Physiological aspects of *Corylus avellana* associated with the French black truffle fungus *Tuber melanosporum* and the consequence for commercial production of black truffles in Western Australia.

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I declare that this thesis is my own account of my research and contains as its main content work which has not previously been submitted for a degree at any tertiary education institution.

B. P. Bradshaw

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Abstract

The black truffle (*Tuber melanosporum* Vitt.) industry in Australia is relatively new and has enormous potential but some truffières (truffle farms) fail to meet anticipated harvest projections. Inappropriate soil conditions and climate, and the management of such factors are suggested as the primary reasons for inadequate yield. In addition, requirements for ascocarp initiation and development and the role of the host plant in such processes are unknown. This study examines interactions between European hazel (*Corylus avellana* L.) and the ectomycorrhizal (ECM) black truffle symbiont in a commercial truffière (Hazel Hill) in south-western Australia. Specific studies were initiated to examine the interactions of host physiology, mycorrhizal infection and the interaction with abiotic factors. The study related specific physiological processes of the host plant to the known life cycle of the black truffle to determine the role of the host plant in ascocarp production, if any. The work also examined the effect of silvicultural treatments intended to increase truffle production.

A review of existing literature was undertaken to determine the key soil and climatic factors required for successful truffle production. Climatic conditions appeared more important than soil chemistry and structure in Western Australia, with significant seasonal variation in air and soil temperatures required plus irrigation to supplement summer rainfall. This information was used to define areas with potential for truffle production in the south-west of Western Australia: the cooler, high rainfall regions (>1000 mm annual rainfall) where there is sufficient seasonal variation in soil temperature and availability of adequate quantities of quality water for irrigation. Subsurface soil acidification and salinity, as well as groundwater salinity, are constraining factors. Lime amendment is necessary to create sufficiently high pH and CaCO$_3$ levels required by the truffle fungus.

A field trial was established to monitor the seasonal C dynamics of European hazel in the context of the life cycle of the black truffle. Maximum translocation of sucrose in the phloem sap coincided with the period of anticipated rapid growth of the truffle ascocarp implicating the use of current photosynthate in C nutrition of the ascocarp. Sampling of non-structural carbohydrates (NC) of above and belowground plant material indicated maximum storage of C in the host coincides with maturation of the
ascocarp. These observations provide evidence of a synchronous growth habit of the plant host and the ascocarp.

The C allocation patterns of European hazel in response to liming a loamy soil, taken from near the Hazel Hill truffière, and inoculation with ECM fungi (T. melanosporum, Hebeloma sp. and Scleroderma sp.) were examined in a glasshouse pot trial. Liming increased biomass allocation to the shoot and induced deficiencies of phosphorus and manganese. Colonisation by ECM fungi significantly increased net photosynthesis, indicating the sink strength of these fungi, but there was no relationship between the level of mycorrhizal infection and fine root NC. The maximum rate (40 g lime kg⁻¹ soil) reduced infection by Hebeloma and Scleroderma and had no impact on T. melanosporum. Further, infection rates of T. melanosporum did not increase in response to lime suggesting lime is not necessary for ECM development in this soil type.

Fertiliser is widely used in commercial truffières in Australia but the consequences for truffle production are unknown. In a field trial, the growth and physiological response of European hazel to forms of phosphorus (34 and 68 kg ha⁻¹ apatite-P and 68 kg ha⁻¹ triple super phosphate-P) and nitrogen (50 kg ha⁻¹ of NO₃⁻ and NH₄-N) were examined as well as the mycorrhizal response to fertiliser. Apatite-P increased phloem sap sucrose concentrations which was attributed to increased root biomass and associated sink capacity. Fertiliser application did not change fine root NC concentrations suggesting no increase in allocation of C to ECM structures. The highest rate of apatite-P decreased mycorrhizal infection rates of T. melanosporum and, most likely, was the result of increased infection rates of Hebeloma. In contrast to the literature relating to indigenous Australian ECM fungi, the highest rate of soluble-P did not decrease ECM infection rates in T. melanosporum. Nitrogen treatments increased foliar N content and improved gas exchange efficiency of plants, and had no adverse impact on the level of ECM infection. Fertilisation with N significantly increased soil respiration rates suggesting N limits mineralisation at this site.

Some truffières manage the canopies of the host tree to ensure maximum exposure of the soil surface in order to increase soil temperatures. As there are no published data on the effect of pruning on black truffle production, a field trial was established to document the impact of canopy pruning on host physiology and soil temperature. The
removal of 65% of canopy leaf area reduced phloem sap sucrose concentrations, soil respiration rate and the soluble: insoluble NC ratio of fine roots in the short term (1-3 weeks). There was no compensatory response of leaf gas exchange parameters as a result of pruning. Generally, there was no long term impact on plant physiological parameters as a result of pruning. Long term effects on soil temperature were observed as a result of pruning. Mean annual temperature and amplitude increased significantly beneath pruned trees and spring, summer and autumn soil temperatures increased as did diurnal variation as a result of pruning. Pruning did not increase winter soil temperatures and therefore would probably not impact on ascocarp maturation during this period.

This research has provided insight into the C physiology of hazel associated with the black truffle and the consequences for truffle production. The results provide anecdotal evidence of direct C transfer between the host and the developing truffle, contrary to the existing paradigm that the ascocarp is saprotrophic for the majority of its growth and development. There is a need to validate this finding as there are consequences for management of commercial truffières. Liming of loam duplex soils can reduce the abundance of the most common competitor ECM fungi and should be encouraged in commercial truffières. Applying phosphorus and nitrogen to commercial truffières will improve growth rates of planted trees without adversely impacting on ECM infection by black truffle fungi, although the impact on truffle production remains unknown. It is anticipated truffle production will improve in the longer term as a result of pruning and prudent canopy management. Management options should include tree removal to reduce planting density and increase soil exposure in truffières. There is a need for longer term trials to be established to determine the C nutrition of the truffle ascocarp and to clearly define the key stages of the black truffle life cycle in Western Australia.
Publications arising from this research


Statement relating to the reporting of the seasonal calendar of the northern and southern hemispheres in this thesis.

Interpretation and quotation of material relating to the northern hemisphere has been adapted for the southern hemisphere throughout this thesis. For literature reporting events occurring during certain months of a season in the northern hemisphere, the corresponding month of the southern hemisphere is reported, unless otherwise stated. Where clarification is required it is indicated in parentheses as to which hemisphere the statement refers e.g. (SH), southern hemisphere or (NH), northern hemisphere.
**List of abbreviations**

<table>
<thead>
<tr>
<th>Abbreviation</th>
<th>Description</th>
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<tbody>
<tr>
<td>$A$</td>
<td>Net photosynthetic rate</td>
</tr>
<tr>
<td>AA</td>
<td>Amino acids</td>
</tr>
<tr>
<td>AgWA</td>
<td>Department of Agriculture Western Australia</td>
</tr>
<tr>
<td>ANOVA</td>
<td>Analysis of variance</td>
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<tr>
<td>$Ap$</td>
<td>Atmospheric pressure</td>
</tr>
<tr>
<td>a.s.l.</td>
<td>Above sea level</td>
</tr>
<tr>
<td>BSTFA</td>
<td>bi(trimethylsilyl)trifluoroacetamide</td>
</tr>
<tr>
<td>C</td>
<td>Carbon</td>
</tr>
<tr>
<td>$C_i$</td>
<td>Sub-stomatal CO$_2$ concentration</td>
</tr>
<tr>
<td>DDI</td>
<td>Distilled deionised water</td>
</tr>
<tr>
<td>DMS</td>
<td>Dimethylsulphoxide</td>
</tr>
<tr>
<td>DNA</td>
<td>Deoxyribonucleic acid</td>
</tr>
<tr>
<td>dS</td>
<td>Decisiemens</td>
</tr>
<tr>
<td>DTPA</td>
<td>Diethylene triamine pentaacetic acid</td>
</tr>
<tr>
<td>DW</td>
<td>Dry weight</td>
</tr>
<tr>
<td>e</td>
<td>Vapour pressure</td>
</tr>
<tr>
<td>$E$</td>
<td>Evapotranspiration</td>
</tr>
<tr>
<td>ECM</td>
<td>Ectomycorrhiza</td>
</tr>
<tr>
<td>ELSD</td>
<td>Evaporative light scattering detector</td>
</tr>
<tr>
<td>$e_s$</td>
<td>Saturation vapour pressure</td>
</tr>
<tr>
<td>exp</td>
<td>Exponential</td>
</tr>
<tr>
<td>FID</td>
<td>Flame ionisation detection</td>
</tr>
<tr>
<td>GC</td>
<td>Gas chromatography</td>
</tr>
<tr>
<td>GLM</td>
<td>General linear model</td>
</tr>
<tr>
<td>$g_s$</td>
<td>Stomatal conductance</td>
</tr>
<tr>
<td>HPLC</td>
<td>High pressure liquid chromatography</td>
</tr>
<tr>
<td>ICP</td>
<td>Inductively coupled plasmospectrometry</td>
</tr>
<tr>
<td>IGEE</td>
<td>Intrinsic gas exchange efficiency</td>
</tr>
<tr>
<td>INRA</td>
<td>Institut National de la Recherche Agronomique</td>
</tr>
<tr>
<td>IRGA</td>
<td>Infra-red gas analyser</td>
</tr>
<tr>
<td>IRMS</td>
<td>Isotope ratio mass spectrometry</td>
</tr>
<tr>
<td>ITS</td>
<td>Internal transcribed spacer</td>
</tr>
<tr>
<td>IWUE</td>
<td>Instantaneous water use efficiency</td>
</tr>
</tbody>
</table>
K
Potassium

LA
Leaf area

LAI
Leaf area index

LSD
Least significant difference

MAFRL
Marine and fresh water research laboratory

meq
Milliequivalents

MS
Mass spectrometry

N
Nitrogen

NC
Non-structural carbohydrate

NH
Northern hemisphere

NMR
Nuclear magnetic resonance

P
Phosphorus

PAI
Periodic annual increment

PAR
Photosynthetically active radiation

PCR
Polymerase chain reaction

PVC
Polyvinyl chloride

rH
Relative humidity

R_s
Soil respiration rate

SE
Standard error of mean

SH
Southern hemisphere

SLA
Specific leaf area

sp., spp.
Species, (singular, plural)

SW
South-west

T
Temperature

TCMS
Trimethylchlorosilane

TNC
Total non-structural carbohydrate

TSP
Triple super-phosphate

T_w
Wet bulb temperature

VOC
Volatile organic compound

VPD
Vapour pressure deficit

WA
Western Australia

YFEL
Youngest fully expanded leaf

δ^{13}C
^{13}C/^{12}C notation relative to the Peedee belemnite standard (%)
Acknowledgements

This work was supported by the Australian Research Council (ARC) through provision of an APA (Industry) scholarship in conjunction with the industry partner, Horticultural Management Ltd. I am indebted to both these organisations for their support and the opportunity to indulge a passion.

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Special thanks to Jean-Marc Olivier and Gérard Chevalier for their insight into French trufficulture and thanks also to Pierre Sourzat for providing climate data and enlightenment on the cultivation of black truffles. I also wish to thank Alessandra Zambonelli for similar enlightenment of the Italian white truffle. I am immensely grateful to these individuals for their time and hospitality and I am humbled by their generosity of wisdom of all things truffle. I thank the Research and Development Board of Murdoch University for financial assistance to visit trufficultural regions of France and Italy.
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To my young truffle dog, Oakey, who has been a willing companion and who provided the exhilaration of a first truffle find - may there be many more!

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“…..a shortage of grain is preferable to a shortage of truffles....”

- Plutarch, AD 46-120