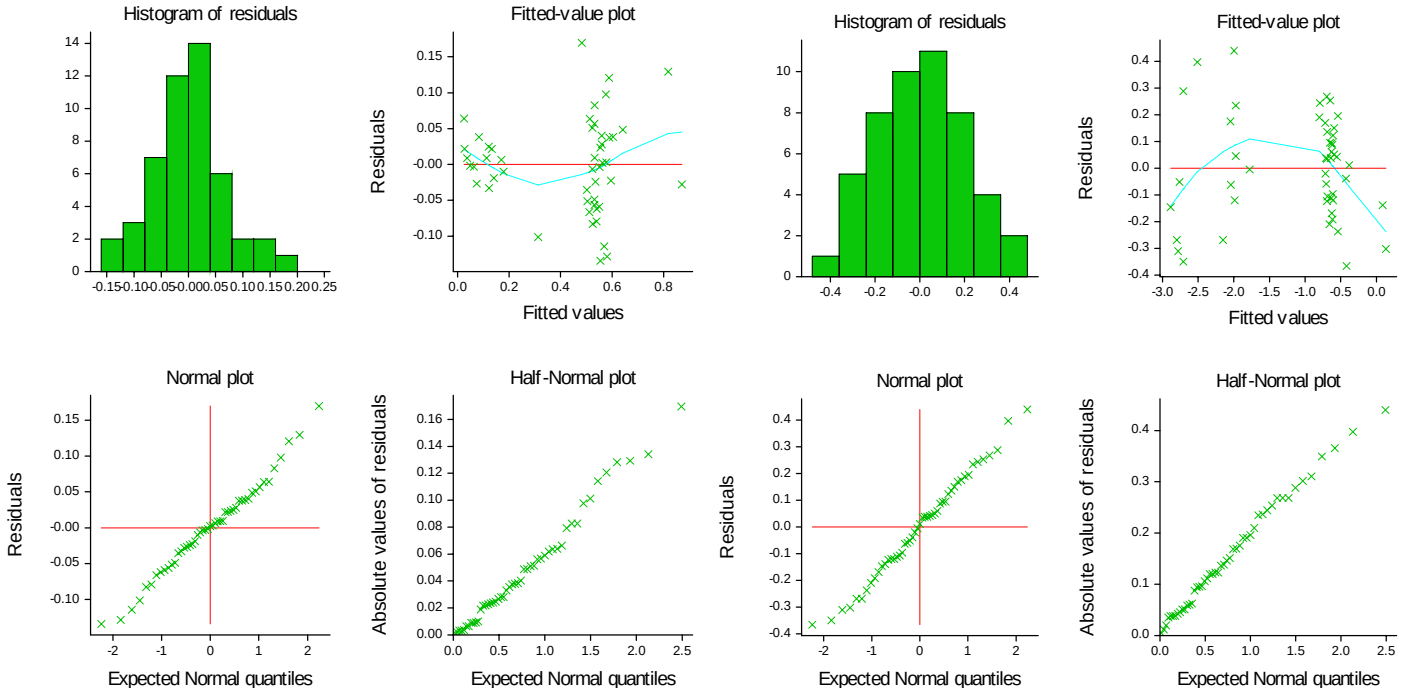
c) Whole plant (%)

Whole plant P was calculated by adding the total P content of the roots and shoots and dividing by whole plant weight (see materials and method). Prior to analysis one outlier was removed due to suspected error in calculation of root P and thus extreme value of whole plant P. Furthermore several whole plant P values could not be calculated due to previous removal of three root P values. Data were log-transformed prior to analysis. Also note that one value was already missing. Addition of a correlated error structure did not significantly improve the analysis.

Change in deviance = -35.78 - -45.11
= 9.33 on 31-26 = 5 df

P = 0.097

Original data **log(PhosphorusWholePlant%)**




```
543  VCOMPONENTS [FIXED=Block+Mycorrhiza*Phosphorous; FACTORIAL=9]
RANDOM=Block.Treatment_Code
 544  REML [PRINT=model,components,means,deviance,waldTests; PSE=alldifferences;
MVINCLUDE=*;\
 545   METHOD=AI; MAXCYCLE=2000] log(Phos%_wholeplant)
```

REML variance components analysis

Response variate: LOG(Phos%_wholeplant)
Fixed model: Constant + Block + Mycorrhiza + Phosphorous + Mycorrhiza.Phosphorous
Random model: Block.Treatment_Code
Number of units: 49 (23 units excluded due to zero weights or missing values)

Block.Treatment_Code used as residual term

Sparse algorithm with AI optimisation

Residual variance model

Term	Factor	Model(order)	Parameter	Estimate	s.e.
Block.Treatment_Code		Identity	Sigma2	0.0562	0.01404

Deviance: -2*Log-Likelihood

Deviance	d.f.
-35.78	31

Note: deviance omits constants which depend on fixed model fitted.

Wald tests for fixed effects

Sequentially adding terms to fixed model

Fixed term	Wald statistic	d.f.	Wald/d.f.	chi pr
Block	73.63	11	6.69	<0.001
Mycorrhiza	9.70	1	9.70	0.002
Phosphorous	530.14	2	265.07	<0.001
Mycorrhiza.Phosphorous	18.73	2	9.36	<0.001

Dropping individual terms from full fixed model

Fixed term	Wald statistic	d.f.	Wald/d.f.	chi pr
Mycorrhiza.Phosphorous	18.73	2	9.36	<0.001
Block	32.28	11	2.93	<0.001

Message: chi-square distribution for Wald tests is an asymptotic approximation (i.e. for large samples) and underestimates the probabilities in other cases.

Table of predicted means for Constant

-1.150 Standard error: 0.0369

Table of predicted means for Block

Block	1	2	3	4	5	6	7	8
	-0.976	-1.229	-1.266	-1.243	-1.247	-1.188	-1.350	-0.463

Block	9	10	11	12
	-1.173	-1.242	-1.174	-1.252

Standard errors of differences between pairs

Block 1	1	*	
Block 2	2	0.208	*

Block 3	3	0.163	0.210	*				
Block 4	4	0.207	0.244	0.209	*			
Block 5	5	0.152	0.205	0.163	0.207	*		
Block 6	6	0.152	0.209	0.163	0.204	0.152	*	
Block 7	7	0.152	0.204	0.160	0.204	0.152	0.152	*
Block 8	8	0.176	0.226	0.187	0.226	0.179	0.179	0.180
Block 9	9	0.152	0.204	0.160	0.204	0.152	0.152	0.150
Block 10	10	0.152	0.209	0.163	0.204	0.152	0.150	0.152
Block 11	11	0.152	0.204	0.160	0.204	0.152	0.152	0.150
Block 12	12	0.176	0.232	0.188	0.226	0.179	0.176	0.180
		1	2	3	4	5	6	7

Block 8	8	*				
Block 9	9	0.180	*			
Block 10	10	0.179	0.152	*		
Block 11	11	0.180	0.150	0.152	*	
Block 12	12	0.198	0.180	0.176	0.180	*
		8	9	10	11	12

Standard errors of differences

Average:	0.1794
Maximum:	0.2444
Minimum:	0.1499

Average variance of differences: 0.03283

Table of predicted means for Mycorrhiza

Mycorrhiza	N	Y
	-1.293	-1.007

Standard errors of differences between pairs

Mycorrhiza N	1	*	
Mycorrhiza Y	2	0.071	*
		1	2

Table of predicted means for Phosphorous

Phosphorous	h	l	o
	-0.579	-0.555	-2.316

Standard errors of differences between pairs

Phosphorous h	1	*		
Phosphorous l	2	0.088	*	
Phosphorous o	3	0.093	0.085	*
		1	2	3

Standard errors of differences

Average:	0.08894
Maximum:	0.09296
Minimum:	0.08540

Average variance of differences: 0.007920

Table of predicted means for Mycorrhiza.Phosphorous

Phosphorous / Mycorrhiza	h	l	o
N	-0.602	-0.594	-2.683
Y	-0.556	-0.517	-1.950

Standard errors of differences between pairs

Mycorrhiza N.Phosphorous h	1	*				
Mycorrhiza N.Phosphorous l	2	0.112	*			
Mycorrhiza N.Phosphorous o	3	0.119	0.121	*		
Mycorrhiza Y.Phosphorous h	4	0.129	0.131	0.139	*	
Mycorrhiza Y.Phosphorous l	5	0.114	0.117	0.120	0.138	*
Mycorrhiza Y.Phosphorous o	6	0.122	0.122	0.129	0.139	0.125
		1	2	3	4	5

Mycorrhiza Y.Phosphorous o	6	*
		6

Standard errors of differences

Average:	0.1251
Maximum:	0.1394
Minimum:	0.1119

Average variance of differences: 0.01573

Standard error of differences for same level of factor:

	Mycorrhiza	Phosphorous
Average:	0.1257	0.1250
Maximum:	0.1394	0.1291
Minimum:	0.1119	0.1171

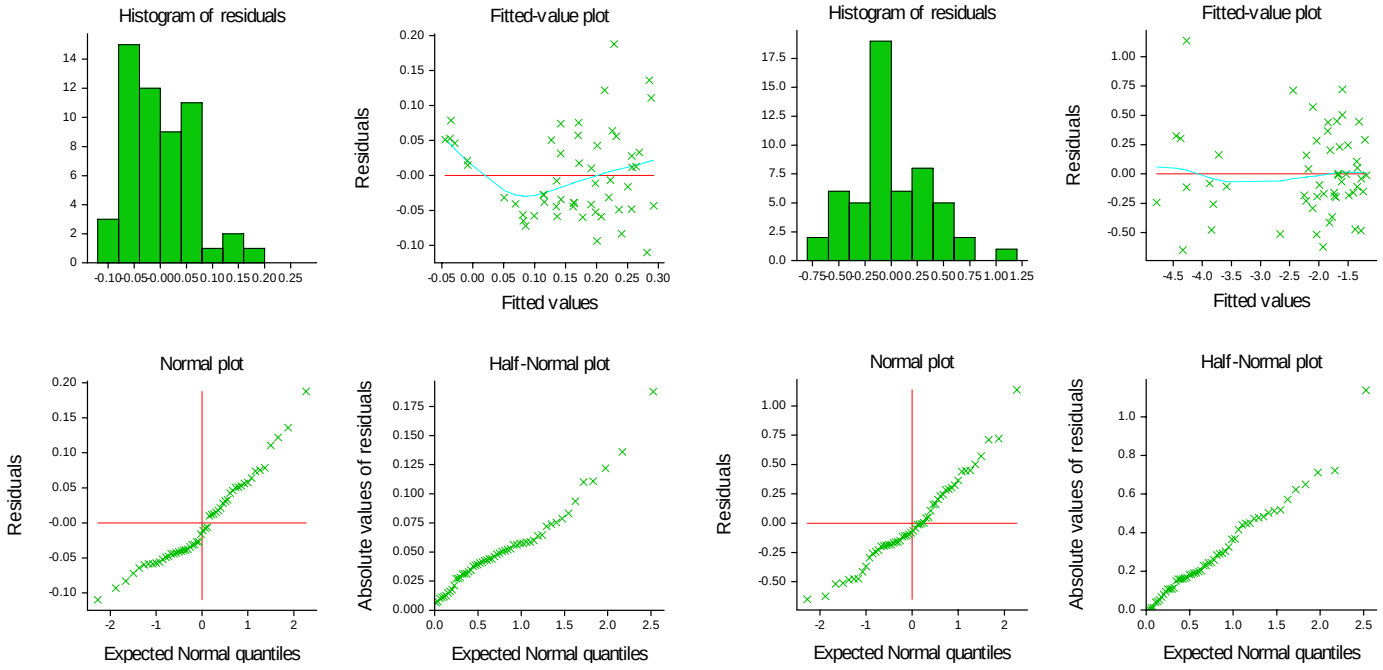
Average variance of differences:

0.01590	0.01565

4. DRY WEIGHT

a) Roots

Data were analysed on a log scale to reduce fanning of residuals




```
797  VCOMPONENTS [FIXED=Block+Mycorrhiza*Phosphorous; FACTORIAL=9]
RANDOM=Block.Treatment_Code;\
 798   INITIAL=1; CONSTRAINTS=positive
 799  REML [PRINT=model,components,means,deviance,waldTests; PSE=alldifferences;
MVINCLUDE=*;\
 800   METHOD=AI; MAXCYCLE=2000] log(Root_DW)
```

REML variance components analysis

Response variate:	LOG(Root_DW)
Fixed model:	Constant + Block + Mycorrhiza + Phosphorous + Mycorrhiza.Phosphorous
Random model:	Block.Treatment_Code
Number of units:	54 (18 units excluded due to zero weights or missing values)

Block.Treatment_Code used as residual term

Sparse algorithm with AI optimisation

Residual variance model

Term	Factor	Model(order)	Parameter	Estimate	s.e.
Block.Treatment_Code	Identity	Sigma2	0.193	0.0449	

Deviance: -2*Log-Likelihood

Deviance	d.f.
2.61	36

Note: deviance omits constants which depend on fixed model fitted.

Wald tests for fixed effects

Sequentially adding terms to fixed model

Fixed term	Wald statistic	d.f.	Wald/d.f.	chi pr
Block	39.88	11	3.63	<0.001
Mycorrhiza	25.83	1	25.83	<0.001
Phosphorous	113.59	2	56.80	<0.001
Mycorrhiza.Phosphorous	112.44	2	56.22	<0.001

Dropping individual terms from full fixed model

Fixed term	Wald statistic	d.f.	Wald/d.f.	chi pr
Mycorrhiza.Phosphorous	112.44	2	56.22	<0.001
Block	28.44	11	2.59	0.003

Message: chi-square distribution for Wald tests is an asymptotic approximation (i.e. for large samples) and underestimates the probabilities in other cases.

Table of predicted means for Constant

-2.132 Standard error: 0.0637

Table of predicted means for Block

Block	1	2	3	4	5	6	7	8
	-2.401	-1.612	-2.298	-2.298	-1.746	-1.968	-1.903	-2.811

Block	9	10	11	12
	-1.871	-2.362	-2.476	-1.839

Standard errors of differences between pairs

Block 1	1	*						
Block 2	2	0.384	*					
Block 3	3	0.302	0.388	*				
Block 4	4	0.302	0.388	0.315	*			
Block 5	5	0.281	0.378	0.301	0.301	*		
Block 6	6	0.281	0.383	0.301	0.301	0.281	*	
Block 7	7	0.283	0.377	0.297	0.297	0.282	0.282	*
Block 8	8	0.297	0.397	0.322	0.317	0.300	0.300	0.302
Block 9	9	0.283	0.377	0.297	0.297	0.282	0.282	0.278

Block 10	10	0.268	0.370	0.288	0.288	0.267	0.267	0.268
Block 11	11	0.283	0.377	0.297	0.297	0.282	0.282	0.278
Block 12	12	0.297	0.397	0.317	0.322	0.300	0.300	0.302
		1	2	3	4	5	6	7

Block 8	8	*				
Block 9	9	0.302	*			
Block 10	10	0.287	0.268	*		
Block 11	11	0.302	0.278	0.268	*	
Block 12	12	0.315	0.302	0.287	0.302	*
		8	9	10	11	12

Standard errors of differences

Average:	0.3075
Maximum:	0.3970
Minimum:	0.2674

Average variance of differences: 0.09587

Table of predicted means for Mycorrhiza

Mycorrhiza	N	Y
	-2.392	-1.872

Standard errors of differences between pairs

Mycorrhiza N	1	*	
Mycorrhiza Y	2	0.125	*
		1	2

Table of predicted means for Phosphorous

Phosphorous	h	l	o
	-1.742	-1.722	-2.932

Standard errors of differences between pairs

Phosphorous h	1	*		
Phosphorous l	2	0.162	*	
Phosphorous o	3	0.156	0.147	*
		1	2	3

Standard errors of differences

Average:	0.1550
Maximum:	0.1623
Minimum:	0.1470

Average variance of differences: 0.02405

Table of predicted means for Mycorrhiza.Phosphorous

Phosphorous	h	l	o
Mycorrhiza			
N	-1.502	-1.572	-4.102
Y	-1.982	-1.872	-1.762

Standard errors of differences between pairs

Mycorrhiza N.Phosphorous h	1	*				
Mycorrhiza N.Phosphorous l	2	0.206	*			
Mycorrhiza N.Phosphorous o	3	0.196	0.203	*		
Mycorrhiza Y.Phosphorous h	4	0.237	0.241	0.235	*	
Mycorrhiza Y.Phosphorous l	5	0.210	0.215	0.202	0.251	*
Mycorrhiza Y.Phosphorous o	6	0.206	0.213	0.203	0.241	0.215
		1	2	3	4	5

Mycorrhiza Y.Phosphorous o	6	*
		6

Standard errors of differences

Average:	0.2184
Maximum:	0.2510
Minimum:	0.1963

Average variance of differences: 0.04799

Standard error of differences for same level of factor:

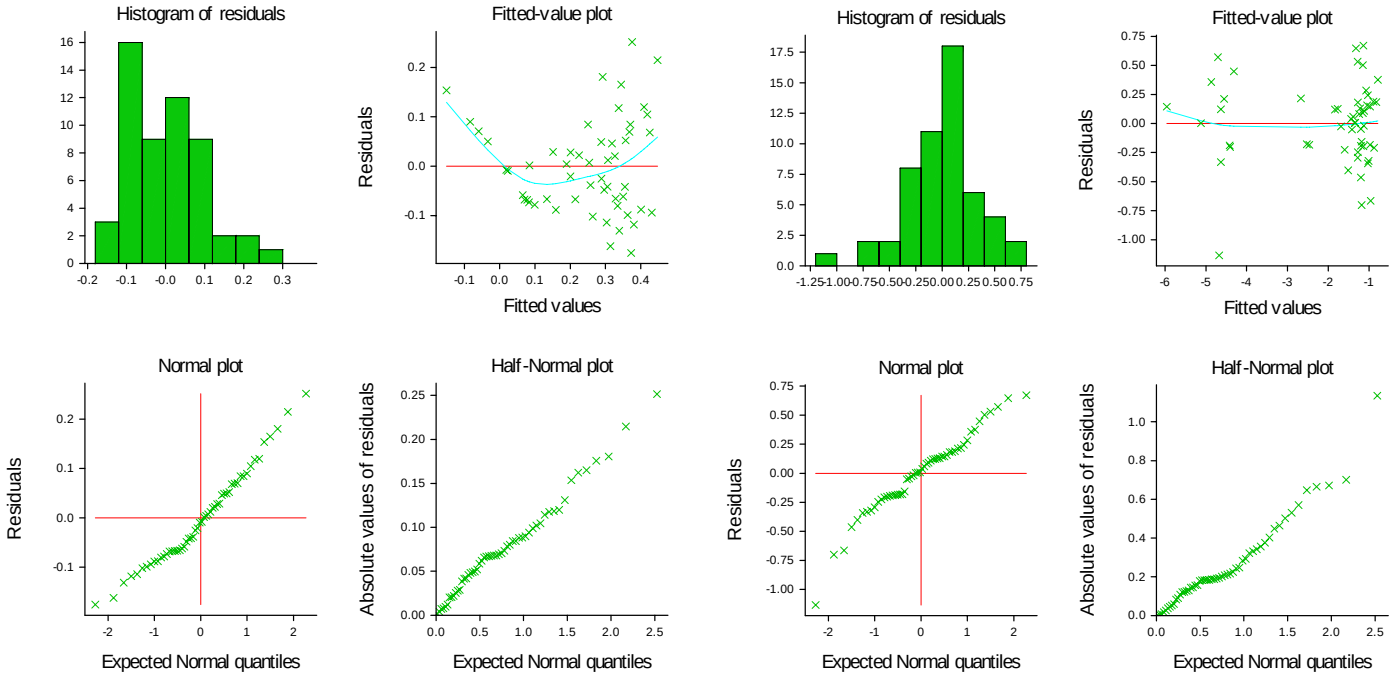
	Mycorrhiza	Phosphorous
Average:	0.2188	0.2184
Maximum:	0.2510	0.2370
Minimum:	0.1963	0.2035

Average variance of differences:

0.04827	0.04791

b) Shoots

Data were analysed on a log scale to reduce fanning




```
701  VCOMPONENTS [FIXED=Block+Mycorrhiza*Phosphorous; FACTORIAL=9]
RANDOM=Block.Treatment_Code;\
 702   INITIAL=1; CONSTRAINTS=positive
 703  REML [PRINT=model,components,means,deviance,waldTests; parameterization=sigmas;\
 704  PSE=alldifferences; MVINCLUDE=*;\
 705   METHOD=AI; MAXCYCLE=2000] log(Shoot_DW)
```

REML variance components analysis

Response variate: LOG(Shoot_DW)
Fixed model: Constant + Block + Mycorrhiza + Phosphorous + Mycorrhiza.Phosphorous
Random model: Block.Treatment_Code
Number of units: 54 (18 units excluded due to zero weights or missing values)

Block.Treatment_Code used as residual term

Sparse algorithm with AI optimisation

Residual variance model

Term	Factor	Model(order)	Parameter	Estimate	s.e.
Block.Treatment_Code	Identity	Sigma2	0.166	0.0386	

Deviance: -2*Log-Likelihood

Deviance	d.f.
-2.98	36

Note: deviance omits constants which depend on fixed model fitted.

Wald tests for fixed effects

Sequentially adding terms to fixed model

Fixed term	Wald statistic	d.f.	Wald/d.f.	chi pr
Block	95.49	11	8.68	<0.001
Mycorrhiza	127.08	1	127.08	<0.001
Phosphorous	283.83	2	141.91	<0.001
Mycorrhiza.Phosphorous	176.04	2	88.02	<0.001

Dropping individual terms from full fixed model

Fixed term	Wald statistic	d.f.	Wald/d.f.	chi pr
Mycorrhiza.Phosphorous	176.04	2	88.02	<0.001
Block	52.16	11	4.74	<0.001

Message: chi-square distribution for Wald tests is an asymptotic approximation (i.e. for large samples) and underestimates the probabilities in other cases.

Table of predicted means for Constant

-1.884 Standard error: 0.0591

Table of predicted means for Block

Block	1	2	3	4	5	6	7	8
	-1.785	-1.570	-2.020	-1.859	-1.465	-1.655	-1.708	-3.107

Block	9	10	11	12
	-1.556	-1.828	-2.272	-1.783

Standard errors of differences between pairs

Block 1	1	*						
Block 2	2	0.356	*					
Block 3	3	0.280	0.360	*				
Block 4	4	0.280	0.360	0.292	*			
Block 5	5	0.261	0.350	0.279	0.279	*		
Block 6	6	0.260	0.355	0.279	0.279	0.260	*	
Block 7	7	0.262	0.349	0.275	0.275	0.262	0.261	*
Block 8	8	0.275	0.368	0.298	0.294	0.279	0.278	0.280
Block 9	9	0.262	0.349	0.275	0.275	0.262	0.261	0.258
Block 10	10	0.248	0.343	0.267	0.267	0.248	0.248	0.249
Block 11	11	0.262	0.349	0.275	0.275	0.262	0.261	0.258
Block 12	12	0.275	0.368	0.294	0.298	0.279	0.278	0.280
		1	2	3	4	5	6	7

Block 8	8	*				
Block 9	9	0.280	*			
Block 10	10	0.266	0.249	*		
Block 11	11	0.280	0.258	0.249	*	
Block 12	12	0.292	0.280	0.266	0.280	*
		8	9	10	11	12

Standard errors of differences

Average:	0.2851
Maximum:	0.3682
Minimum:	0.2479

Average variance of differences: 0.08242

Table of predicted means for Mycorrhiza

Mycorrhiza	N	Y
	-2.470	-1.298

Standard errors of differences between pairs

Mycorrhiza N	1	*		
Mycorrhiza Y	2	0.116	*	
		1	2	

Table of predicted means for Phosphorous

Phosphorous	h	l	o
	-1.270	-1.293	-3.089

Standard errors of differences between pairs

Phosphorous h	1	*		
Phosphorous l	2	0.151	*	
Phosphorous o	3	0.144	0.136	*
		1	2	3

Standard errors of differences

Average:	0.1437
Maximum:	0.1505
Minimum:	0.1363

Average variance of differences: 0.02068

Table of predicted means for Mycorrhiza.Phosphorous

Phosphorous	h	l	o
Mycorrhiza			
N	-1.301	-1.379	-4.731
Y	-1.240	-1.207	-1.447

Standard errors of differences between pairs

Mycorrhiza N.Phosphorous h	1	*				
Mycorrhiza N.Phosphorous l	2	0.191	*			
Mycorrhiza N.Phosphorous o	3	0.182	0.189	*		
Mycorrhiza Y.Phosphorous h	4	0.220	0.224	0.218	*	
Mycorrhiza Y.Phosphorous l	5	0.195	0.199	0.188	0.233	*
Mycorrhiza Y.Phosphorous o	6	0.191	0.198	0.189	0.224	0.199
		1	2	3	4	5

Mycorrhiza Y.Phosphorous o	6	*
		6

Standard errors of differences

Average:	0.2025
Maximum:	0.2328
Minimum:	0.1820

Average variance of differences: 0.04126

Standard error of differences for same level of factor:

	Mycorrhiza	Phosphorous
Average:	0.2028	0.2025
Maximum:	0.2328	0.2197
Minimum:	0.1820	0.1887

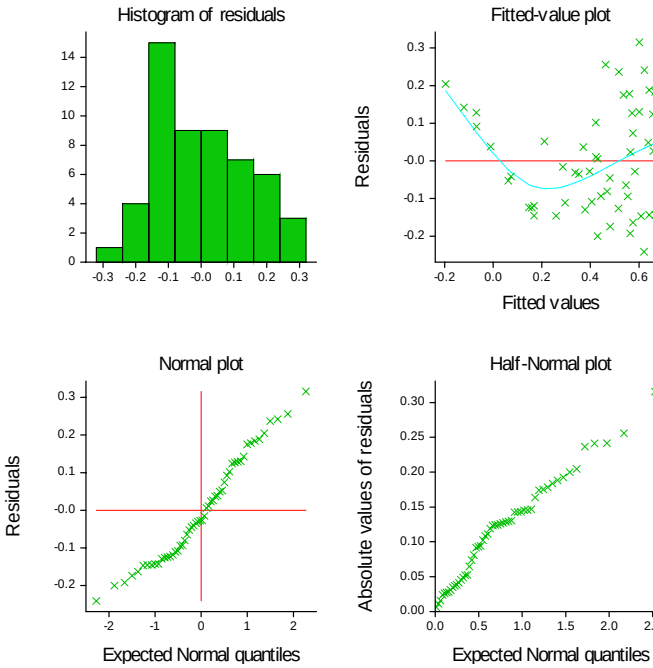
Average variance of differences:

0.4150	0.04119

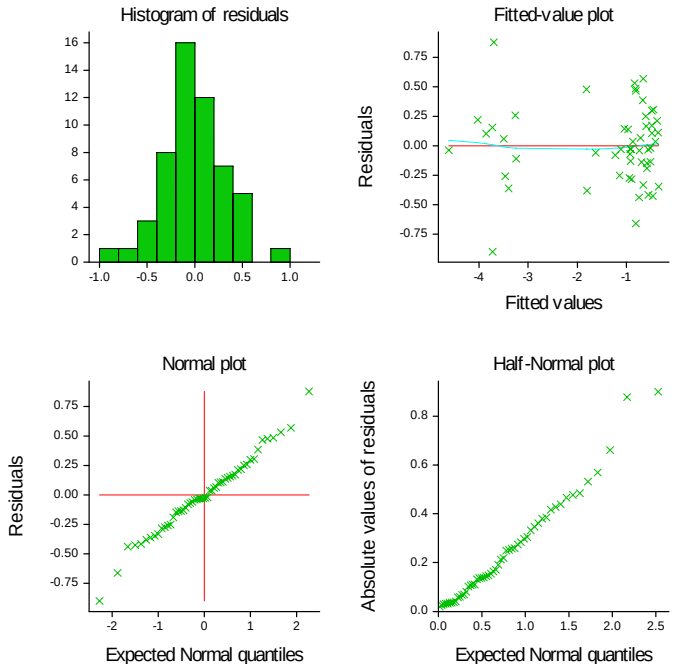
c) Dry Weight of the Whole Plant

Analysed on a log scale to reduce fanning of residuals:

Original data:



Log(DWwholeplant)




```
821  VCOMPONENTS [FIXED=Block+Mycorrhiza*Phosphorous; FACTORIAL=9]
RANDOM=Block.Treatment_Code;\
 822   INITIAL=1; CONSTRAINTS=positive
 823  REML [PRINT=model,components,means,deviance,waldTests; PSE=alldifferences;
MVINCLUDE=*;\
 824   METHOD=AI; MAXCYCLE=2000] log(WholeDW)
```

REML variance components analysis

Response variate:	LOG(WholeDW)
Fixed model:	Constant + Block + Mycorrhiza + Phosphorous + Mycorrhiza.Phosphorous
Random model:	Block.Treatment_Code
Number of units:	54 (18 units excluded due to zero weights or missing values)

Block.Treatment_Code used as residual term

Sparse algorithm with AI optimisation

Residual variance model

Term	Factor	Model(order)	Parameter	Estimate	s.e.
Block.Treatment_Code	Identity	Sigma2	0.149	0.0346	

Deviance: -2*Log-Likelihood

Deviance	d.f.
-7.04	36

Note: deviance omits constants which depend on fixed model fitted.

Wald tests for fixed effects

Sequentially adding terms to fixed model

Fixed term	Wald statistic	d.f.	Wald/d.f.	chi pr
Block	71.38	11	6.49	<0.001
Mycorrhiza	81.44	1	81.44	<0.001
Phosphorous	221.95	2	110.97	<0.001
Mycorrhiza.Phosphorous	164.67	2	82.33	<0.001

Dropping individual terms from full fixed model

Fixed term	Wald statistic	d.f.	Wald/d.f.	chi pr
Mycorrhiza.Phosphorous	164.67	2	82.33	<0.001
Block	41.91	11	3.81	<0.001

Message: chi-square distribution for Wald tests is an asymptotic approximation (i.e. for large samples) and underestimates the probabilities in other cases.

Table of predicted means for Constant

-1.265 Standard error: 0.0559

Table of predicted means for Block

Block	1	2	3	4	5	6	7	8
	-1.328	-0.844	-1.455	-1.298	-0.862	-1.069	-1.095	-2.212

Block	9	10	11	12
	-0.998	-1.321	-1.627	-1.067

Standard errors of differences between pairs

Block 1	1	*						
Block 2	2	0.337	*					
Block 3	3	0.265	0.340	*				
Block 4	4	0.265	0.340	0.277	*			
Block 5	5	0.247	0.332	0.264	0.264	*		
Block 6	6	0.247	0.336	0.264	0.264	0.246	*	
Block 7	7	0.248	0.331	0.260	0.260	0.248	0.247	*
Block 8	8	0.260	0.348	0.282	0.278	0.264	0.263	0.265
Block 9	9	0.248	0.331	0.260	0.260	0.248	0.247	0.244
Block 10	10	0.235	0.325	0.253	0.253	0.235	0.235	0.235
Block 11	11	0.248	0.331	0.260	0.260	0.248	0.247	0.244
Block 12	12	0.260	0.348	0.278	0.282	0.264	0.263	0.265

		1	2	3	4	5	6	7
Block 8	8	*						
Block 9	9	0.265	*					
Block 10	10	0.252	0.235	*				
Block 11	11	0.265	0.244	0.235	*			
Block 12	12	0.277	0.265	0.252	0.265	*		
		8	9	10	11	12		

Standard errors of differences

Average: 0.2699
Maximum: 0.3485
Minimum: 0.2347

Average variance of differences: 0.07384

Table of predicted means for Mycorrhiza

Mycorrhiza	N	Y
	-1.698	-0.831

Standard errors of differences between pairs

Mycorrhiza N	1	*		
Mycorrhiza Y	2	0.110	*	
		1	2	

Table of predicted means for Phosphorous

Phosphorous	h	l	o
	-0.767	-0.764	-2.263

Standard errors of differences between pairs

Phosphorous h	1	*		
Phosphorous l	2	0.142	*	
Phosphorous o	3	0.137	0.129	*
		1	2	3

Standard errors of differences

Average: 0.1360
Maximum: 0.1425
Minimum: 0.1290

Average variance of differences: 0.01853

Table of predicted means for Mycorrhiza.Phosphorous

Phosphorous	h	l	o
Mycorrhiza			
N	-0.680	-0.751	-3.663
Y	-0.854	-0.777	-0.864

Standard errors of differences between pairs

Mycorrhiza N.Phosphorous h	1	*				
Mycorrhiza N.Phosphorous l	2	0.181	*			
Mycorrhiza N.Phosphorous o	3	0.172	0.179	*		
Mycorrhiza Y.Phosphorous h	4	0.208	0.212	0.206	*	
Mycorrhiza Y.Phosphorous l	5	0.185	0.189	0.178	0.220	*
Mycorrhiza Y.Phosphorous o	6	0.181	0.187	0.179	0.212	0.189
		1	2	3	4	5

Mycorrhiza Y.Phosphorous o	6	*
		6

Standard errors of differences

Average:	0.1917
Maximum:	0.2203
Minimum:	0.1723

Average variance of differences: 0.03696

Standard error of differences for same level of factor:

	Mycorrhiza	Phosphorous
Average:	0.1920	0.1917
Maximum:	0.2203	0.2080
Minimum:	0.1723	0.1786

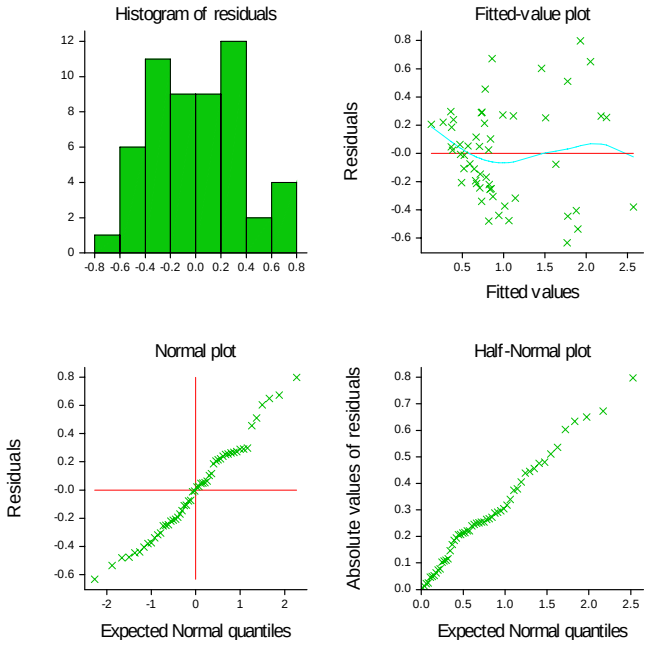
Average variance of differences:

0.03718	0.03690

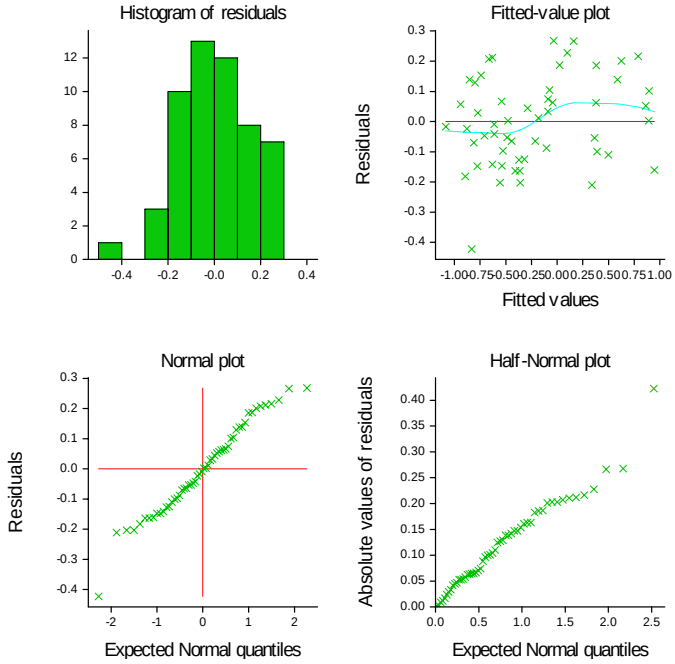
5. ROOT TO SHOOT DRY WEIGHT RATIO

Analysed on a log scale to reduce fanning

Original data:



log(RootShootDWRatio):




```
916  VCOMPONENTS [FIXED=Block+Mycorrhiza*Phosphorous; FACTORIAL=9]
RANDOM=Block.Treatment_Code;\
 917   INITIAL=1; CONSTRAINTS=positive
 918  REML [PRINT=model,components,means,deviance,waldTests; PSE=alldifferences;
MVINCLUDE=*;\
 919   METHOD=AI; MAXCYCLE=2000] log(Root_shoot_DW)
```

REML variance components analysis

Response variate: LOG(Root_shoot_DW)
Fixed model: Constant + Block + Mycorrhiza + Phosphorous + Mycorrhiza.Phosphorous
Random model: Block.Treatment_Code
Number of units: 54 (18 units excluded due to zero weights or missing values)

Block.Treatment_Code used as residual term

Sparse algorithm with AI optimisation

Residual variance model

Term	Factor	Model(order)	Parameter	Estimate	s.e.
Block.Treatment_Code	Identity	Sigma2	0.133	0.0310	

Deviance: -2*Log-Likelihood

Deviance	d.f.
-11.10	36

Note: deviance omits constants which depend on fixed model fitted.

Wald tests for fixed effects

Sequentially adding terms to fixed model

Fixed term	Wald statistic	d.f.	Wald/d.f.	chi pr
Block	28.60	11	2.60	0.003
Mycorrhiza	41.78	1	41.78	<0.001
Phosphorous	35.69	2	17.85	<0.001
Mycorrhiza.Phosphorous	4.30	2	2.15	0.117

Dropping individual terms from full fixed model

Fixed term	Wald statistic	d.f.	Wald/d.f.	chi pr
Mycorrhiza.Phosphorous	4.30	2	2.15	0.117
Block	20.55	11	1.87	0.038

Message: chi-square distribution for Wald tests is an asymptotic approximation (i.e. for large samples) and underestimates the probabilities in other cases.

Table of predicted means for Constant

-0.2480 Standard error: 0.05294

Table of predicted means for Block

Block	1	2	3	4	5
	-0.6161	-0.0417	-0.2776	-0.4386	-0.2809

Block	6	7	8	9	10
	-0.3133	-0.1944	0.2964	-0.3143	-0.5349

Block	11	12
	-0.2037	-0.0568

Standard errors of differences between pairs

Block 1	1	*			
Block 2	2	0.3192	*		
Block 3	3	0.2508	0.3222	*	
Block 4	4	0.2508	0.3222	0.2620	*

Block 5	5	0.2336	0.3139	0.2503	0.2503	*		
Block 6	6	0.2334	0.3182	0.2503	0.2503	0.2333	*	
Block 7	7	0.2347	0.3129	0.2466	0.2466	0.2343	0.2343	*
Block 8	8	0.2466	0.3299	0.2672	0.2635	0.2496	0.2494	0.2511
Block 9	9	0.2347	0.3129	0.2466	0.2466	0.2343	0.2343	0.2310
Block 10	10	0.2224	0.3073	0.2391	0.2391	0.2222	0.2222	0.2229
Block 11	11	0.2347	0.3129	0.2466	0.2466	0.2343	0.2343	0.2310
Block 12	12	0.2466	0.3299	0.2635	0.2672	0.2496	0.2494	0.2511
		1	2	3	4	5	6	7

Block 8	8	*						
Block 9	9	0.2511	*					
Block 10	10	0.2386	0.2229	*				
Block 11	11	0.2511	0.2310	0.2229	*			
Block 12	12	0.2620	0.2511	0.2386	0.2511	*		
		8	9	10	11	12		

Standard errors of differences

Average:	0.2555
Maximum:	0.3299
Minimum:	0.2222

Average variance of differences: 0.06618

Table of predicted means for Mycorrhiza

Mycorrhiza	N	Y
	0.0779	-0.5739

Standard errors of differences between pairs

Mycorrhiza N	1	*	
Mycorrhiza Y	2	0.1038	*
		1	2

Table of predicted means for Phosphorous

Phosphorous	h	l	o
	-0.4719	-0.4290	0.1570

Standard errors of differences between pairs

Phosphorous h	1	*		
Phosphorous l	2	0.1349	*	
Phosphorous o	3	0.1293	0.1221	*
		1	2	3

Standard errors of differences

Average: 0.1288
Maximum: 0.1349
Minimum: 0.1221

Average variance of differences: 0.01660

Table of predicted means for Mycorrhiza.Phosphorous

Phosphorous	h	l	o
Mycorrhiza			
N	-0.2010	-0.1938	0.6285
Y	-0.7427	-0.6643	-0.3145

Standard errors of differences between pairs

Mycorrhiza N.Phosphorous h	1	*				
Mycorrhiza N.Phosphorous l	2	0.1710	*			
Mycorrhiza N.Phosphorous o	3	0.1631	0.1690	*		
Mycorrhiza Y.Phosphorous h	4	0.1969	0.2004	0.1955	*	
Mycorrhiza Y.Phosphorous l	5	0.1747	0.1785	0.1681	0.2086	*
Mycorrhiza Y.Phosphorous o	6	0.1710	0.1772	0.1690	0.2004	0.1785
		1	2	3	4	5

Mycorrhiza Y.Phosphorous o	6	*
		6

Standard errors of differences

Average: 0.1815
Maximum: 0.2086
Minimum: 0.1631

Average variance of differences: 0.03313

Standard error of differences for same level of factor:

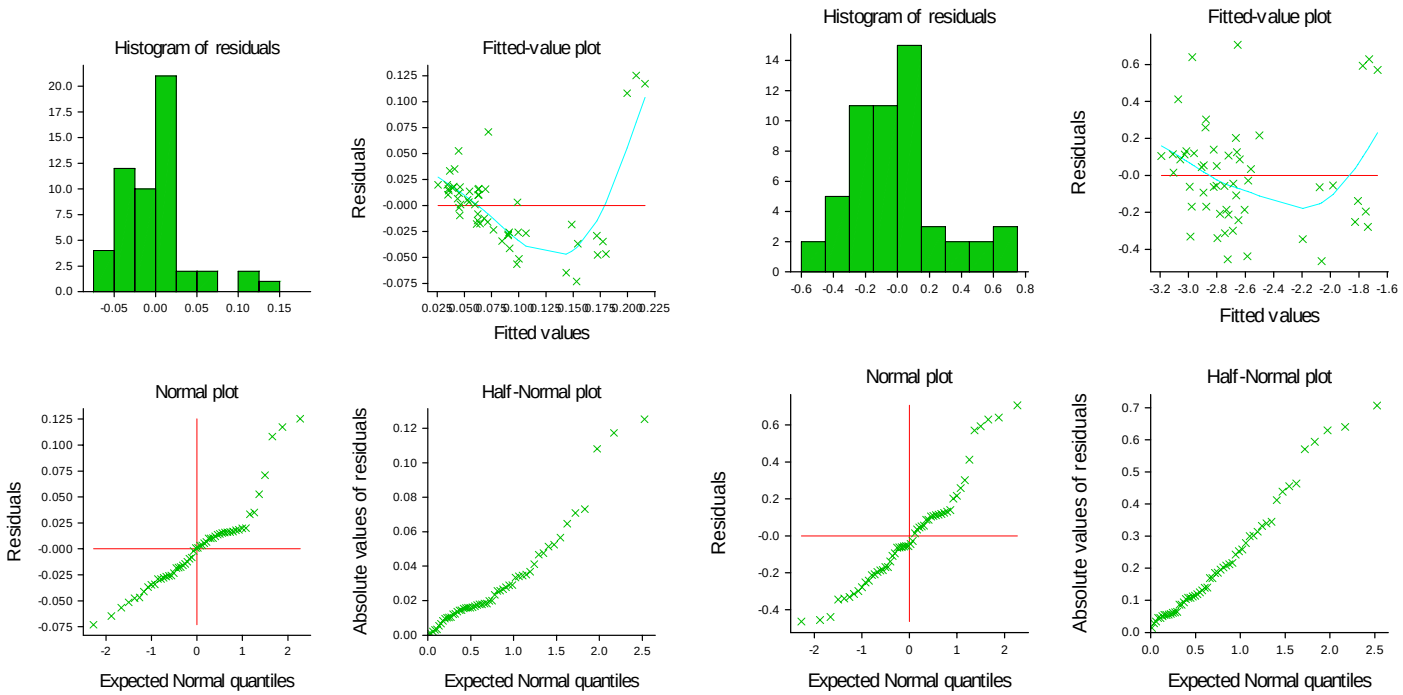
	Mycorrhiza	Phosphorous
Average:	0.1818	0.1815
Maximum:	0.2086	0.1969
Minimum:	0.1631	0.1690

Average variance of differences:

0.03332	0.03307

6. LEAVES PER MM

Data were analysed on a log scale to reduce fanning:




```
802  VCOMPONENTS [FIXED=Block+Mycorrhiza*Phosphorous; FACTORIAL=9]
RANDOM=Block.Treatment_Code;\
 803   INITIAL=1; CONSTRAINTS=positive
 804  REML [PRINT=model,components,means,deviance,waldTests; PSE=alldifferences;
MVINCLUDE=*;\
 805   METHOD=AI; MAXCYCLE=2000] log(leaves_mm)
```

REML variance components analysis

Response variate: LOG(leaves_mm)
Fixed model: Constant + Block + Mycorrhiza + Phosphorous + Mycorrhiza.Phosphorous
Random model: Block.Treatment_Code
Number of units: 54 (18 units excluded due to zero weights or missing values)

Block.Treatment_Code used as residual term

Sparse algorithm with AI optimisation

Residual variance model

Term	Factor	Model(order)	Parameter	Estimate	s.e.
Block.Treatment_Code	Identity	Sigma2	0.114	0.0264	

Deviance: -2*Log-Likelihood

Deviance	d.f.
-17.06	36

Note: deviance omits constants which depend on fixed model fitted.

Wald tests for fixed effects

Sequentially adding terms to fixed model

Fixed term	Wald statistic	d.f.	Wald/d.f.	chi pr
Block	16.45	11	1.50	0.125
Mycorrhiza	10.94	1	10.94	<0.001
Phosphorous	37.94	2	18.97	<0.001
Mycorrhiza.Phosphorous	16.91	2	8.46	<0.001

Dropping individual terms from full fixed model

Fixed term	Wald statistic	d.f.	Wald/d.f.	chi pr
Mycorrhiza.Phosphorous	16.91	2	8.46	<0.001
Block	12.64	11	1.15	0.317

Message: chi-square distribution for Wald tests is an asymptotic approximation (i.e. for large samples) and underestimates the probabilities in other cases.

Table of predicted means for Constant

-2.665 Standard error: 0.0488

Table of predicted means for Block

Block	1	2	3	4	5	6	7	8
	-2.611	-2.766	-2.521	-2.591	-2.556	-2.748	-2.978	-2.512

Block	9	10	11	12
	-2.846	-2.453	-2.859	-2.534

Standard errors of differences between pairs

Block 1	1	*						
Block 2	2	0.294	*					
Block 3	3	0.231	0.297	*				
Block 4	4	0.231	0.297	0.242	*			
Block 5	5	0.216	0.290	0.231	0.231	*		
Block 6	6	0.215	0.294	0.231	0.231	0.215	*	
Block 7	7	0.217	0.289	0.227	0.227	0.216	0.216	*
Block 8	8	0.228	0.304	0.246	0.243	0.230	0.230	0.232
Block 9	9	0.217	0.289	0.227	0.227	0.216	0.216	0.213
Block 10	10	0.205	0.283	0.221	0.221	0.205	0.205	0.206
Block 11	11	0.217	0.289	0.227	0.227	0.216	0.216	0.213
Block 12	12	0.228	0.304	0.243	0.246	0.230	0.230	0.232

		1	2	3	4	5	6	7
Block 8	8	*						
Block 9	9	0.232	*					
Block 10	10	0.220	0.206	*				
Block 11	11	0.232	0.213	0.206	*			
Block 12	12	0.242	0.232	0.220	0.232	*		
		8	9	10	11	12		

Standard errors of differences

Average: 0.2357
Maximum: 0.3043
Minimum: 0.2050

Average variance of differences: 0.05633

Table of predicted means for Mycorrhiza

Mycorrhiza	N	Y
	-2.518	-2.811

Standard errors of differences between pairs

Mycorrhiza N	1	*		
Mycorrhiza Y	2	0.096	*	
		1	2	

Table of predicted means for Phosphorous

Phosphorous	h	l	o
	-2.903	-2.794	-2.297

Standard errors of differences between pairs

Phosphorous h	1	*		
Phosphorous l	2	0.124	*	
Phosphorous o	3	0.119	0.113	*
		1	2	3

Standard errors of differences

Average: 0.1188
Maximum: 0.1244
Minimum: 0.1127

Average variance of differences: 0.01413

Table of predicted means for Mycorrhiza.Phosphorous

Phosphorous	h	l	o
Mycorrhiza			
N	-2.876	-2.796	-1.881
Y	-2.930	-2.791	-2.712

Standard errors of differences between pairs

Mycorrhiza N.Phosphorous h	1	*				
Mycorrhiza N.Phosphorous l	2	0.158	*			
Mycorrhiza N.Phosphorous o	3	0.150	0.156	*		
Mycorrhiza Y.Phosphorous h	4	0.182	0.185	0.180	*	
Mycorrhiza Y.Phosphorous l	5	0.161	0.165	0.155	0.192	*
Mycorrhiza Y.Phosphorous o	6	0.158	0.163	0.156	0.185	0.165
		1	2	3	4	5

Mycorrhiza Y.Phosphorous o	6	*
		6

Standard errors of differences

Average: 0.1674
Maximum: 0.1924
Minimum: 0.1505

Average variance of differences: 0.02819

Standard error of differences for same level of factor:

	Mycorrhiza	Phosphorous
Average:	0.1677	0.1674
Maximum:	0.1924	0.1816
Minimum:	0.1505	0.1560

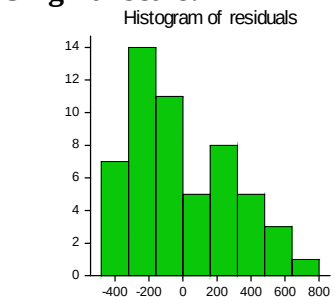
Average variance of differences:

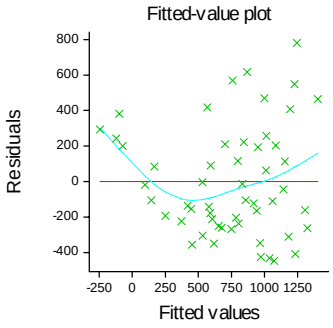
0.02836 0.02815

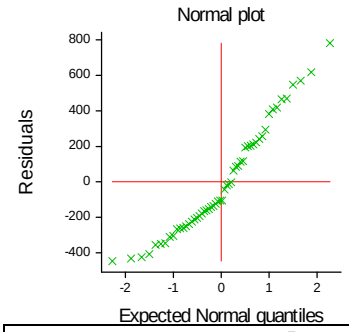
7. ROOT LENGTH

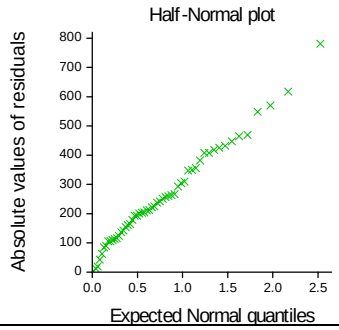
Data were analysed on a log scale to make variance more consistent across fitted values (as indicated by fanning of residual plots), although this situation is still not ideal.

Original scale:

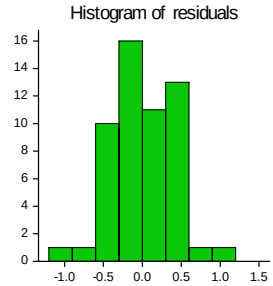


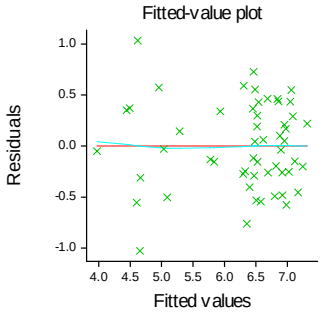


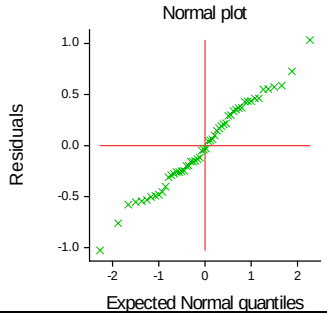


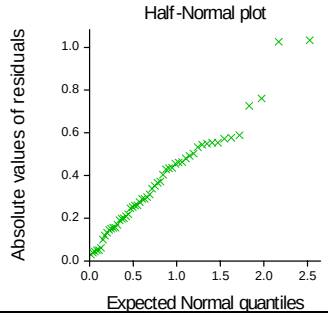


Log scale:










```
275  VCOMPONENTS [FIXED=Block+Mycorrhiza*Phosphorous; FACTORIAL=9]
RANDOM=Block.Treatment_Code
 276  REML [PRINT=model,components,means,deviance,waldTests; PSE=alldifferences;
MVINCLUDE=*;\
 277    METHOD=AI; MAXCYCLE=2000] log(root_length)
```

REML variance components analysis

Response variate:	LOG(root_length)
Fixed model:	Constant + Block + Mycorrhiza + Phosphorous + Mycorrhiza.Phosphorous
Random model:	Block.Treatment_Code
Number of units:	54 (18 units excluded due to zero weights or missing values)

Block.Treatment_Code used as residual term

Sparse algorithm with AI optimisation

Residual variance model

Term	Factor	Model(order)	Parameter	Estimate	s.e.
Block.Treatment_Code	Identity	Sigma2	0.254	0.0590	

Deviance: -2*Log-Likelihood

Deviance	d.f.
12.73	36

Note: deviance omits constants which depend on fixed model fitted.

Wald tests for fixed effects

Sequentially adding terms to fixed model

Fixed term	Wald statistic	d.f.	Wald/d.f.	chi pr
Block	38.95	11	3.54	<0.001
Mycorrhiza	22.39	1	22.39	<0.001
Phosphorous	49.61	2	24.81	<0.001
Mycorrhiza.Phosphorous	49.01	2	24.50	<0.001

Dropping individual terms from full fixed model

Fixed term	Wald statistic	d.f.	Wald/d.f.	chi pr
Mycorrhiza.Phosphorous	49.01	2	24.50	<0.001
Block	23.59	11	2.14	0.015

Message: chi-square distribution for Wald tests is an asymptotic approximation (i.e. for large samples) and underestimates the probabilities in other cases.

Table of predicted means for Constant

6.324 Standard error: 0.0730

Table of predicted means for Block

Block	1	2	3	4	5	6	7	8
	6.083	6.192	6.625	6.209	6.875	6.584	6.547	5.564

Block	9	10	11	12
	6.680	6.246	6.032	6.252

Standard errors of differences between pairs

Block 1	1	*						
Block 2	2	0.440	*					
Block 3	3	0.346	0.445	*				
Block 4	4	0.346	0.445	0.362	*			
Block 5	5	0.322	0.433	0.345	0.345	*		
Block 6	6	0.322	0.439	0.345	0.345	0.322	*	
Block 7	7	0.324	0.432	0.340	0.340	0.323	0.323	*
Block 8	8	0.340	0.455	0.369	0.364	0.344	0.344	0.346
Block 9	9	0.324	0.432	0.340	0.340	0.323	0.323	0.319
Block 10	10	0.307	0.424	0.330	0.330	0.307	0.307	0.308
Block 11	11	0.324	0.432	0.340	0.340	0.323	0.323	0.319
Block 12	12	0.340	0.455	0.364	0.369	0.344	0.344	0.346
		1	2	3	4	5	6	7

Block 8	8	*				
Block 9	9	0.346	*			
Block 10	10	0.329	0.308	*		
Block 11	11	0.346	0.319	0.308	*	
Block 12	12	0.362	0.346	0.329	0.346	*
		8	9	10	11	12

Standard errors of differences

Average: 0.3525
Maximum: 0.4552
Minimum: 0.3066

Average variance of differences: 0.1260

Table of predicted means for Mycorrhiza

Mycorrhiza	N	Y
	6.032	6.617

Standard errors of differences between pairs

Mycorrhiza N	1	*	
Mycorrhiza Y	2	0.143	*
		1	2

Table of predicted means for Phosphorous

Phosphorous	h	l	o
	6.567	6.692	5.714

Standard errors of differences between pairs

Phosphorous h	1	*		
Phosphorous l	2	0.186	*	
Phosphorous o	3	0.178	0.168	*
		1	2	3

Standard errors of differences

Average: 0.1777
Maximum: 0.1861
Minimum: 0.1685

Average variance of differences: 0.03161

Table of predicted means for Mycorrhiza.Phosphorous

Phosphorous Mycorrhiza	h	l	o
N	6.597	6.763	4.734
Y	6.537	6.620	6.693

Standard errors of differences between pairs

Mycorrhiza N.Phosphorous h	1	*				
Mycorrhiza N.Phosphorous l	2	0.236	*			
Mycorrhiza N.Phosphorous o	3	0.225	0.233	*		
Mycorrhiza Y.Phosphorous h	4	0.272	0.276	0.270	*	
Mycorrhiza Y.Phosphorous l	5	0.241	0.246	0.232	0.288	*
Mycorrhiza Y.Phosphorous o	6	0.236	0.244	0.233	0.276	0.246
		1	2	3	4	5

Mycorrhiza Y.Phosphorous o	6	*
		6

Standard errors of differences

Average:	0.2504
Maximum:	0.2878
Minimum:	0.2250

Average variance of differences: 0.06307

Standard error of differences for same level of factor:

	Mycorrhiza	Phosphorous
Average:	0.2508	0.2504
Maximum:	0.2878	0.2717
Minimum:	0.2250	0.2333

Average variance of differences:

0.06344	0.06296

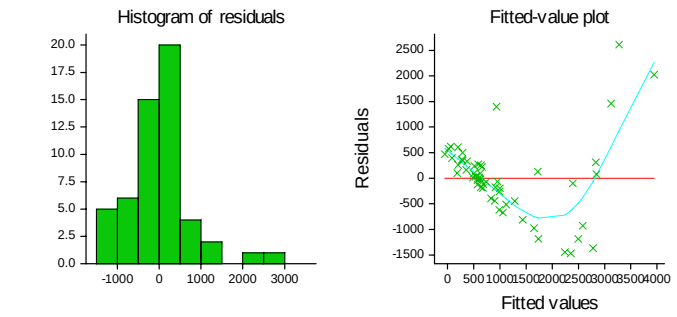
Note – there was no significant improvement to the model with addition of correlated error structure:
Change in deviance = 12.73 – 8.13 = 4.6
This is on 36-31 = 5df

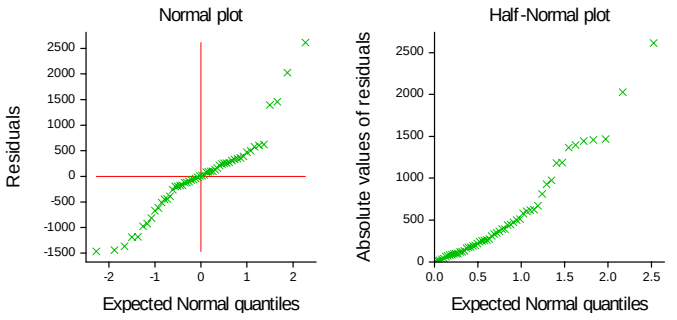
P = 0.467

8. ROOT LENGTH TO WEIGHT RATIO

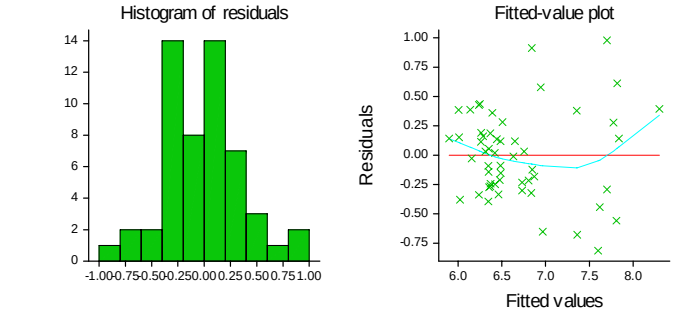
Data were analysed on a log scale to make variance more consistent across fitted values (as indicated by fanning of residual plots) and with a diagonally correlated error structure by treatment code (improvement indicated by change in deviance).

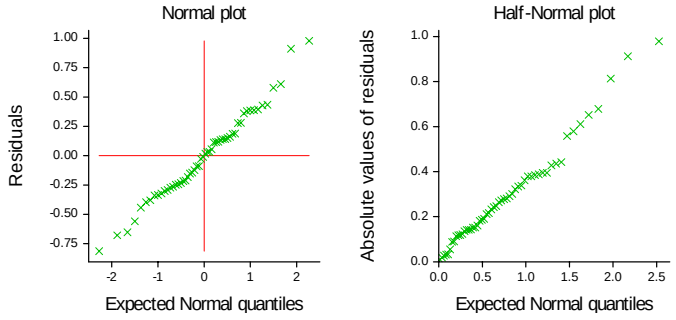
Original scale:





Log scale:






```
329  VCOMPONENTS [FIXED=Block+Mycorrhiza*Phosphorous; FACTORIAL=9]
RANDOM=Block.Treatment_Code
 330  VSTRUCTURE [TERMS=Block.Treatment_Code; FORMATION=direct]
MODEL=identity,diagonal;\
 331   ORDER=*,*; FACTOR=Block,Treatment_Code
 332  REML [PRINT=model,components,means,deviance,waldTests; PSE=alldifferences;
MVINCLUDE=*;\
 333   METHOD=AI; MAXCYCLE=2000] log(root_length_weight)
```

REML variance components analysis

Response variate:	LOG(root_length_weight)
Fixed model:	Constant + Block + Mycorrhiza + Phosphorous + Mycorrhiza.Phosphorous
Random model:	Block.Treatment_Code
Number of units:	54 (18 units excluded due to zero weights or missing values)

Residual term has been added to model

Sparse algorithm with AI optimisation

Covariance structures defined for random model

Covariance structures defined within terms:

Term	Factor	Model	Order	No. rows
Block.Treatment_Code	Block	Identity	0	12
	Treatment_Code			
		Diagonal	6	6

Estimated parameters for covariance models

Random term(s)	Factor	Model(order)	Parameter	Estimate	s.e.
Block.Treatment_Code					
	Block	Identity	-	-	-
	Treatment_Code	Diagonal	d_1	16.02	7.92
			d_2	4.409	3.013
			d_3	0.000	bound
			d_4	0.000	bound
			d_5	3.202	2.427
			d_6	1.363	1.664

Note: the covariance matrix for each term is calculated as G or R where
var(y) = Sigma2(ZGZ'+R), i.e. relative to the residual variance, Sigma2.

Residual variance model

Term	Factor	Model(order)	Parameter	Estimate	s.e.
Residual		Identity	Sigma2	0.0355	aliased

Deviance: -2*Log-Likelihood

Deviance	d.f.
-9.30	31

Note: deviance omits constants which depend on fixed model fitted.

Wald tests for fixed effects

Sequentially adding terms to fixed model

Fixed term	Wald statistic	d.f.	Wald/d.f.	chi pr
Block	20.29	11	1.84	0.042
Mycorrhiza	0.88	1	0.88	0.348
Phosphorous	30.29	2	15.14	<0.001
Mycorrhiza.Phosphorous	13.68	2	6.84	0.001

Dropping individual terms from full fixed model

Fixed term	Wald statistic	d.f.	Wald/d.f.	chi pr
Mycorrhiza.Phosphorous	13.68	2	6.84	0.001
Block	21.92	11	1.99	0.025

Message: chi-square distribution for Wald tests is an asymptotic approximation (i.e. for large samples) and underestimates the probabilities in other cases.

Table of predicted means for Constant

6.623 Standard error: 0.0578

Table of predicted means for Block

Block	1	2	3	4	5	6	7	8
	6.506	5.926	6.957	6.670	6.738	6.652	6.582	6.907

Block	9	10	11	12
	6.578	6.654	6.841	6.468

Standard errors of differences between pairs

Block 1	1	*						
Block 2	2	0.316	*					
Block 3	3	0.193	0.318	*				
Block 4	4	0.195	0.318	0.206	*			
Block 5	5	0.185	0.317	0.207	0.209	*		
Block 6	6	0.165	0.308	0.186	0.188	0.179	*	
Block 7	7	0.187	0.315	0.199	0.201	0.201	0.180	*
Block 8	8	0.174	0.319	0.198	0.199	0.190	0.170	0.192
Block 9	9	0.187	0.315	0.199	0.201	0.201	0.180	0.193
Block 10	10	0.164	0.307	0.185	0.187	0.178	0.157	0.179
Block 11	11	0.187	0.315	0.199	0.201	0.201	0.180	0.193
Block 12	12	0.173	0.318	0.196	0.199	0.189	0.169	0.191
		1	2	3	4	5	6	7

Block 8	8	*				
Block 9	9	0.192	*			
Block 10	10	0.169	0.179	*		
Block 11	11	0.192	0.193	0.179	*	
Block 12	12	0.178	0.191	0.168	0.191	*
		8	9	10	11	12

Standard errors of differences

Average: 0.2089
Maximum: 0.3185
Minimum: 0.1572

Average variance of differences: 0.04603

Table of predicted means for Mycorrhiza

Mycorrhiza	N	Y
	6.769	6.478

Standard errors of differences between pairs

Mycorrhiza N	1	*		
Mycorrhiza Y	2	0.113	*	
		1	2	

Table of predicted means for Phosphorous

Phosphorous	h	l	o
	6.254	6.403	7.213

Standard errors of differences between pairs

Phosphorous h	1	*			
Phosphorous l	2	0.101	*		
Phosphorous o	3	0.149	0.161	*	
		1	2	3	

Standard errors of differences

Average:	0.1369
Maximum:	0.1611
Minimum:	0.1006

Average variance of differences: 0.01941

Table of predicted means for Mycorrhiza.Phosphorous

Phosphorous	h	l	o
Mycorrhiza			
N	6.221	6.365	7.720
Y	6.286	6.441	6.706

Standard errors of differences between pairs

Mycorrhiza N.Phosphorous h	1	*					
Mycorrhiza N.Phosphorous l	2	0.144	*				
Mycorrhiza N.Phosphorous o	3	0.244	0.270	*			
Mycorrhiza Y.Phosphorous h	4	0.109	0.157	0.252	*		
Mycorrhiza Y.Phosphorous l	5	0.122	0.166	0.255	0.141	*	
Mycorrhiza Y.Phosphorous o	6	0.160	0.197	0.278	0.171	0.180	
		1	2	3	4	5	

Mycorrhiza Y.Phosphorous o	6	*	
		6	

Standard errors of differences

Average:	0.1897

Maximum:	0.2783
Minimum:	0.1086

Average variance of differences: 0.03892

Standard error of differences for same level of factor:

	Mycorrhiza	Phosphorous
Average:	0.1917	0.1845
Maximum:	0.2696	0.2783
Minimum:	0.1411	0.1086

Average variance of differences:

0.03910 0.03899

Deviance change (with addition of correlated error structure):

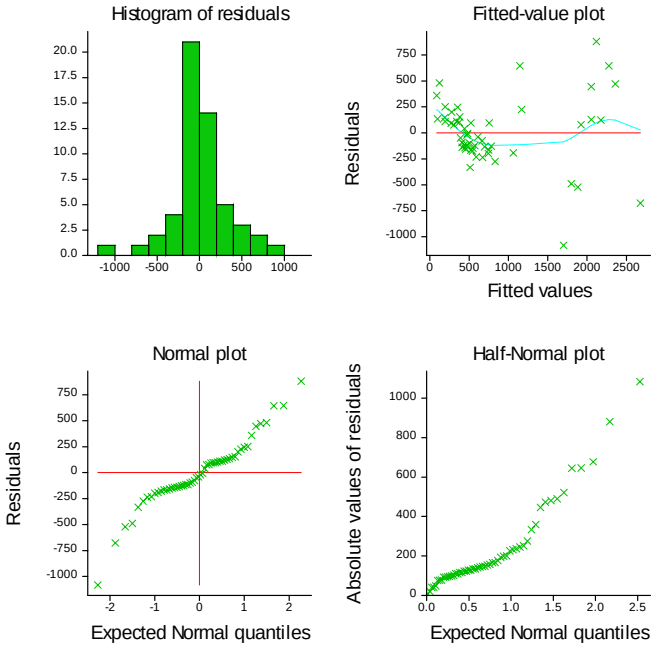
-9.30 – 3.70 = -13.00
This is on 36-31 = 5 df

P = 0.023

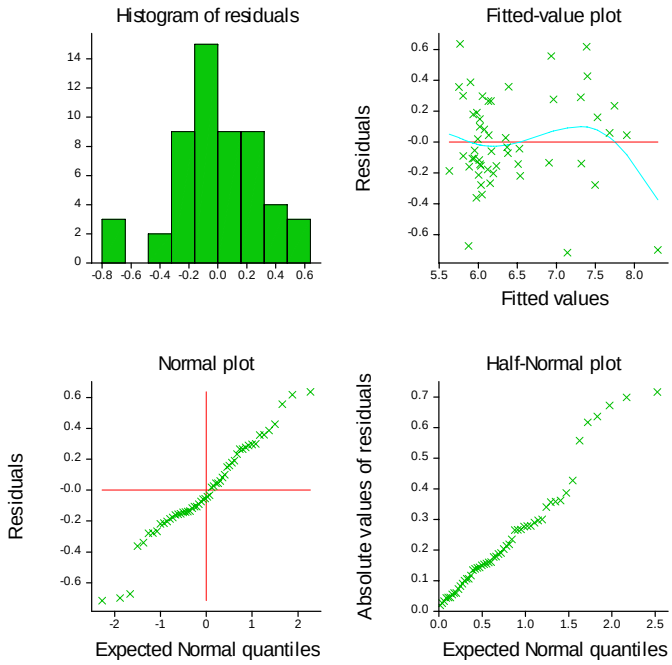
9. PLANT HEIGHT:SHOOT DRY WEIGHT RATIO

Data were analysed on a log scale to increase equality of variance.

Original scale:



Log(height_DW_ratio)




```
293  VCOMPONENTS [FIXED=Block+Mycorrhiza*Phosphorous; FACTORIAL=9]
RANDOM=Block.Treatment_Code
 294  REML [PRINT=model,components,means,deviance,waldTests; PSE=alldifferences;
MVINCLUDE=*;\
 295   METHOD=AI; MAXCYCLE=2000] log(Height_ShootDW_ratio)
```

REML variance components analysis

Response variate: LOG(Height_ShootDW_ratio)
Fixed model: Constant + Block + Mycorrhiza + Phosphorous + Mycorrhiza.Phosphorous
Random model: Block.Treatment_Code
Number of units: 54 (18 units excluded due to zero weights or missing values)

Block.Treatment_Code used as residual term

Sparse algorithm with AI optimisation

Residual variance model

Term	Factor	Model(order)	Parameter	Estimate	s.e.
Block.Treatment_Code	Identity	Sigma2	0.127	0.0295	

Deviance: -2*Log-Likelihood

Deviance	d.f.
-12.95	36

Note: deviance omits constants which depend on fixed model fitted.

Wald tests for fixed effects

Sequentially adding terms to fixed model

Fixed term	Wald statistic	d.f.	Wald/d.f.	chi pr
Block	50.44	11	4.59	<0.001
Mycorrhiza	31.60	1	31.60	<0.001
Phosphorous	57.78	2	28.89	<0.001
Mycorrhiza.Phosphorous	41.37	2	20.69	<0.001

Dropping individual terms from full fixed model

Fixed term	Wald statistic	d.f.	Wald/d.f.	chi pr
Mycorrhiza.Phosphorous	41.37	2	20.69	<0.001
Block	36.70	11	3.34	<0.001

Message: chi-square distribution for Wald tests is an asymptotic approximation (i.e. for large samples) and underestimates the probabilities in other cases.

Table of predicted means for Constant

6.388 Standard error: 0.0516

Table of predicted means for Block

Block	1	2	3	4	5	6	7	8
	6.364	6.517	6.332	6.154	5.980	6.249	6.581	7.135

Block	9	10	11	12
	6.232	6.225	6.740	6.147

Standard errors of differences between pairs

Block 1	1	*						
Block 2	2	0.311	*					
Block 3	3	0.245	0.314	*				
Block 4	4	0.245	0.314	0.256	*			
Block 5	5	0.228	0.306	0.244	0.244	*		
Block 6	6	0.228	0.310	0.244	0.244	0.228	*	
Block 7	7	0.229	0.305	0.240	0.240	0.229	0.228	*
Block 8	8	0.241	0.322	0.261	0.257	0.243	0.243	0.245
Block 9	9	0.229	0.305	0.240	0.240	0.229	0.228	0.225
Block 10	10	0.217	0.300	0.233	0.233	0.217	0.217	0.217

Block 11	11	0.229	0.305	0.240	0.240	0.229	0.228	0.225
Block 12	12	0.241	0.322	0.257	0.261	0.243	0.243	0.245
		1	2	3	4	5	6	7

Block 8	8	*				
Block 9	9	0.245	*			
Block 10	10	0.233	0.217	*		
Block 11	11	0.245	0.225	0.217	*	
Block 12	12	0.256	0.245	0.233	0.245	*
		8	9	10	11	12

Standard errors of differences

Average: 0.2492
Maximum: 0.3217
Minimum: 0.2167

Average variance of differences: 0.06294

Table of predicted means for Mycorrhiza

Mycorrhiza	N	Y
	6.638	6.138

Standard errors of differences between pairs

Mycorrhiza N	1	*	
Mycorrhiza Y	2	0.101	*
		1	2

Table of predicted means for Phosphorous

Phosphorous	h	l	o
	6.202	6.107	6.856

Standard errors of differences between pairs

Phosphorous h	1	*		
Phosphorous l	2	0.132	*	
Phosphorous o	3	0.126	0.119	*
		1	2	3

Standard errors of differences

Average: 0.1256
Maximum: 0.1315
Minimum: 0.1191

Average variance of differences: 0.01579

Table of predicted means for Mycorrhiza.Phosphorous

Mycorrhiza \ Phosphorous	h	l	o
N	6.187	6.176	7.552
Y	6.217	6.038	6.159

Standard errors of differences between pairs

Mycorrhiza N.Phosphorous h	1	*				
Mycorrhiza N.Phosphorous l	2	0.167	*			
Mycorrhiza N.Phosphorous o	3	0.159	0.165	*		
Mycorrhiza Y.Phosphorous h	4	0.192	0.195	0.191	*	
Mycorrhiza Y.Phosphorous l	5	0.170	0.174	0.164	0.203	*
Mycorrhiza Y.Phosphorous o	6	0.167	0.173	0.165	0.195	0.174
		1	2	3	4	5

Mycorrhiza Y.Phosphorous o	6	*
		6

Standard errors of differences

Average:	0.1770
Maximum:	0.2034
Minimum:	0.1591

Average variance of differences: 0.03151

Standard error of differences for same level of factor:

	Mycorrhiza	Phosphorous
Average:	0.1773	0.1770
Maximum:	0.2034	0.1920
Minimum:	0.1591	0.1649

Average variance of differences:

0.03169	0.03145

10. NUMBER OF LEAVES

a) 5/8/06 (1 week before harvest)

Data were severely underdispersed and thus the dispersion parameter was estimated from the data as 0.491.

```
807  "Log-linear modelling."
 808  MODEL [DISTRIBUTION=poisson; LINK=log; DISPERSION=*] leaves_2_8
 809  FITINDIVIDUALLY [PRINT=model,summary,estimates,accumulated; CONSTANT=estimate;
FPROB=yes;\
 810   TPROB=yes; FACT=9] Block+Mycorrhiza*Phosphorous
```

Regression analysis

Response variate: leaves_2_8
Distribution: Poisson
Link function: Log
Fitted terms: Constant + Block + Mycorrhiza + Phosphorous + Mycorrhiza.Phosphorous

Summary of analysis

Source	d.f.	deviance	mean deviance	deviance ratio	approx F pr.
Regression	16	32.05	2.0028	4.08	<.001
Residual	41	20.15	0.4915		
Total	57	52.20	0.9157		
Change	-2	-7.10	3.5516	7.23	0.002

Dispersion parameter is estimated to be 0.491 from the residual deviance.

Message: the following units have large standardized residuals.

Unit	Response	Residual
21	2.000	-2.77
22	6.000	2.85
39	2.000	-2.71

Estimates of parameters

Parameter	estimate	s.e.	t(41)	t pr.	antilog of estimate
Constant	1.873	0.152	12.35	<.001	6.510
Block 2	-0.095	0.221	-0.43	0.670	0.9097
Block 3	-0.252	0.192	-1.31	0.197	0.7774
Block 4	-0.231	0.206	-1.12	0.271	0.7941
Block 5	-0.212	0.187	-1.13	0.264	0.8090
Block 6	-0.160	0.176	-0.91	0.368	0.8524
Block 7	-0.285	0.181	-1.57	0.123	0.7521
Block 8	-0.426	0.217	-1.96	0.056	0.6530
Block 9	-0.060	0.181	-0.33	0.743	0.9418
Block 10	-0.048	0.171	-0.28	0.779	0.9527
Block 11	-0.449	0.201	-2.23	0.031	0.6380
Block 12	-0.056	0.197	-0.28	0.777	0.9454

Mycorrhiza Y	0.068	0.141	0.49	0.630	1.071
Phosphorous l	-0.002	0.133	-0.01	0.990	0.9983
Phosphorous o	-0.682	0.152	-4.48	<.001	0.5056
Mycorrhiza Y .Phosphorous l	0.244	0.196	1.24	0.220	1.277
Mycorrhiza Y .Phosphorous o	0.785	0.211	3.72	<.001	2.193

Message: s.e.s are based on the residual deviance.

Parameters for factors are differences compared with the reference level:

Factor	Reference level
Block	1
Mycorrhiza	N
Phosphorous	h

Accumulated analysis of deviance

Change	d.f.	deviance	mean deviance	deviance ratio	approx F pr.
+ Block	11	5.6638	0.5149	1.05	0.425
+ Mycorrhiza	1	11.6548	11.6548	23.71	<.001
+ Phosphorous	2	7.6237	3.8119	7.76	0.001
+ Mycorrhiza.Phosphorous	2	7.1032	3.5516	7.23	0.002
Residual	41	20.1500	0.4915		
Total	57	52.1955	0.9157		

Block was dropped from the model to simplify calculation of anti-logs:

```
291  "Log-linear modelling."
 292  MODEL [DISTRIBUTION=poisson; LINK=log; DISPERSION=*] leaves_2_8
 293  FITINDIVIDUALLY [PRINT=model,summary,estimates,accumulated; CONSTANT=estimate;
FPROB=yes;\
 294   TPROB=yes; FACT=9] Mycorrhiza*Phosphorous
```

Regression analysis

Response variate:	leaves_2_8
Distribution:	Poisson
Link function:	Log
Fitted terms:	Constant + Mycorrhiza + Phosphorous + Mycorrhiza.Phosphorous

Summary of analysis

Source	d.f.	deviance	mean deviance	deviance ratio	approx F pr.
Regression	5	26.33	5.2669	10.59	<.001
Residual	52	25.86	0.4973		
Total	57	52.20	0.9157		
Change	-2	-7.07	3.5374	7.11	0.002

Dispersion parameter is estimated to be 0.497 from the residual deviance.

Message: the following units have large standardized residuals.

Unit	Response	Residual

21	2.000	-2.57
22	6.000	2.51
39	2.000	-2.81

Estimates of parameters

Parameter	estimate	s.e.	t(52)	t pr.	antilog of estimate
Constant	1.6964	0.0910	18.64	<.001	5.455
Mycorrhiza Y	0.074	0.137	0.54	0.591	1.077
Phosphorous l	0.008	0.132	0.06	0.950	1.008
Phosphorous o	-0.685	0.153	-4.48	<.001	0.5042
Mycorrhiza Y .Phosphorous l	0.213	0.188	1.13	0.262	1.238
Mycorrhiza Y .Phosphorous o	0.767	0.209	3.67	<.001	2.152

Message: s.e.s are based on the residual deviance.

Parameters for factors are differences compared with the reference level:

Factor	Reference level
Mycorrhiza	N
Phosphorous	h

Accumulated analysis of deviance

Change	d.f.	deviance	mean deviance	deviance ratio	approx F pr.
+ Mycorrhiza	1	11.2560	11.2560	22.63	<.001
+ Phosphorous	2	8.0040	4.0020	8.05	<.001
+ Mycorrhiza.Phosphorous	2	7.0747	3.5374	7.11	0.002
Residual	52	25.8608	0.4973		
Total	57	52.1955	0.9157		

Estimates of parameters

Parameter	estimate	s.e.	t(52)	t pr.	antilog of estimate
Constant	1.7047	0.0950	17.94	<.001	5.500
Mycorrhiza Y	0.288	0.129	2.23	0.030	1.333
Phosphorous h	-0.008	0.132	-0.06	0.950	0.9917
Phosphorous o	-0.693	0.155	-4.47	<.001	0.5000
Mycorrhiza Y .Phosphorous h	-0.213	0.188	-1.13	0.262	0.8078
Mycorrhiza Y .Phosphorous o	0.553	0.203	2.72	0.009	1.739

Message: s.e.s are based on the residual deviance.

Parameters for factors are differences compared with the reference level:

Factor	Reference level
Mycorrhiza	N
Phosphorous	l

Estimates of parameters

Parameter	estimate	s.e.	t(52)	t pr.	antilog of estimate
Constant	1.012	0.123	8.25	<.001	2.750
Mycorrhiza Y	0.841	0.157	5.34	<.001	2.318
Phosphorous h	0.685	0.153	4.48	<.001	1.983
Phosphorous l	0.693	0.155	4.47	<.001	2.000
Mycorrhiza Y .Phosphorous h	-0.767	0.209	-3.67	<.001	0.4646
Mycorrhiza Y .Phosphorous l	-0.553	0.203	-2.72	0.009	0.5752

Message: s.e.s are based on the residual deviance.

Parameters for factors are differences compared with the reference level:

Factor	Reference level
Mycorrhiza	N
Phosphorous	o

Estimates of parameters

Parameter	estimate	s.e.	t(52)	t pr.	antilog of estimate
Constant	1.771	0.103	17.23	<.001	5.875
Mycorrhiza N	-0.074	0.137	-0.54	0.591	0.9284
Phosphorous l	0.222	0.135	1.65	0.105	1.248
Phosphorous o	0.082	0.143	0.57	0.569	1.085
Mycorrhiza N .Phosphorous l	-0.213	0.188	-1.13	0.262	0.8078
Mycorrhiza N .Phosphorous o	-0.767	0.209	-3.67	<.001	0.4646

Message: s.e.s are based on the residual deviance.

Parameters for factors are differences compared with the reference level:

Factor	Reference level
Mycorrhiza	Y
Phosphorous	h

Estimates of parameters

Parameter	estimate	s.e.	t(52)	t pr.	antilog of estimate
Constant	1.9924	0.0868	22.96	<.001	7.333
Mycorrhiza N	-0.288	0.129	-2.23	0.030	0.7500
Phosphorous h	-0.222	0.135	-1.65	0.105	0.8011
Phosphorous o	-0.140	0.131	-1.07	0.292	0.8693
Mycorrhiza N .Phosphorous h	0.213	0.188	1.13	0.262	1.238
Mycorrhiza N .Phosphorous o	-0.553	0.203	-2.72	0.009	0.5752

Message: s.e.s are based on the residual deviance.

Parameters for factors are differences compared with the reference level:

Factor	Reference level
Mycorrhiza	Y
Phosphorous	l

Estimates of parameters

Parameter	estimate	s.e.	t(52)	t pr.	antilog of estimate
Constant	1.8524	0.0987	18.77	<.001	6.375
Mycorrhiza N	-0.841	0.157	-5.34	<.001	0.4314
Phosphorous h	-0.082	0.143	-0.57	0.569	0.9216
Phosphorous l	0.140	0.131	1.07	0.292	1.150
Mycorrhiza N .Phosphorous h	0.767	0.209	3.67	<.001	2.152
Mycorrhiza N .Phosphorous l	0.553	0.203	2.72	0.009	1.739

Message: s.e.s are based on the residual deviance.

Parameters for factors are differences compared with the reference level:

Factor	Reference level
Mycorrhiza	Y
Phosphorous	o

b) 9/8/06

Data were severely underdispersed and thus the dispersion parameter was estimated from the data as 0.454.

```
820  MODEL [DISTRIBUTION=poisson; LINK=log; DISPERSION=*] leaves_9_8
 821  FITINDIVIDUALLY [PRINT=model,summary,estimates,accumulated; CONSTANT=estimate;
FPROB=yes;\
 822   TPROB=yes; FACT=9] Block+Mycorrhiza*Phosphorous
```

Regression analysis

Response variate:	leaves_9_8
Distribution:	Poisson
Link function:	Log
Fitted terms:	Constant + Block + Mycorrhiza + Phosphorous + Mycorrhiza.Phosphorous

Summary of analysis

Source	d.f.	deviance	mean deviance	deviance ratio	approx F pr.
Regression	16	54.02	3.3763	7.43	<.001
Residual	38	17.26	0.4542		
Total	54	71.28	1.3200		
Change	-2	-14.87	7.4373	16.37	<.001

Dispersion parameter is estimated to be 0.454 from the residual deviance.

Message: the following units have large standardized residuals.

Unit	Response	Residual
45	0.000	-4.75

Message: the following units have high leverage.

Unit	Response	Leverage
12	13.000	0.79

Estimates of parameters

Parameter	estimate	s.e.	t(38)	t pr.	antilog of estimate
Constant	2.139	0.133	16.10	<.001	8.488
Block 2	0.307	0.218	1.41	0.167	1.359
Block 3	-0.115	0.180	-0.64	0.526	0.8911
Block 4	-0.321	0.189	-1.70	0.098	0.7256
Block 5	-0.037	0.160	-0.23	0.818	0.9637
Block 6	-0.122	0.157	-0.78	0.443	0.8853
Block 7	-0.077	0.164	-0.47	0.639	0.9255
Block 8	-0.721	0.194	-3.72	<.001	0.4865
Block 9	-0.077	0.164	-0.47	0.639	0.9255
Block 10	-0.019	0.151	-0.12	0.902	0.9815
Block 11	-0.346	0.176	-1.97	0.057	0.7077
Block 12	-0.048	0.172	-0.28	0.784	0.9535
Mycorrhiza Y	0.070	0.132	0.53	0.598	1.073
Phosphorous l	-0.085	0.115	-0.74	0.467	0.9187
Phosphorous o	-1.066	0.151	-7.05	<.001	0.3444
Mycorrhiza Y .Phosphorous l	0.056	0.182	0.31	0.761	1.057
Mycorrhiza Y .Phosphorous o	1.018	0.202	5.04	<.001	2.768

Message: s.e.s are based on the residual deviance.

Parameters for factors are differences compared with the reference level:

Factor	Reference level
Block	1
Mycorrhiza	N
Phosphorous	h

Accumulated analysis of deviance

Change	d.f.	deviance	mean deviance	deviance ratio	approx F pr.
+ Block	11	13.7339	1.2485	2.75	0.010
+ Mycorrhiza	1	10.6637	10.6637	23.48	<.001
+ Phosphorous	2	14.7489	7.3745	16.23	<.001
+ Mycorrhiza.Phosphorous	2	14.8746	7.4373	16.37	<.001
Residual	38	17.2610	0.4542		
Total	54	71.2821	1.3200		

Block was removed from the model to simplify the analysis of effects, particularly considering that only block 8 is significantly different to the other blocks.

```
118  factor[modify=yes;referencelevel=1]Phosphorous
 119  "Log-linear modelling."
 120  MODEL [DISTRIBUTION=poisson; LINK=log; DISPERSION=*] leaves_9_8
 121  FITINDIVIDUALLY [PRINT=model,summary,estimates; CONSTANT=estimate; FPROB=yes;
TPROB=yes;\
 122   FACT=9] Mycorrhiza*Phosphorous
```

Regression analysis

Response variate: leaves_9_8
Distribution: Poisson
Link function: Log
Fitted terms: Constant + Mycorrhiza + Phosphorous + Mycorrhiza.Phosphorous

Summary of analysis

Source	d.f.	deviance	mean deviance	deviance ratio	approx F pr.
Regression	5	40.17	8.0333	12.65	<.001
Residual	49	31.12	0.6350		
Total	54	71.28	1.3200		
Change	-2	-12.40	6.2022	9.77	<.001

Dispersion parameter is estimated to be 0.635 from the residual deviance.

Message: the following units have large standardized residuals.

Unit	Response	Residual
45	0.000	-4.88

Estimates of parameters

Parameter	estimate	s.e.	t(49)	t pr.	antilog of estimate
Constant	1.9741	0.0939	21.02	<.001	7.200
Mycorrhiza Y	0.105	0.148	0.71	0.481	1.111
Phosphorous l	-0.057	0.135	-0.42	0.673	0.9445
Phosphorous o	-1.005	0.175	-5.73	<.001	0.3662
Mycorrhiza Y .Phosphorous l	0.085	0.200	0.42	0.674	1.088
Mycorrhiza Y .Phosphorous o	0.918	0.231	3.97	<.001	2.503

Message: s.e.s are based on the residual deviance.

Parameters for factors are differences compared with the reference level:

Factor	Reference level
Mycorrhiza	N
Phosphorous	h

Accumulated analysis of deviance

Change	d.f.	deviance	mean deviance	deviance ratio	approx F pr.
+ Mycorrhiza	1	11.7046	11.7046	18.43	<.001
+ Phosphorous	2	16.0576	8.0288	12.64	<.001
+ Mycorrhiza.Phosphorous	2	12.4043	6.2022	9.77	<.001

Residual	49	31.1156	0.6350	
Total	54	71.2821	1.3200	

Estimates of parameters

Parameter	estimate	s.e.	t(49)	t pr.	antilog of estimate
Constant	1.9169	0.0964	19.87	<.001	6.800
Mycorrhiza Y	0.190	0.134	1.42	0.162	1.209
Phosphorous h	0.057	0.135	0.42	0.673	1.059
Phosphorous o	-0.948	0.177	-5.37	<.001	0.3877
Mycorrhiza Y .Phosphorous h	-0.085	0.200	-0.42	0.674	0.9189
Mycorrhiza Y .Phosphorous o	0.833	0.222	3.75	<.001	2.300

Message: s.e.s are based on the residual deviance.

Parameters for factors are differences compared with the reference level:

Factor	Reference level
Mycorrhiza	N
Phosphorous	l

Estimates of parameters

Parameter	estimate	s.e.	t(49)	t pr.	antilog of estimate
Constant	0.969	0.148	6.55	<.001	2.636
Mycorrhiza Y	1.023	0.177	5.76	<.001	2.782
Phosphorous h	1.005	0.175	5.73	<.001	2.731
Phosphorous l	0.948	0.177	5.37	<.001	2.579
Mycorrhiza Y .Phosphorous h	-0.918	0.231	-3.97	<.001	0.3994
Mycorrhiza Y .Phosphorous l	-0.833	0.222	-3.75	<.001	0.4347

Message: s.e.s are based on the residual deviance.

Parameters for factors are differences compared with the reference level:

Factor	Reference level
Mycorrhiza	N
Phosphorous	o

Estimates of parameters

Parameter	estimate	s.e.	t(49)	t pr.	antilog of estimate
Constant	2.079	0.115	18.08	<.001	8.000
Mycorrhiza N	-0.105	0.148	-0.71	0.481	0.9000
Phosphorous l	0.027	0.148	0.19	0.854	1.028
Phosphorous o	-0.087	0.151	-0.58	0.567	0.9167
Mycorrhiza N .Phosphorous l	-0.085	0.200	-0.42	0.674	0.9189

Mycorrhiza N .Phosphorous o	-0.918	0.231	-3.97	<.001	0.3994

Message: s.e.s are based on the residual deviance.

Parameters for factors are differences compared with the reference level:

Factor	Reference level
Mycorrhiza	Y
Phosphorous	h

Estimates of parameters

Parameter	estimate	s.e.	t(49)	t pr.	antilog of estimate
Constant	2.1068	0.0926	22.75	<.001	8.222
Mycorrhiza N	-0.190	0.134	-1.42	0.162	0.8270
Phosphorous h	-0.027	0.148	-0.19	0.854	0.9730
Phosphorous o	-0.114	0.135	-0.85	0.400	0.8919
Mycorrhiza N .Phosphorous h	0.085	0.200	0.42	0.674	1.088
Mycorrhiza N .Phosphorous o	-0.833	0.222	-3.75	<.001	0.4347

Message: s.e.s are based on the residual deviance.

Parameters for factors are differences compared with the reference level:

Factor	Reference level
Mycorrhiza	Y
Phosphorous	l

Estimates of parameters

Parameter	estimate	s.e.	t(49)	t pr.	antilog of estimate
Constant	1.9924	0.0981	20.32	<.001	7.333
Mycorrhiza N	-1.023	0.177	-5.76	<.001	0.3595
Phosphorous h	0.087	0.151	0.58	0.567	1.091
Phosphorous l	0.114	0.135	0.85	0.400	1.121
Mycorrhiza N .Phosphorous h	0.918	0.231	3.97	<.001	2.503
Mycorrhiza N .Phosphorous l	0.833	0.222	3.75	<.001	2.300

Message: s.e.s are based on the residual deviance.

Parameters for factors are differences compared with the reference level:

Factor	Reference level
Mycorrhiza	Y
Phosphorous	o

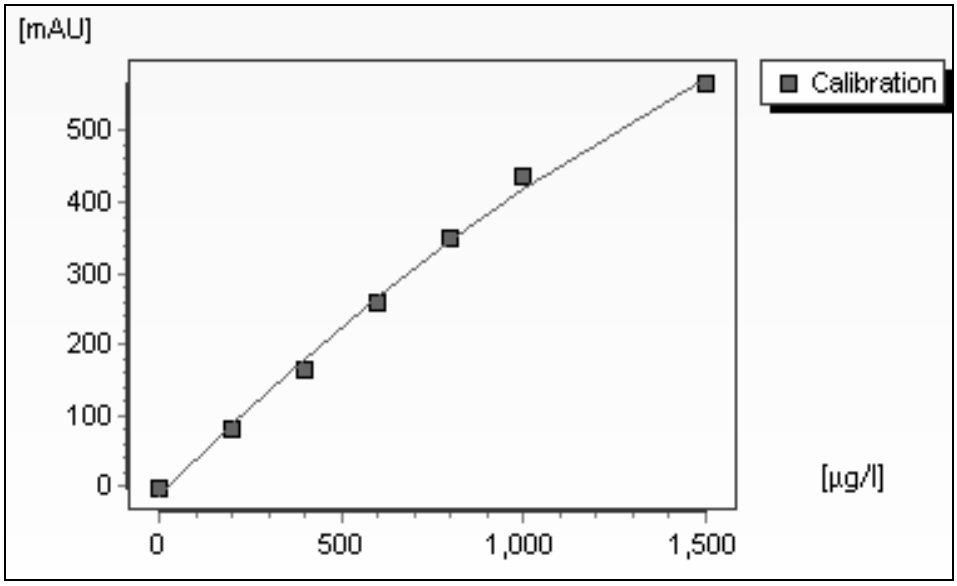


Figure A1 Calibration curve for phosphorus analysis of *Solanum centrale* roots, shoots, seeds and lateral roots. Standard curve for this calibration is mAU = -13.161118 + $0.514315x - 0.000082x^2$