THE EFFECT OF THE ARBUSCULAR MYCORRHIZAL SYMBIOSIS ON THE PRODUCTION OF PHYTOCHEMICALS IN BASIL

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Summary

The overall objective of this thesis was to investigate how the arbuscular mycorrhizal (AM) symbiosis can affect the production of phytochemicals (antioxidants; rosmarinic and caffeic acid, RA & CA) in the shoots of basil (*Ocimum basilicum* L.). As a result of an increasing interest in natural/herbal medicines, more effort is now needed to produce herbal products of better quality, *i.e.* higher and standardised phytochemical concentrations. Thus, it was hypothesised that the naturally occurring AM fungi (AMF) could play an important role in improving the growth and phytochemical concentrations in medicinal herbs such as basil, as organic methods of cultivation are increasingly sought after to grow such plants.

Despite a reasonable amount of information available in the literature on the changes of phytochemical concentrations in the roots of host plants following AM colonisation, very little is known about such processes in the aerial part of such plants. Furthermore, basil has hardly been studied as a host plant in AM research, and very little is known of its responsiveness to AM colonisation. As AMF are well known to improve phosphorus (P) uptake in their host plant, the first objective of this work was to obtain AM and non-mycorrhizal (NM) plants matched for tissue P concentrations and growth rates. Only under such conditions would it then be possible to separate benefits derived from improved plant P uptake from non-nutritional benefits.

It was found that basil is highly responsive to P, and that under low or little P supply it is quite dependent on the AM symbiosis in order to grow. However,

growth depressions were observed when growing basil in winter with *Glomus intraradices*, suggesting that the fungal symbiont can act as a strong sink of carbon (C) under such conditions. Thus, in order to obtain AM and NM plants with matched growth rates and tissue P concentrations, it was found that basil needed to be cultivated in summer in a soil/sand mixture with a ratio of 1:3 (w/w), along with 0.2 g/kg $CaHPO_4$ and 25% of AM inoculum (AM plants). Under these conditions, AM plants grew as well as NM plants and *G. caledonium* and *G. mosseae* were shown to increase the concentrations of RA and CA in the shoots of basil, but not in roots. Such results were not an indirect effect of improved P uptake.

In order to understand the mechanisms by which AMF increased RA and CA concentrations in basil, further experiments were set up to investigate the effect of 1) AM developmental stages, 2) nitrogen (N) supply and 3) phytohormone changes on the production of RA and CA in the shoots. None of these factors was found to contribute to increases in antioxidants in basil under AM symbiosis. Therefore, the mechanisms by which AMF affect RA and CA concentrations in basil still remain unknown. A final experiment was carried out to investigate the potential of an AM fungus to improve the growth of basil when challenged with a specific pathogen *Fusarium oxysporum* f.sp. *basilici* (*Fob*), which causes significant production losses. The results showed that inoculation of basil with *G. mosseae* not only improved plant growth compared to NM plants, but also conferred a protective effect against *Fob*. However, shoot antioxidant concentrations (RA, CA, total phenolics and essential oils) were not increased in

AM plants compared to NM plants, and the mechanism of protection against *Fob* could not be elucidated.

Due to the high variability of RA and CA concentrations obtained in AM plants in different experiments, it cannot be concluded that AMF confer an absolute advantage over uninoculated plants if the main concern is to obtain standardised concentrations of phytochemical in basil. On the other hand, the key results presented in this thesis do indicate that inoculating basil with AMF can be beneficial to improve its growth as well as antioxidant concentrations, compared to NM plants grown under similar conditions. Such results could be of potential interest to basil growers who wish to cultivate this medicinal herb organically (*i.e.* low P supply and no chemical fertilisers added).

Publications and presentations from the thesis

Journal papers

Toussaint JP, Kraml M, Nell M, Smith SE, Smith FA, Steinkellner S, Schmiderer C, Vierheilig H and Novak J. **2008**. Effect of *Glomus mosseae* on the concentrations of rosmarinic and caffeic acids and essential oil compounds in basil inoculated with *Fusarium oxysporum* f.sp. *basilici*. Accepted in *Plant Pathology*.

Toussaint JP. **2007**. Investigating physiological changes in the aerial parts of AM plants: what do we know and where should we be heading? *Mycorrhiza* **17**: 349-353.

Toussaint JP, Smith FA and Smith SE. **2007**. Arbuscular mycorrhizal fungi can induce the production of phytochemicals in sweet basil irrespective of phosphorus nutrition. *Mycorrhiza* **14**: 291-297.

Conference talks and posters

Toussaint JP, Smith FA, Smith SE. **2006**. The influence of AM on the production of phytochemicals in sweet basil*. *5th International Conference On Mycorrhizas* (ICOM5), Granada, Spain.
*BIOTISA student award for best presentation in biotechnology
http://www.mycorrhizas.org/files/NEWSLETTER_2006_NOVEMBER.PDF

Toussaint JP, Smith FA, Smith SE. **2006**. Investigating the effects of AM fungi on phytochemical production in sweet basil. *Centre for Soil and Plant Interaction workshop* (CSPI), Adelaide, Australia.

Toussaint JP, Smith FA, Smith SE. **2005**. Improving the yield of active compounds in medicinal plants through the arbuscular mycorrhizal symbiosis. *CSPI workshop*, Adelaide, Australia.

Toussaint JP, Smith FA, Smith SE. **2005**. Can the AM symbiosis improve the yield of active compounds in medicinal plants? *Australian Society for Biochemistry and Molecular Biology meeting* (COMBIO), Adelaide, Australia. (poster)

Declaration

This work contains no material which has been accepted for the award of any other degree or diploma in any university or other tertiary institution and, to the best of my knowledge and belief, contains no material previously published or written by another person, except where due reference has been made in the text.

I give consent to this copy of my thesis being made available in the University Library.

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Acknowledgments

Doing a Ph.D. is not an easy dish to prepare. Although the recipe might vary from one person to the next, I would say that it takes 1 cup of commitment, 1 cup of perseverance and approximately ½ cup of wits. To this, you can add some fun-times, according to your taste…though not too much, otherwise the whole thing might turn sour. However, one key ingredient that does not always appear in the cook book is the people that you meet during this journey. They are the "secret ingredient" that will make the difference in creating a "Chef-d'oeuvre" (or so to speak)! Therefore, I would like to take this opportunity to acknowledge and thank all of those who made this thesis possible – I would not dare say it is a "Chef-d'oeuvre", but hopefully good enough to be a "Main dish".

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Le Nord est devant toi…