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SHORT NOTE

Investigating physiological changes in the aerial parts of AM plants: what do we know and where should we be heading?

J.-P. Toussaint

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Abstract Research in the field of arbuscular mycorrhizal (AM) symbiosis has taken a giant leap in the past two decades, as demonstrated by the large amount of literature being published every year. Most of the research efforts have been put towards the understanding of the mechanisms of this symbiosis. However, there are still several unknowns on the systemic effects of the AM symbiosis, and our understanding of non-nutritional effects on the physiological changes occurring in the aerial parts of the host plant is yet quite limited. In this short note, I briefly address the question, if there are any changes in metabolic activities that are triggered by AM fungi, and assess the importance of such changes for mycorrhizal research and application.

Keywords Arbuscular mycorrhizas · Aerial parts of plants · Physiological changes · Secondary metabolism

The understanding of the arbuscular mycorrhizal (AM) symbiosis has greatly improved in the past decades mainly due to the broadening and amalgamating of research in plant physiology, plant–microbe interactions, molecular and cellular biology, as well as ecology. This research has one main goal: to understand the biology of the AM symbiosis, particularly at the root level, which is where the symbiosis occurs. Hence, some of the main physiological mechanisms underlying this important and widespread symbiosis are now better understood (e.g. Harrison 2005; Bucher 2007). However, there is still a lack of information on the physiological implications of the symbiosis in the aerial parts (referred here as “shoots”) of host plants.

J.-P. Toussaint (✉)
School of Earth and Environmental Sciences,
Waite Campus DP 636, The University of Adelaide,
Adelaide, South Australia 5005, Australia
e-mail: jeanpatrick.toussaint@adelaide.edu.au

Research activities in the field of AM symbioses have been conducted under a variety of conditions ranging from laboratory studies, including root-organ cultures (ROC; Declerck et al. 2005), to glasshouse and field trials (Fitter 1985; Klironomos et al. 2000; Smith et al. 2003). Recent advances in bio-technology are now providing many molecular tools to elucidate the intricate mechanisms of this symbiosis. ROC systems have been extensively used in the past decade (Fortin et al. 2002) and, more recently, have been used as a tool to understand the mechanisms of the signalling processes involved in the host recognition by the fungus (Buée et al. 2000; Akiyama et al. 2005). Some researchers argue that the growth environment of the ROC system is too artificial, as it totally lacks the shoot input, which is likely to affect the functioning of the symbiosis (i.e. photosynthates, hormonal balance and other signals), therefore “masking” the true physiological and whole-plant nature of the symbiosis. However, the purpose of this communication is not to focus on the advantages and disadvantages of any particular experimental system but rather to assess the importance and relevance of focusing on the physiological (metabolic) changes occurring in the shoots of an AM plant, which is often neglected in AM research, except for obvious effects on growth and nutrient concentrations.

Focusing on the shoots

Mycorrhizal research has largely focused on the uptake of mineral nutrients in AM plants (Marschner and Dell 1994;

Smith and Read 1997), investigating the effects of the symbiosis in increasing nutrients concentrations such as phosphorus (P), nitrogen and zinc (Johansen et al. 1994; Zhu et al. 2001; Smith et al. 2003; Toussaint et al. 2004). This nutritional improvement in AM plants often (but not always) results in positive growth responses in host plants, most evidently at the shoot level. Research on primary metabolism of AM plants has also been partly addressed in studies investigating changes in photosynthetic rates (Wright et al. 1998b) as well as carbon assimilation and allocation in AM plants (Wright et al. 1998a). It has been suggested that the symbiosis can increase photosynthesis through morphological adaptations such as increase in specific leaf area (Harris and Paul 1987). Other less well-defined effects of AM fungi (AMF) on host plant's primary metabolism include drought tolerance and water relations and the changes it induce (e.g. higher enzyme activities, leaf water potential, sugar content; Cui and Nobel 1992; Subramanian et al. 1995, 1997; Ruiz-Lozano 2003; Caravaca et al. 2005).

There are also indirect physiological effects of the symbiosis that are not quite clear yet. AMF have been reported to induce changes in phytohormone balance in host plants (Allen et al. 1980, 1982; Esch et al. 1994; Torelli et al. 2000; Fitze et al. 2005), but there is only limited evidence of such effects in the shoots of AM plants. There also have been several studies related to acquired bio-protective effects through AMF, especially focusing on the activation of plant defence mechanisms in roots of host plants (see reviews by Garcia-Garrido and Ocampo 2002 and Harrier and Watson 2004 and references therein). Although there is no clear pattern of bio-protection of AMF against pathogens (see review by Borowicz 2001), AMF seem to have an impact on host plants physiology. It is believed that the first steps of colonisation by AMF induce the production of secondary plant compounds in the roots of the host plant, such as low molecular weight antimicrobial flavonoids [e.g. phytoalexins (VanEtten et al. 1994; Scharff et al. 1997)] or pathogenesis-related proteins [e.g. chitinase and β-1,3-glucanase (Bonfante and Perotto 1995; Benhamou 1996)]. There also have been other reports on the effect of AMF on accumulation of phenolics (Grandmaison et al. 1993; Larose et al. 2002 and references therein), but again, these were focused on roots.

Systemic effects of AMF in host plants have been partly addressed in the past regarding pathogen resistance (Cordier et al. 1998) and, more recently, in relation to biochemical changes and the induction of plant defences (Singh et al. 2004; Bezemer and van Dam 2005). AMF can have a direct impact in below- and above-ground interactions, although these interactions are quite complex and not well understood. Relevant questions that need addressing include the following: Are there any true systemic effects of this particular symbiosis on the host plant? In that regard, it was recently demonstrated that AM tomato plants show different gene expression patterns in leaf and root tissues (Taylor and Harrier 2003). The authors suggested that these regulation patterns could be the result of a different nutritional status or the alteration of hormonal balance in the host plant. Studies such as the latter can provide important insights on the mechanisms by which the AM symbiosis influences the host plant metabolism and physiology. However, as a word of caution, despite the advantages of new molecular techniques, it is imperative to incorporate both basic physiological investigations and novel technical approaches in AM research, as it is not always the case in molecular-focused studies. On the other hand, another question that, to my belief, could be quite captivating and worth investigating is: Are new bio-chemical pathways or changes in metabolic activities triggered by AMF in the aerial parts of a host plant?

New perspectives

Until recently, very little attention had been paid on the accumulation of secondary compounds in the aerial parts of AM plants. There have been reports on the activity of antioxidants in AM plants, but mainly in response to abiotic stresses (Schutzendubel and Polle 2002; Porcel and Ruiz-Lozano 2004). It has also been known for several years that different species of AMF can contribute to higher production and yield of essential oils in plants with medicinal virtues such as mint (Sirohi and Singh 1983). However, no clear mechanism, other than an improvement of the nutritional status (mainly P), has been put forward to explain such observations. A recent study showed that increases of essential oils in sweet basil colonised by *Gigaspora rosea* are correlated to a larger number of peltate glandular trichomes per surface area (Copetta et al. 2006). It was suggested that the increase in number of trichomes could have been regulated by changes in hormonal balance in AM plants as previously reported (Allen et al. 1980, 1982). Another study reported increases of essential oil concentrations in oregano plants colonised by *Glomus mosseae*, which was not related to improved P nutrition (Khaosaad et al. 2006). Furthermore, we recently demonstrated that *Glomus caledonium* and *G. mosseae* induce the production of antioxidants in the aerial parts of sweet basil (Toussaint et al. 2007). These results were obtained under matched P tissue concentrations for both AM and non-AM plants; therefore, these were not solely the indirect effect of improved P nutrition, but a fungal-mediated enhancement of active compounds in the shoots. Altogether, these reports illustrate that there can be accumulations of secondary plant compounds in the aerial parts of AM plants, which are "non-nutritional-related", and therefore highlight the need for further research aiming at elucidating the mechanisms by which AMF affect plant metabolism in shoots.

In search of an answer...where to next?

Plant pathogens and herbivores are known to directly or indirectly influence defence responses in roots and shoots of plants (Bezemer and van Dam 2005). There is evidence that defence responses involve similar compounds against root pathogens and herbivores (e.g. 2-*b*-*O*-D-glucopyranosyl-4-hydroxy-7-methoxy-1,4-benzoxazin-3-one, DIMBOA-Glc; Collantes et al. 1998). Ectomycorrhizal fungi are thought to produce their own secondary metabolites that can protect the host plant against root pathogens (Jones and Last 1991). If such compounds can be translocated to the shoots of the plant, this could enhance plant defences against herbivores. Likewise, AMF could confer some protection to host plants by inducing the production secondary compounds that would make the plant less palatable to herbivores. However, such a hypothesis has not been properly investigated. There have been reports of the reduction in survival of Lepidopteran (*Noctuidea*) larvae when fed with the leaves of AM plants compared to the leaves of non-AM plants (Rabin and Pacovsky 1985). The effect was not correlated to any increase in total phenolic content, and no clear mechanism was suggested that would explain how the AMF rendered the leaves less palatable. Perhaps such a mechanism could not be revealed due to the experimental methods used at the time, which might not have allowed detecting subtle, yet significant, changes in plant secondary compounds. New techniques (e.g. liquid and gas chromatography, molecular tools) might help circumvent this problem. Recent and novel analytical techniques/approaches such as metabolomics, gene expression studies and proteomics will most definitely help us understand and gain a “global picture” of plant–microbe interactions, including AMF (Cánovas et al. 2004; Bezemer and van Dam 2005). Therefore, if we are to understand the effects of the AM symbiosis at the shoot level, and the molecular mechanisms involved behind it, we need to carefully combine new high throughput techniques, such as the ones mentioned above, with classical physiological investigations (i.e. nutritional status of the plants, growth/morphological characteristics, etc.). Only then will we be able to fill some of the gaps left in this area of AM research and be able to answer the “how’s” and “why’s”.

There is increasing interest in the use of AMF as biological control agents (Whipps 2004) as well as in organic farming (Gosling et al. 2006). This was particularly illustrated at the latest International Conference on Mycorrhiza (ICOM), held in Granada, Spain, where an entire session was devoted to “Mycorrhiza in alternative production systems”. There were six oral presentations relating to this subject and more than 30 posters of original research topics, confirming the growing interest in AM research related to practical and holistic approaches. As an example, the production of commercially important crops might benefit from knowledge gained through investigations of the effect of AMF on the production of phytochemicals or secondary compounds. This is especially true as the harvested product is quite often the aerial part. For instance, by the year 2000, the herbal medicine industry was generating \$4 billion in the USA, about £31 million in the UK and £1.3 billion in Germany (Ernst 2000). As there is a need to develop herb varieties with better growth and increased levels of active compounds (Rai et al. 2001), the exploitation of the AM symbiosis could greatly benefit such an industry; providing that further attention is paid towards the understanding of the effects of AMF in the bio-chemical pathways occurring in the shoots of host plants. The research in this area would also be relevant in the sector of research and development, especially when focusing on sustainable management systems (see Lumpkin 2005). Ultimately, it should be highly valuable to further acknowledge the influence of AMF on the bio-chemical pathways in the aerial parts of the host plant and scrutinise this symbiosis in a more holistic way.

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References

Akiyama K, Matsuzaki K, Hyashi H (2005) Plant sesquiterpenes induce hyphal branching in arbuscular mycorrhizal fungi. Nature 435:824–827

Allen MF, Moore TS, Christensen M (1980) Phytohormone changes in *Bouteloua gracilis* infected by vesicular–arbuscular mycorrhizae. I. Cytokinin increases in the host plant. Can J Bot 58:371–374

Allen MF, Moore TS, Christensen M (1982) Phytohormone changes in *Bouteloua gracilis* infected by vesicular–arbuscular mycorrhizae. II. Altered levels of gibberellin-like substances and abscisic acid in the host plant. Can J Bot 60:468–471

Benhamou N (1996) Elicitor-induced plant defence pathways. Trends Plant Sci 1:233–240

Bezemer TM, van Dam NM (2005) Linking aboveground and belowground interactions via induced plant defenses. Trends Ecol Evol 20:617–624

Bonfante P, Perotto S (1995) Strategies of arbuscular mycorrhizal fungi when infecting host plants. New Phytol 130:3–21

Borowicz VA (2001) Do arbuscular mycorrhizal fungi alter plant–pathogen relations? Ecology 82:3057–3068

Bucher M (2007) Functional biology of plant phosphate uptake at root and mycorrhiza interfaces. New Phytol 173:11–26

Buée M, Rossignol M, Jauneau A, Ranjeva R, Bécard G (2000) The pre-symbiotic growth of arbuscular mycorrhizal fungi is induced by a branching factor partially purified from plant root exudates. Mol Plant Microbe Interact 13:693–698

Cánovas FM, Dumas-Gaudot E, Recorbet G, Jorrin J, Mock HP, Rossignol M (2004) Plant proteome analysis. Proteomics 4:285–298

Caravaca F, Alguacil MM, Hernandez JA, Roldan A (2005) Involvement of antioxidant enzyme and nitrate reductase activities during water stress and recovery of mycorrhizal *Myrtus communis* and *Phillyrea angustifolia* plants. Plant Sci 169:191–197

Collantes HG, Gianoli E, Niemeyer HM (1998) Changes in growth and chemical defences upon defoliation in maize. Phytochemistry 49:1921–1923

Copetta A, Lingua G, Berta G (2006) Effects of three AM fungi on growth, distribution of glandular hairs, and essential oil production in *Ocimum basilicum* L. var. Genovese. Mycorrhiza 16:485–494

Cordier C, Pozo MJ, Barea JM, Gianinazzi S, Gianinazzi-Pearson V (1998) Cell defence responses associated with localized and systemic resistance to *Phytophthora parasitica* induced in tomato by an arbuscular mycorrhizal fungus. Mol Plant Microbe Interact 11:1017–1028

Cui M, Nobel PS (1992) Nutrient status, water uptake and gas exchange for three desert succulents infected with mycorrhizal fungi. New Phytol 122:643–649

Declerck S, Strullu DG, Fortin JA, Ed EDS (2005) In vitro culture of mycorrhizas. Soil biology. Springer, Berlin

Ernst E (2000) Herbal medicines: where is the evidence? Growing evidence of effectiveness is counterbalanced by inadequate regulation. Br Med J 321:395–396

Esch H, Hundeshagen B, Schneiderpoetsch H, Bothe H (1994) Demonstration of abscisic acid in spores and hyphae of the arbuscular mycorrhizal fungus Glomus and in the N2 fixing cyanobacterium *Anabaena variabilis*. Plant Sci 99:9–16

Fitter AH (1985) Functioning of vesicular–arbuscular mycorrhizas under field conditions. New Phytol 99:257–265

Fitze D, Wiepning A, Kaldorf M, Ludwig-Muller J (2005) Auxins in the development of an arbuscular mycorrhizal symbiosis in maize. J Plant Physiol 162:1210–1219

Fortin JA, Bécard G, Declerck S, Dalpé Y, St-Arnaud M, Coughlan AP, Piché Y (2002) Arbuscular mycorrhiza on root-organ cultures. Can J Bot 80:1–20

Garcia-Garrido JM, Ocampo JA (2002) Regulation of the plant defence response in arbuscular mycorrhizal symbiosis. J Exp Bot 53:1377–1386

Gosling P, Hodge A, Goodlass G, Bending GD (2006) Arbuscular mycorrhizal fungi and organic farming. Agric Ecosyst Environ 113:17–35

Grandmaison J, Olah GM, Vancalsteren MR, Furlan V (1993) Characterization and localization of plant phenolics likely involved in the pathogen resistance expressed by endomycorrhizal roots. Mycorrhiza 3:155–164

Harrier LA, Watson CA (2004) The potential role of arbuscular mycorrhizal (AM) fungi in the bioprotection of plants against soil-borne pathogens in organic and/or other sustainable farming systems. Pest Manag Sci 60:149–157

Harris D, Paul EA (1987) Carbon requirements of vesicular–arbuscular mycorrhizae. In: Ecophysiology of VA mycorrhizal plants. CRC, Boca Raton, FL, pp 93–105

Harrison MJ (2005) Signaling in the arbuscular mycorrhizal symbiosis. Annu Rev Microbiol 59:19–42

Johansen A, Jakobsen I, Jensen ES (1994) Hyphal N transport by a vesicular–arbuscular mycorrhizal fungus associated with cucumber grown at three nitrogen levels. Plant Soil 160:1–9

Jones CG, Last FT (1991) In: Barbosa P, Krischik VA, Jones CG (eds) Microbial mediation of plant–herbivore interactions. Wiley, New York, pp 65–103

Khaosaad T, Vierheilig H, Nell M, Zitterl-Eglseer K, Novak J (2006) Arbuscular mycorrhiza alter the concentration of essential oils in oregano (*Origanum* sp., Lamiaceae). Mycorrhiza 16:443–446

Klironomos JN, McCune J, Hart M, Neville J (2000) The influence of arbuscular mycorrhizae on the relationship between plant diversity and productivity. Ecol Lett 3:137–141

Larose G, Chenevert R, Moutoglis P, Gagné S, Piché Y, Vierheilig H (2002) Flavonoid levels in roots of *Medicago sativa* are modulated by the developmental stage of the symbiosis and the root colonizing arbuscular mycorrhizal fungus. J Plant Physiol 159:1329–1339

Lumpkin HM (2005) A comparison of lycopene and other phytochemicals in tomatoes grown under conventional and organic management systems. In: Kalb T (ed) Technical bulletin no. 34. AVRDC—The World Vegetable Center, Shanhua, Taiwan, p 48

Marschner H, Dell B (1994) Nutrient uptake in mycorrhizal symbiosis. Plant Soil 159:89–102

Porcel R, Ruiz-Lozano JM (2004) Arbuscular mycorrhizal influence on leaf water potential, solute accumulation, and oxidative stress in soybean plants subjected to drought stress. J Exp Bot 55:1743–1750

Rabin LB, Pacovsky RS (1985) Reduced larva growth of two Lepidoptera (Noctuidae) on excised leaves of soybean infected with a mycorrhizal fungus. J Econ Entomol 78:1358–1363

Rai M, Acharya D, Singh A, Varma A (2001) Positive growth responses of the medicinal plants *Spilanthes calva* and *Withania somnifera* to inoculation by *Piriformospora indica* in a field trial. Mycorrhiza 11:123–128

Ruiz-Lozano JM (2003) Arbuscular mycorrhizal symbiosis and alleviation of osmotic stress. New perspectives for molecular studies. Mycorrhiza 13:309–317

Scharff AM, Jakobsen I, Rosendahl L (1997) The effect of symbiotic microorganisms on phytoalexin contents of soybean roots. J Plant Physiol 151:716–723

Schutzendubel A, Polle A (2002) Plant responses to abiotic stresses: heavy metal-induced oxidative stress and protection by mycorrhization. J Exp Bot 53:1351–1365

Singh DP, Srivastava JS, Bahadur A, Singh UP, Singh SK (2004) Arbuscular mycorrhizal fungi induced biochemical changes in pea (*Pisum sativum*) and their effect on powdery mildew (*Erysiphe pisi*). Journal of Plant Diseases and Protection 111:266–272

Sirohi SS, Singh OS (1983) Relationship of endomycorrhizal association of unsterilized soils with available soil phosphorus, plant growth, phosphorus uptake and oil synthesis in peppermint. Sci Hortic 20:185–191

Smith SE, Read DJ (1997) Mycorrhizal symbiosis, 2nd edn. Academic, London

Smith SE, Smith FA, Jakobsen I (2003) Mycorrhizal fungi can dominate phosphate supply to plants irrespective of growth responses. Plant Physiol 133:16–20

Subramanian KS, Charest C, Dwyer LM, Hamilton RI (1995) Arbuscular mycorrhizas and water relations in maize under drought stress at tasselling. New Phytol 129:643–650

Subramanian KS, Charest C, Dwyer LM, Hamilton RI (1997) Effects of arbuscular mycorrhizae on leaf water potential, sugar content, and P content during drought and recovery of maize. Can J Bot 75:1582–1591

Taylor J, Harrier LA (2003) Expression studies of plant genes differentially expressed in leaf and root tissues of tomato colonised by the arbuscular mycorrhizal fungus *Glomus mosseae*. Plant Mol Biol 51:619–629

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

Toussaint JP, Smith F, Smith S (2007) Arbuscular mycorrhizal fungi can induce the production of phytochemicals in sweet basil irrespective of phosphorus nutrition. Mycorrhiza (in press). DOI 10.1007/s00572-006-0104-3

Toussaint JP, St-Arnaud M, Charest C (2004) Nitrogen transfer and assimilation between the arbuscular mycorrhizal fungus *Glomus intraradices* Schenck & Smith and Ri T-DNA roots of *Daucus carota* L. in an in vitro compartmented system. Can J Microbiol 50:251–260

VanEtten HD, Mansfield JW, Bailey JA, Farmer JA (1994) Two classes of plant antibiotics: phytoalexins versus "phytoanticipins". Plant Cell 6:1191–1192

Whipps JM (2004) Prospects and limitations for mycorrhizas in biocontrol of root pathogens. Can J Bot 82:1198–1227

Wright DP, Read DJ, Scholes JD (1998a) Mycorrhizal sink strength influences whole plant carbon balance of *Trifolium repens* L. Plant Cell Environ 21:881–891

Wright DP, Scholes JD, Read DJ (1998b) Effects of VA mycorrhizal colonization on photosynthesis and biomass production of *Trifolium repens* L. Plant Cell Environ 21:209–216

Zhu Y-G, Christie P, Laidlaw AS (2001) Uptake of Zn by arbuscular mycorrhizal white clover from Zn-contaminated soil. Chemosphere 42:193–199