



AperTO - Archivio Istituzionale Open Access dell'Università di Torino

The future has roots in the past: the ideas and scientists that shaped mycorrhizal research

This is the author's manuscript
Original Citation:
Availability:
This version is available http://hdl.handle.net/2318/1677006 since 2018-09-21T16:42:42Z
Published version:
DOI:10.1111/nph.15397
Terms of use:
Open Access
Anyone can freely access the full text of works made available as "Open Access". Works made available under a Creative Commons license can be used according to the terms and conditions of said license. Use of all other works requires consent of the right holder (author or publisher) if not exempted from copyright protection by the applicable law.

(Article begins on next page)

$\| [SA per TO]$



UNIVERSITÀ DEGLI STUDI DI TORINO

- 1 2
- -
- 3
- 4 This is the author's final version of the contribution published as:
- 5 Paola Bonfante
- 6 The future has roots in the past: the ideas and scientists that shaped mycorrhizal
- 7 research
- 8 New Phytologist, 2018 doi: 10.1111/nph.15397
- 9
- 10 The publisher's version is available at:
- 11 https://nph.onlinelibrary.wiley.com/doi/abs/10.1111/nph.15397
- 12
- 13 When citing, please refer to the published version.
- 14
- 15
- 16 Link to this full text:
- 17 [http://hdl.handle.net/ 2318/1677006]
- 18
- 19
- 20 This full text was downloaded from iris-Aperto: <u>https://iris.unito.it/</u>
- 21 iris-AperTO
 22 University of Turin's Institutional Research Information System and Open Access Institutional Repository
 24

25	Tansley 2018
26	The future has roots in the past: the ideas and scientists that shaped mycorrhizal research
27	Paola Bonfante ¹
28	
29	
30	
31	
32	
33	
34	
35	
36	
37	
38	
39	
40	
41	
42	
43	
44	1: University of Torino, Department of Life Sciences and Systems Biology, Viale Mattioli, 25 -
45	10125 Torino, Italy. Tel: +39 011 6705965 E-mail address: paola.bonfante@unito.it ORCID ID:

http://orcid.org/0000-0003-3576-8530 46

47 Summary

Our knowledge of mycorrhizas dates back to at least 150 years ago, when the plant pathologists A.B. Frank and G. Gibelli described the surprisingly morphology of forest tree roots surrounded by a fungal mantle. Compared with this history, our molecular study of mycorrhizas remains a young science. To trace the history of mycorrhizal research, from its roots in the distant past, to the present and the future, this review outlines a few topics that were already central in the nineteenth century and were seminal in revealing the biological meaning of mycorrhizal associations. These include investigations of nutrient exchange between partners, plant responses to mycorrhizal fungi, and the identity and evolution of mycorrhizal symbionts, as just a few examples of how the most recent molecular studies of mycorrhizal biology sprouted from the roots of past research. In addition to clarifying the ecological role of mycorrhizas, some of the recent results have changed the perception of the relevance of mycorrhizas in the scientific community, and in the whole society. Looking to past knowledge while foreseeing strategies for the next steps can help us catch a glimpse of the future of mycorrhizal research.

73 Introduction

There are many ways to speak about the past. In this review, rather than presenting a detailed analysis of the minutiae of ever-changing scientific results, I chose to speak at a more general level, to address mycorrhizal research from a historical and conceptual perspective, based on my experience in the field.

Starting at the end of the nineteenth century, researchers in the traditional fields of mycology and botany became more and more aware of the relevance of plant–fungal symbioses. One hundred and fifty years later, researchers still discuss issues in plant–fungal interactions and pose substantial questions in ecology, evolution, microbiology, plant pathology, agronomy and forestry sciences, as well as in applied economy and bioinformatics, just to name some of the relevant fields.

83 This interdisciplinary interest, fed by different experimental approaches, mirrors the awareness that 84 the umbrella term 'mycorrhiza' covers a huge number of biological systems that include most plant 85 species. According to Brundrett and Tedersoo (2018), 72% of vascular plants are arbuscular 86 mycorrhizal (where Glomeromycotina fungi form inter-intracellular hyphal networks within the 87 roots), 2.0% are ectomycorrhizal (where fungi of the Ascomycota or Basidiomycota produce a mantle surrounding the root tip as well as an intercellular hyphal network between the root 88 89 epidermal and cortical cells), 1.5% are ericoid mycorrhizal (where mostly Ascomycota form coils 90 inside the epidermal cells of the thin roots of Ericales) and 10% are orchid mycorrhizal (where 91 mostly Basidiomycota colonize the cortical cells of orchid protocorms and roots). Just 8% of plants 92 are completely nonmycorrhizal, and 7% have inconsistent nonmycorrhizal-arbuscular mycorrhizal 93 associations. The "State of the World's Plants" report (2017, https://www.kew.org/science/who-we-94 are-and-what-we-do/strategic-outputs-2020/state-of-the-worlds-plants) lists about 391,000 species 95 of vascular plants currently known to science; therefore, we can conclude that the number of 96 mycorrhizal plant species ranges from 320,000 to 340,000, also taking into account that many non-97 vascular plants, like liverworts, interact with mycorrhizal fungi. All these plants associate with 98 more than 50,000 fungal species (van der Heijden et al, 2015) and appear equally successful in 99 colonizing different environments, from alpine and boreal zones to tropical forests and grasslands.

100 The impressive biodiversity revealed by these figures makes mycorrhizal association one of the 101 most relevant biological processes of our planet, opening the question of how to understand and 102 explore the complexities of fungal-plant interactions. Traditionally, mycorrhizas have been 103 investigated following two main trajectories (O. Ferlian et al., unpublished): first, the ecological 104 one, which has developed crucial concepts, *i.e.* the demonstration that mycorrhizal symbiosis is a 105 determinant of plant biodiversity, and ecosystem variability (van der Heijden et al, 1998), controls 106 plant productivity, nitrogen, and phosphorus cycles, as well as soil aggregation and seedling 107 survival (van der Heijden et al, 2015). These concepts have produced potential applications such as 108 using mycorrhizas as powerful tools for sustainable agriculture focused on lower chemical inputs 109 and improved food security (Rodriguez and Sanders, 2015), as well as for preserving forest 110 ecosystems (Courty et al., 2010). In a second trajectory, another enormous wealth of data has been 111 developed under laboratory conditions trying to understand the mechanisms behind the complexity 112 of in-field associations. According to the classical biological reductionistic methods, cellular, 113 molecular, and physiological approaches have been applied to simpler biological systems, usually represented by one plant species colonized by one fungal species. 114

The aim of this review is to draw up a map of the ideas (and to highlight the scientists) that changed mycorrhizal research, searching for topics that were central in the nineteenth century, became seminal in revealing the biological meaning of mycorrhizal associations along the decades, and are still crucial today in the 'omics' era. The selection of these issues is the fruit of a personal perspective, which mostly mirrors my main scientific interests.

120

121 The portraits of our ancestors: a gallery of ideas from more than one hundred years of 122 mycorrhizal research

123 A journey to the past of mycorrhizal research must start with the scientists who produced the basis 124 of our current knowledge; as in all other scientific fields, the most recent molecular studies of 125 mycorrhizal biology were developed on the shoulders of work by researchers in the past. In this 126 context, Albert Bernard Frank (1839–1900) is probably the most famous: he was the first to recognize the widespread nature of the associations between plant roots and ectomycorrhizal fungi 127 128 (Trappe, 2005 for original quotations), even if other scientists provided contributions on other 129 plant- fungal associations in the same period. Franz Kamienski (1851-1912) showed that 130 Monotropa hypopitys was nourished by fungi associated with the roots of neighboring trees (Berch 131 et al., 2005), while Noël Bernard (1874-1911) established that Neottia nidus-avis needs for a 132 fungus, which is also forming mycorrhizae in adults, for seed germination (Selosse, 2017). Surely 133 Giuseppe Gibelli (1831–1898), professor of Botany at the University of Modena and then of 134 Torino, is worthy of being acknowledged: he provided beautiful drawings of chestnuts roots (Fig.

135 1), where the features of the mycorrhizal structures are finely described. In Torino some years later,

- 136 Oreste Mattirolo (1856-1947) hypothesized that truffles were ectomycorrhizal fungi, as witnessed
- 137 by a painting in the Botanical Garden of Torino (Fig. 1). Many years passed before truffles so
- 138 loved in the cuisines of Mediterranean countries were acknowledged as true symbionts (Fontana
- and Palenzona, 1969; Martin et al 2010).

140 Inspired by the forests of larches and birches in the north of Europe, Elias Melin (1889–1979) laid 141 the foundation for study of the physiology of ectomycorrhizas in trees: his observations on drained 142 peat bogs led to the conclusion that coniferous and dicot seedlings require ectomycorrhizal 143 associations for normal growth and development. After this discovery, Melin devoted his life to 144 studying the structure, function, and importance of the ectomycorrhizal fungi (Lindeberg, 1989 and references quoted therein), moving from a description of the process in nature to experimental 145 146 validation (Melin and Nilsson, 1950). Thanks to a protocol that allowed him to produce 147 ectomycorrhizal symbioses under sterile conditions, he examined the competition for available 148 nitrogen between soil microorganisms and plant roots, and suggested that ectomycorrhizal fungi 149 primarily facilitate nitrogen uptake.

150 Under the direction of Professor Melin, Uppsala became an attractive centre for many students and 151 researchers. Among them, Erik Bjorkman (1912-1973) developed his carbohydrate theory: under 152 controlled conditions, he found a positive correlation between carbohydrate concentration in the 153 roots and frequency of mycorrhizas in conifers. Increased illumination, combined with moderate 154 deficiencies in nitrogen and phosphorus, resulted in increased carbohydrate concentrations in the 155 pine roots and increased mycorrhizal infection. Bjorkman's theory, although criticised, inspired 156 many other researchers (Lindeberg, 1989), such as Edward Hacskaylo (1925-2018) and Sagar 157 Krupa. Indeed, the source–sink relationships in mycorrhizas represent an issue that has been often investigated (Allen et al, 1981; Reid et al, 1983), but photosynthesis in mycorrhizal plants remains 158 159 to be fully clarified using advanced molecular techniques or computational models (Norby et al, 160 2016).

In the fifties, a new star was rising in the world of mycorrhizas: John Laker Harley (1911–1990), Professor of Forest Science at the University of Oxford from 1969 to 1979, and Fellow of St. John's College, Oxford. He was the giant who developed the biology of mycorrhizas, wrote the first book devoted to mycorrhiza, *The Biology of Mycorrhiza* (1959), and who, as the editor of *New Phytologist*, launched the journal as *the* place for publication of work on mycorrhizas. Thanks to his unforgettable personality and his productive school (just to drop a couple of names, his students included Sir David Read and Prof. Sally Smith, editors of the reference book Smith and Read, 168 1997), mycorrhizas were acknowledged all over the world, and interest in these plant-fungus 169 interactions no longer remained restricted to a limited community. The transfer of phosphate from 170 ectomycorrhizal fungi to their tree host was one of his main interests and many publications bear 171 witness to his experimental vision, which has provided strong experimental support to the concept 172 of nutrient exchange between mycorrhizal partners (Harley and Mccready 1952; Harley and 173 Brierley 1955). Indeed, with today's eyes, it seems that David Read transferred the wealth of 174 experimental data of his laboratory into an ecological vision, where different plant communities 175 have a dominant mycorrhizal type, depending on the physiological capacity of the symbiotic fungi 176 (Read 1991).

177 At the end of the 19th century, the roots of forest trees were not the only ones to be dug, studied, and characterised. Many herbs and flowering plants were investigated, as detailed by Koide and 178 179 Mosse in their excellent "A history of research on arbuscular mycorrhiza" (Koide and Mosse, 180 2004). Pioneering observations on the so-called endotrophic mycorrhizas were conducted, initially 181 by P.A. Dangeard (1862-1947), J.M. Janse (1860-1938), L.Petri (1875-1946) and J. Gallaud (1904) 182 (Fig. 2), and later continued by MH Rayner (1890-1948). The terminology we still use today comes 183 from those observations: for example, J. Gallaud was the first to distinguish between Arum and 184 Paris types of arbuscules (Smith and Smith 2007, Dickson 2004). Beniamino Peyronel (1890-1975) 185 also played a crucial role, as he was one of the first to detect the so-called double infection, the 186 presence of the endomycorrhizal fungus coupled with other endophytic fungi. I refer the interested 187 reader to Koide and Mosse (2004), which contains a full reference list and extensive original 188 quotations.

189 Irrespective of the huge number of descriptions, many years were required before researchers 190 produced an experimental demonstration of the role played by these endophytic fungi we now 191 identify as arbuscular mycorrhizal fungi. Barbara Mosse (1962) was one of the first to reach this 192 objective; she used sporocarps of the fungus that now bears her name (Funneliformis mosseae) to 193 inoculate plants growing in autoclaved soil, and demonstrated a growth effect. Similarly, 194 Gerdemann (1964) grew plants in steamed soil, inoculated them with sporocarps (and treated the 195 control plants with sporocarp washings), setting up a protocol that became the standard for 196 mycorrhization experiments. It was becoming clear that arbuscular mycorrhizal fungi were not 197 parasites, as previously suggested (see Koide and Mosse, 2004).

A meeting organized in Leeds in 1974 (Fig. 3) by Sanders, Mosse, and Tinker (1975) brought together, for the first time, scientists who were active in research on arbuscular mycorrhizal fungi, as well as in ericoid and orchid mycorrhizas. Looking at the titles of the presentations, we 201 immediately perceive that the main issues under debate today (evolution and classification of the 202 endophytes, nutrient exchange, systemic effects, multiple interactions) were already a major focus. 203 The transfer of phosphate from the fungus to the plant was one of the first topics; more than one 204 hundred pages of the book are devoted to this matter, covering theoretical aspects related to 205 membrane structure, the accumulation of phosphate at the tips of mycorrhizal clover, as well as the 206 quantification of phosphate flow in the extraradical hyphae. Many of these reports had their 207 foundation in a very important paper published by Sander and Tinker in Nature (1973), where the 208 authors calculated the phosphate inflow in mycorrhizal vs. control onion roots.

209 Twenty years had to pass before another seminal step occurred: the identification of the phosphate 210 transporter in Glomus versiforme by Maria Harrison and Marianne van Buuren (1995). This study 211 revealed the molecular basis of phosphate uptake by the fungus and described the first step of 212 phosphate's journey from the soil to the fungus to the plant. The further physiological and 213 molecular characterization of the arbuscular mycorrhizal-inducible plant phosphate transporters represented another milestone; thanks to many groups (among them, those led by Marcel Bucher, 214 215 Sally Smith, Iver Jakobsen, and again Maria Harrison), we learnt about the symbiotic phosphate 216 pathway (Harrison et al, 2002; Bucher 2007, Smith et al, 2011). This road is still open: phosphate is 217 a hub where symbiotic fungal needs (Ezawa and Saito, 2018), mycorrhization, plant development, 218 regulation of transporters, and hormones are interconnected, and such networks have yet to be 219 completely deciphered.

In another important meeting held in Quebec some years later (1980) and organized by a scientist with a large vision of biology, J. Andre Fortin, in the context of the North American Conference on Mycorrhizas, an accurate mycorrhizal atlas was already available. The main mycorrhizal typologies were associated to specific pairs of partners with a strong, increasing interest in the ecological aspects of mycorrhizal symbiosis.

225 Even if largely incomplete, this gallery of scientists reflects the evolution of some ideas in 226 mycorrhizal research: in less than one hundred years, times were already mature for discussing not 227 only the descriptive aspects, but also for trying to understand the mechanisms operating at the 228 ecological and molecular levels. A good example of the ideas stemmed from the knowledge already 229 available in the eighties is provided by the powerful concept of interaction networks (Toju et al 230 2014, van der Heijden et al., 2015): plants associate with their dominant mycorrhizal fungi forming separate underground networks. However, borders are not so clear-cut: in temperate forests, 231 232 ectomycorrhizal trees often harbour an understory of shrubs (e.g. Vaccinium) that bear ericoid 233 mycorrhizal fungi. In these forests, fungi may form both ectomycorrhizal and ericoid mycorrhizal

associations, meaning that there might be linkages between the different fungal networks (van der
Heijden et al., 2015). Ecology has put the taxonomic, anatomical, and physiological data into a
novel, broader context.

237

238 Mycorrhizal fungi in the 'omics' era: first puzzle, how to name mycorrhizal fungi

239 Giving a name to the object of your biological investigation is a substantial starting point. For many 240 years, ectomycorrhizal fungi were identified on the basis of the fruiting bodies from which they had 241 been isolated, while collecting mycorrhizas in nature often led to a situation resembling the famous 242 Latin expression "mater semper certa est, pater numquam" (the mother is always certain, but not 243 the father). Indeed, there were no doubts on the name of the plant, while the name of the associated 244 ectomycorrhizal fungus was always a difficult gamble. There were exceptions: Cenococcum 245 geophilum with its black mycorrhizal mantle was very easy, even for mycologists with little 246 experience, even if Douhan and colleagues (2007) later revealed that C. geophilum is indeed a 247 species complex.

248 Applying molecular techniques, which were first focused on RNA ribosomal genes, allowed 249 researchers to identify fungal symbionts with greater confidence (White et al, 1989; Gardes and 250 Bruns, 1993), quickly moving from identification and diagnostic aims to more general issues of 251 biodiversity and molecular ecology. A further crucial step was the genome sequencing of 252 mycorrhizal fungi. The Laccaria bicolor genome (Martin et al, 2008) opened a window to an 253 unknown world: the 65-megabase genome revealed unexpected features, most notably a battery of 254 effector-type small secreted proteins, several of which are only expressed in symbiotic tissues. 255 Another noteworthy finding was the lack of carbohydrate-active enzymes involved in degradation 256 of plant cell walls. The capacity to degrade non-plant cell wall polysaccharides suggested a dual 257 saprotrophic and biotrophic lifestyle of the mycorrhizal fungus, enabling it to grow in the soil and in 258 living plant roots. The availability of a fungal genome provided tools to understand for the first time 259 the processes by which symbionts interact with plants within their ecosystem.

260 On the wave of success of the *Laccaria* and truffle (Martin et al, 2010) genomes, Francis Martin 261 promoted the Joint Genome Institute Mycorrhizal Genomics Initiative, which aimed to sequence 262 phylogenetically and ecologically diverse mycorrhizal fungi (Basidiomycota and Ascomycota), 263 including the major clades of symbiotic species associating with trees and woody shrubs 264 (https://genome.jgi.doe.gov/Mycorrhizal_fungi/Mycorrhizal_fungi.info.html). Thanks to this huge 265 wealth of data, many mechanistic answers were offered to explain the strategies of ectomycorrhizal fungi, *i.e.* the loss of genes involved in plant cell wall degradation, and the acquisition of many small secreted proteins, as a tool to interact with the host (Martin et al., 2016).

268 Phylogenomics was also used to reconstruct the evolution of plant-interacting fungal groups and to 269 trace their common origins. For example, Kohler et al. (2015) demonstrated how ectomycorrhizal 270 fungi evolved to depend on their green hosts, but in order to exploit the protective plant niche, they 271 had to lose the potential to degrade lignocellulose compounds. Interestingly, these ectomycorrhizal 272 features emerged with similar genetic adaptations across multiple clades, including symbiotic fungi with fully diverse strategies, as those colonizing heathers and orchids. So, thanks to this balance 273 274 between losses and gains, they acquired evolutionary stability. Parallel to the evolution of ideas, the 275 Linnean names of many sequenced fungi changed; for example, Pezizella ericae, which I 276 investigated 40 years ago together with Vivienne Gianinazzi Pearson (1979), is now called 277 Rhizoscyphus ericae.

278 Giving a name to arbuscular mycorrhizal fungi has been an endless tale that has stretched over 279 many years. As Koide and Mosse (2004) summarize, these symbiotic fungi were first called 280 'phycomycetoid fungi' to distinguish them from those living inside the Ericaceae or Orchidaceae. 281 The name was related to the morphology of the fungi, which are aseptate and coenocytic. Peyronel 282 (1923, 1924) was one of the first to assign arbuscular mycorrhizal fungi to a defined taxon. He was 283 successful in tracing the extraradical hyphae from mycorrhizal roots to spores of Endogone 284 fuegiana, E. vesiculifera, and other Endogone species. As a consequence, the hypothesis was that 285 phycomycetoid fungi were Zygomycetes-related, since on the basis of their zygospores and 286 chlamydospores, Endogonaceae were placed inside the Mucorales (Koide and Mosse, 2004). The 287 name was successful; for example, in the Leeds meeting many years later, arbuscular mycorrhizal 288 fungi were listed as Endogone-related (Sanders et al., 1975). Gerdeman and Trappe (1974) provided 289 a very detailed list of the more common arbuscular mycorrhizal fungal genera and wisely 290 commented that their revision of Endogonaceae (Endogonales, Zygomycetes) offered a temporary 291 solution to a difficult taxonomic problem.

Many new genera and families were proposed over the years, but only the advent of molecular tools enabled the development of new ideas. Schußler et al. (2001) established the relationships among arbuscular mycorrhizal fungi and between them and the other fungi: arbuscular mycorrhizal fungi were identified as a phylum (Glomeromycota), which was a sister group to Ascomycota and Basidiomycota. Even if we have used these concepts for years, many aspects were not fully clear: for example, *Endogone* did not group with the Glomeromycota, but *Geosyphon pyriforme*, a very peculiar fungus, was added to the Glomeromycota, even if it does not produce arbuscular mycorrhizal symbioses. Lastly, Zygomycetes turned out to be a polyphyletic group, without adefined taxonomic meaning.

301 As for the ectomycorrhizal fungi, the sequenced genomes gave the green light to clear analysis of 302 phylogenetic relationships. As already suggested by the mitochondrial genomes (Lee and Young, 303 2009; Pelin et al 2012), examination of the genome of Rhizophagus irregularis (Lin et al 2014; 304 Tisserant et al, 2013) revealed closer relationships with Mucoromycota than with the Dikarya. The 305 decisive word arrived with a phylogenomics analysis performed by Spatafora and colleagues 306 (2016). Thanks to the sequencing of new genomes, their study clearly indicated that Mucoromycota constitutes a phylum with three subphyla, Mucoromycotina, Mortierellomycotina, and 307 308 Glomeromycotina: this study unambiguously defines the phylogenetic position of arbuscular 309 mycorrhizal fungi. It is true that our arbuscular mycorrhizal fungi are now relegated from phylum 310 status to a lower subphylum level, but many shared phenotypic features among the three subphyla, 311 for example the presence of endobacteria (Bonfante and Desiro 2017), have provided a good 312 rationale for explaining similarities (hyphal morphology) and dissimilarities (nutritional styles) 313 among these enigmatic fungi.

We can conclude this endless tale by claiming that one hundred years ago (1923) Beniamino Peyronel, who looked at the coenocitic hyphae running between mycorrhizal roots and the spores of *Endogone*-like fungi, was not so far from our current views.

317 Signalling: a central question of our time?

318 One of the major questions of the community studying plant-microbe interactions is the nature of 319 the signals exchanged between the partners and how they are perceived. Oliveira Chagas and 320 colleagues (2018) compiled an exhaustive list of the molecules so far identified as involved in 321 plant-microbe interactions. However, and probably differently from our expectations, the scientists 322 previously quoted in the portrait gallery were already well aware of the crucial role played by the 323 early events where unknown molecules act as the driving factors. For example, one of Elias Melin's 324 interests focussed on the root exudates that stimulate the growth of ectomycorrhizal fungi, *i.e.* the 325 so called 'M-factor' (Melin, 1954). The effect of this factor was demonstrated in experiments where 326 excised pine roots, cultivated in tissue culture, were placed on the surface of nutrient agar that 327 contained suspensions of finely divided mycelia of different ectomycorrhizal fungi. The stimulating 328 effect of the root exudate on the fungi was very evident. Several attempts were made to purify and 329 characterize the active principle but, much to Melin's disappointment, these attempts produced no 330 definitive results (Lindeberg 1989).

331 With a very similar approach, Hepper and Mosse (1974) studied the interaction between their 332 arbuscular mycorrhizal fungi (F. mosseae, at that time called Endogone) and root organ cultures of 333 Trifolium pratense. The pre-germinated spores were stimulated by exudates diffusing from the 334 growing roots in the absence of any physical contact (Fig. 4) The stimulation of hyphal branching 335 was impressive. While similar observations were nicely confirmed by the group of Manuela 336 Giovannetti in Pisa (Giovannetti et al, 1993), only many years later did other studies identify the 337 plant bioactive molecules that stimulate the branching and metabolism of pre-symbiotic hyphae in 338 arbuscular mycorrhizal fungi as strigolactones (Akiyama et al., 2005; Besserer et al., 2006).

Strigolactones derive from carotenoid metabolism (Al-Babili and Bouwmeester, 2015) and were first studied as root-exuded molecules that elicit the germination of parasitic plants (Cook et al., 1966). Later, strigolactones emerged as key plant hormones that control several aspects of plant biology and physiology, such as the repression of shoot branching (Waters et al. 2017). Strigolactone production is conserved from Charales to Embryophytes (Delaux et al., 2012), suggesting that their function in the rhizosphere probably came about as a secondary feature of their active release from the roots into the soil (Kretzschmar et al., 2012, Bonfante and Genre, 2015).

346 Despite the emerging understanding of the role of strigolactones, the molecular mechanisms 347 underlying the hyphal branching of arbuscular mycorrhizal fungi, first observed by Hepper and 348 Mosse (1974), remain poorly known (Lanfranco et al., 2018b). Data from RNA sequencing of 349 germinated spores of Gigaspora margarita after a treatment with the synthetic strigolactone 350 analogue GR24 confirmed the findings of Besserer and colleagues (2006, 2008), revealing the up-351 upregulated expression of mitochondrial genes (Salvioli et al., 2016) as well as of some genes 352 related to cell wall components (encoding chitin deacetylase, chitin synthase). The data suggest that 353 not only the mitochondrion, but also other fungal compartments are sensitive to strigolactones. 354 However, despite the availability of sequenced genomes of arbuscular mycorrhizal fungi, the fungal 355 strigolactone receptor has not yet been identified.

356 Along the same line, after many years, the M factor acting on ectomycorrhizal fungi is still waiting 357 to be identified, even if some flavonoids, such as rutin and quercetin, have been hypothesized to be 358 sensed by ectomycorrhizal fungi (Lagrange et al, 2001). By contrast, many molecules of fungal 359 origin that are perceived by the host plant have been investigated in both ectomycorrhizal and 360 arbuscular mycorrhizal fungi. Ditengou and colleagues (2015) demonstrated that Laccaria bicolor 361 emits volatiles, identified as sesquiterpenes, during the interaction with host and non-host plants 362 (poplar and Arabidopsis, respectively). The main effect of the volatiles, together with fungal auxin, 363 is to induce lateral root formation, which is the first developmental cue to induce mycorrhization.

As reviewed by Martin et al (2016), other active components of the molecular dialogue in ectomycorrhizal symbioses are the fungal mycorrhiza-induced small secreted proteins, which interact with plant hormone receptors, thus altering root development.

367 The molecules that are released by arbuscular mycorrhizal fungi and interact with the plant host 368 during the presymbiotic phase are mainly chitin-related. Chitin is a crucial cell wall component of 369 arbuscular mycorrhizal fungi, and changes its structural organization during the fungal life cycle 370 (Bonfante-Fasolo 1988). In most spores of arbuscular mycorrhizal fungi, chitin is laid down in 371 fibrils that are spatially organized with a particular helicoidal form (Fig 5), but this organization is 372 lost during germination, when chitin morphology changes together with alterations of the cell wall. 373 Moving from the extra to the intraradical phase, the wall becomes progressively thinner and thinner, 374 and at the end in the arbuscular branches, the wall is barely present, with an amorphous structure, 375 and chitin fibrils are no longer detectable, even if chitooligomers are still present (Bonfante et al 376 1990 a).

377 To explain these observations, I hypothesized, with the self-confidence of a young researcher 378 (Bonfante-Fasolo 1988), that i) the fungal wall might release chitooligomers that act as signals for 379 the plant during the intraradical phase, and ii) that the fungal wall was thinning due to the activity of 380 a plant chitinase. The second hypothesis was surely wrong, but thanks to the collaboration of 381 Thomas Boller in Basel, an outstanding expert in chitinases, and of Pietro Spanu, at that time an 382 undergraduate student in Torino, we performed a detailed study of plant chitinase expression, 383 revealing that it was limited to the early moments of the interaction (Spanu et al, 1989). This 384 observation has been largely confirmed by the detection of many chitinases and pathogenesis-385 related proteins in transcriptomic studies (Giovannetti et al, 2015, Fiorilli et al., 2015) and has 386 provided the basis of novel ideas, *i.e.* that the fungus induces priming in the host plant, thus 387 activating a range of molecules related to innate immunity (including chitinases), thereby raising 388 the basal level of defences in the plant (Pozo and Azcón-Aguilar, 2007; Martinez et al, 2017; 389 Chialva et al, 2018).

Currently, we know that lipochitooligosaccharides and chitooligomers act as signalling molecules in the pre-symbiotic phase, eliciting calcium spiking, a key part of the symbiotic pathway involved in the initial stages of root colonization (Maillet et al 2011, Genre et al., 2013; Sun et al., 2015). The discovery that GR24 treatment led to an increase in the release of chitin oligomers (Genre et al., 2013) by arbuscular mycorrhizal fungi and, subsequently, to amplification of the calcium spiking response, offered the first experimental evidence of the interaction between the signalling molecules released by the fungal and plant partners (Bonfante and Genre, 2015). However, the origin of the 397 fungal bioactive chitooligomers and lipochitooligomers is largely unknown. Are they the 398 degradation product of chitin thanks to fungal/plant chitinases, or are they the product of specific 399 catabolic pathways that lead to short oligosaccharides?

400 Chitin is a double-faced molecule: on the one hand it may act as signal for symbiosis, but on the 401 other, pathogenic fungi also release chitooligomers (Sánchez-Vallet et al 2015). The way in which 402 plants manage to understand the origin and the length of the chitin fragments thanks to a plethora of 403 chitin receptors is the focus of many recent reviews (Barker et al 2017, Zipfel and Oldroyd 2017). 404 In the context of arbuscular mycorrhizal fungi signalling, Miyata et al., 2014 Zhang et al., 2015 405 Carotenuto et al (2017) revealed that the rice lysin-motif receptor-like kinase OsCERK1 plays a 406 central role in perceiving the short-chain chitooligomer signals and activating the downstream 407 conserved symbiotic signal transduction pathway. Could chitooligomers have a role in modulating 408 plant responses also during the intracellular colonization? At the moment, we lack experimental 409 evidence. Small secreted proteins are the likely candidates for the signals that modulate plant 410 responses to arbuscular mycorrhizas and ectomycorrhizas (Kloppholz et al, 2011, Kamel et al, 411 2017; Plett et al 2011), but this does not exclude a role for other bioactive molecules.

Looking at the observations produced by Melin and Mosse, we can conclude that their inspiring experiments with plant exudates and their observation of the fungal phenotype have opened one of the crucial chapters of plant–fungal interactions in our time. The issue is still open: Bonfante and Genre (2015) commented that the molecules involved in interkingdom symbiotic signalling, such as strigolactones, and chitin-related molecules, also have key roles in plant and fungal development, originally unrelated to symbiosis. Therefore, the symbiotic role of these molecules relies on the coevolved capacity of the arbuscular mycorrhizal partners to perceive them as symbiotic signals.

419 Despite the striking effects of strigolactones, it is hard to believe that plants seeking arbuscular 420 mycorrhizal symbiosis rely exclusively on released strigolactones to capture the fungi that are 421 present in the soil. The discovery of a plant transporter of N-acetylglucosamine in maize and rice 422 that is also required during the pre-contact phase (Nadal et al., 2017) suggests that other signalling 423 molecules could be important during the pre-contact phase. Lastly and surprisingly, 424 ectomycorrhizal plants have been poorly investigated in this context. Looking at the fungal factors, 425 chitin-related molecules seem to be shared by pathogenic and arbuscular mycorrhizal fungi, 426 opening the question of whether they could also function in signalling in ectomycorrhizal 427 symbioses.

428

429 The colonization process: how cellular studies predicted future 'omics' data

14

430 If we stop and look at the old drawings by G. Gibelli, J. Gallaud, or B. Peyronel, and compare them 431 with the beautiful schemes of ectomycorrhizas in the publications by Martin and colleagues (2016), 432 or with the iconic arbuscules shown in the Brundrett et al (1984) or by Maria Harrison's group 433 (Bravo et al 2017), we will have no doubt of the beauty and richness of the details in the recent 434 publications. However, many basic points of information, *i.e.* fungal structure, host anatomy, and 435 plant cell specificity, were already correctly identified at the dawn of research on mycorrhizas. The 436 finding that fossils of the Rhynie chert have root associations similar to modern arbuscular 437 mycorrhizal fungi reflects the excellent knowledge of our colleagues of the past (Kidston & Lang 438 1921).

439 Moving from morphology to physiology, the functional ideas that are at the basis of our current 440 concepts mostly stemmed from transmission electron microscopy observations. Transmission 441 electron microscopes became operative at the end of the fifties. In Torino, Arturo Ceruti (1911-442 2000) funded one of the first Centres of Electron Microscopy, providing an important tool to look at 443 mycorrhiza with a new technique. One of the major and longstanding concepts that developed at 444 that time was the existence and importance of the interface, *i.e.* the area of physical contact between 445 the plant cell and the mycorrhizal fungus (Fig. 6). Silvano Scannerini (1940-2005) was one of the 446 first to adopt the terminology proposed for plant-pathogen interactions (Bracker and Littlefield, 447 1973). At the 1974 Leeds meeting, the different types of interfaces originating during the 448 interaction between Ornithogalum umbellatum and its endogenous arbuscular mycorrhizal fungi 449 were carefully described and listed. Moreover, they were assigned different numbers: the plant cell 450 wall-fungal wall contact was named IT8, and at the moment of fungal penetration and plant 451 membrane invagination, the interface was named IT24 (live-walled hosts and endophyte with 452 matrix in the middle). Finally, when the fungus was collapsing, the interface was identified as IT25 453 (Scannerini et al, 1974). Luckily, no one today speaks about the interface numbers, but indeed this 454 catalogue provided the basis for understanding the structure and the nature of the material laid down 455 between the fungus and the invaginated surrounding membrane.

456 Cytochemical approaches allowed us to detect polysaccharides and proteins, revealing that the 457 composition of the matrix is related to the plant cell wall (Scannerini and Bonfante-Fasolo, 1979). 458 Indeed, years later work using more sophisticated *in situ* techniques detected many plant cell wall 459 components, *i.e.* pectins, cellulose, hemicellulose, and the hydroxyproline-rich protein extensin 460 (Bonfante et al 1990a, b; Balestrini et al, 1996 a,b; Balestrini and Bonfante 2014 for a review) in the 461 interface compartment, observations that are still valid today. The activation of genes involved in 462 cell wall synthesis was nicely confirmed by many transcriptomic studies in both arbuscular mycorrhizal and ectomycorrhizal symbioses, as well as all the events that lead to new synthesis of
plant membranes required for accommodation of the fungus (Balestrini et al., 2017, Guether et al.,
2009; Genre et al, 2005; Genre et al, 2012).

466 The eighties were also the years in which researchers compiled a detailed atlas of all mycorrhizal 467 associations. For example, Larry Peterson provided beautiful descriptions of ectomycorrhizas and 468 orchid associations (Peterson et al 2004) and, in the same period, together with Vivienne Gianinazzi 469 Pearson, we provided an accurate description of the morphology of ericoid mycorrhizas. The hair-470 roots of Calluna showed a peculiar anatomy, consisting of a few epidermal cells filled up by the 471 coiled fungal symbiont (Bonfante- Fasolo and Gianinazzi-Pearson, 1979, 1982). Interestingly, the 472 ultrastructure of ericoid plant cells was very similar to that of arbuscular mycorrhizal fungi-473 colonized cortical cells (Scannerini and Bonfante, 1983), Fig. 6. However, by using in situ 474 techniques and antibodies against pectin and polygalacturonase, we observed that the fungal cell 475 walls of the two endosymbiotic fungi had very diverse compositions and that the ericoid fungus 476 releases polygalacturonase enzymes mostly at the contact with the thin hair roots. We concluded 477 that, in contrast to arbuscular mycorrhizal fungi, ericoid fungi produce cell wall-degrading enzymes 478 and that this capacity was probably modulated during the interaction with host and non-host plants 479 (Perotto et al., 1995). Indeed, genomics and transcriptomics of arbuscular mycorrhizal fungi 480 (Tisserant et al, 2013; Chen et al 2018, Salvioli et al 2016) and of ericoid fungi (Kohler et al 2015, 481 Martino et al 2018, Perotto et al., 2018) have demonstrated that arbuscular mycorrhizal fungi do not 482 possess genes coding for cell-wall degrading enzymes, in contrast to ericoid fungi, largely 483 confirming the hypotheses advanced about 25 years before!

484 In the absence of molecular data, ultrastructural observations allowed researchers to look beyond 485 the hedge: the deep re-organization of the cortical cells following the fungal colonization suggested 486 a reprogramming of the molecular plant machinery which has been largely confirmed by RNA-487 sequencing studies in all the mycorrhizal symbioses (Martino et al, 2018, Veneault-Fourrey et al, 488 2014; Peter et al., 2016; Fochi et al, 2017; Fiorilli et al, 2015; Sugimura and Saito, 2017). The 489 interface was identified as a constant feature present in all the mycorrhizal associations; this led to 490 the concept that the interface is a novel compartment, and a cellular marker of compatible 491 interactions (Bonfante 2001). Going further, the enlarged nucleus of the host arbusculated cells as 492 well as its loose chromatin organization (Fig. 7) suggested reduplication events, a hypothesis that 493 has already received partial experimental confirmation (Fusconi et al, 2005.; Genre et al., 2008).

as already received partial experimental confirmation (Fusconi et al, 2005.; Genre et al., 2008)

494 Cellular studies between the seventies and the eighties gave therefore the foundation for the 495 functional concepts that are at the forefront of research today: mineral or organic nutrients have to 496 cross plant-fungal interfaces, and the nutrient transporters have to be located at the fungal-plant 497 membranes. The functional characterization of such nutrient transporters (transporting molecules 498 from minerals to organic compounds), as well as the events that allow new membrane biogenesis, 499 and the regulatory machinery, already belongs to the molecular era of mycorrhizal research, as 500 summarized in many recent reviews (Mclean et al, 2017; Pimpryka and Gutjahr 2018, Lanfranco et al., 2018a).

502

503 The genetics underlying colonization events

504 At the end of the eighties we already had a good deal of knowledge of mycorrhizal morphology, but 505 a crucial bit was missing: the genetic control that plants exert on entry by the fungus. This 506 important discovery was made in Dijon, when the plant geneticist Gerard Duc, collaborating with 507 two "mycorrhizal" colleagues, Vivienne Gianinazzi-Pearson and Silvio Gianinazzi, discovered that 508 mutant plants that were not successful in producing active nodules were also resistant to 509 mycorrhization. This finding was validated for pea and fava bean (Duc et al., 1989) and represented 510 a paradigm shift, since for the first time it demonstrated that mycorrhization is under the genetic 511 control of the host plant.

512 The second conclusion we have come to with Duc and colleagues discovery, was that a common 513 genetic basis governs root symbioses in legumes. Indeed, this has been one of the most deeply 514 investigated issues in mycorrhizal research. During the years we have learned that a common 515 genetic pathway operates for the establishment of root symbioses; work since 2000 has identified a 516 common set of genes, found in studies conducted by many groups (among them, Martin Parniske in 517 Germany, and J. Stougaard in Denmark; see Parniske, 2008 for a review). For example, these 518 studies found that nuclear calcium spiking was one of the first detectable events in root symbioses 519 (see Oldroyd, 2013). Very interestingly from an evolutionary point of view, these molecular 520 determinants are also present in non-leguminous plants (Gutjahr et al 2008).

In the context of a mycorrhizal excursus, we have to note that the Dijon discovery by Gerard Duc and colleagues on the one hand reinforced the plant-centric view that has dominated mycorrhizal research for many years, and on the hand other introduced mutants as crucial tools to understand mycorrhizal functioning. Thanks to them, we have learned that many genes control the signalling/early phase, while others are directly related to mycorrhizal functioning. Interestingly, most of these mutants share a similar phenotype: the arbuscules are stunted and not fully developed, suggesting that plant genetic determinants control arbuscule morphology (Gutjahr and Parniske,2013). An exhaustive list of the plant genes that play a crucial role in a functioning
mycorrhiza (from lipid transfer, to phosphate uptake and ATPase activity), as well as in
transcriptional regulation, can be found in Pimpryka and Gutjahr (2018).

These molecular data provide some explanations for the popular advertisement "mycorrhizal plants, bigger plants", but indeed they do not offer convincing explanations on the mechanisms: how AM fungi, which exclusively colonize hypogeous organs in vascular plants, may have an impact on epigeous organs? Which is the basis of the systemic effects? Are microRNAs and hormonal balance the main drivers?

536 What else is missing from our understanding? In the genomic era, we have learned how important 537 genetic variation in different lines of the same plant species can be. To better investigate the genes 538 that are responsible for efficient mycorrhization, natural variation studies have to be undertaken, as 539 has already been done in some pioneering work (Dreher et al., 2017). Natural variation is also 540 relevant for the fungal partners. We already have some diffuse information telling us that different 541 isolates of the same mycorrhizal species lead to different effects at the systemic level (Roger et al., 542 2013). It will be crucial to identify the roles of such fungal factors, to have a more balanced view of 543 mycorrhizal interactions.

544 In the context of sustainable agricultural and forestry practices, in a moment in which some of the 545 innovations of the Green Revolution are seen as no longer affordable, we may need to come back to 546 plant varieties that were not selected as responding to fertilizers and to test whether they can 547 achieve high productivity under a lower-fertilization regimen, but with the help of an efficient 548 mycorrhizal fungus. Therefore, one of the next challenges will be to identify the plant and fungal 549 genes that lead to a highly compatible couple that produces the best yield for crop plants. This could 550 be a novel trait to be selected when using breeding approaches to generate new crop varieties 551 (Sawers et al, 2008). Lastly, it will be crucial to remember that arbuscular mycorrhizal fungi do not 552 work alone, but they belong to a complex microbiota (Chialva et al., 2018). Tailoring the 553 interactions of crop plants and their associated microbiota may provide a crucial advance for

- sustainable agriculture.
- 555

556 Concluding thoughts: chance and needs in mycorrhizal symbioses

A walk through mycorrhizal research from the middle of 1800 to today reveals that many of the crucial questions we are facing now were first asked many years ago. Interestingly, our colleagues of the past provided many replies (exudates as sources of signalling molecules, transport of mineral nutrients investigated at the whole organism level, attempts to give a name to the fungal symbionts) and maybe (possibly by chance) many of these replies are still the right ones. Of course, the replies mirror the technical tools that were available at the moment. Moreover, not every question may have a reply, especially if it is not asked at the right time. For example, Barbara Mosse (1973) described the presence of bacteria-like organisms inside the spores of *Funneliformis mosseae*; some years later I observed similar organisms the first time that I looked at a mycorrhizal section under the electron microscope (Bonfante 2014), but the invention of PCR was needed for successful naming of these organisms (Bianciotto et al 1994, Bonfante and Desiro 2017).

568 This is a great time for the mycorrhizal scientific community: this is not only due to the powerful 569 tools that are now available, but also to a crucial change in the perception of mycorrhizal symbiosis. 570 Today mycorrhizas are perceived as relevant not only by researchers, but also by society. We can 571 illustrate the societal impact of our research putting a strong emphasis on the role of our fungi as 572 biofertilizers and bio-protectors. In a moment in which the environment is seen as a precious gift 573 that has to be preserved, we can take many positive actions. It has been a wonderful opportunity to 574 work on mycorrhizas. Over more than 40 years, I have seen that biology is a river in flood and that 575 mycorrhizal research is a part of it. In looking to the future of mycorrhizal studies, we can learn 576 much by examining their roots in the past-and I look forward to future developments in our 577 understanding of these remarkable biological systems.

578 Acknowledgements

My sincere thanks to: Guillaume Becard and David Baker for the invitation to the IMMM in 579 580 Toulouse (2017) stimulating me in thinking of the past in mycorrhizas; Dr Vivienne Gianinazzi-581 Pearson for her help in giving a name to some of the scientists attending the Leeds meeting in 1974 582 and shown in the congress picture (Fig 3); the team of Library of Department of Life Sciences and 583 Systems Biology (Dr. Franco Estivi with Professor Guglielmo Pandolfo and Dr. Alessandra 584 Fenoglio) for their competent and kind assistance in the search of the old volumes; Dr. Mara 585 Novero for her continuous help, also in the plate preparation; and Luisa Lanfranco, Silvia Perotto, 586 and Andrea Genre for reading the draft. A lot of thanks to Jennifer Mach for the generous text 587 editing. Lastly, I would like to thanks the three reviewers, and apologize to all the colleagues whose 588 work has not been quoted here for space reasons.

589

- 590
- 591

592 **References**

- Allen MF, Smith WK, Moore TS, Christensen M. 1981. Comparative Water Relations and
 Photosynthesis of Mycorrhizal and Non-Mycorrhizal Bouteloua-Gracilis HBK Lag Ex Steud. *New Phytol* 88: 683-693.
- 596 Akiyama K, Matsuzaki K, Hayashi H. 2005. Plant sesquiterpenes 739 induce hyphal
- 597 branching in arbuscular mycorrhizal fungi. *Nature* **435**: 824-827.
- Al-Babili S, Bouwmeester HJ. 2015. Strigolactones, a novel carotenoid- derived plant hormone. *Annual Review of Plant Biology* 66:161–186.
- Barker DG, Chabaud M, Russo G, Genre A. 2017. Nuclear Ca2+ signalling in arbuscular
 mycorrhizal and actinorhizal endosymbioses: on the trail of novel underground signals. *New Phytol*214: 533-538. doi:10.1111/nph.14350
- Balestrini R, Bonfante P. 2014. Cell wall remodeling in mycorrhizal symbiosis: a way towards
 biotrophism. *Front Plant Sci* 5: 237.doi: 10.3389/fpls.2014.00237
- Balestrini R, Hahn MG, Bonfante P. 1996a. Location of cell-wall components in ectomycorrhizae
 of *Corylus avellana* and *Tuber magnatum*. Protoplasma 191: 55–69. doi: 10.1007/BF01280825
- Balestrini R, Hahn MG, Faccio A, Mendgen K, Bonfante P. 1996b. Differential localization of
 carbohydrate epitopes in plant cell walls in the presence and absence of arbuscular mycorrhizal
 fungi. *Plant Physiol* 111: 203–213. doi: 10.1104/pp.111.1.203
- Balestrini R, Salvioli A, Dal Molin A, Novero M, Gabelli G, Paparelli E, Marroni F, Bonfante
 P. 2017. Impact of an arbuscular mycorrhizal fungus versus a mixed microbial inoculum on the
 transcriptome reprogramming of grapevine roots. *Mycorrhiza*. 27: 417-430.
- Berch S.M., Massicotte H.B., Tackaberry L.E. 2005. Re-publication of a translation of 'The
 vegetative organs of *Monotropa hypopitys* L.' published by F. Kamienski in 1882, with an update
 on *Monotropa* mycorrhizas. *Mycorrhiza* 15: 323-332. https://doi.org/10.1007/s00572-004-03341
- 617 Besserer A, Bécard G, Jauneau A, Roux C, Séjalon-Delmas N. 2008. GR24, a synthetic analog
 618 of strigolactones, stimulates the mitosis and growth of the arbuscular mycorrhizal fungus *Gigaspora*619 *rosea* by boosting its energy metabolism. *Plant Physiology* 148: 402–413.

- 620 Besserer A, Puech-Pages V, Kiefer P, Gomez-Roldan V, Jauneau A, Roy S, Portais JC, Roux
- 621 C, Becard G, S.jalon-Delmas N. 2006. Strigolactones stimulate arbuscular mycorrhizal fungi by 622 activating mitochondria. *PLoS Biology* 4: 1239–1247.
- Bianciotto V, Bandi C, Minerdi D, Sironi M, Tichy HV, Bonfante P. 1996. An obligately
 endosymbiotic fungus itself harbors obligately intracellular bacteria. *Appl Environ Microbiol* 62:
 3005–3010.
- Bonfante P. 2001. At the interface between mycorrhizal fungi and plants: the structural
 organization of cell wall, plasma membrane and cytoskeleton, in *The Mycota IX: Fungal Associations*, ed. B. Hock (Berlin: Springer), 45–61.
- Bonfante P. 2014. The endless tale of endobacteria: a conversation with Paola Bonfante *Trends in Plant Science* 19: 744-746
- Bonfante P, Faccio A, Perotto S, Schubert A. 1990a. Correlation between chitin distribution and
 cell wall morphology in the mycorrhizal fungus *Glomus versiforme*. *Mycol Res* 94: 157–165. doi:
 10.1016/S0953-7562(09)80 607-2
- Bonfante P, Vian B, Perotto S, Faccio A, Knox JP.1990b. Cellulose and pectin localization in
 roots of mycorrhizal *Allium porrum*: labeling conti- nuity between host cell wall and interfacial
 material. *Planta* 180: 537–547. doi: 10.1007/BF02411452
- Bonfante P, Genre A. 2010. Mechanisms underlying beneficial plant– fungus interactions in
 mycorrhizal symbiosis. *Nat. Commun* 1: 48. doi: 10.1038/ncomms1046
- Bonfante P, Genre A. 2015. Arbuscular mycorrhizal dialogues: do you speak 'plantish' or
 'fungish'? *Trends in Plant Science* 20: 150-4.
- Bonfante P, Desirò A. 2017. Who lives in a fungus? The diversity, origins and functions of fungal
 endobacteria living in Mucoromycota. *ISME J* 11: 1727–1735.
- Bonfante-Fasolo P. 1988. The role of the cell wall as a signal in mycorrhizal associations. In: *Cell to Cell signals in Plant, Animal and Microbial Symbiosis*, eds Scannerini et al., Springer Verlag
 Berlin, 219-235.
- 646 Bonfante-Fasolo P, Gianinazzi-Pearson V. 1979. Ultrastructural aspects of endomycorrhiza in the

- 647 Ericaceae I. Naturally infected hair roots of *Calluna vulgaris* L. Hull. *New Phytol* 83: 739-744.
- Bonfante-Fasolo P, Gianinazzi-Pearson V. 1982. Ultrastructural Aspects of Endomycorrhiza in
 the Ericaceae. III. Morphology of the Dissociated Symbionts and Modifications Occurring during
 their Reassociation in Axenic Culture. *New Phytol* 91, 691-704
- Bracker CE, Littlefield LJ. 1973. Structural concepts of host-pathogen interfaces. In: *Fungal Pathogenicity and the Plant's Response*, eds R. J. W. Byrde and C. V. Cutting (London, NY:
 Academic Press), 159–317.
- 654 **Bravo A, Brands M, Wewer V, Dormann P, Harrison MJ**. 2017. Arbuscular mycorrhiza-655 specific enzymes FatM and RAM2 fine-tune lipid biosynthesis to promote development of 656 arbuscular mycorrhiza. *New Phytol* **214**: 1631–1645.
- 657 Brundrett MC, Tedersoo L. 2018. Evolutionary history of mycorrhizal symbioses and global host
 658 plant diversity. *New Phytol* doi:10.1111/nph.14976
- Brundrett MC, Piché Y, Peterson RL. 1984 A new method for observing the morphology of
 vesicular-arbuscular mycorrhizas. *Canadian J Botany* 62: 2128-2134.
- Bucher M. 2007. Functional biology of plant phosphate uptake at root and mycorrhiza interfaces.
 New Phytol 173:11-26.
- 663 Carotenuto G, Chabaud M, Miyata K, Capozzi M, Takeda N, Kaku H, Shibuya N, Nakagawa
 664 T, Barker DG, Genre A. 2017. The rice LysM receptor-like kinase OsCERK1 is required for the
 665 perception of short-chain chitin oligomers in arbuscular mycorrhizal signaling. *New Phytol* 214:
 666 1440-1446.
- 667 Chen ECH, Morin E, Beaudet D, Noel J, Yildirir G, Ndikumana S, Charron P, St-Onge C,
 668 Giorgi J, Krüger M et al. 2018. High intraspecific genome diversity in the model arbuscular
 669 mycorrhizal symbiont *Rhizophagus irregularis*. *New Phytol* doi: 10.1111/nph.14989.
- 670 Chialva M, Salvioli A, Daghino S, Ghignone S, Bagnaresi P, Chiapello M, Novero M, Spadaro
- 671 D, Perotto S, Bonfante P. 2018. Native Soils with Their Microbiotas Elicit a State of Alert in
- Tomato Plants. *New Phytol* https://doi.org/10.1111/nph.15014.
- 673 Cook CE, Whichard LP, Turner B, Wall ME, Egley GH. 1966. Germination of witchweed
- 674 (*Striga lutea* Lour.): isolation and properties of a potent stimulant. *Science* **154**: 1189–1190.

- 675 Courty P-E, Buee M, Diedhiou AG, Frey-Klett P, Le Tacon F, Rineau F, Turpault M-P, Uroz
- 676 S, Garbaye J. 2010. The role of ectomycorrhizal communities in forest ecosystem processes: New
- 677 perspectives and emerging concepts. Soil Biology & Biochemistry. 42: 679-698
- 678 Delaux PM, Xie X, Timme RE, Puech-Pages V, Dunand C, Lecompte E, Delwiche CF,
- 679 Yoneyama K, Bécard G, Séjalon-Delmas N. 2012. Origin of strigolactones in the green lineage.
- 680 *New Phytol* **195**: 857–871.
- 681 Dickson S. 2004. The Arum–Paris continuum of mycorrhizal symbioses. New Phytol. 63: 187–200.
- 682 Ditengou FA, Muller, A., Rosenkranz M, Felten J, Lasok H, van Doorn, MM, Legue V, Palme
- K, Schnitzler JP, Polle A. 2015. Volatile signalling by sesquiterpenes from ectomycorrhizal fungi
 reprogrammes root architecture. *Nature Communications* 6: 6279.
- Douhan GW, Huryn KL, Douhan LI. 2007. Significant diversity and potential problems
 associated with inferring population structure within the *Cenococcum geophilum* species complex.
 Mycologia 99:812–819, doi:10.3852/mycologia.99.6.812
- Dreher D, Yadav H, Zander S, Hause B. 2017. Is there genetic variation in mycorrhization of
 Medicago truncatula? PeerJ 5: e3713 https://doi.org/10.7717/peerj.3713
- 690 Duc G, Trouvelot A, Gianinazzi-Pearson V, Gianinazzi S, 1989. First report of non-mycorrhizal
- 691 plant mutants (Myc-) obtained in pea (*Pisum sativum* L.) and fababean (*Vicia faba* L.)
- 692 Plant Science 60:215-222
- Ezawa T, K Saito, 2018. How do arbuscular mycorrhizal fungi handle phosphate? New insight into
 fine-tuning of phosphate metabolism. *New Phytol* https://doi.org/10.1111/nph.15187
- Fiorilli V, Vallino M, Biselli C, Faccio A, Bagnaresi P, Bonfante P. 2015. Host and non-host
 roots in rice: cellular and molecular approaches reveal differential responses to arbuscular
 mycorrhizal fungi. *Front Plant Sci* 6: 636.
- 698 Fochi V, Chitarra W, Kohler A, Voyron S, Singan VR, Lindquist EA, Barry KW, Girlanda
- 699 M, Grigoriev IV, Martin F. 2017. Fungal and plant gene expression in the Tulasnella calospora-
- *Serapias vomeracea* symbiosis provides clues about nitrogen pathways in orchid mycorrhizas. *New Phytol* 213: 365-379.
- 702 Fontana A, Palenzona M. 1969. Sintesi micorrizica di Tuber albidum in coltura pura con Pinus

- strobus e Pioppo euroamericano. Allionia 15: 99-104.
- Fusconi A, Lingua G., Trotta A. Berta G. 2005. Effects of arbuscular mycorrhizal colonization
 and phosphorus application on nuclear ploidy in Allium porrum plants. *Mycorrhiza* 15: 313–321.
- Gallaud J. 1905. Etudes sur le Mycorrhizas endotrophs. *Revue generale de Botanique* pages 1-48;
 66-85; 223-239; 313-324, 479-500
- Gardes M, Bruns TD. 1993. ITS primers with enhanced specificity for basidiomycetes application to the identification of mycorrhizae and rusts. *Molecular Ecology*https://doi.org/10.1111/j.1365-294X.1993.tb00005.x
- Genre A, Chabaud M, Timmers T, Bonfante P, Barker DG. 2005. Arbuscular mycorrhizal fungi
 elicit a novel intracellular apparatus in *Medicago truncatula* root epidermal cells before infection.
 Plant Cell 17: 3489–3499. doi: 10.1105/tpc.105.035410
- Genre A, Chabaud M, Faccio A, Barker DG, Bonfante P. 2008. Prepenetration Apparatus
 Assembly Precedes and Predicts the Colonization Patterns of Arbuscular Mycorrhizal Fungi within
 the Root Cortex of Both *Medicago truncatula* and *Daucus carota*. *Plant Cell* 20: 1407-1420.
- Genre A, Ivanov S, Fendrych M, Faccio A, Zársky V, Bisseling T, Bonfante P. 2012. Multiple
 exocytotic markers accumulate at the sites of perifungal mem- brane biogenesis in arbuscular
 mycorrhizas. Plant Cell Physiol. 53: 244–255. doi: 10.1093/pcp/pcr170
- Genre A, Chabaud M, Balzergue C, Puech-Pagès V, Novero M, Rey T, Fournier J, Rochange
 S, Bécard G, Bonfante P et al. 2013. Short-chain chitin oligomers from arbuscular mycorrhizal
 fungi trigger nuclear Ca2+ spiking in *Medicago truncatula* roots and their production is enhanced
 by strigolactone. New Phytol 198: 190-202.
- Gerdemann JW 1964. The effect of mycorrhiza on the growth of maize. *Mycologia* 56: 342–349.
- Gerdemann JW. 1968. Vesicular-arbuscular mycorrhizae and plant growth. *Annu Rev Phytopathol*6: 397–418.
- Gerdemann JW, Trappe JM. 1974. The Endogonaceae in the Pacific Northwest. The New York
 Botanical Garden, New York
- Gibelli G. 1883. Nuovi studi sulla malattia del castagno detta dell'inchiostro. *Mem Accad Sci Ist*Bologna 4: 287–314.

- Giovannetti M, Sbrana C, Avio L, Citernesi AS, Logi C. 1993. Differential hyphal
 morphogenesis in arbuscular mycorrhizal fungi during preinfection stages. *New Phytol*125: 587593.
- Giovannetti M, Mari A, Novero M, Bonfante P. 2015. Early *Lotus japonicus* root transcriptomic
 responses to symbiotic and pathogenic fungal exudates. *Front. Plant Sci* 6: 480. doi:
 10.3389/fpls.2015.00480
- Guether M, Balestrini R, Hannah M, He J, Udvardi M, Bonfante P. 2009. Genome-wide
 reprogramming of regulatory networks, transport, cell wall and membrane biogenesis during
 arbuscular mycorrhizal sym- biosis in *Lotus japonicus*. New Phytol 182: 200–212.
- 740 Gutjahr C, Banba M, Croset V, An K, Miyao A, An G, Hirochika H, Imaizumi-Anraku H,
- 741 Paszkowski U. 2008. Arbuscular mycorrhiza-specific signaling in rice transcends the common
- symbiosis signaling pathway. *Plant Cell* **20**: 2989–3005.
- Gutjahr C, Parniske M. 2013 Cell and developmental biology of the arbuscular mycorrhiza
 symbiosis. *Annu. Rev Cell Dev Biol* 29: 593–617.
- 745 Harley JL. 1959. The Biology of Mycorrhiza Leonard Hill, London
- Harley J, Mcready CC. 1952. The uptake of phosphate by excised mycorrhizal roots of the beech. *New Phytologist*, 51: 56-64. doi:10.1111/j.1469-8137.1952.tb06115.x
- Harley J, Brierley JK. 1955. The uptake of phosphate by excised mycorrhizal roots of the beech. *New Phytologist*, 54: 296-301. doi:10.1111/j.1469-8137.1955.tb06184.x
- Harrison MJ, van Buuren ML. 1995. A phosphate transporter from the mycorrhizal fungus *Glomus versiforme. Nature* 378: 626-629.
- Harrison M, Dewbre G, Liu J. 2002. A phosphate transporter of *Medicago truncatula* involved in
 the acquisition of phosphate released by arbuscular mycorrhizal fungi. *Plant Cell* 14: 2413–2429.
- Hepper C, Mosse B. 1975. Techniques used to study the interaction between Endogone and Plant
 roots. In *Endomycorrhizas*, FE Sanders, B. Mosse, PB Tinker (Eds.), Academic Press, London 65756
- Kamel L, Tang N, Malbreil M, San Clemente H, Le Marquer M, Roux C, Frei dit Frey N.
 2017. The comparison of expressed candidate secreted proteins from two arbuscular mycorrhizal

- fungi unravels common and specific molecular tools to invade different host plants. *Frontiers in Plant Science* 8:124. doi:10.3389/fpls.2017.00124
- Kidston R, Lang WH. 1921. On Old Red Sandstone plants showing structure, from the Rhynie
 Chert bed, Aberdeenshire. V. *Trans. Roy. Soc. Edinburgh* 52: 855-902.
- 763 Kohler A, Kuo A, Nagy LG, Morin E, Barry KW, Buscot F, Canbäck B, Choi C, Cichocki N,
- 764 Clum A et al. 2015. Convergent losses of decay mechanisms and rapid turnover of symbiosis genes

in mycorrhizal mutualists. *Nature GeNetics* **47**: 410-5. doi:10.1038/ng.3223

766

767 Koide RT, Mosse B. 2004. A history of research on arbuscular mycorrhiza. *Mycorrhiza* 14:145-63.

- 768 Kloppholz S, Kuhn H, Requena N. 2011. A secreted fungal effector of Glomus intraradices
- 769 promotes symbiotic biotrophy. *Cur Biol* **21**: 1204–1209. doi: 10.1016/j.cub.2011.06.044
- 770 Kretzschmar T, Kohlen W, Sasse J, Borghi L, Schlegel M, Bachelier JB, Reinhardt D, Bours
- **R, Bouwmeester HJ, Martinoia E**. 2012. A petunia ABC protein controls strigolactone-dependent
 symbiotic signalling and branching. *Nature* 483: 341–44.
- Lagrange H, Jay-Allgmand C, Lapeyrie F. 2001. Rutin, the phenolglycoside from eucalyptus
 root exudates, stimulates *Pisolithus* hyphal growth at picomolar concentrations *New Phytol* 149:
 349–355.
- Tranco L, Fiorilli V, Gutjahr C. 2018a. Partner communication and role of nutrients the
 arbuscular mycorrhizal symbiosis. *New Phytol* in press
- Lanfranco L, Fiorilli V, Venice F, Bonfante P. 2018b. Strigolactones cross the kingdoms: plants,
 fungi, and bacteria in the arbuscular mycorrhizal symbiosis *Journal of Experimental Botany*doi:10.1093/jxb/erx432
- Lee J, Young JPW. 2009. The mitochondrial genome sequence of the arbuscular mycorrhizal
 fungus *Glomus intraradices* isolate 494 and implications for the phylogenetic placement of *Glomus*. *New Phytol* 183: 200–211.
- 784
- Lin KL, Limpens E, Zhang Z, Ivanov S, Saunders DGO, Mu D, Pang E, Cao H, Cha H, Lin T,
 et al. 2014. Single Nucleus Genome Sequencing Reveals High Similarity among Nuclei of an
 Endomycorrhizal Fungus. *PLOS Genetics* https://doi.org/10.1371/journal.pgen.1004078

- Lindeberg G. 1989. Elias Melin: Pioneer Leader in Mycorrhizal research. *Annual Review of Phytopathology* 27: 49-58
- 790
- 791 Maillet F, Poinsot V, Andre O, Puech-Pages V, Haouy A, Gueunier M, Cromer L, Giraudet
- 792 D, Formey D, Niebel A, et al. 2011 Fungal lipochitooligosaccharide symbiotic signals in
 793 arbuscular mycorrhiza. *Nature* 469: 58–63.
- 794 Martin F, Aerts A, Ahren D, Brun A, Danchin EGJ, Duchaussoy F, Gibon J, Kohler A,
- Lindquist E, Pereda V et al. 2008. Symbiosis insights from the genome of the mycorrhizal
 basidiomycete *Laccaria bicolor*. *Nature* 452: 88–92. doi: 10.1038/nature06556
- 797 Martin F, Kohler A, Murat C, Balestrini R, Coutinho PM, Jaillon O, Montanini B, Morin E,
- 798 Noel B, Percudani R et al. 2010. Périgord black truffle genome uncovers evolutionary origins and
- 799 mechanisms of symbiosis. *Nature* 464: 1033–1038. doi: 10.1038/nature08867
- Martin F, Kohler A, Murat C, Veneault-Fourrey C, Hibbett DS. 2016. Unearthing the roots of
 ectomycorrhizal symbiosis Nature Reviews 14:760-773. doi:10.1038/nrmicro.2016.149
- 802 Martínez-Medina A, Flors V, Heil M, Mauch-Mani B, Pieterse CM, Pozo MJ, Ton J, van Dam
- 803 NM, Conrath U. 2016. Recognizing plant defense priming. *Trends in Plant Science* 21: 818-822
- Martino E, Morin E, Grelet G, Kuo A, KohlerA, Daghino S, Barry KW, Cichocki N, Clum A,
 Dockter RB, et al. 2018. Comparative genomics and transcriptomics depict ericoid mycorrhizal
 fungi as versatile saprotrophs and plant mutualists. *New Phytol.* 217: 1213-1229.
 doi:10.1111/nph.14974
- McLean AM, Bravo A, Harrison MJ. 2017. Plant signaling and metabolic pathways enabling
 arbuscular mycorrhizal symbiosis. *Plant Cell* 29: 2319–2335. doi:10.1105/tpc.17.00555.
- Melin E, Nilsson H. 1950. Transfer of radioactive phosphorus to pine seedlings by means of
 mycorrhizal hyphae. *Physiol Plant* 3:88-92.
- 812
- 813 Melin E. 1954. Growth factor requirements of mycorrhizal fungi of trees. Sv Bot Tidskr 48: 86-94.
- 814 Miyata K, Kozaki T, Kouzai Y, Ozawa K, Ishii K, Asamizu E, Okabe Y, Umehara Y,

- Miyamoto A, Kobae Y et al. 2014. The bifunctional plant receptor, OsCERK1, regulates both
 chitin-triggered immunity and arbuscular mycorrhizal symbiosis in rice. *Plant and Cell Physiology*55: 1864–1872.
- 818
- 819 Mosse B. 1962. The establishment of vesicular-arbuscular mycorrhiza under aseptic conditions. J
 820 *Gen Microbiol* 27: 509–520
- Mosse B. 1970. Honey-coloured, sessile Endogone spores: II. Changes in fine structure during
 spore development. *Archiv für Mikrobiologie* 74: 129-145.
- Nadal M, Sawers R, Naseem S, Bassin B, Kulicke C, Sharman A, An G, An K, Ahern KR,
 Romag A et al. 2017. An N-acetylglucosamine transporter required for arbuscular mycorrhizal
 symbioses in rice and maize. *Nature Plants*, 3 (17073)https://doi.org/10.1038/nplants.2017.73
- Norby RJ, De Kauwe MG, Domingues TF, Duursma RA, Ellsworth DS, Goll DS, Lapola DM,
 Luus KA, MacKenzie AR, Medlyn BE et al. 2016. Model–data synthesis for the next generation
 of forest free-air CO2 enrichment (FACE) experiments. *New Phytol* 209: 17-28.
 doi:10.1111/nph.13593
- 830 Oldroyd GE. 2013. Speak, friend, and enter: signalling systems that promote beneficial symbiotic
 831 associations in plants. *Nat Rev Microbiol* 11: 252–263.
- 832 Oliveira Chagas F, Pessotti RC, Caraballo-Rodríguez AM, Pupo MT. 2018. Chemical signaling
 833 involved in plant-microbe interactions. *Chem Soc Rev* 47: 1652-1704.
- Parniske M. 2008. Arbuscular mycorrhiza: the mother of plant root endosymbioses. *Nat Rev Microbiol* 6: 763-75.
- Pelin A, Pombert JF, Salvioli A, BoneL, Bonfante P, Corradi N. 2012. The mitochondrial
 genome of the arbuscular mycorrhizal fungus *Gigaspora margarita* reveals two unsuspected transsplicing events of group I introns. *New Phytol* 194:836–845.
- 839 Perotto S, Daghino S, Martino E. 2018. Ericoid mycorrhizal fungi and their genomes: another
 840 side to the mycorrhizal symbiosis? *New Phytol* in press
- Perotto S, Peretto R, Faccio A, Schubert A, Bonfante P, Varma A. 1995. Ericoid mycorrhizal
 fungi: cellular and molecular basis of their interactions with the host plant. *Canadian Journal of Botany* 73: 557–568.

- 844 Peter M, Kohler A, Ohm RA, Kuo A, Kruzmann J, Morin E, Arend M, Barry KW, Binder M,
- Choi A, Clum A. et al. 2016. Ectomycorrhizal ecology is imprinted in the genome of the dominant
 symbiotic fungus *Cenococcum geophilum*. *Nature Communications* 7:12662 | DOI:
 10.1038/ncomms12662
- Peterson RL, Massicotte HB, Melville LH. 2004. Mycorrhizas: Anatomy and Cell Biology.
 Toronto: CABI.
- 850 Peyronel B. 1923. Fructification de l'endophyte arbuscules et vesicules des mycorhizes
 851 endotrophes. *Bull Soc Mycol Fr* 39:119–126.
- 852 Peyronel B. 1924. Specie di "Endogone" produttrici di micorize endotrofiche. *Boll Stn Patol Veg*853 Roma 5:73–75.
- Pimprikar P, Gutjahr C. 2018. Transcriptional regulation of arbuscular mycorrhiza development.
 Plant Cell Physiology doi.org/10.1093/pcp/pcy024 (Review)
- Plett J M, Kemppainen M, Kale SD, Kohler A, Legué V, Brun A, Tyler BM, Pargo AG,
 Martin F. 2011. A secreted effector protein of *Laccaria bicolor* is required for symbiosis
 development. *Curr Biol* 21: 1197–1203. doi: 10.1016/j.cub.2011.05.033
- Pozo MJ, Azcón-Aguilar C. 2007. Unravelling mycorrhiza-induced resistance. *Current Opinion in Plant Biology* 10: 393-398.
- **Read DJ.** 1991. Mycorrhizas in ecosystems *Experientia* **47**: 376-391.
- Reid CPP, Kidd FA, Ekwebelam SA. 1983. Nitrogen nutrition, photosynthesis and carbon
 allocation in ectomycorrhizal pine. *Plant and Soil* 71:415-431.
- Rodriguez A, Sanders IR. 2015. The role of community and population ecology in applying
 mycorrhizal fungi for improved food security. *ISME Journal* 9: 1053-1061.
- Roger A, Colard A, Angelard C, Sanders IR. 2013. Relatedness among arbuscular mycorrhizal
 fungi drives plant growth and intraspecific fungal coexistence. *ISME Journal* 7: 2137-2146.
- Salvioli A, Ghignone S, Novero M, Navazio L, Venice F, Bagnaresi P, Bonfante P. 2016.
 Symbiosis with an endobacterium increases the tness of a mycorrhizal fungus, raising its
 bioenergetic potential. *ISME Journal* 10:130-144.

- Sánchez-Vallet A, Mesters JR, Thomma BPHJ. 2015. The battle for chitin recognition in plant microbe interactions. *FEMS Microbiology Reviews* 39:171–183.
 https://doi.org/10.1093/femsre/fuu003
- 874 Sanders FE, Tinker PB. 1971. Mechanism of Absorption of Phosphate from Soil by *Endogone*875 Mycorrhizas. *Nature* 233: 278–279. doi:10.1038/233278c0
- 876 Sanders FE, Mosse B, Tinker PB. 1975 *Endomycorrhizas*. Proceedings of the Symposium held at
 877 the University of Leeds, 22–25 July 1974. Academic Press, London
- 878 Sawers RJH, Gutjahr C, Paszkowski U. 2008. Cereal mycorrhiza: an ancient symbiosis in
 879 modern agriculture. *Trends in Plant Science* 13:93-97
- Scannerini S, Bonfante-Fasolo P, Fontana A. 1975. An ultrastructural model for the hostsymbiont interaction in the endotrophic mycorrhizae of *Ornithogalum umbellatum* L. In *Endomycorrhizas*. Edited by F. E. Sanders, B. Mosse, and P. B. Tinker. Academic Press, N.Y.
 pp.313-324.
- Scannerini S, Bonfante-Fasolo P. 1979. Utrastructural cytochemical demonstration of
 polysaccharides and proteins within host-arbuscule interfacial matrix in an endomycorrhiza. *New Phytol* 83: 87–94.
- 887 Scannerini S, Bonfante-Fasolo P. 1983. Comparative ultrastructural analysis of mycorrhizal
 888 associations. Can J Bot 61:917–943. doi: 10.1139/b83-104
- 889 Selosse MA, Minasiewicz J., Boullard, B. 2017. An annotated translation of Noël Bernard's
- 890 1899 article 'On the germination of *Neottia nidus-avis*' *Mycorrhiza* 27: 611-618
- 891 https://doi.org/10.1007/s00572-017-0774-z
- Schußler A, Schwarzott D, Walker C. 2001. A new fungal phylum, the Glomeromycota:
 phylogeny and evolution. *Mycol Res* 105:1413–1421.
- 894 Smith SE, Read DJ. 1997. Mycorrhizal symbiosis, 2nd edn. Academic Press, San Diego
- 895 Smith FA, Smith SE. 1997. Structural diversity in (vesicular)-arbuscular mycorrhizal symbiosis.
 896 New Phytol 137: 373–388.
- Smith SE, Jakobsen I, Grønlund M, Smith FA. 2011. Roles of Arbuscular Mycorrhizas in Plant
 Phosphorus Nutrition: Interactions between Pathways of Phosphorus Uptake in Arbuscular
 Mycorrhizal Roots Have Important Implications for Understanding and Manipulating Plant

- 900 Phosphorus Acquisition. Plant Physiology 156: 1050-1057; DOI: 10.1104/pp.111.174581
- 901 Spanu P, Boller T, Ludwig A, Wiemken A, Faccio A, Bonfante P. 1989. Chitinase in roots of
 902 mycorrhizal *Allium porrum*: regulation and localization. *Planta* 177: 447–455.
- 903 Spatafora JW, Chang Y, Benny GL, Lazarus K, Smith ME, Berbee ML et al. 2016. A phylum904 level phylogenetic classification of zygomycete fungi based on genome- scale data. *Mycologia* 108:
 905 1028–1046.
- Sun J, Miller J, Granqvist E, Wiley-Kalil A, Gobbato E, Maillet F, Cottaz S, Samain E,
 Venkateshwaran M, Fort S et al. 2015. Activation of symbiosis signaling by arbuscular
 mycorrhizal fungi in legumes and rice. *Plant Cell* 27:823-38.
- Sugimura Y, Saito K. 2017. Transcriptional profiling of arbuscular mycorrhizal roots exposed to
 high levels of phosphate reveals the repression of cell cycle-related genes and secreted protein
 genes in *Rhizophagus irregularis Mycorrhiza* 27:139-146.
- 912 Tisserant E, Malbreil M, Kuo A, Kohler A, Symeonidi A, Balestrini R, Charron P, Duensing
- N, Frei dit Frey N, Gianinazzi-Pearson V, et al. 2013. Genome of an arbuscular mycorrhizal
 fungus provides insight into the oldest plant symbiosis. *Proceedings of the National Academy of Sciences* 110: 20117-22.
- 916 Toju H, Guimarães PR, Olesen JM, Thompson JN. 2014. Assembly of complex plant–fungus
 917 networks. *Nature Communications* 5: 5273. doi:10.1038/ncomms6273
- 918 Trappe JM. 2005 AB Frank and mycorrhizae: the challenge to evolutionary and ecologic theory
 919 *Mycorrhiza* 15: 277-81. https://doi.org/10.1007/s00572-004-0330-5
- van der Heijden MG, Boller T, Wiemken A, Sanders IR. 1998. Different arbuscular mycorrhizal
 fungal species are potential determinants of plant community structure. *Ecology* 79: 2082-2091.
 doi:10.1890/0012-9658(1998)079[2082:DAMFSA]2.0.CO;2
- van der Heijden MG, Martin FM, Selosse M, Sanders IR. 2015. Mycorrhizal ecology and
 evolution: the past, the present, and the future. *New Phytol* 205: 1406-1423. doi:10.1111/nph.13288
- 925 Veneault-Fourrey C, Commun C, Kohler A, Morin E, Balestrini R, Plett J, Danchin E,
 926 Coutinho P, Wiebenga A, deVries RPet al. 2014. Genomic and transcriptomic analysis of

927	Laccaria bicolor CAZome reveals insights into polysaccharides remodelling during symbiosis
928	establishment. Fungal Genet Biol 72: 168-181.
929	Waters MT, Gutjahr C, Bennett T, Nelson DC. 2017. Strigolactone signaling and evolution.
930	Annual Review of Plant Biology 68: 291-322
931	
932	White TJ, Bruns TD, Lee SB, Taylor JW. 1989. Amplification and direct sequencing of fungal
933	ribosomal RNA Genes for phylogenetics PCR - Protocols and Applications - A Laboratory Manual,
934	Eds: Sninsky J and White T. San Diego: CA Academic Press: 315-322
935	
936	Zhang X, Dong W, Sun J, Feng F, Deng Y, He Z, Oldroyd GED, Wang E. 2015. The receptor
937	kinase CERK1 has dual functions in symbiosis and immunity signalling. <i>Plant Journal</i> 81: 258–267
938	
939	Zipfel C, Oldroyd GED. 2017. Plant signalling in symbiosis and immunity. <i>Nature</i> 543: 328-335.
940	http://dx.doi.org/10.1038/nature22009
941	
942	
943	
944	
945	
946	
947	
948	
949	
950	
951	Keywords: Mycorrhizas, History, "Omics" approaches, Plant Microbiota, Evolution, Mutants,

952 Signalling molecules, Colonization Process

953 Legends

Fig. 1 a. The drawings from Giuseppe Gibelli (1883) illustrate chestnut roots colonized by anunknown fungus leading to the typical rounded mycorrhizal tip.

956 Fig. 1b. Oreste Mattirolo had a painting made by an unknown artist on the occasion of an 957 international exhibition held in Torino in 1911. The painting illustrates how a pig and a dog are 958 looking for truffles under an oak. The root systems bear the typical ectomycorrhizal roots. 959 Reproduced with the permission of the Department of Life Science and Systems Biology- Library 960 of the Botanical Garden, University of Torino.

961 Fig. 2. Top line: In his 1905 reports, J. Gallaud provided beautiful illustrations of endomycorrhizas 962 depicting the different morphologies of arbuscules as reproduced here and showing details of the 963 root symbioses in Sequoia and Arum, respectively. The drawing on the left side illustrates an Allium 964 arbuscule where some branches are already collapsing; according to the terminology of the time 965 these fungal structures were defined as sporangioles. The same morphology can be appreciated in a 966 section of a root of Lotus japonicus colonized by Gigaspora margarita and stained with wheat germ 967 agglutinin conjugated with a fluorescent probe to reveal the fungal wall and seen under confocal 968 microscopy (Courtesy of Dr. Mara Novero)

Fig. 3 The group picture illustrates the scientific community who convened in Leeds (1974) for the
first meeting on endomycorrhizas. In the first row: from right, third position: Silvano Scannerini,
Jack Harley, Mrs. Harley, Bernard Tinker, Vivienne Pearson (not yet Gianinazzi); from left, first
position: Francis Sanders; Geff Hadley; Barbara Mosse; Glynn Bowen, and after one person, C.
Hepper. David Read is in the last line, the third from the left. Paola Bonfante is in the second row.

Fig. 4. On the left side: Drawing by C. Hepper and B. Mosse (1975) showing the effect of *Trifolium*exudates on the hyphal branching of *F. mosseae* (a) when compared to the untreated fungus(b). On
the right side, an experiment, similar to the one developed by Akyama *et al.* 30 years later (2005),
illustrates how the germinating spore of *Gigaspora margarita* is triggered to branch by GR24, a
synthetic strigolactone analogue (courtesy of Dr. Mara Novero).

979 Fig. 5. A look at the wall of an AM fungus under transmission electron microscope. A detail of the 980 cell wall of a spore of *Glomus versiforme* reveals a 3D helicoidal architecture created by highly 981 cristalline nano-chitin fibrils (arrows), which are laid down in planes which progressively rotate of a 982 certain angle (a). The optical effects of this 3D architecture after sectioning are fibrillar arcs. This 983 architecture provides strength and resistance to the spores. Such organization is lost during the 984 germination. However, the nanocrystals of fibrillar chitin (arrow heads) are easily detected in the 985 wall of extraradical (b) but not of intracellular (c) hyphae, and of thin arbuscule branches (d). Here, 986 the cell wall structure is amorphous, but Wheat Germ Agglutinin coupled to gold granules still 987 reveals the presence of chitooligosaccharides. The molecular control of such hierarchical 988 organization is at the moment not known.

Fig. 6. Schematic view of the interface zone in an ectomycorrhizal (A) and AM (B) symbiosis. In C
several of the molecules so far determined through in situ labeling experiments are listed. Modified
from Bonfante 2001 and Balestrini and Bonfante 2014.

992 Fig. 7. Electron microscopy observations in the seventies revealed the cellular re-organization of 993 plant cells following the fungal colonization. On the right: a Lotus japonicus cortical cell is 994 colonized by Gigaspora margarita. The cortical cell is dominated by an enlarged nucleus with a 995 prominent nucleolus and a loose chromatin. The fungal branches of different size (from the trunk to 996 the collapsed terminal branches) fill up the plant cell lumen. At the left, an epidermal Calluna cell is 997 colonized by Pezizella ericae, which forms a coil. Also in this case the coil is surrounded by the 998 invaginated host membrane and the plant nucleus occupies a central position. These observations 999 reveal a similar cell-reorganization, irrespectively of the different fungal physiology.