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.

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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 NO_3- and NH_4-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

Dell, B. **Bradshaw, B.P.** Dunstan, W.A. and Malajczuk, N. (2003) Turner Review. Truffles: their biology and cultivation from an Australian perspective. *Australian Journal of Botany* In review

Bradshaw, B.P. Dell, B. and Malajczuk, N. (2003) The effect of lime on competitor dynamics in a commercial truffiere in SW Australia. *Proceedings of the 3rd international workshop on edible mycorrhizal mushrooms: Ecology, physiology and cultivation.* August 17-19th, University of Victoria, BC, Canada.

Bradshaw, B.P. Dell, B. and Malajczuk, N. (2003) Seasonal Carbon dynamics of *Corylus avellana* in a commercial truffiere in south-western Australia. *Proceedings of the 3rd international workshop on edible mycorrhizal mushrooms: Ecology, physiology and cultivation.* August 17-19th, University of Victoria, BC, Canada.

Bradshaw, B.P. Dell, B. and Malajczuk, N. (2003) Mycorrhizal response to carbon partitioning in *Corylus avellana*. *Proceedings of the third international symposium on dynamics of physiological processes in woody roots.* September 29 – October 3, 2003 Perth, Western Australia.

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

A	Net photosynthetic rate
AA	Amino acids
AgWA	Department of Agriculture Western Australia
ANOVA	Analysis of variance
A*p*	Atmospheric pressure
a.s.l.	Above sea level
BSTFA	bi(trimethylsilyl)trifluoroacetamide
C	Carbon
C_i	Sub-stomatal CO_2 concentration
DDI	Distilled deionised water
DMS	Dimethylsulphoxide
DNA	Deoxyribonucleic acid
dS	Decisiemens
DTPA	Diethylene triamine pentaacetic acid
DW	Dry weight
e	Vapour pressure
E	Evapotranspiration
ECM	Ectomycorrhiza
ELSD	Evaporative light scattering detector
e_s	Saturation vapour pressure
exp	Exponential
FID	Flame ionisation detection
GC	Gas chromatography
GLM	General linear model
g_s	Stomatal conductance
HPLC	High pressure liquid chromatography
ICP	Inductively coupled plasmospectrometry
IGEE	Intrinsic gas exchange efficiency
INRA	Institut National de la Recherche Agronomique
IRGA	Infra-red gas analyser
IRMS	Isotope ratio mass spectrometry
ITS	Internal transcribed spacer
IWUE	Instantaneous water use efficiency

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
$\delta^{13}C$	$^{13}C/^{12}C$ notation relative to the Peedee belemnite standard (‰)

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

- Plutarch, AD 46-120