RURAL INDUSTRIES RESEARCH
& DEVELOPMENT CORPORATION

French Black Truffle

Establishment and Production In Tasmania

A report for the Rural Industries Research and Development Corporation

by DC Garvey and PB Cooper

June 2001

RIRDC Publication No 01/084
RIRDC Project No. PTT-1A, PTT-2A

ISBN 0 642 58306 4
ISSN 1440-6845

French Black Truffle - Establishment and Production in Tasmania
Publication No. 01/084
Project No. PTT-1A, PTT-2A

Researcher Contact Details
Mr Duncan Garvey
Perigord Truffles of Tasmania
Tasman Highway
GROVE TAS 7109

Phone: 03 62664213
Fax: 03 62664012
Email: duncan@perigord.com.au

RIRDC Contact Details
Rural Industries Research and Development Corporation
Level 1, AMA House
42 Macquarie Street
BARTON ACT 2600
PO Box 4776
KINGSTON ACT 2604

Phone: 02 6272 4539
Fax: 02 6272 5877
Email: rirdc@rirdc.gov.au.
Website: http://www.rirdc.gov.au

Published in June 2001
Printed on environmentally friendly paper by Canprint

Foreword

French black truffles *(Tuber melanosporum)* have traditionally been produced in France, Italy and Spain. The fungus forms a symbiotic relationship with hazel *(Corylus avellana)* and oak *(Quercus spp*) trees. The truffles are detected by their unique aroma when mature. They are located using trained detector dogs in the northern hemisphere winter months of December January and February. Tasmania's position with respect to latitude, climate and temperatures are very similar to the principal production areas of Europe and afford the opportunity to produce and supply traditional markets out of season.

This publication considers some factors involved in transferring and applying the technology developed by the French. It analyses the traditional production systems and markets and addresses some factors considered potential impediments to establishment and production in Tasmania.

This project was funded from RIRDC Core Funds which, are provided by the Federal Government.

This report, a new addition to RIRDC's diverse range of over 700 research publications, forms part of our new plant products R&D program, which aims to facilitate the development of new industries based on plants or plant products that have commercial potential for Australia.

Most of our publications are available for viewing, downloading or purchasing online through our website:

- downloads at www.rirdc.gov.au/reports/Index.htm
- purchases at www.rirdc.gov.au/eshop

Peter Core
Managing Director
Rural Industries Research and Development Corporation

Acknowledgements

Perigord Truffles of Tasmania would like to thank the following people for their assistance and support:

- To our joint venture growers who have supported a project to produce a product that in many cases they had not seen touched or tasted prior to their commitment;
- Dr. Daryl Brown for his assistance in data analysis;
- University of Tasmania for allowing Dr. Brown to study and complete his thesis;
- RIRDC for sponsoring and supporting the project; and
- Members of the French truffle industry including scientists, producers and marketers for their assistance and support.

Contents

Executive Summary

Perigord Truffles of Tasmania Pty Ltd (PTT) was formed in 1992 to exploit the opportunity of producing and marketing the highly valued and sought after gourmet food product known as the French black truffle or Perigord truffle.

The French black truffle, which is the fruiting body of the fungus *Tuber melanosporum*, grows in a symbiotic relationship with oak and hazel trees and has been traditionally produced in regions of France, Italy and Spain for over a millennium.

The truffles which are formed in the top 20 cm of the soil are harvested manually after using dogs to detect their presence by the perfume they emit at maturity which occurs in the northern hemisphere winter months of December, January and February.

PTT recognised the opportunity to produce and market fresh French black truffles *T. melanosporum* in the southern hemisphere winter months of June, July and August.

An initial study trip to France in 1992 by the directors highlighted areas of research, which were important to achieving the objective of successfully producing and marketing French black truffles.

These were;

- Identification and examination of some introduced and endemic Australian ectomycorrhizal fungi and their ability to compete with *T. melanosporum* in establishing truffières in Tasmania;
- Developing an understanding of the market both domestically and internationally, by investigating the supply chain and relationships between producers, wholesalers and consumers as either a fresh or value added product; and
- Identifying impediments or limiting factors to production in Europe, such as irrigation methods and scheduling weed control in truffières and manipulation of some nutrients.

Since 1993 PTT have been conducting a program to address these key research issues.

Study Trips to France

The directors determined that if a French truffle industry was to be successfully established in Tasmania it was essential to gain a complete understanding of the industry in France.

Three research trips to France were made by the directors of PTT to confirm the reality of the opportunity, assess the likelihood of obtaining the technology necessary to produce trees inoculated with *T. melanosporum* and develop distribution channels for marketing truffles.

PhD Thesis

A PhD study was undertaken by Dr. Daryl Brown addressed the following key areas of research:

- Ectomycorrhizal fungi associated with hazel in Tasmania, Australia;
- Effect of applied lime on *T. melanosporum* and other introduced ectomycorrhizal fungi;
- Effect of applied lime on *T. melanosporum* and other ectomycorrhizal fungi forming mycorrhiza with hazel;

- The effect of pH and calcium on competition between *T. melanosporum* and other ectomycorrhizal fungi;
- The effects of the interaction between lime and phosphorus on competition between *T. melanosporum* and other ectomycorrhizal;
- Survey of ectomycorrhizal fungi in a Tasmanian truffière; and
- Identification of (*Tuber*) mycorrhiza using DNA finger printing.

The key findings from the thesis were:

- The research indicated that there is a range of endemic fungi in Tasmania soils that may be potential competitors to *T. melanosporum*;
- The study showed that a percentage of the endemic fungi have a reduced activity and competitiveness at higher soil pH levels; and
- pH has a more significant affect on the colonisation of *T. melanosporum* than calcium.

Herbicide Evaluation

The objective in Tasmanian truffières is to establish a strip of grass and clover between the rows of trees rather than having bare earth. Herbicide use is necessary to control the weeds around the trees to avoid competition for nutrients and soil moisture.

PTT established a trial to assess the effects of the use of two herbicides on *T. melanosporum* mycorrhiza on hazels in Tasmania. The research indicated that applying relatively high rates of the herbicides to the soil at the base of the hazel seedling did not impact the level of colonisation by *T. melanosporum*. Furthermore, soil type did not appear to influence the effect of the herbicides on *T. melanosporum* colonisation.

The findings of the experiment have enabled Perigord Truffles of Tasmania to develop a weed control strategy that gives flexibility both in total weed control and in the manipulation of desirable species within truffières.

Irrigation Trial

PTT conducted a trial on two commercial truffières in Tasmania to determine if there is correlation between soil moisture and mycorrhizal root development. If a relationship could be determined it would have a significant impact on the irrigation scheduling of the commercial truffières established in Tasmania. Potentially it could be a significant factor in the commencement of early truffle production.

The trial conducted over two season's 1996-98 used two irrigation monitoring systems, to determine the soil moisture levels under three different water application rates and the effect on mycorrhizal activity.

Three irrigation application rates were used using three different output Dan 2001 micro jet low pressure sprinklers. The three applications were 35 litres/hour, 70 litres/hour and 105 litre/hour respectively.

The results of the trial showed no significant difference between the mycorrhizal development under the three different treatments.

Although the results of the experimentation were inconclusive the information generated by recording soil moisture levels was very important.

In both truffières the data clearly indicated that there were wide variations in soil moisture levels particularly in the first year. The trial strongly indicated the benefits of using either form of monitoring equipment. Perigord has through the experience gained been able to convey to it's joint venture farmers the importance of timely reading and recording of probes and tensiometers with respect to irrigation scheduling.

Tree Growth Trial

The objective of the tree growth trial was to investigate the effects of increasing tree growth rate on the level of mycorrhization and therefore reducing the time from out-planting to the commencement of truffle production.

The key aims of the experimentation which were established on two commercial truffières in Tasmania were:

- To determine if increased tree growth would result in increased competition from endemic fungi;
- To investigate the effects of increasing tree growth rate on the level of mycorrhization; and
- To reduce the time from out-planting to truffle production

In August 1998 replicates of four rows of ten trees were selected in each truffière.
Three treatments of phosphorus and nitrogen were applied in split applications to the trees. In addition an artificial windbreak was constructed to assess the effect of wind on the growth rates of the trees.

The key findings of the trial were:

- No significant difference in the rate of mycorrhizal growth under any of the treatments;
- No reduction in the mycorrhiza as a result of applying the phosphorus and nitrogen;
- Whilst a small number of other unidentified mycorrhiza were observed, there was no significant effect between the treatments;
- There was no significant response in tree growth to the phosphorus and nitrogen treatments compared to the controls at either site; and
- A limited response in tree growth to the windbreak treatment in one truffière.

The above findings have allowed PTT to proceed with confidence in further assessing the relationship between fertiliser applications and the response in tree growth rates.

The production of 3 kg of French truffles on multiple sites in the winter of 2000 has confirmed PTT's success in achieving its goals. The company has confirmed its ability to transfer and apply the necessary technology and had its theories with respect to climate, geography and geology tested and confirmed. PTT has seen the creation of a unique production agreement with its joint venture growers that has withstood the test of time and has successfully harvested and marketed a premium quality product to an excited and receptive market at a price almost double its budget.

The nature of the joint venture agreement between PTT and truffle growers results in both the company and farmers sharing equally in any of the benefits gained from the research and development program.

1. Introduction

Perigord Truffles of Tasmania Pty Ltd (PTT) was formed in 1992 to exploit the opportunity of producing and marketing the highly valued and sought after gourmet food product known as the French black truffle or Perigord truffle.
An initial study trip to France in 1992 by the directors highlighted areas of research which were important to achieving the objective of successfully producing and marketing French black truffles and since 1993 PTT have been conducting a program to address these key research issues.

The following report covers the research undertaken.

Study Trips to France

The directors determined that if a French truffle industry was to be successfully established in Tasmania it was essential to gain a complete understanding of the industry in France.

Three research trips to France were made by the directors of PTT to confirm the reality of the opportunity, assess the likelihood of obtaining the technology necessary to produce trees inoculated with *T. melanosporum* and develop distribution channels for marketing truffles.

PhD Thesis

A PhD study titled "The Effect of Applied Lime and Phosphorus on the Competitiveness of *Tuber melanosporum* and other Ectomychorrizal Fungi found in Tasmania" was completed by Dr. Daryl Brown in 1998.

Herbicide Evaluation

PTT established a trial to assess the effects of the use of two herbicides on *T. melanosporum* mycorrhiza on hazels in Tasmania.

Irrigation Trial

A trial was conducted on two commercial truffières in Tasmania to determine if there is correlation between soil moisture and mycorrhizal root development

Tree Growth Trial

The objective of the tree growth trial was to investigate the effects of increasing tree growth rate on the level of mycorrhization and therefore reducing the time from out-planting to the commencement of truffle production.

The production of 3 kg of French truffles on multiple sites in the winter of 2000 has confirmed PTT's success in achieving its goals. The company has confirmed its ability to transfer and apply the necessary technology and had its theories with respect to climate, geography and geology tested and confirmed. PTT has seen the creation of a unique production agreement with its joint venture growers that has withstood the test of time and has successfully harvested and marketed a premium quality product to an excited and receptive market at a price almost double its budget.

2. Research Trips to France

Introduction

Perigord Truffles of Tasmania (PTT) have recognised the opportunity to produce and market fresh French black truffles (*Tuber melanosporum)* in the southern hemisphere winter months of June, July and August. To capitalise on this opportunity, PTT has had to achieve the following goals:

- Acquire and apply the technology to produce trees inoculated with *T. melanosporum*;
- Prove that the fungus would proliferate in Tasmanian soils;
- Produce and harvest the truffles; and
- Develop distribution channels and market the truffles.

With France being the traditional and major producer of French truffles, three research trips were made to France to confirm the reality of the opportunity and the likelihood of being able to obtain the necessary technology.

The key objective of the research trips to France undertaken by the directors of PTT (Duncan Garvey and Peter Cooper) was to pursue the goals outlined above. The directors determined that if a French truffle industry was to be successfully established in Tasmania it was essential to gain a complete understanding of the industry in France, from the scientific research and distribution channels through to the end users of the truffles.

Overview of French Industry

The French black truffle has arrived from a past of grandeur. The ancient Greeks and Romans attributed therapeutic and aphrodisiac powers to them, qualities still in vogue last century. Brillat Savarin referred to them as "the jewel of cookery", saying they aroused "erotic and gastronomic memories among the skirted sex, and memories gastronomic and erotic among the bearded sex." Alexandre Dumas described them as "the gastronomes holy of holies."

Towards the end of the last century, France annually produced up to 1000 tonnes of French black truffles from more than 20 regions located in southern France. Since this period output has fallen continuously, with some slight variations, to a level of combined production from France, Spain and Italy of 50-80 tonnes annually. The causes of this decline in production are many including; abandonment of land cultivation, wartime destruction of trees, planned de-forestation and acid rain.

During the mid 1960's the French Government research institution Institut National de la Recherche Agronomique (INRA) undertook a massive research program aimed at saving the industry. By the late1970's a technique to artificially inoculate oak and hazel trees with spores of *T. melanosporum* was developed. Using the technology under licence, a private company, Agritruffe, produced and marketed inoculated trees, resulting in a renewed interest in producing truffles. The technology has been successful with hazel trees producing truffles after 5 years and oaks producing after 6-8 years, allowing for the establishment of artificial truffières in many regions of France.

.

Agritruffe remain the principal supplier and industry standard for inoculated trees in France with an annual production of 100,000 trees. They have continued to work closely with INRA in further

developing and improving the technology, however a long-standing agreement between Agritruffe and INRA has meant much of the methodology and research is still regarded as commercially sensitive.

There are currently a number of smaller organisations in France and parts of Spain producing inoculated trees. In recent years legislation has been enacted in an attempt to implement some quality control measures, however due to the cash nature of the truffle industry the effectiveness of these controls remains questionable.

Inoculated trees are also being produced in the USA and in New Zealand. The New Zealand Ministry of Agriculture and Fisheries developed their own technology during the mid 1980's, which has resulted in their first harvest of truffles on a small number of sites in recent years.

A group of Americans based in Oregon who initially purchased inoculated trees from the French produced their first French black truffle in the Northern Hemisphere winter of 1991. Recent news releases also indicate successful attempts have been made to produce the French black truffle in California.

Discussions have been held with the key scientists involved in the French truffle industry and strong relationships have been formed with the French scientists working with the INRA and those involved in private research. The level of interest among the French scientists in the Tasmanian truffle project has been very high. As southern hemisphere production is seen as complementary to their industry, rather than a threat, the level of communication with the French has been excellent and they have been very supportive.

French Truffle Production

The annual harvest of French black truffles from France, Italy and Spain combined is currently 50-80 tonnes. Truffle production in France occurs in the south east and south west areas namely the Provence and Perigord regions. Truffle production has increased in the Provence area and now accounts for up to 75% of total French production.

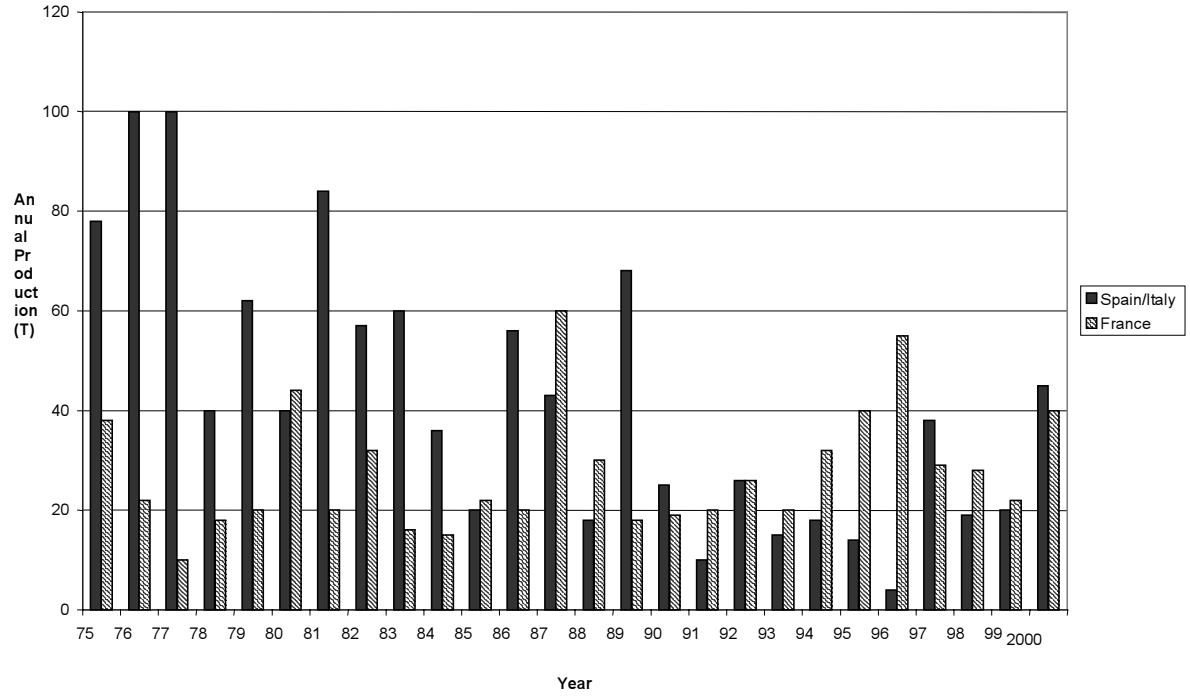
Up to 70% of the annual harvest continues to be collected from the native oak and hazel forests and not from specially planted truffières. Droughts and other adverse conditions do have a significant affect on the level of production, with many French producers adopting horticultural management systems such as irrigation scheduling and soil testing, resulting in more consistent yields from their plantations.

The only northern hemisphere production is in the USA where industry sources suggest a total production of less than 100kg.

It should be noted that a portion of production is consumed without ever entering the market and, due to the cash nature of the industry, total production figures cannot be totally relied upon for accuracy.

Production figures for the French truffle are given in Figure 1.

Figure 1. Annual truffle production in France and Spain/Italy from 1975 to 2000.



Time was spent with the leading French producers in the Perigord and Provence regions discussing all aspects of their production and management systems for their plantations.

Markets

Fresh Truffles

Fresh French black truffles are sold to all the finest restaurants in all corners of the globe. Through the northern hemisphere winter they are served in the great dining rooms of the world. The French are by far the largest consumers of French black truffles (up to 40% of the fresh truffles) with the balance being exported.

The proportion sold fresh each year varies but generally is up to 60% of annual production with the remaining 40% sold in a processed or value added form. (Source: Souzart pers.com)

Other EU countries such as the UK, Belgium, Germany and Switzerland are major importers of French black truffles. The USA is also a large importer of truffles, however Japan is the largest outside the EU and imports large quantities of fresh and preserved truffles annually.

The Japanese market for truffles is very strong and, when in season, are on the menus of many leading restaurants (source Phillipe Mochel, formerly Paul Bocuse Restaurant, Osaka, Japan).

Table 1 below illustrates the size of the Japanese market for fresh and preserved truffles.

Table 1. Japanese imports of fresh and preserved truffles (source: JETRO)

Year	**Fresh (kg)**	**Preserved (kg)**	**Total (kg)**
1998	3184	1563	4747
1997	4249	1233	5482
1996	4805	1581	6386
1995	3342	1767	5109
1994	2354	2022	4376
1993	2675	1730	5405

In Australia current annual consumption of imported fresh truffles is between 100 and 150kg annually, with preserved or value added truffle product consumption estimated at 200kg.

The data from the Australian Bureau of Statistics (ABS) on the importation of truffles and truffle products to Australia is not accurate due to the classifications of other mushroom products within the data. However, the figures for the imported fresh truffles are in line with PTT estimates of the market size for their importing.

PTT directors have attended a number of the small truffle markets in the Perigord and Provence regions and observed the unusual selling system and the presentation of the truffles. On one occasion, Garvey and Cooper attended a market in the Vauclause region at which 1000kg of truffles were sold in less than two hours early on a Saturday morning. The truffles were unwashed, with soil accounting for about 10% of the truffle weight, making it the most expensive soil in the world. Roughly $80,000 worth of soil was sold that morning at the market.

Preserved and Value Added Truffles

In France between 40 and 50% of the harvest is sold in a processed or value added form, which in a typical year could total 30 tonnes. There is a wide broad range of truffle products on the market ranging from whole truffles in jars to truffle purees, and truffle pieces in oils and juices. To complement the preserved truffles there is a selection of value added truffle products available including truffles with foie gras, alcohol products, butter and other traditional French foods.

The truffle products are widely distributed through France and the rest of the world. PTT's research indicates that a percentage of the preserved truffles are sold to restaurants when the fresh truffles are not available. However a very large proportion of the preserved and value added products are marketed in small cans and jars and are distributed through a wide range of wholesale and retail outlets.

In Australia a number of fine food outlets including David Jones and Simon Johnson stock a range of truffle products. Import data from ABS suggests that up to 200kg of truffle products are imported into Australia annually.

A large number of the companies involved in the preserving of the truffles market their product range through the Internet. An example of such is given in Table 2 below.

Table 2. Range of truffle products on the website of truffle wholesaler J Gulliot of France

Product	Weight (grams)	$ AUD	$ per kg equivalent
Whole brushed truffles in jars	28	$92	$3,270
Whole brushed truffles in jars	56	$150	$2,680
Whole brushed truffles in jars	196	$417	$2,120
Whole brushed truffles in jars	476	$667	$1,400
Truffle Peelings	28	$42	$1,490
Truffle Puree	28	$33	$1,320

Over a number of years PTT have conducted extensive research into the French truffle and its markets. PTT directors and staff have spent a total of 11 months over the past 7 years in Europe undertaking study of the truffle industry.

During the regular trips to France during the harvest season, PTT directors participated in many truffle hunts with the French farmers and have observed closely the post harvest procedures of washing, grading and storage of the truffles in preparation for the market.

On one such trip Tim Pak Poy, a leading Sydney chef, accompanied Duncan Garvey on an extensive visit to France for the express purpose of investigating current methods of preservation and value adding of truffles and truffle products. The primary objective of the visit was for Pak Poy to gain a greater knowledge and understanding of current processes in order to aid PTT in the development of this area of its business. PTT sees Pak Poy's role in this area as one of great importance and commissioned him to begin trial recipes and techniques with a small amount of truffles imported from France.

Distribution

The first point of sale for the truffles is the traditional truffle market, which spread across the main production areas in the Perigord and the Provence. The key markets in the areas are held weekly in the small villages. In the Perigord region the markets are in Lalbenque and St Alvere, and in the Provence region, where up to 70% of production occurs, the market towns are Richerenches, Vaucluse, Carpentras and Vars.

The truffles are presented in plastic bags or small baskets and generally are unwashed and not graded. The quality of the truffles sold at these markets varies considerably from the perfect shaped fresh truffles to broken, badly frosted types.

At the markets, agents for processors, restaurateurs and wholesalers buy directly from the producers. A large percentage of the truffles are bought and sold for cash, hence accurate details of volumes sold are almost impossible to obtain. The majority of the truffles purchased from these markets are by the wholesalers and processors, who then transported back to premises where they are cleaned and graded. The major truffle processing and export companies, Pebeyre, Plantin and Comptoire Sud Est de la Truffe have been visited. It has been an excellent opportunity to see the infrastructure and equipment, which will be required by PTT in the future.

In France the restaurants either purchase their fresh truffles directly from the markets or through the wholesalers. Due to the limited shelf life of the product the wholesalers distribute the truffles very quickly.

The fresh truffles, which are exported, are distributed through importing agents in the respective countries. The importing agents handle all the importing protocols and distribute to the individual restaurants.

The value added or processed truffles are distributed through fine food outlets in both France and other countries. Similarly to the fresh truffles, the importers handle the distribution in their countries. In the French market the value added truffle products are sold directly from the processor to wholesale agents and from there distributed to the retail outlets.

Apart from the traditional cash orientated markets for the truffles in the small villages of the Perigord and the Provence, the distribution of the fresh and value added products is similarly structured to many other fine foods. There are an increasing number of export companies in France and other countries selling a range of truffle products via the Internet. Initially the focus was on marketing a range of processed truffle products, but now there is the opportunity to purchase fresh truffles on the Internet during the season.

Truffle Importing

The directors of PTT decided in 1996 that they needed to create an opportunity to gain a better understanding of the French truffles, how to handle and distribute them and also the truffle from the chef's perspective.

PTT commenced importing truffles to leading Sydney restaurants during the northern hemisphere harvest in December, January and February. The imported truffles are purchased directly from a number of truffle growers in the Provence, which further cements PTT's relationship with these growers. The small importing division of PPT have been able to better appreciate the intricacies of the supply chain and the potential problems that can occur in the transportation of a perishable product, both domestically and internationally. This has given the directors a greater understanding of the French black truffles before starting commercial production in Tasmania.

During the past four years PTT has become one of the major importers of fresh French truffles into Australia, supplying truffles to all of Sydney's finest restaurants. The knowledge gained from working closely with some of these chefs will prove invaluable when PTT targets Sydney with fresh truffles produced in Australia.

Truffle Prices

French truffles are considered one of the great foods of the world and when fresh in season prices can be as high as $2,500 per kg at some of the exclusive fine food outlets.

In years of poor production wholesale prices to restaurants can approach $2,000 per kg and in years of good production wholesale prices can be as low as $1,400 per kg.

PTT's research in France pointed to four factors which influence price. These are:

1. Supply

The majority of truffles harvested in France are from native forests, hence the annual production is very much determined by seasonal conditions. In years of poor rainfall prices for good quality truffles can be expected to be considerably higher.

2. Quality

Higher prices are achieved for truffles of good shape and perfume.

3. Seasonal demand

Truffle prices usually peak over the Christmas period. This is a reflection of increased demand and lower supply in the earlier part of the season.

4. Freshness

It appears some of the wholesalers achieve a premium for freshness, however surprisingly, this does not have a significant impact on prices.

During the past four years of importing, PTT believe that supply has the largest influence on price. This is reflected in Table 3 below.

Table 3. The relationship between world supply of truffles and the wholesale price in Sydney.

Year	Wholesale Price Sydney	Total Production
1996/97	$1,400	67 tonnes
1997/98	$1,800	47 tonnes
1998/99	$1,900	42 tonnes
1999/2000	$1,400	85 tonnes

The yearly prices paid to the farmers in France vary considerably. In the typical French truffle market where the truffles are sold unwashed and ungraded the farmers receive between $600-900 per kg.

Discussions with the major wholesaler, Pebeyre in Cahors, suggested that the wholesale mark up is between 50 and 100% on the price paid to the producer. The high wholesale mark up allows for the typical loss of weight after cleaning of 10-15%.

During the visits to France, PTT have gained a good understanding of the structure of the truffle marketing and distribution channels. Along with discussions with the farmers and the truffle wholesalers, PTT have visited a number of the fine food outlets in both France and the UK where fresh truffles are sold.

Peter Cooper, while in the UK and France on his Nuffield Farming Scholarship, made contact with a number of companies importing and retailing fresh and preserved truffle products in London. The management at both Harrods and Harvey Nichols were excited about the prospect of purchasing fresh truffles from Australia through June to September.

In recent years PTT have initiated market research in the Japanese market, as it is planned to be PTT's first export market. Duncan Garvey visited Japan when returning from France in 1997. Facilitated through contacts in Austrade, Garvey had the opportunity to meet a small number of leading Japanese chefs in the Tokyo area.

Truffle Harvesting

The traditional method of harvesting in France is to use a trained dog to indicate the presence of a truffle to its handler. The animal is directed along the rows of trees and upon detecting the scent of a mature truffle is taught to indicate its presence by scratching on the soil surface above the truffle, which is then carefully excavated by the handler.

It is usually the case that dogs used by truffle harvesters in France are household pets, but due to the scale of operations and the contractual arrangements of the joint-venture, PTT has adopted a strategy of owning, training and housing all dogs used for its harvesting operations.

As is the case with most organisations employing large numbers of dogs and handlers (eg. Australian Quarantine Inspection Service (AQIS), Police and Armed Forces), it is imperative that dogs be trained to a standard that will allow them to perform efficiently with a number of trained handlers as distinct from a permanent team.

PTT has contracted one of Australia's leading dog trainers, Mr S. Austin, to advise and assist in the selection and training of both dogs and their handlers. He is highly experienced in this field, having held the contract to train all AQIS detector dogs and their handlers for the past 5 years.

Handlers are introduced to the principles of handling and are then allocated a dog 2 to 3 months prior to the start of the season, during which they reinforce the training procedures required. They are assessed on their ability and relationship with their dog regularly, and then accompany an experienced handler and dogs through the season. The new team is given first opportunity to search a truffière, followed by the proven team who provides a check on the progress and ability of the new team, until the new team is detecting truffles with the same efficiency and reliability as the proven team.

PTT currently has six trained and proven dogs and will expand the number as required to service truffières, as they become mature enough to begin production. It is anticipated that PTT will require 60 dogs and 30 handlers over a 4-month season from May to September to harvest 300ha of truffières on a schedule of weekly harvests.

During the off-season all dogs are kept at kennels owned and operated by PTT and located near its nursery. Handlers are currently employed on an hourly basis, but as yields increase this arrangement will change to a mixture of hourly rate and a piecework rate based on area searched and truffles harvested.

Once harvested, truffles are weighed and transported daily to a central location where they are cleaned and graded ready for dispatch.

PTT have had several meetings and discussions with Dr Thierry Talou at the University of Paul Sabatier in Toulouse. Dr Talou is studying the organoleptic qualities of the truffles and has also participated in the design of a gas detector for the truffle harvest.

Subsequent travel to France, Italy and Spain by directors has resulted in PTT being able to establish its credibility and persistence with a wide range of producers and researchers both in the government and private sectors. The relationship with the French has undoubtedly been a major contributing factor in the success of the venture.

3. PhD Study

The following is the abstract of the thesis prepared by Dr Daryl Brown. The thesis is subject to a full and complete embargo until July 2003.

The Effect of Applied Lime and Phosphorus on the Competitiveness of *Tuber melanosporum* and other Ectomycorrhizal Fungi Found in Tasmania

Hazel (*Corylus avellana* L.) seedlings inoculated with the Perigord black truffle fungus (*Tuber melanosporum* Vitt.) are being planted in Tasmania in an attempt to culture truffles. Competition from other ectomycorrhizal fungi has a significant impact on truffle production in Europe and can be expected to have some effect on the Tasmanian industry. This thesis examines ectomycorrhizal fungi occurring in Tasmania with respect to their ability to form mycorrhizas with hazel and compete with *T. melanosporum* under various soil treatments.

Stands of hazel previously established for nut production or as ornaments were surveyed for the sporocarps of ectomycorrhizal fungi. Several species were found including species that are new either to Australia or Tasmania. The endemic species *Descomyces albus* (Klotzsch) Bougher & Castellano and *Podohydnangium* sp., previously thought to be *Eucalyptus* specific were fruiting under hazel.

T. melanosporum occurs naturally on calcareous soils in Europe. Truffières in Tasmania are heavily limed to create a calcareous soil environment. The response of selected introduced and endemic ectomycorrhizal fungi to applied lime was studied in a glasshouse experiment. Some of the endemic species, which would normally inhabit acidic soils, were unable to survive high rates of lime application and therefore should not pose a threat to the truffle industry. The introduced species were generally more tolerant to lime application.

A subsequent glasshouse experiment sought to separate the effects of pH and calcium on colonisation by *T. melanosporum*. Seedling hazels were inoculated with *T. melanosporum*. After twelve months, they were transplanted using soil amended with fourteen rates of either $CaCO_3$, $CaSO_4$, or $MgCO_3$. The seedlings were then exposed to spores of two endemic fungal species. Applied $CaCO_3$ and $MgCO_3$ increased level of colonisation by *T. melanosporum*, whereas $CaSO_4$ had little or no effect. Soil pH appears to have a stronger influence on colonisation by *T. melanosporum* than the level of applied or exchangeable calcium. Colonisation by endemic species was low and sporadic across all treatments.

Another glasshouse experiment of similar design to that above was established to observe the effect of lime and phosphorus interaction. Applied lime significantly increased the level of colonisation of *T. melanosporum*, but phosphorus had no effect, even at very high rates of application (150 mg P / Kg soil).

A commercial truffière was surveyed for the level of colonisation by *T. melanosporum* and other ectomycorrhizal fungi.

Descriptions were compiled of the mycorrhizas of fungal species found in the glasshouse and field experiments to assist in their future identification.

The morphological identification of *Tuber* mycorrhizas was confirmed using PCR and RFLP of DNA extracted from single mycorrhizal tips.

4. The Effect of Roundup and Basta on Truffles

Introduction

The control of broadleaf and grass weeds in Tasmanian truffières has become an increasing problem. The major weed problems are subterranean clover (*Trifolium spp*), dock (*Rumex spp*), slender thistle (*Carduus tenuiflorus*), perennial thistle (*Cirsium arvense*) and shepherds purse (*Capsella busa-pastoris*) and a range of perennial and annual rye grasses.

The objective in Tasmanian truffières is to establish a strip of grass and clover between the rows of trees rather than having bare earth. Herbicide use is necessary to control the weeds around the trees to avoid competition for nutrients and soil moisture. There are few mechanical devices available for weed control close to the tree and there is concern such an implement may create soil compaction in the long term.

The grasses that will be used between the rows are dwarf growing fescues and the clover will be a prostrate growing white clover. It is expected that the mixed sward of grass and white clover in truffières will have many benefits to the trees and to truffle production. These include:

- increased microbiological activity as a result of organic matter being returned to the soil;
- better soil moisture levels at the soil's surface as a result of the plant cover and slashed plant material forming a mulch on the surface;
- improved soil structure; and
- a strong grass and clover sward will suppress other problem weeds.

Literature reviews of all the French information confirms that very little research has been undertaken into the use of herbicides in truffières in France. Roundup (Glyphosate) and Gesatop (simazine) are the only two herbicides being recommended in France. Simazine has applied in limited situations because of the difficulty in predicting the chemical behaviour in different soil types. Roundup is widely used and is considered safe with respect to the trees and the mycorrhizal roots. However our literature review indicated that there has been no quantitative research to assess whether the use of the herbicides has a detrimental effect on the mycorrhized roots.

The objective of the trial is to evaluate two herbicides that can be applied in existing truffières without any detrimental affect on the growth of the tree or the level of mycorrhizal activity on the roots and minimum impact on the environment. If the evaluation of the two chemicals is successful a herbicide program can be developed which will be of great benefit to controlling the weeds in the truffières.

Material and Methods

Overview

An experiment was undertaken at Perigord Truffles of Tasmania's commercial tree production nursery at Plenty, Tasmania. The experiment was conducted as a pot experiment in a green house where soil moisture levels and other environmental factors could be controlled and the level of mycorrhizal colonisation can be determined more readily than in the field.

In September 1996, ninety hazel seedlings (*Corylus avellana*), which had been inoculated with *Tuber melanosporum* 12 months previously, were transplanted into 250 mm diameter pots. Plants were potted with one of 3 soil treatments. Thirty of the plants were potted with a sandy loam; another thirty plants were potted with a clay loam, and the remaining 30 plants were potted with a kraznozem soil.

The plants were left to grow for six months to ensure good new root development before applying the herbicide treatments. Prior to the herbicide application, the plants were non-destructively sampled to determine the level of mycorrhization. Two herbicide treatments and a control treatment were applied. For each soil treatment, 10 plants were treated with Roundup, another 10 plants were treated with Basta and the remaining 10 plants were not treated. These treatments are summarised in Table 4.

Table 4. Treatments applied.

	Roundup	**Basta**	**Control**
Clay Loam	10 trees	10 trees	10 trees
Sandy Loam	10 trees	10 trees	10 trees
Kraznozem	10 trees	10 trees	10 trees

Herbicide treatment involved spraying the soil at the base of the plant. Care was taken not to apply any herbicide to the plant itself. Each tree was non-destructively harvest to determine the level of mycorrhization at 1 week and 8 weeks after the herbicide treatment.

Soil Treatments

Truffières in Tasmania have been established on a range of different soil types. To gain the maximum information from the evaluation of the herbicides, the trial was replicated on three soil types with a different level of clay and organic matter.

The soil was collected from three commercial truffières that have been established in southern and northern Tasmania. The chemical analysis of the soil is given in Table 5.

Table 5. Chemical analysis of the soil.

Soil Property	Unit	Clay Loam	Sandy loam	Kraznozem
pH(1:5 water)		7.5	7.74	7.4
Exchangeable calcium	ppm	2129	2643	3280
Total calcium	%	3.6	4.17	3.4
Free $CaCO_3$	%	3.0	1.62	0.4
Organic matter	%	6.9	5.5	10.7
Organic carbon	%	4.0	3.19	6.22
Potassium	ppm	298	241	224
Phosphorus	ppm	25	21.2	8
Total phosphorus	ppm	300	789	600
Magnesium	ppm	226	383	36
Total nitrogen	ppm	2580	2300	4600
Carbon/Nitrogen	ppm	15.6	13.8	13.5

Soil analysis was conducted by Ag Lab Services Moolap, Victoria. The methodology for each extraction is given in Appendix 1.

Herbicide Treatments

Roundup and Basta were selected for evaluation for the following reasons:

- They are non-selective herbicides that control a broad range of plant species,
- They are relative safe to the environment, and
- They are relatively safe to the person applying the herbicide.

Roundup (450g/l glyphosate) is a non selective herbicide, which translocates through the plant's tissue. It was selected for the trial because of its activity on a wide range of plants and its non-residual characteristics in the soil.

Basta (200g/l glufosinate ammonium) is also a non- selective herbicide, but is a desiccant with very limited translocative action in the plant tissue.

The two herbicides were applied as per Table 6.

Table 6. Rate of herbicide application

Herbicide	Active constituent	Equiv rate per Ha
Roundup CT	450 g/l Glyphosate	3 litres
Basta	200 g/l Glufosinate Ammonium	4 litres

Herbicide was applied using a calibrated hand sprayer.

Mycorrhizal Root Assessment

Roots samples were collected using a stainless steel corer, 25 mm in diameter and 100 mm in length. Two cores were taken from each pot. The roots were removed from the soil by immersing the cores in water until they collapsed with gentle manipulation to allow the roots to float free. The roots were then gathered using a 0.1 mm sieve. The 2 cores from each pot were combined to make one sample.

The percent colonisation of the fine roots by *T. melanosporum* was calculated on the basis of length. The lengths of each root type were determined using the grid-line intersection method (Giovannette and Mossem, 1980: Tennant. 1975). For each sample, roots were counted as either fine roots or coarse roots. Fine roots were defined as those capable of forming mycorrhizal roots (generally less than 160μm in diameter).

For the fine root portion, the length of mycorrhizal roots and the length of non-mycorrhizal roots were determined. These figures enabled the calculation of percent colonisation by *T. melanosporum* using the following formula:

Percentage colonisation by *T. melanosporum* =
Length of Mycorrhizal Roots / Length of Fine Roots*100

Timetable

Table 7. Timetable of Events

Date	Activity
September 1996	• Collection and analysis of the soil from the three commercial truffières. • Transplantation of the inoculated hazel seedlings into the 250mm pots.
April 1997	• Taking of root cores one week prior to the application of the herbicides • The application of the two herbicides. • Taking of root cores one week following the application of the herbicides.
July 1997	• Root sampling 8 weeks after the application of herbicide.

Statistical analysis

Treatment effects were analysed using 2-way analysis of variance.

Results

The results for the harvest taken immediately prior to the herbicide application are shown in Table 8. There was no significant difference between the herbicide treatments (P=0.45) or between the soil treatments (P=0.07). Therefore there was no need to consider this data when analysing the harvests taken after the herbicide application.

Table 8. Results for the harvest taken 1 week prior to herbicide application. Figures are the mean for each treatment. The standard deviation for the mean is given in brackets.

Herbicide	Sandy Loam	Clay Loam	Kraznozem	Mean for each herbicide
Roundup	53.0 (19.5)	60.5 (22.3)	61.6 (17.8)	58.4 (19.6)
Basta	56.2 (21.4)	53.5 (21.1)	67.1 (22.4)	58.9 (21.7)
Control	55.5 (24.4)	65.1 (23.1)	73.1 (15.9)	64.6 (22.0)
Mean for each soil type	54.9 (21.1)	59.7 (21.9)	67.3 (18.8)	

The results for the harvest taken one week after herbicide application are shown in Table 9. There was no significant difference between the herbicide treatments (P=0.54) or between the soil treatments (P=0.09), nor was there an interaction between the herbicide and soil treatments (P=0.97).

Table 9. Results for the harvest taken 1 week after herbicide application. Figures are the mean for each treatment. The standard deviation for the mean is given in brackets.

Herbicide	Sandy Loam	Clay Loam	Kraznozem	Mean for each herbicide
Roundup	43.7 (20.6)	51.1 (19.0)	58.3 (21.0)	51.0 (20.4)
Basta	52.9 (20.5)	58.0 (21.4)	59.5 (21.7)	56.8 (20.7)
Control	46.9 (23.6)	52.9 (17.1)	60.5 (16.2)	53.4 (19.4)
Mean for each soil type	47.8 (21.2)	54.0 (18.8)	59.4 (19.1)	

The results for the harvest taken 8 weeks after herbicide application are shown in Table 10. There was no significant difference between the herbicide treatments (P=0.11) or between the soil treatments (P=0.25), nor was there an interaction between the herbicide and soil treatments (P=0.95).

Table 10. Results for the harvest taken 8 weeks after herbicide application. Figures are the mean for each treatment. The standard deviation for the mean is given in brackets.

Herbicide	Sandy Loam	Clay Loam	Kraznozem	Mean for each herbicide
Roundup	55.8 (22.9)	59.5 (19.4)	67.7 (18.5)	61.0 (20.3)
Basta	57.9 (23.1)	56.0 (24.2)	66.6 (17.5)	60.2 (21.5)
Control	50.7 (21.5)	48.6 (22.5)	53.1 (16.7)	50.8 (19.8)
Mean for each soil type	54.8 (21.9)	54.7 (21.8)	62.5 (18.2)	

Discussion

The application of relatively high rates of Roundup and Basta to the soil at the base of the hazel seedling did not impact the level of colonisation by *T. melanosporum*. Furthermore, soil type did not appear to influence the effect of the herbicides on *T. melanosporum* colonisation.

From these findings it would appear that applying Roundup or Basta to a truffière, at rates up to 3 and 4 L/Ha respectively, should not adversely affect *T. melanosporum*. There is the possibility that while these herbicides do not impact on colonisation, they may impact on truffle production. This may warrant further investigation, however, it is considered unlikely. Furthermore, herbicide applications are usually made after the truffle harvest in late winter.

While there was no significant difference in the level of colonisation between the soil types, the kraznozem had a consistently higher average level of colonisation than the other soil types. Future experimentation on soil types with a greater number of replicates may be warranted.

Implications

The findings of the experiment have enabled Perigord Truffles of Tasmania to develop a weed control strategy. The strategy gives flexibility both in total weed control and in the manipulation of desirable species within the truffières. For the first 18–24 months after planting the trees into the field, all trees are enclosed in a protective plastic tree guard. This enables the application of Roundup and Basta to control weeds with minimal risk of getting spray drift onto the trees.

During the winter months when the trees are inactive, Roundup can be used for total weed control, both around the trees and between the rows. Basta, although more expensive than Roundup, offers additional advantages in that it is non- translocating and can be used with a greater degree of safety around trees that have foliage intact. This option gives some flexibility should the need arise to control weeds in late autumn in preparation for the winter harvest period. Too much vegetation beneath the trees can make it difficult for the dogs and handlers to find and retrieve the truffles.

The use of these two herbicides by virtue of their differing levels of efficacy against some weed species enables the manipulation of the ground cover. For example, Roundup has relatively low efficacy against some clover species. Timely application of Roundup enables growers to control broadleaf and grass species whilst at the same time allowing clover species to dominate, resulting in a more manageable cover that has the added advantage of nitrogen fixation. Should there be a need to control a vigorous stand of clover, Basta affords an excellent option for control. Additionally, being able to use both products benefits the industry by avoiding building up resistance and tolerance to Roundup by some species.

Recommendations

Basta is registered for use in Australia for control of primo-canes in *Rubus spp.* Perigord Truffles of Tasmania intends to conduct further evaluation to determine the efficacy of this product in providing a means of chemical control of suckers on hazel trees. Current control measures are limited to hand pruning which can be an expensive and time consuming operation for growers especially in sites of high vigor. Should the results prove positive, Basta will provide a valuable option to growers and may well be able to be incorporated into the program for weed control by altering the time of application to simultaneously control suckers.

5. The Effect of Soil Moisture Levels on the Colonisation of Truffles in Commercial Truffières

Introduction

French literature on the French truffle considers the development of the mycorrhizal root as one of the most significant factors affecting the formation of fruiting bodies (truffles). Unfortunately, as is the case with many other factors that are likely to affect the formation of fruiting bodies of the French truffle, the subject of soil moisture has not been extensively researched. During many discussions with French scientists and truffle producers, it is apparent that there is a lack of any quantifiable data with respect to the ideal soil moisture levels required for the maximisation of mycorrhizal root development.

Limited research has been undertaken to assess the relationship between mycorrhizal root development and soil moisture levels under laboratory conditions (Mamoun and Oliver 1993). A number of papers have also been presented relating annual rainfall to truffle production in particular departments in France (Le Tacon, *et al* 1982, and Jean Demerson 1996).

The papers indicate that there is a relationship between dry summers and lower production of truffles. However due to the large variability of soil types within a particular area and the fact that up to 70 % of the truffles are harvested from non-irrigated native forests, it is very difficult to draw any conclusions from the research.

PTT's aim is to conduct a trial on two commercial truffières in Tasmania to determine if there is correlation between soil moisture levels and mycorrhizal root development. If a relationship were determined it would have a significant impact on the irrigation scheduling of the commercial truffières established in Tasmania.

Methodology

Overview

A field irrigation trial was conducted on two truffières in Tasmania over two summers from late 1996 to early 1998. One truffière is located at Rosevears, near Launceston and the other at Bothwell in the Central Highlands. In both truffières, three rates of irrigation were applied using micro jet low-pressure sprinklers with three different output rates; 35 litres/hour, 70 litres/hour and 105 litres/hour. Each irrigation rate was applied to a row of 10 hazel trees. The remainder of the truffières were also irrigated with 70 litres/hour microjet low pressure sprinklers.

The trees with the 35 L/hr and 105 L/hr sprinklers were irrigated at the same time and for the same duration as those trees with the 70 L/hr sprinklers. Therefore trees with the 35 L/hr sprinklers received 50% of the water applied to the trees with 70 L/hr sprinklers and conversely, the trees with the 105 L/hr sprinklers received 150% of the water applied to the trees with 70 L/hr sprinklers.

The soil moisture content for the three irrigation treatments was monitored using an Enviroscan magnetic probe at the Bothwell truffière and tensiometers at the Rosevears truffière. The Enviroscan magnetic probe measures the percentage soil moisture content. Tensiometers measure soil moisture tension in KPA.

At the Rosevears truffière, the truffle grower was instructed to attempt to keep the soil moisture content under the 70 L/hr sprinklers between 20 and 40 KPA at 10 to 20 cm depths. At the Bothwell truffière, the truffle grower was instructed to attempt to keep the percentage soil moisture between 20 and 30% at 10cm and to try not to exceed 40% at 20cm. Due to a practical problem of the truffle grower not being able to download the data from the Enviroscan on a regular basis, in the second year, tensiometers were installed at Bothwell to assist with the irrigation scheduling.

Prior to the commencement of the trial, the tree's roots were non-destructively sampled to determine the level of colonisation. The trees were sampled again at the end of the first and second irrigation seasons. The results were analysed to determine if the irrigation treatments had significantly affected the level of mycorrhization.

The Truffières

The Rosevears truffière was established in the spring of 1995. The truffière covers an area of 1.25 hectares of clay loam soil with good moisture retention characteristics. Prior to the establishment of the truffière, it had been sown to pasture, and before that, a market garden.

The Bothwell truffière was established in the autumn 1994. The truffière covers an area of 3 Hectares of clay loam soil. Prior to the establishment of the truffière the area had been sown down to pasture for a considerable number of years.

In both truffières, fescue and white clover has been established between the tree rows and is regularly slashed during the growing season. The soil analysis for the truffières is given in Table 11.

Table 11. Chemical analysis of the soil.

Soil Property	Unit	Rosevears	Bothwell
pH(1:5 water)		7.84	7.74
Exchangeable calcium	PPM	3124	2643
Total calcium	%	3.6	4.17
Free $CaCO_3$	%	2.7	1.62
Organic matter	%	3.9	5.5
Organic carbon	%	2.3	3.19
Potassium	ppm	124	241
Phosphorus	ppm	27	21.2
Total phosphorus	ppm	1050	789
Magnesium	ppm	265	383
Total nitrogen	ppm	1900	2300
Carbon/Nitrogen	ppm	12.1	13.9

Soil analysis conducted by Ag Lab Services Moolap, Victoria.

Soil Moisture Monitoring Systems

Two different moisture monitoring systems were used in the trial, tensiometers and the Enviroscan magnetic probe.

A tensiometer is an instrument designed to measure the tension or suction that plants' roots must exert to extract water from the soil. This tension is a direct measure of the availability of water to a plant.

The tensiometer consists of airtight, water filled tube with a porous ceramic tip at the bottom. When buried in the soil the ceramic tip allows water to move freely in or out of the tube. As the soil dries out, water is drawn through the porous wall of the ceramic tip, creating a partial vacuum inside the tensiometer, which can then be read via a vacuum gauge.

At the Rosevears truffière, four tensiometers were placed in each of the three irrigation treatments. Two of the tensiometers were placed at 10cm depth and the remaining two at 20cm depth. The tensiometers were placed 20 cm from the base of the trees.

Enviroscan magnetic probes have a series of sensors, which are placed at different depths in the soil. The sensors measure the percent soil moisture level. The data is collected in a recording unit, which is powered by a solar panel and then down loaded onto a computer.

At the Bothwell truffière, one Enviroscan probe was placed in each irrigation treatment, 20 cm from the base of one of the trees. The four sensors on the probe were set at a soil depth of 10, 20, 30 and 50 cm.

Tensiometer levels recorded twice weekly and Enviroscan data was downloaded fortnightly.

Harvesting and Mycorrhizal Root Assessment

The tree roots were non-destructively harvested using a stainless steel corer, 100 mm in diameter and 200 mm in length. Two cores were taken from each tree at a distance of 40cm from the base.

The root cores were collected on three occasions:
1. October 1996 prior to commencement of the irrigation treatments;
2. April 97 at the end of the first irrigation season; and
3. April 98 at the completion of the trial.

Roots were removed from the soil by immersing the cores in water until they collapsed with gentle manipulation to allow the roots to float free. The roots were then gathered using a 0.1 mm sieve. The two cores from the trees were combined to make one sample.

The percent colonisation of the fine roots by *T. melanosporum* was calculated on the basis of length. The lengths of each root type were determined using the grid-line intersection method (Giovannette and Mossem, 1980; Tennant. 1975). For each sample, roots were counted as either fine roots or coarse roots. Fine roots were defined as those capable of forming mycorrhizal roots (generally less than 160µm in diameter).

For the fine root portion, the length of mycorrhizal roots and the length of non-mycorrhizal roots were determined. These figures enabled the calculation of percent colonisation by *T. melanosporum* using the following formula:

Percentage colonisation by *T. melanosporum* =
Length of Mycorrhizal Roots / Length of Fine Roots*100

Statistical analysis

Treatment effects at each harvest were analysed using analysis of variance.

Results

The results for the harvest taken prior to the commencement of the irrigation treatments are shown in Table 12As there was no significant difference between the irrigation treatments (P=0.86) there was no need to consider this data when analysing subsequent harvest.

Table 12. Results of the harvest taken prior to the commencement of the irrigation treatments. Figures are the mean for each treatment. The standard deviation for the mean is given in brackets.

Truffière	35 L/hr	70 L/hr	105 L/hr	Mean for each truffière
Bothwell	45.9 (25.0)	62.4 (21.9)	47.9 (24.2)	52.1 (24.1)
Rosevears	62.1 (22.8)	52.0 (22.6)	59.2 (21.3)	57.8 (21.9)
Mean for each irrigation rate	54.0 (24.7)	57.2 (22.3)	53.55 (22.9)	

The results for the harvest taken at the end of the first irrigation season are shown in Table 13. The was no significant difference (P=0.09) between the irrigation treatments, although the mean for the 105 L/hr treatment was lower than the two other irrigation treatments at both sites.

Table 13. Results for the harvest taken at the end of the first irrigation season. Figures are the mean for each treatment. The standard deviation for the mean is given in brackets.

Truffière	35 L/hr	70 L/hr	105 L/hr	Mean for each truffière
Bothwell	60.3 (12.8)	61.5 (20.2)	47.9 (22.5)	56.6 (19.3)
Rosevears	58.5 (19.6)	56.8 (18.7)	47.6 (17.4)	54.3 (18.6)
Mean for each irrigation rate	59.4 (16.2)	59.2 (19.1)	47.8 (19.6)	

Results for the final harvest at the end of the second irrigation season are shown in Table 14. The means were not significantly different (P=0.08), but the mean level of colonisation for the 70 L/hr treatment was higher at both sites.

Table 14. Results for the final harvest at the end of the second irrigation season. Figures are the mean for each treatment. The standard deviation for the mean is given in brackets.

Truffière	35 L/hr	70 L/hr	105 L/hr	Mean for each truffière
Bothwell	51.5 (24.1)	67.2 (16.1)	55.4 (21.1)	58.0 (21.1)
Rosevears	50.7 (20.8)	61.8 (19.9)	52.4 (15.9)	55.0 (19.0)
Mean for each irrigation rate	51.1 (21.9)	64.5 (17.8)	53.9 (18.2)	

Soil moisture levels recorded at both sites are given in Appendix 2.

Discussion

The results of the trial showed there was no significant difference ($P>0.05$) in the mycorrhizal root development between the irrigation treatments.

The graphs for the first year of the trial indicate that there was a large variation in the soil moisture level which made it difficult to demonstrate that a treatment had been imposed.. This problem was more apparent on the Rosevears site where the tensiometers were used. This was largely due to difficulties in obtaining accurate readings and being able to adjust the irrigation scheduling accordingly. The effect of wind and inaccuracies of the micro jet sprinklers could have also contributed to the inability to provide even distribution of moisture.

The results in the second year show that the irrigation treatments had a more observable effect on soil moisture. However, these irrigation treatments did not influence the level of colonisation by *T. melanosporum*.

Implications

Although the results of the experimentation were inconclusive the information generated by recording soil moisture levels was important.

In both truffières the data clearly indicated that were wide variations in soil moisture levels particularly in the first year. The truffières suffered periods of moisture stress and water logging when the larger volume sprinklers were used. The trial strongly indicated the benefits of using either form of monitoring equipment.

Perigord has through the experience gained, been able to convey to it's joint venture partners the importance of timely reading and recording of probes and tensiometers with respect to irrigation scheduling.

Further benefits to growers are in the form of a greater level of understanding of apparent versus actual soil moisture levels, the rate of dispersion of moisture through the soil profile, the rate of evaporation according to climatic conditions and the ability of measuring equipment to assist in the conservation of irrigation water.

The ability to accurately determine soil moisture levels has proven extremely beneficial to those growers reliant on stored water during 1999, which was the driest year on record in Tasmania. Although some sites had used available water by mid summer, those affected growers were able to utilise their available resources to their maximum advantage.

There is no question that the knowledge gained from this trial and disseminated to growers during 1999 was of great benefit and a number of sites would have suffered more severely than they otherwise did without the information gained.

Recommendations

Perigord Truffles of Tasmania has now provided at the companies cost, tensiometers and reading units to all growers with established truffières. Growers have been vigorously encouraged to monitor tensiometers twice weekly during spring/summer and most now provide details of readings by e-mail to Perigord. This system enables Perigord to provide assistance with scheduling whilst at the same time contributing to a database of information that will become increasingly more valuable over time.

With the harvest of truffles on three truffières in 2000 the impetus for further research with soil moisture has become paramount. Perigord now have the opportunity of attempting to find a correlation between soil moisture levels and truffle yield.

6. The Effect of Tree Nutrition and Wind on Tree Growth Rate, and Subsequent Impact on the Level of Colonisation by Truffles

Introduction

Typically in France truffle production commences in truffières in years 5-8. The time lag from the planting to production remains a significant question in the production of French truffles. If the period of time can be reduced then there would be enormous economic benefits to the French truffle industry.

Perigord Truffles of Tasmania's aim is to reduce the time from establishment of the truffière to production. PTT budget on a production of 3 kg per hectare in year 5, increasing to 60kg per hectare in Year 8. If earlier and more significant production can be achieved it will have significant impact on the financial returns and growth of the industry.

Very little work has been done in France on assessing the benefits of increasing the fertility of the truffières and obtaining the subsequent increase in tree growth and truffle production earlier in life of the truffière. The results of a trial undertaken by Shaw *et al* (1996) in the Provence region of France indicated that there was a strong correlation between basal diameter as an indicator of tree growth and the level of fruiting bodies produced. The trial was done only on *Quercus ilex,* and did not include *Corylus avellana.*

Research into the effects of increasing the fertility of French truffle plantations has been hampered by the general consensus held by the industry in France that accelerated tree growth optimises the opportunity for invasion by competitive fungi. In France, many of the truffières are contaminated by a range of other fungi which occur naturally in the soils.

Survey work carried out by Dr. Daryl Brown in his PhD studies indicated that the cultural conditions prevailing in Tasmanian truffières ie. high pH levels due to the application of large quantities of limestone, has the effect of making some endemic fungi significantly less competitive.

Because of this unique situation, it may be possible to accelerate tree growth in Tasmanian truffières without the impediment of promoting competition from invading endemic fungi. Should the results of the proposed experiments mirror the results of a trial by Shaw *et al* (1996) previously referred to, the most significant benefit would be from plantations producing truffles on younger trees and in greater quantities.

The objective of the tree growth trial is to investigate the effects of increasing tree growth rate on the level of mycorrhization and therefore reducing the time from out-planting to the commencement of truffle production.

The key aims of the experimentation are:

- To investigate the effects of increasing tree growth rate on the level of mycorrhization
- To reduce the time from out-planting to truffle production

- Dissemination of the results to the farmers so they can reap the commercial benefits.

Methodology

Overview

A field experiment was established on two commercial truffières in Tasmania. Four fertilizer treatments were applied. The four treatments were:

- Control
- 100 kg/Ha P, 100kg/ha N (split application) and a windbreak,
- 100 kg/Ha P, 100kg/ha N (split application) and no windbreak,
- 50 kg/Ha P, 50kg/ha N and no windbreak,

Each treatment was applied to a row of ten trees. The phosphorus and nitrogen were applied to 1.5m either side of the trees. This gave a 32.7m long by 3m wide strip with a total area per treatment of 98 m^2 (.01Ha). An artificial windbreak using 75% shade cloth material was constructed for one of the treatments to assess the effect of wind on the growth rates of the trees. This was done as it was thought that wind at the Bothwell truffière may have been affecting tree growth.

Prior to the application of fertilser, the plants were non-destructively sampled to determine the level of mycorrhizal colonisation. Basal stem diameter and tree height were also recorded. These measurements were repeated in May 1999 at the end of the first growing season and May 2000 at the completion of the trial.

The Truffières

The two truffières selected for the trial are at Bream Creek in South Eastern Tasmania and Bothwell in the Central Highlands region of Tasmania.

The Bream Creek truffière was established in October 1993. The truffière covers an area of 2.2 hectares. Prior to the establishment of the truffière the area had been sown to pasture and grazed by dairy cows. The soil, a podsolic on sandstone had a pH (1; 5 H_2O) of 6.3. Limestone was applied at 37 tonnes per hectare and incorporated to a depth of 200mm prior to planting the trees.

The Bothwell truffière was established in the autumn 1994. The truffière covers an area of 3 Hectares. Prior to the establishment of the truffière, the area had been sown down to pasture for a considerable number of years. The soil, a clay loam had a pH (1; 5 H2O) of 6.0. Since the initial soil test, lime has been applied at the rate of 45 tonnes per hectare.

The soil was sampled and analysed from both truffières (Table 15).

Table 15. Chemical analysis of the soil.

Soil Property	Unit	Bream Creek	Bothwell
pH(1:5 water)		7.5	7.74
Exchangeable calcium	ppm	2129	2643
Total calcium	%	3.6	4.17
Free $CaCO_3$	%	3.0	1.62
Organic matter	%	6.9	5.5
Organic carbon	%	4.0	3.19
Potassium	ppm	298	241
Phosphorus	ppm	25	21.2
Total phosphorus	ppm	300	789
Magnesium	ppm	226	383
Total nitrogen	ppm	2580	2300
Carbon/Nitrogen	ppm	15.6	13.8

Soil analysis conducted by Ag Lab Services Moolap, Victoria.

Measurements

Tree growth
Basal diameter and tree height was measured using callipers and measuring tapes. Basal stem diameter was used to calculate the stem cross-sectional area for each tree.

Mycorrhizal root assessment
Roots samples were collected using a stainless steel corer, 100 mm in diameter and 200 mm in length. Two cores were taken from each tree at 70cm from the base. The roots were removed from the soil by immersing the cores in water until they collapsed with gentle manipulation to allow the roots to float free. The roots were then gathered using a 0.1 mm sieve. The 2 cores from the trees were combined to make one sample.

The percent colonisation of the fine roots by *Tuber melanosporum* was calculated on the basis of length. The lengths of each root were determined using the grid-line intersection method (Giovannette and Mossem, 1980: Tennant. 1975). For each sample, roots were counted as either fine roots or coarse roots. Fine roots were defined as those capable of forming mycorrhizal roots (generally less than 160μm in diameter).

For the fine root portion, the length of mycorrhizal roots and the length of non-mycorrhizal roots were determined. These figures enabled the calculation of percent colonisation by *T. melanosporum* using the following formula:

Percentage colonisation by *T. melanosporum* =
Length of Mycorrhizal Roots / Length of Fine Roots*100

Statistical Analysis
The results were analysed using analysis of variance.

Results

Growth rate in the height and stem cross-sectional area are given in Tables 16, 17, 18 and 19. There was no significant effect (P>0.05) of any of the treatments on the measured growth rate of the trees. The windbreak appeared to favour the trees at the Bothwell site, but the difference was not significant.

Table 16. Tree height and height growth rate at the Bothwell truffière. Tree heights are in centimetres. Growth rate was determined by dividing the current tree height by the tree height in Sept 98.

	Control	50 kg/Ha P 50 Kg/Ha N	100 kg/Ha P 100 kg/Ha N	100 kg/Ha P 100 kg/Ha N + Windbreak
Tree Height Sept 98	132	123	132	127
Tree Height May 99	125	129	133	136
Growth Rate to May 99	0.95	1.05	1.01	1.09
Tree Height May 2000	134	134	139	151
Growth Rate to May 2000	1.02	1.09	1.05	1.19

Table 17. Total stem cross-sectional area (stem area) and growth rate of the stem cross-sectional area at the Bothwell truffière. The units for cross-sectional area are mm^2. The growth rate of the stem cross-sectional area was calculated by dividing the current stem cross-sectional area by the stem cross-sectional area in Sept 98.

	Control	50 kg/Ha P 50 Kg/Ha N	100 kg/Ha P 100 kg/Ha N	100 kg/Ha P 100 kg/Ha N + Windbreak
Stem Area Sept 98	1649	1371	1544	1177
Stem Area May 99	2580	1900	2477	1915
Growth Rate May 99	1.57	1.49	1.67	1.72
Stem Area May 2000	3497	2652	3112	2645
Growth Rate May 2000	212	193	202	225

Table 18. Tree height and height growth rate at the Bream Creek truffière. Tree heights are in centimetres. Growth rate was determined by dividing the current tree height by the tree height in Sept 98.

	Control	50 kg/Ha P 50 Kg/Ha N	100 kg/Ha P 100 kg/Ha N	100 kg/Ha P 100 kg/Ha N + Windbreak
Tree Height Sept 98	170	166	148	175
Tree Height May 99	186	184	180	197
Growth Rate to May 99	1.09	1.11	1.22	1.13
Tree Height May 2000	206	219	228	241
Growth Rate to May 2000	121	132	154	138

Table 19. Total stem cross-sectional area (stem area) and growth rate of the stem cross-sectional area at the Bream Creek truffière. The units for cross-sectional area are mm^2. The growth rate of the stem cross-sectional area was calculated by dividing the current stem cross-sectional area by the stem cross-sectional area in Sept 98.

	Control	50 kg/Ha P 50 Kg/Ha N	100 kg/Ha P 100 kg/Ha N	100 kg/Ha P 100 kg/Ha N + Windbreak
Stem Area Sept 98	1564	1852	1807	2288
Stem Area May 99	2546	3213	3000	3496
Growth Rate May 99	1.63	1.78	1.71	1.54
Stem Area May 2000	3189	4211	3987	4785
Growth Rate May 2000	2.04	2.27	2.21	2.09

The results for the harvest taken in September 98, May 99 and May 2000 are shown in Table 20, Table 21 and Table 22 respectively. There was no significant difference between the fertiliser treatments (P=0.78) for either of the truffières at any of the harvests.

Table 20. The results for the harvest taken in September 98, prior to the application of fertiliser. Figures are the mean for each treatment. The standard deviation for the mean is given in brackets.

	Control	50 kg/Ha P 50 Kg/Ha N	100 kg/Ha P 100 kg/Ha N	100 kg/Ha P 100 kg/Ha N + Windbreak	Mean for each Truffière
Bothwell	51.5 (22.5)	54.5 (18.3)	57.4 (17.9)	57.7 (11.6)	55.3 (17.5)
Bream Creek	59.5 (25.5)	60.0 (19.1)	52.5 (18.1)	53.8 (21.1)	56.5 (20.6)
Mean for each treatment	55.5 (23.7)	57.3 (18.5)	55.0 (17.7)	55.8 (16.7)	

Table 21. The results for the harvest taken in May 99. Figures are the mean for each treatment. The standard deviation for the mean is given in brackets.

	Control	50 kg/Ha P 50 Kg/Ha N	100 kg/Ha P 100 kg/Ha N	100 kg/Ha P 100 kg/Ha N + Windbreak	Mean for each Truffière
Bothwell	59.1 (18.5)	51.4 (19.3)	58.7 (20.7)	48.2 (18.7)	54.4 (19.1)
Bream Creek	44.4 (19.6)	43.4 (20.6)	52.1 (21.8)	63.3 (24.1)	50.8 (22.3)
Mean for each treatment	51.8 (20.0)	47.4 (19.9)	55.4 (20.9)	55.8 (22.4)	

Table 22. The results for the harvest taken in May 2000. Figures are the mean for each treatment. The standard deviation for the mean is given in brackets.

	Control	50 kg/Ha P 50 Kg/Ha N	100 kg/Ha P 100 kg/Ha N	100 kg/Ha P 100 kg/Ha N + Windbreak	Mean for each Truffière
Bothwell	51.5 (22.5)	54.5 (18.3)	57.4 (17.9)	57.7 (11.6)	55.3 (17.5)
Bream Creek	59.5 (25.5)	60.0 (19.1)	52.5 (18.1)	53.8 (21.1)	56.5 (20.6)
Mean for each treatment	55.5 (23.7)	57.3 (18.5)	55.0 (17.7)	55.8 (16.7)	

Discussion

The results of the trial showed no significant difference in the rate of mycorrhizal growth under any of the treatments. The results also indicated that there was no reduction in the mycorrhiza as a result of applying the phosphorus and nitrogen.

Whilst a small number of other unidentified mycorrhiza were observed, there was no correlation between the treatments.

There was no significant response in tree growth to the phosphorus and nitrogen treatments compared to the control.

Whilst the wind break treatment at both the Bream Creek and Bothwell sites had no significant effect on tree growth, other observations recorded at the Bothwell site were:

1. Trees sheltered by the windbreak displayed a significant improvement in health and vigour;

2. Trees were not subject to the same degree of wind damage and burning of leaf edges that was apparent in other areas of the truffière exposed to the wind.

3. Subsequent soil analysis of the site indicated low levels of iron and a significant increase in the degree of soil salinity. Most likely as a result of drought, which required irrigation water to be sourced from an artesian bore containing saline water.

Implications

Commercial truffières in Tasmania are established on a number of sites with a range of soils displaying variations in type, structure and fertility. The knowledge that phosphorus and nitrogen applications will not have a direct effect in decreasing the levels of *T. melanosporum* mycorrhiza, will allow PTT to proceed with confidence to conduct further trials on a number of truffières.

Recommendations

This trial addressed the effect of treatments on mycorrhizal roots, but did not address the effects on production of fruiting bodies, as none had been detected at the time of the trial.

With the production of *T. melanosporum* fruiting bodies on three sites in the 2000 season. PTT now has the opportunity to investigate the effects of nutrient application on fruiting body production in addition to assessment of mycorrhizal root development and further research in this area is necessary and contemplated.

7. References

Demerson, J., Les pluies de printemps et d'automne ont-elles un effect sur la production de truffes. *Compte Rendu des Interventions des Jornees Nationales (1996).*

Le Tacon, F Delmas, J.,Gleyze, R., and Bouchard,D. !1982). Influence du regime hydrique du sol et de la fertilisation sur la frutification de la truffe noire du Perigord (*Tuber melanosporum* Vitt.) dans Sud-Est de la France. Acta oecologica., Oecol. Applicata 3,291-306

Mamoun, M., and Oliver, J.M. !1993a) Competition between *Tuber melanosporum* and other ectomycorrhizal fungi under two different irrigation regimes. 1. Competition with *Tuber brumale*. *Plant and Soil* **149**, 211-218.

Shaw, P.J.A., Lankey, K., and Jourdan, A (1996). Factors affecting the yield of *Tuber melanosporum* in a *Quercus ilex* plantation in southern France. *Mycological Research 100, 1176-1178.*

Personal Communications:

Chevalier, G INRA Clermond Ferrand, France.

G Callot, G INRA, Montpellier, France.

Eitenne, P. Truffle producer. Provence, France.

Pebeyre. JP Truffle exporter Cahors, France.

Souzart, P Station d'Experimentations sur la Truffe, Montant, France.

8. Appendices

Appendix 1. AgLab Soil analysis procedure

- Electrical Conductivity - EC of 1:5 soil/water extract.
- Soil pH - pH of 1:5 soil/water suspension
- Organic Carbon - Walkley & Black.
- Nitrogen - Total Nitrogen (Combustion - Dumus)
- Carbon/Nitrogen Ratio - Total organic carbon/total nitrogen ratio.
- Phosphorous - Ammonium Acetate + 0.03M EDTA extractable phosphorous,
- Ion Exchange Properties - Exchangeable bases (Ca2+,Mg2+,Na+,K+),
  - 1M ammonium acetate at pH 7.0,
  - Cation exchange capacity measurement - automated determination of ammonium ions,

Appendix 2. Soil moisture levels recorded at the Bothwell and Rosevears truffières during the 1996/97 and 1997/98 irrigation seasons.

Figure 2. Percent soil moisture content at 10cm depth for the Bothwell truffière in 1996/97 irrigation season.

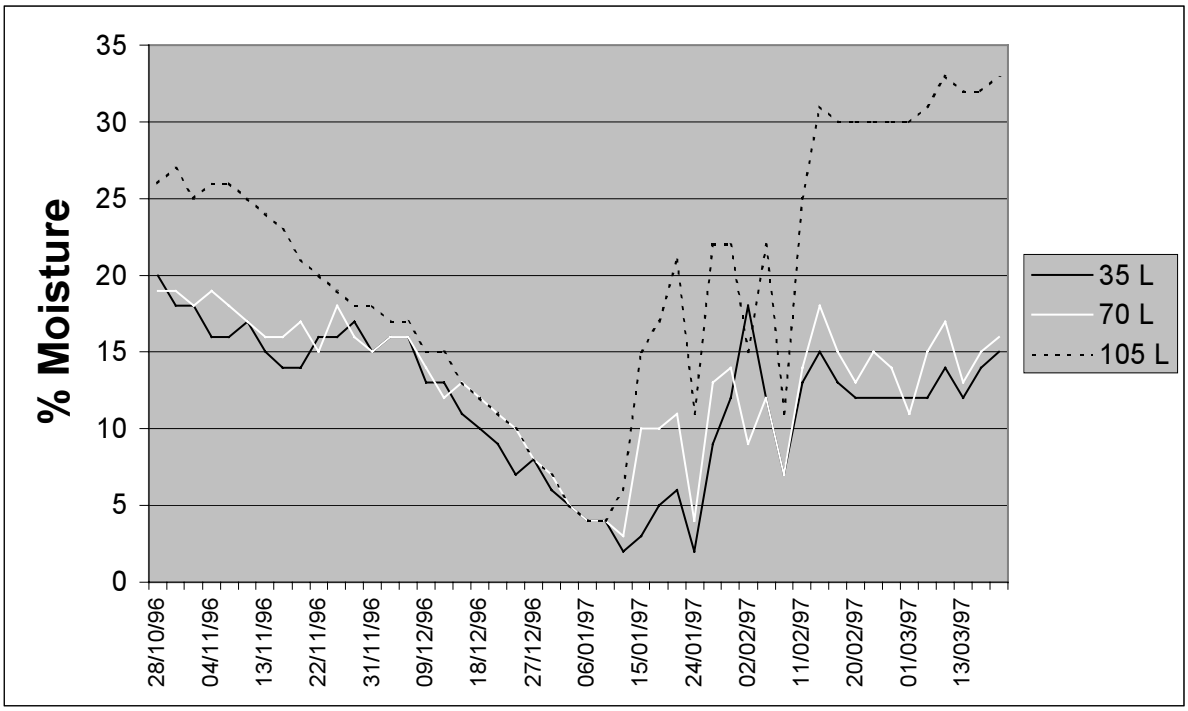


Figure 3. Percent soil moisture content at 20cm depth for the Bothwell truffière in 1996/97 irrigation season.

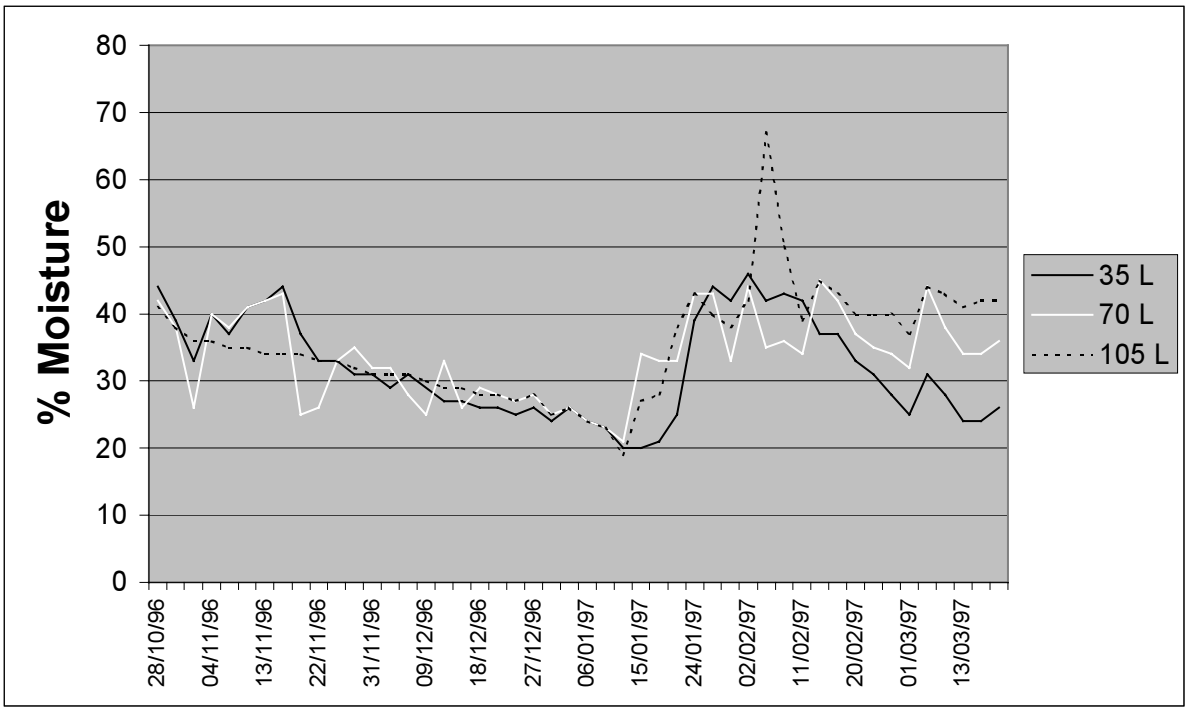


Figure 4. Percent soil moisture content at 30cm depth for the Bothwell truffière in 1996/97 irrigation season.

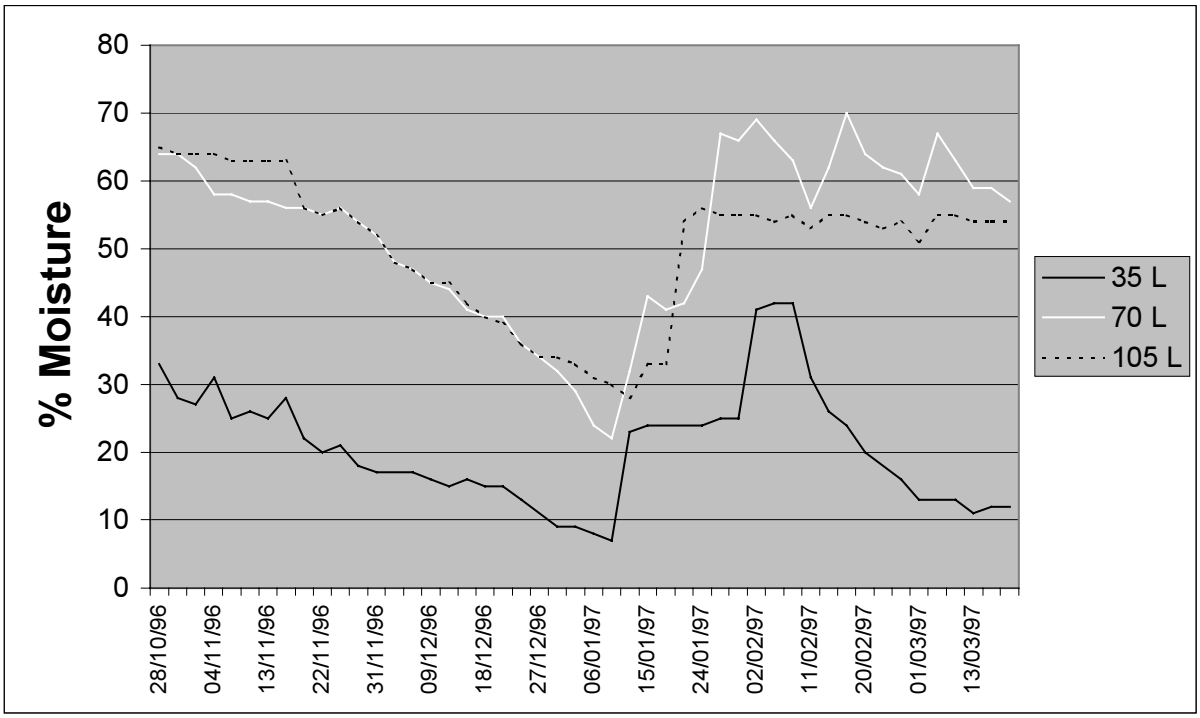


Figure 5. Percent soil moisture content at 50cm depth for the Bothwell truffière in 1996/97 irrigation season.

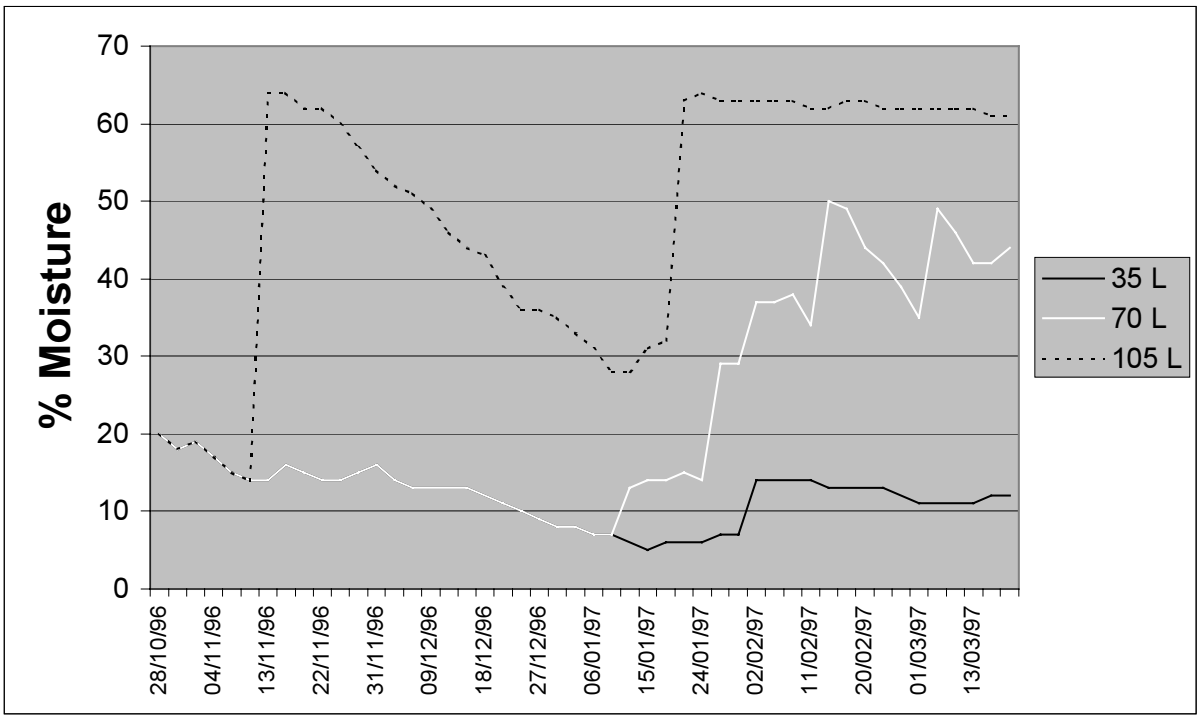


Figure 6. Percent soil moisture content at 10cm depth for the Bothwell truffière in 1997/98 irrigation season.

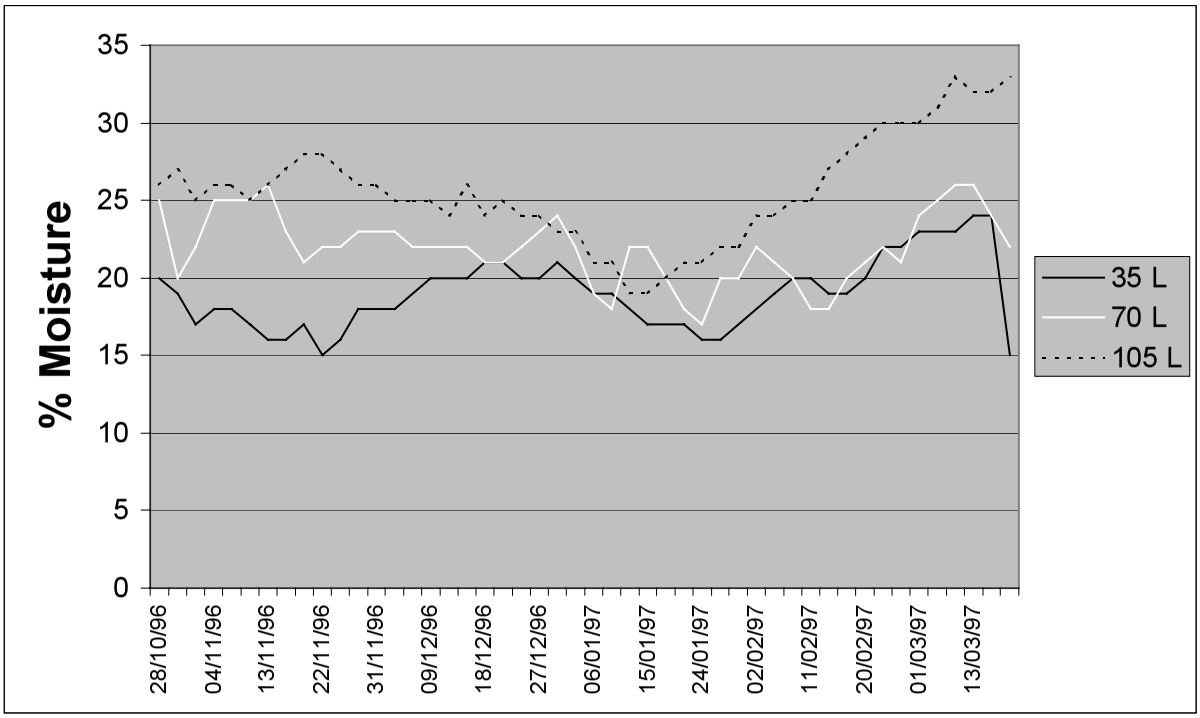


Figure 7. Percent soil moisture content at 20cm depth for the Bothwell truffière in 1997/98 irrigation season.

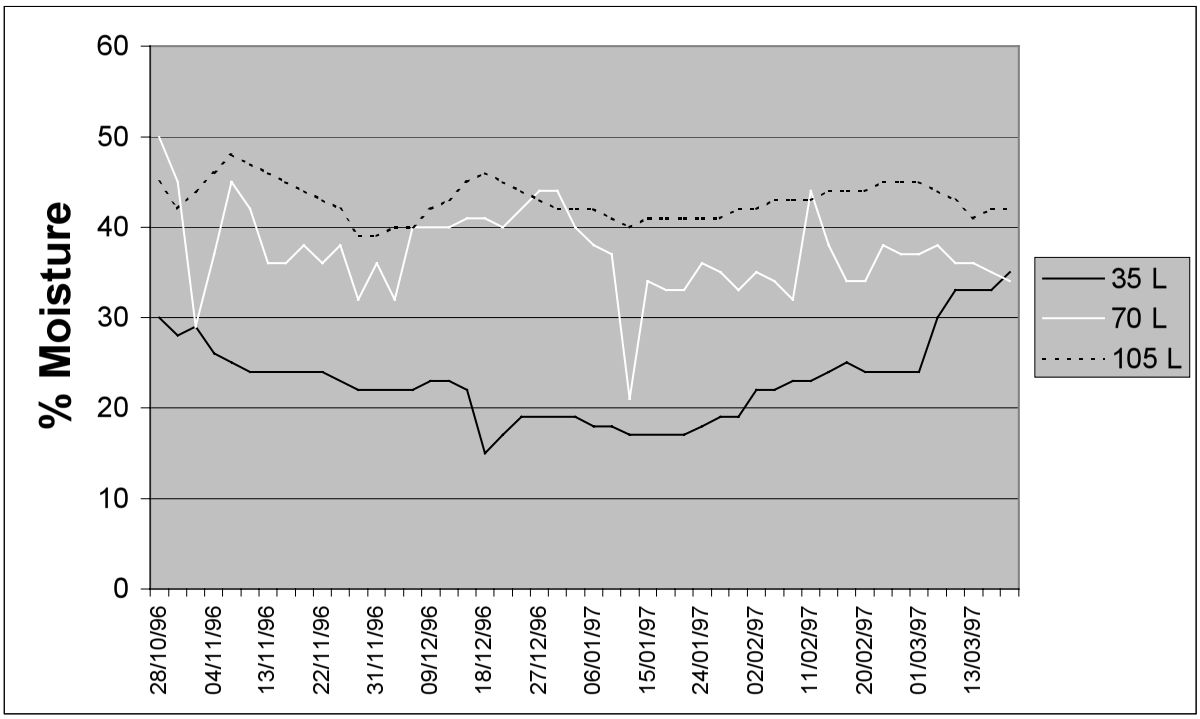


Figure 8. Percent soil moisture content at 30cm depth for the Bothwell truffière in 1997/98 irrigation season.

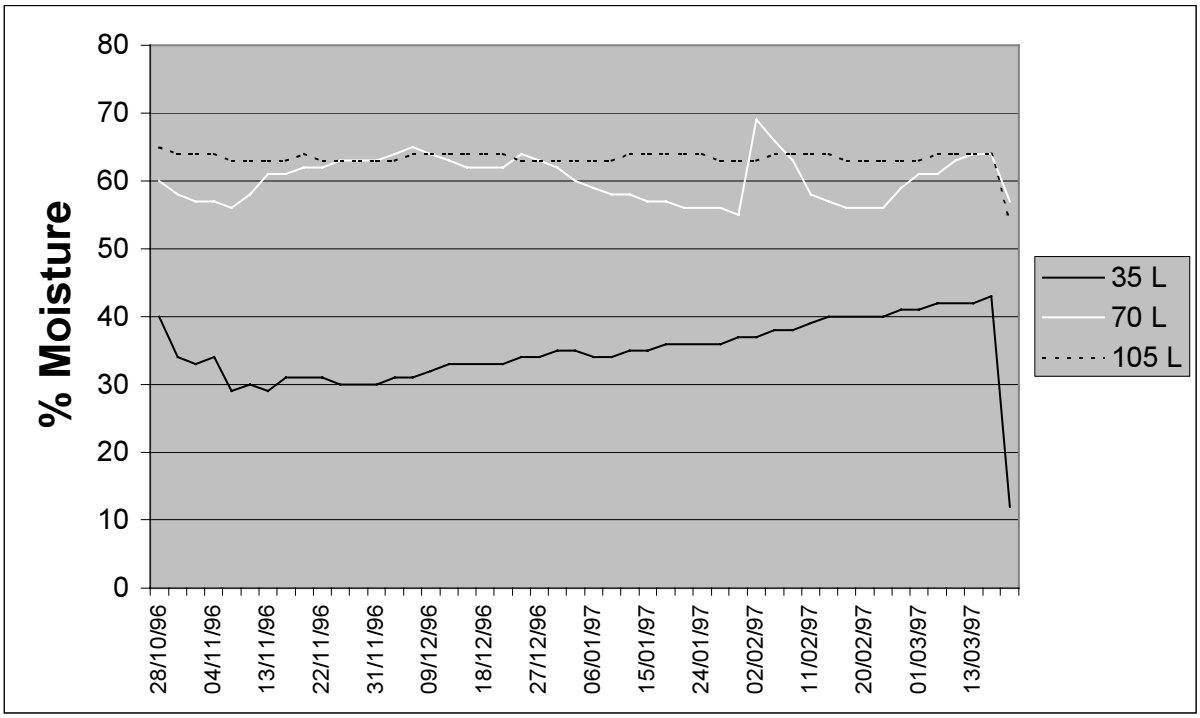


Figure 9. Percent soil moisture content at 50cm depth for the Bothwell truffière in 1997/98 irrigation season.

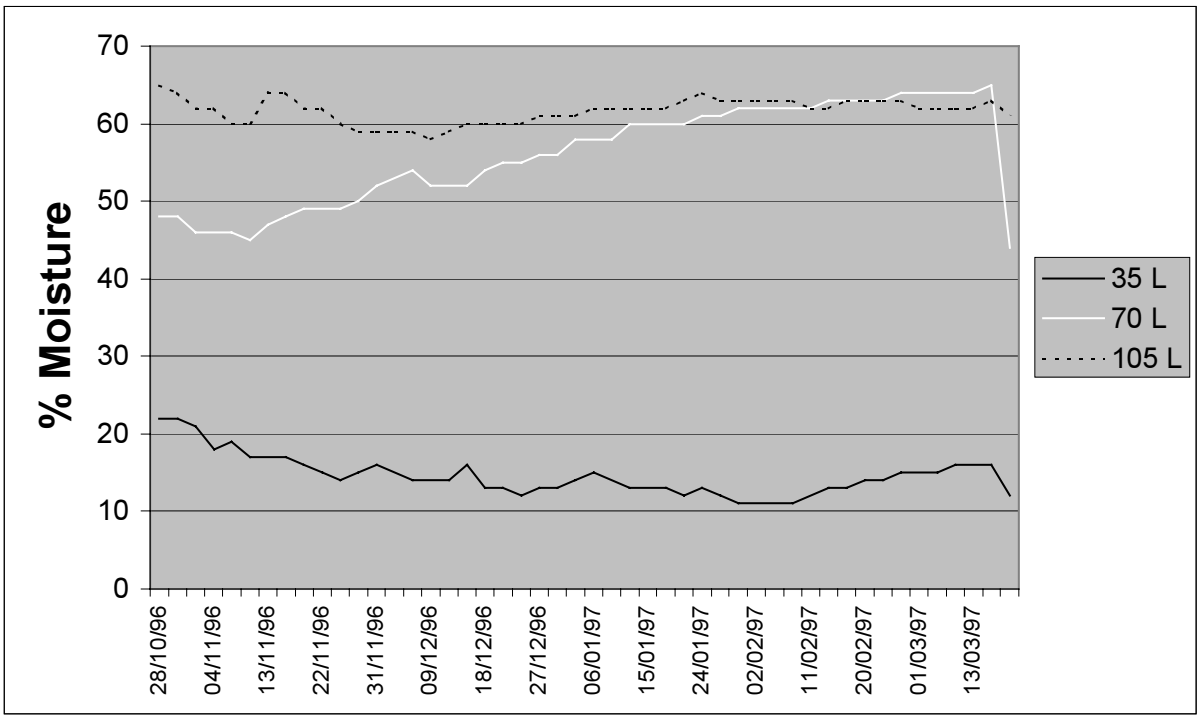


Figure 10. Soil moisture tension (KPA) at 10cm depth for the Rosevears truffière in 1996/97 irrigation season.

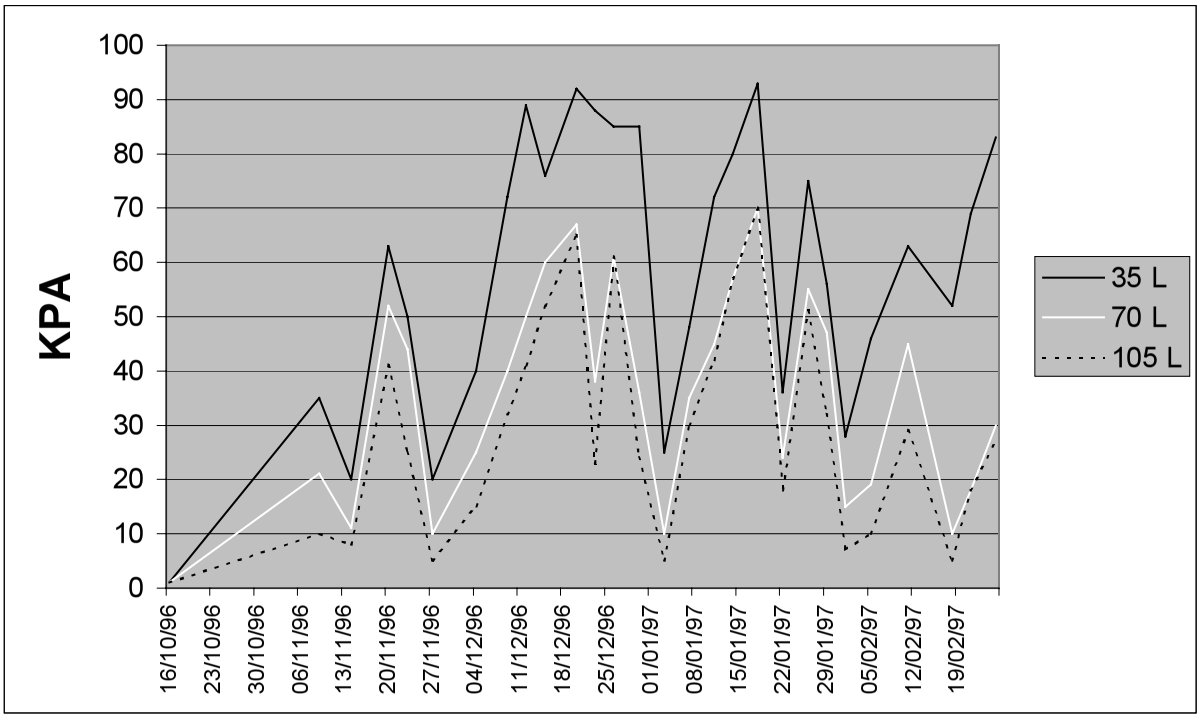


Figure 11. Soil moisture tension (KPA) at 20cm depth for the Rosevears truffière in 1996/97 irrigation season.

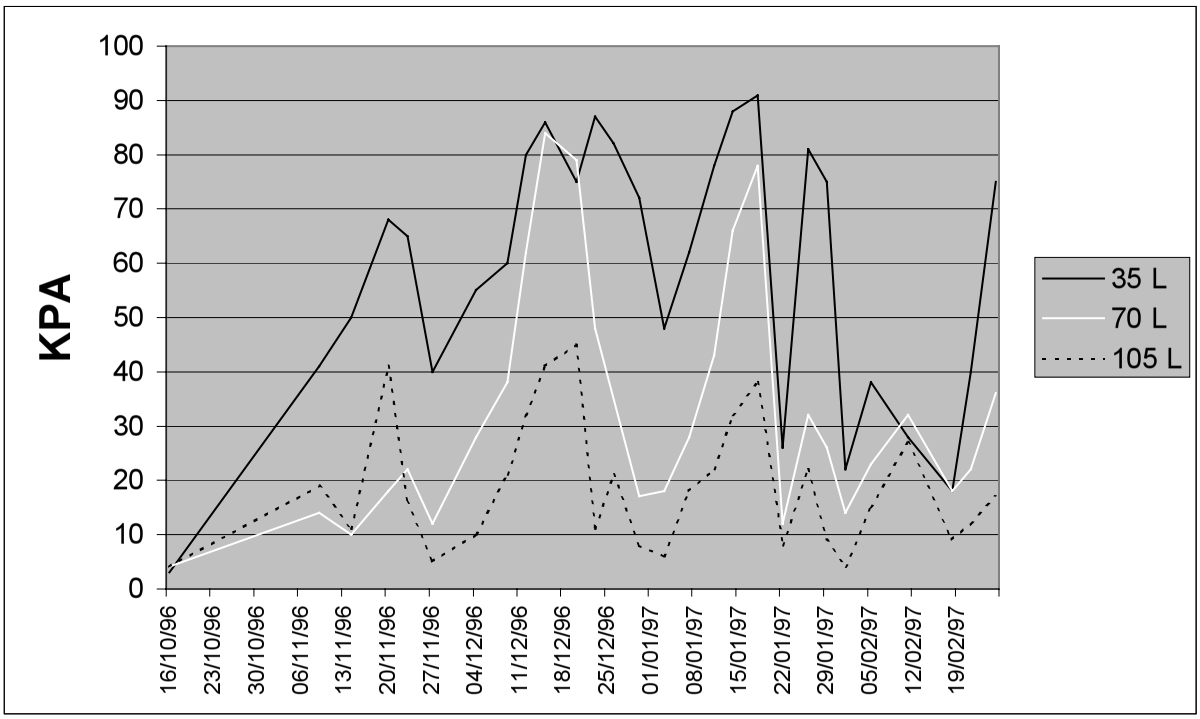


Figure 12. Soil moisture tension (KPA) at 10cm depth for the Rosevears truffière in 1997/98 irrigation season.

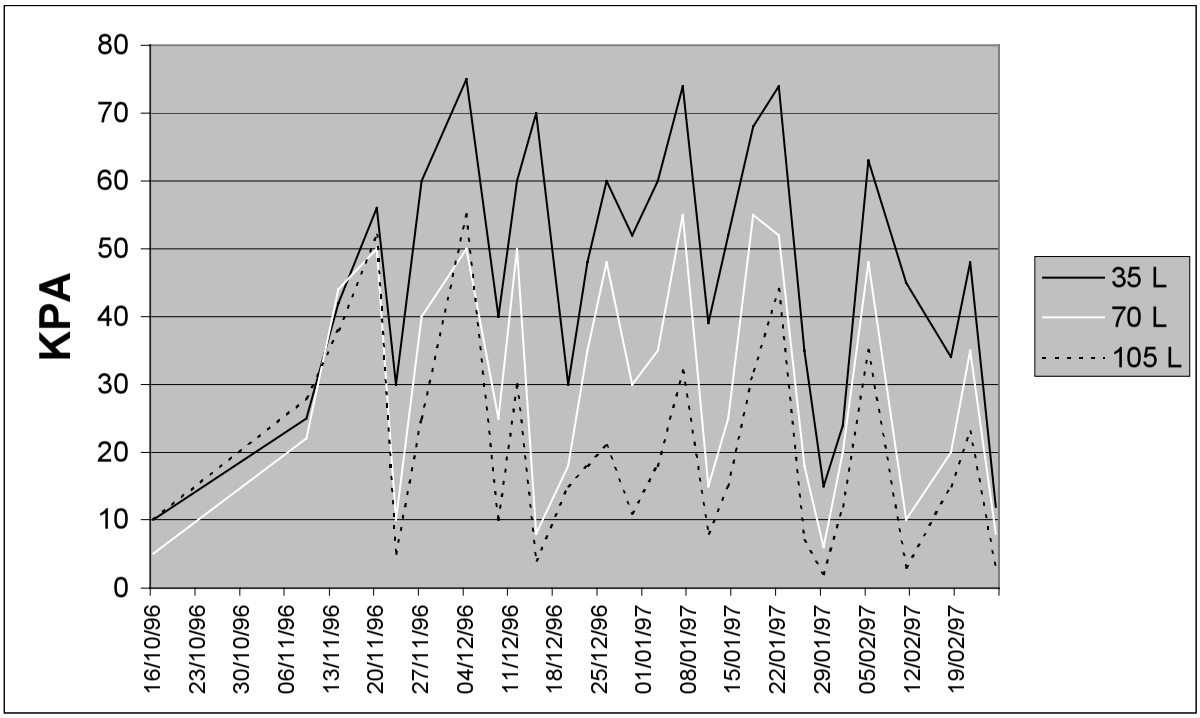


Figure 13. Soil moisture tension (KPA) at 20cm depth for the Rosevears truffière in 1997/98 irrigation season.