

1 **Biological disease control by beneficial (micro)organisms: Selected**
2 **breakthroughs in the past 50 years**

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7 **Summary**

8 Biological control of plant disease by beneficial (micro)organisms is one of the main
9 tools available to preserve plant health within the wider context of One Health and in
10 line with the goals of the Agenda 2030 for Sustainable Development. The commercial
11 development of biocontrol agents, together with a new perspective on the resident
12 microbial community, all supported by innovative “omics” technologies, continues to
13 gain in prominence in plant pathology, addressing the need to feed the increasing world
14 population and to assure a safe and secure access to food. The present review considers
15 selected advances within the last 50 years, highlighting those that can be considered as
16 breakthroughs for the biological control research field. Selected examples of successful
17 biocontrol agents and strategies are reported, including the history of the progress in
18 researching *Trichoderma* isolates as commercial biocontrol agents, the exploitation of
19 mycoviruses to confer hypovirulence to plant pathogenic fungi, the role of microbial
20 communities in the suppressiveness of soils, and evolving approaches including the
21 establishment of Synthetic Microbial Communities (SynCom).

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23 **Key words:** Biological control; Food security; Climate Change; Microbiome;

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25

26 **Plant pathology and sustainable plant disease management in the 21st century**

27 Being plant pathologists today means a need to be aware of developments throughout
28 the biological world that can be applied to solve the grand challenges facing the planet
29 and humanity. The world population is increasing and estimated to reach 10.5 billion
30 by 2050 (FAO, 2013). As a consequence, the total global food demand is expected to
31 increase by 35 to 56% between 2010 and 2050 (van Dijk et al., 2021). This raises major
32 concerns for both food security and safety. Many different strategies encompassing
33 various domains of expertise are needed to address these challenges. These include
34 reducing food waste and the unequal distribution of food among countries and among
35 people within the same country, but at the same time both the quantity and the quality
36 of agricultural products need to be increased. Crop losses due to diseases and pests, and
37 food contamination by mycotoxins are key issues plant pathologists must face in the
38 present and, even more so, in the near future to offer efficient and sustainable solutions
39 to guarantee sufficient and safe food for all (*Codex Alimentarius*, 2022), in line with
40 public demands and needs.

41 The main threat to crop production is the plethora of biotic and abiotic stresses
42 quantitatively and qualitatively affecting yield of commodities destined for human and
43 animal nutrition. Climate change arguably represents the most challenging issue facing
44 humankind today, and the associated rapid and significant changes in environmental
45 conditions are affecting agriculture by changing the geographic distribution of crops as
46 well as pests and pathogens associated with cultivates species (Burdon and Zhan, 2020).
47 Several strategies and action plans have been adopted at a global level, such as the
48 Agenda 2030, a plan of action for people, planet and prosperity based on 17 Sustainable
49 Development Goals and 169 targets that, among others, aim to taking urgent (and
50 sustainable) actions on climate change.

51 In this context, ecologically inspired control of diseases perfectly fits within the Agenda
52 2030 and is in line with the One Health concept, an integrated, unifying approach
53 aiming to balance and optimize the health of people, animals and ecosystems
54 sustainably. This approach involves multiple sectors and disciplines to create long-
55 term, sustainable solutions in the public health, veterinary and environmental sectors.
56 The One Health approach is particularly relevant for food safety and nutrition, with
57 Plant Health occupying a pivotal place in this paradigm (WHO, 2021). Indeed, the One
58 Health perspective also encompasses the complex interplay between human well-being
59 and plant health by focusing on the environmental dimension directly underpinning
60 human health (Hoffmann et al., 2022).

61 One strategy to meet the need for new sustainable strategies in agriculture is the use of
62 biological control agents (BCAs). Biological control using products containing one or
63 more (micro)organisms - such as filamentous fungi and yeasts, fungal-like species,
64 bacteria and mycoviruses - as active ingredients increasingly represents a valid
65 alternative to chemical pesticides, especially in those European countries where the use
66 of transgenic plants is not permitted at present (Collinge and Sarrocco, 2022; Collinge
67 et al., 2022).

68 The authorship of the term “biological control” should be attributed to Carl Freiherr
69 von Tubeuf who pioneered biocontrol of plant diseases and introduced this term to plant
70 pathology over a century ago in 1914 (Maloy and Lang, 2003). However, historically,
71 the foundation for modern biocontrol research was laid during the 1970s, with 1974 as
72 a milestone, when Baker and Cook published the first book wholly devoted to the
73 subject of biological control of plant pathogens or, more precisely, “*the first publication*
74 *of sufficient length to do full justice to this large and expanding field of research*” thus
75 giving voice to more than 50 years of research and observations (Baker and Cook,

76 1974).

77 In the 50 subsequent years, many investigations have focused on the selection,
78 evaluation and use of beneficial (micro)organisms to be developed as biological control
79 agents for the management of plant diseases affecting cultivated plants for feed, food
80 and non-food purposes. During the same time, the definition of “biological control” has
81 experienced many changes over time. According to the recent review by Collinge et al.
82 (2022), and in line with what was originally stated by Cook and Baker (1983),
83 biological control should be defined as “direct or indirect inhibition of a disease, or the
84 pathogen causing the disease, by another organism (antagonist) or group of organisms”.

85 However, a modern broader definition now also includes specialized metabolites (such
86 as signaling, antibiotic or attractant substances) often termed biopesticides (Roberts and
87 Taylor, 2016). Collinge and co-authors recommended to use the new term
88 bioprotectants, as indicated by Stenberg et al. (2021), to avoid the misleading term
89 biopesticides, while the term bioprotection (including the use of non-living extracts and
90 natural products) should replace the wider use of the term biological control that should
91 be reserved to situations where a living BCA is used (Stenberg et al., 2021).

92 This review does not have the aim to list all the events that occurred from the first case
93 of the exploitation of a (micro)organism for the biological management of plant
94 diseases (Tromso et al., 2020; Collinge et al., 2022); rather, it chronicles breakthroughs
95 that led us from the first report of an isolate of *Trichoderma lignorum* (*T. viride*) capable
96 of reducing the symptoms caused by *Rhizoctonia solani* on citrus (Weindling, 1932) to
97 the present; the evolving approaches in biological control that take advantage, *inter*
98 *alia*, of the exploitation of mycoviruses conferring hypovirulence to plant pathogenic
99 fungi (the case of *Chryphonectria parasitica*); and of the increasing knowledge of the
100 microbiota, exploited as Synthetic Microbial Community (SynCom) and supported by

101 new “omics” technologies, to confer more efficient plant protection than individual
102 strains (Vannier et al., 2019).

103

104 ***Trichoderma* from 1930 (1980) to 2022: evolution and revolution in biological**
105 **disease control**

106 When, in 1985, George C. Papavizas wrote the first review on the biology, ecology and
107 potential for biocontrol by *Trichoderma* (Papavizas, 1985), more than half a century
108 had passed from the description of the tri-partite interaction *T. lignorum*/citrus/*R. solani*
109 (Weindling, 1930). These 50 years were marked by strong efforts to promote the idea
110 that *Trichoderma* has all the credentials to be considered the most promising biocontrol
111 agent, as demonstrated by the massive amount of papers on this topic as well as by the
112 commercial success of products containing these organisms as active ingredient. It can
113 be argued that the *Trichoderma* “age” began in 1984 when the First International
114 Workshop on *Trichoderma* and *Gliocladium* was held, thanks to Papavizas, at the
115 USDA Beltsville Agricultural Research Center in 1984 (Figure 1). This workshop
116 brought together almost all the scientists who were beginning to write the history of
117 *Trichoderma* and whose work became a milestone for scientists working on the
118 biological control of plant diseases.

119

120 **Figure 1.** Participants at the First International Workshop on *Trichoderma* and *Gliocladium*, USDA
121 Beltsville Agricultural Research Center, 3-5 April 1984 (*Phytopathology News*, 18(7), July 1984).

122

123 The message given by Papavizas was positively accepted by the plant pathology and
124 crop protection community to the point that *Trichoderma* is still to this day the main
125 actor of many papers (Figure 2). The starting date is set at 1930, with the pioneering
126 work of Weindling. Since then, over 85,000 papers (with around 80% of these as peer-

127 reviewed research papers) have been published - with a significant increase from 2018
128 - all dealing with this important fungal genus (www.scopus.com), its biology,
129 physiology, ecology, mechanisms of action as biocontrol agent, their role as plant
130 growth stimulators as well as genome evolution within the genus.

131 Studies with *Trichoderma* do not only concern agriculture (20.1%) but are included in
132 several other subject areas such as biochemistry (21.1%), environmental science
133 (8.6%), immunology (13.1%) and chemistry (6.6%), thus demonstrating the
134 multidisciplinary approach characterizing investigations of one of the most used
135 beneficial organisms as active ingredient of commercial biological control (Collinge et
136 al., 2022).

137

138 **Figure 2.** Statistics concerning available literature focusing on *Trichoderma* spp. (Source:
139 www.scopus.com).
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141

142 Despite the intense research activity focusing on isolates and species belonging to the
143 genus *Trichoderma* as beneficial fungi, very few commercial products intended for
144 agriculture were on the market by the end of the last century (Harman, 2000), and these
145 were commercialized for very specific purposes, such as the control of a particular
146 pathogen or applied to a narrow range of plant species.

147 The first real breakthrough in the application of biocontrol came in the 1990s when
148 Gary E. Harman decided that his research efforts should be transferred from the lab to
149 the market. During those years, he devoted his research activity toward the development
150 of biocontrol systems to be used in commercial agriculture, thus including additional
151 efforts such as patenting, registration, commercial production and development of
152 biological control products. In line with this new approach, he cofounded the company

153 TGT Inc. (in 2000 called BioWorks, Inc.) with the aim of transferring biocontrol
154 research results to a tool available to farmers (Harman, 2000). This was a successful
155 action that promoted products containing *Trichoderma harzianum* T-22 as active
156 ingredient to the top of the BCA market. With its first appearance on the U.S. market
157 in 1993, T-22 has been used as active ingredient of almost 20 different commercial
158 products (Woo et al., 2022), taking the lion's share of the market.

159 Noteworthy, the way T-22 was produced, having been obtained by using protoplast
160 fusion of two isolates, *T. harzianum* T-95, a rhizosphere-competent strain and *T.*
161 *harzianum* T-12, a strain adapted to iron-limiting conditions, in order to select a highly
162 rhizosphere-competent strain also able to compete with spermosphere bacteria (Stasz
163 et al., 1988). As a result, T-22 was more effective than both parental strains being able
164 to control several diseases in diverse environmental conditions (Harman 2000).
165 Protoplast fusion allows creating stable haploid recombinants even in not closely
166 related species (Annè, 1983). This technique still represents an important tool to
167 combine complex traits in a single individual, thus obtaining, after a massive selection
168 of fusion products, hybrid strains with desired properties (Stasz et al., 1988). Compared
169 with other established genetic manipulation techniques, a high frequency of
170 recombination can be achieved by fusing complete protoplast genomes (Strom and
171 Bushley, 2016). Hybrids obtained by the protoplast fusion technique are not considered
172 genetically modified organisms (GMOs), thus allowing their exploitation also in those
173 countries where GMOs are not permitted (Collinge and Sarrocco, 2022).
174 Recombination of polygenic traits, such as those connected with the beneficial
175 mechanisms of action of BCAs, in stable hybrids is difficult to obtain even if more
176 targeted methods of genetic modification are used (Xu et al., 2011), including genome
177 editing (Muñoz et al., 2019).

178 This success was also the fruit of the new concept highlighted by Harman who
179 revolutionized the myths and dogmas until then universally accepted within the
180 biocontrol community. He concluded that ideas such as that a BCA would be
181 necessarily less effective and less reliable than agro-chemicals and that, if added to
182 roots or inoculated into the soil, it could not affect the microbial community or control
183 a root pathogen for a long period of time, were not generally applicable to all biocontrol
184 systems, such as those he was working with. Similarly at that time, the biocontrol
185 community accepted that a single BCA could not be effective in multiple environments,
186 on different crops or against a wide range of pathogens, and also that BCAs have single
187 mechanism of action controlled by one or a few genes. His message was that the
188 acceptance of these statements as largely and wholly true by biocontrol researchers
189 could have represented an obstacle for biocontrol progress (Harman, 2000).

190 Fortunately, history tells us that things went in another direction to the point that
191 biocontrol in general, and *Trichoderma* in particular, forcefully entered the Genomic
192 Era (Mukherjee et al., 2013). The translational research performed on these fungi
193 allowed moving from “omics” to the field (Lorito et al., 2010). Structural and functional
194 genomics, as well as transcriptomics, proteomics and metabolomics expanded the
195 understanding and application of *Trichoderma* used as BCA for plant disease control.
196 Not only as an efficient mycoparasite, but also as a “hacker” of plant gene expression
197 (Shoresh et al., 2010). *Trichoderma* can interact with plant roots activating plant
198 immune responses by the induction of defense mechanisms, a mechanism known as
199 priming (Conrath et al., 2015). *Trichoderma* recognition by the plant triggers a
200 modulation of defense-related genes, in addition to the activation of antioxidant systems
201 and phytohormone signalling, that results in an increase of the systemic immune
202 responses of the host to the attack by plant pathogens and improves the tolerance to

203 abiotic stresses (Woo et al., 2022). Modulation of defense responses in plants have been
204 described in several cases as the result of the intimate relationships between
205 *Trichoderma* isolates and the host (Mendoza-Mendoza et al. 2018; Moran-Diez et al.,
206 2021; Sarrocco et al., 2017; Risoli et al., 2021; Sarrocco et al., 2021). *Trichoderma*
207 species are also prolific producers of specialized metabolites whose ecological and
208 biological significance play an important role in biocontrol (Vicente et al., 2022).

209 However, if Harman represented a milestone in the revolution of *Trichoderma* spp. as
210 active ingredient of commercial products for the control of plant disease in the field,
211 one must not forget Christian P. Kubicek, whose profuse research activity resulted in
212 around 300 papers dealing with the biology, ecology, and evolution of *Trichoderma*, in
213 addition to patents and books, activities that earned him several honors at the
214 international level. He must be considered the driver who pushed the evolution of
215 research on these fungi, with his review “*Trichoderma*: the genomics of opportunistic
216 success” as the most cited (Druzhinina et al., 2021). Kubicek’s studies are a milestone
217 in understanding the chemistry, metabolic regulation, ecology, and functional gene
218 characterization aimed to use *Trichoderma* strains in industry and agriculture
219 (Druzhinina et al., 2011; Druzhinina and Kubicek, 2016; Kubicek et al., 2019). He was
220 the first Chair of the International Subcommittee on *Trichoderma* Taxonomy (ICTT;
221 formerly International Subcommittee on *Trichoderma* and *Hypocrea* - ISTH) and
222 made the first and most significant comparative genomic studies for this genus,
223 implementing the DNA Barcoding and Genealogical Concordance Phylogenetic
224 Species Recognition concept in *Trichoderma* taxonomy.

225 None of the targets reached in *Trichoderma* R&D would have been achieved without
226 the contribution of Kubicek and, possibly, *Trichoderma* would not have been the major
227 tool used in biological control of plant diseases.

228 The success of *Trichoderma* as commercial biocontrol agent is now confirmed by the
229 growing trend of *Trichoderma*-based products in the global market, from 21 strains
230 worldwide registered in 2014 (Woo et al., 2014) to 144 registrations in 40 countries in
231 2022, including 11 *Trichoderma* species and 44 strains, as summarized in a recent
232 review by Woo et al. (2022). In their survey, Latin America has the most active market,
233 with Brazil (28% of total registrations) at the top of the list, while the European Union
234 includes the 15% of registrations in 22 countries. Even if the information provided is
235 not fully complete due to the diverse registration procedures used by the different
236 countries (Woo et al. 2022), these data are a robust example of how *Trichoderma* still
237 occupies, as single or combined ingredient, a relevant position among commercial
238 biocontrol agents across a wide field of crop and disease applications (Collinge et al.,
239 2022; Woo et al., 2022),

240

241 ***Cryphonectria parasitica* vs. *Cryphonectria parasitica***

242 Within the range of mechanisms of action that biocontrol of plant diseases can be based
243 on, hypovirulence is one of the most fascinating, not only in view of its specificity but
244 also for its evolutionary implications. In fungal plant pathogens, hypovirulence refers
245 to the reduced ability of fungal isolates belonging to a pathogenic population to infect,
246 colonize or kill a specific plant host (Boland, 2003). Associated with double- or single-
247 stranded RNA fungal viruses (mycoviruses), hypovirulence has been described in many
248 pathogenic fungi such as *Fusarium* spp. (Lee et al., 2011), *Sclerotinia sclerotiorum*
249 (Boland et al., 2004), *Ophiostoma novo-ulmi*, *Heterobasidion* spp. (Vainio et al., 2018),
250 and *Alternaria alternata* (Li et al., 2019), just to cite a few (Garcia-Pedrajas et al.,
251 2019). Two main hypotheses have been formulated to explain the origin of
252 mycoviruses: the “ancient coevolution hypothesis” stating that mycoviruses evolved

253 from the association between viruses and fungi, and the “plant virus hypothesis”
254 suggesting that mycoviruses evolved - relatively recently - from plant viruses (Son et
255 al., 2015).

256 Undoubtedly, the first example of hypovirulence applied to biocontrol of plant disease
257 was the pathosystem *Castanea* spp./*Cryphonectria parasitica*, the causal agent of
258 chestnut blight (Figure 3A). Originating in Asia, chestnut blight is a disease affecting
259 American and European chestnut trees (first record is from 1904 in New York, U.S.)
260 and is still considered as a textbook example of an introduced pathogen that caused
261 devastating disease epidemics on native tree species (Anderson and Anderson, 1912).

262 Although classified as a quarantine pathogen by EPPO in 2005 to limit its spread in
263 Europe (EPPO, 2005), asymptomatic infected plants, which cannot be detected through
264 visual inspections, are still a major problem today (Rigling and Prospero, 2018). In *C.*
265 *parasitica*, hypovirulence refers to a viral disease caused by ssRNA(+) mycoviruses
266 located in the fungal cytoplasm (Choi and Nuss, 1992). The virus, *Cryphonectria*
267 *hypovirus* (CHV), belongs to the family *Hypoviridae* and was renamed in 2021 to
268 *Alphahypovirus chryphonectriae*, according to the proposal to divide the family
269 *Hypoviridae* into three new genera (Suzuki et al., 2018). First observed in Italy in the
270 1950s (Heiniger and Rigling, 1994), it was only in 1965 that Jean Grente fully
271 understood the biological potential of hypovirulence (Grente, 1965), thus giving way
272 to several studies that marked a significant breakthrough in the control of chestnut
273 blight. Transferred to other fungal individuals by horizontal transmission (via
274 anastomosis or during mating) or by vertical transmission (via asexual sporogenesis),
275 CHV-1 - probably introduced to Europe together with its fungal host (Feau et al., 2014,
276 Ježić et al., 2021) - can infect a large proportion of the pathogen population (Prospero
277 et al., 2006). Vegetative compatibility barriers narrowly regulate the diffusion among

278 *C. parasitica* populations (Grente, 1975). In areas where the incidence of hypovirulence
279 is very low or does not exist, the hypovirus (usually CHV-1) can be artificially
280 introduced by treating chestnut cankers with a hypovirulent *C. parasitica* strain, which
281 is then transmitted to virulent pathogenic isolates via hyphal anastomosis (Prospero and
282 Rigling, 2016), thus resulting in a reduction or arrest of canker expansion (healing
283 canker, Figure 3B).

284

285 **Figure 3.** *Castanea sativa* shoot showing symptoms of chestnut blight (A): chestnut shoot showing a
286 healing canker (B) as result of hypovirulence (kindly provided by Giovanni Vannacci).
287

288 In Europe, the therapeutic use of hypovirulence effectively contributes to contain
289 chestnut blight (Prospero and Rigling, 2016). However, in North America the
290 application of hypovirulent strains resulted in an almost complete failure mainly due to
291 the high diversity of vegetative incompatibility group types of the pathogen, which
292 presents a barrier to anastomosis, and therefore the most important obstacle to
293 hypovirulence transmission (Springer et al., 2013). Another contributing factor is the
294 higher susceptibility of the North American chestnut (*Castanea dentata*) compared with
295 that of the European chestnut (*Castanea sativa*).

296 In Europe, biocontrol of chestnut blight is largely a natural phenomenon. The ability of
297 mycoviruses to cause alterations and abnormalities in their fungal host, affecting their
298 morphology, spore production, growth, pigmentation and toxin production (Nuss,
299 2005) and the ability to modulate fungal virulence (Zhang et al., 2020) renders their use
300 as virocontrol agents deserving of further investigation (Bocos-Asenjo et al., 2022).

301 Next Generation Sequencing (NGS) techniques, such as RNAseq, are providing
302 revolutionary support to better understand the mechanisms underpinning the
303 hypovirulent effects of mycoviruses on their fungal hosts. Such studies investigated the

304 mechanisms involved in the way mycoviruses regulate fungal genes at a transcriptome
305 level (Chun et al., 2020), and allowed the identification of an unexpected number -
306 destined to increase in the future - of new mycoviruses. As an example,
307 metatranscriptomics provided information about the viromes of five plant pathogenic
308 fungi and identified 66 previously unknown mycoviruses (Lee et al., 2016). Double-
309 stranded RNA-seq and total RNA-seq have been used to study several fungal specimens
310 discovering that more than 20% possessed one or more mycoviruses (Myers et al.,
311 2020), such as in *B. cinerea*, whose mycovirome has been profusely investigated (Ruiz-
312 Padilla et al., 2021) or in *S. sclerotiorum* where two new mycoviruses were discovered
313 in a hypovirulent strain (Wang et al., 2019). Results derived from the use of these new
314 high-throughput technologies could represent a breakthrough to widen the exploitation
315 of this biocontrol strategy by exploiting other mycoviruses against fungal pathogens
316 affecting horticultural, agricultural, agroforestry, or viticultural crops and in other
317 pathosystems, even where transmission is reduced due to the horizontal transmission
318 handicap (Bocos-Asenjo et al., 2022).

319 The example of *Chryphonectria* highlights once more the importance of the knowledge
320 of the biology and ecology of both the pathogen and the corresponding BCA, a concept
321 that will emerge even stronger in the following sections.

322

323 **Suppressive soils: a natural microbial make-up to control soilborne diseases**

324 When in 1983 David Hornby published his contribution on suppressive soils in the
325 *Annual Review of Phytopathology* (Hornby, 1983), he introduced his manuscript by
326 reporting how the word “suppressive soil(s)” appeared more and more frequently
327 during the previous 10 years as an ever-expanding area of interest in the field of
328 biological control. Being himself an expert of a topic directly involving suppressive

329 soils (take-all decline disease of wheat caused by *Gaeumannomyces* and suppressed by
330 *Pseudomonas* spp.), Hornby explored critically what writers and researchers meant by
331 suppressive soils in order to decide whether it was a new topic or merely represented
332 old ideas under a new name. By considering the difficulties of giving an exact definition
333 for suppressive soils, he highlighted two extreme positions, namely “soils which were
334 unfit for the development of certain diseases” (Mangenot and Diem, 1979) and “soils
335 in which there was a natural reduction of disease incidence” (Papavizas and Lumsden,
336 1980). Since suppressiveness could not be considered as an absolute phenomenon,
337 Claude Alabouvette (the pioneer of research on soils suppressive to Fusarium wilt)
338 proposed to use the term "disease-reducing soils", as suggested previously by David W.
339 Burke (Alabouvette et al., 1979).

340 However this phenomenon (or perhaps these phenomena) have been defined, the most
341 important point that revolutionized the biological control of soilborne diseases was the
342 centralization of the role played by soil, acting not as a neutral milieu, where pathogens
343 can freely establish a negative relationship with their host plants, but where soil
344 determines how these plant-pathogen interactions develop to the point of being able to
345 modify the outcome. As discussed in detail by Sagova-Mareckova et al. (2023) in this
346 issue, suppressive soils can act under two models, namely in a general or in a specific
347 way. In the former case, the activities of several resident soil organisms, usually a
348 natural and pre-existing characteristic community, act against most, if not all,
349 pathogens. In contrast, in the latter case, suppression is directed toward specific
350 pathogenic organisms, as exemplified by the cases of Fusarium wilts, caused by *F.*
351 *oxysporum*, as well as in take-all disease caused by *Gaeumannomyces graminis* var.
352 *tritici* (Chandrashekara et al., 2012). The hypothesis is that pathogen infection triggers
353 host signals to recruit beneficial microbes, the “cry for help” hypothesis which states

354 that roots can selectively enrich plant-protective microbes when they are diseased or
355 under attack by pathogens (Bakker et al., 2018). This hypothesis fits what happens in
356 the rhizosphere for *Pseudomonas* spp., found to be enriched in several disease -
357 suppressive soil systems (Haas and Defago, 2005). Although several antagonistic
358 species have been reported as potential BCAs against take-all decline, evidence
359 confirmed the major role played by *Pseudomonas* spp. (Schlatter et al., 2017),
360 particularly those able to produce the antibiotic 2,4-diacetylphloroglucinol (DAPG]
361 (Landa et al., 2006). DAPG producers have been deeply studied, revealing significant
362 genetic diversity among isolates (Weller et al. 2007) in the *P. fluorescens* complex
363 (Loper et al. 2012). However it was only with the application of whole-genome
364 sequencing that the diversity within the DAPG-producing pseudomonads was clarified
365 with at least three species (*P. fluorescens*, *P. protegens*, and *P. brassicacearum*)
366 indicated as the major producers (Loper et al. 2012). This is in line with what was stated
367 by Weller et al. (2002), *i.e.*, that take-all suppressive soils require shifts in specific
368 antibiotic-producing *Pseudomonas* populations.

369 Studies applied to this context moved from investigations focusing on the inundative
370 application of specific BCAs aimed to improve the suppressive performance of soils,
371 to the understanding of the ways in which indigenous microbial populations could
372 reduce disease, even in the presence of virulent pathogens and susceptible hosts, and
373 when environmental conditions are favorable to the disease. Microbiome investigations
374 confirmed this drastic shift of the microbiota upon infection (Schlatter et al., 2017).

375 Hornby in 1983 concluded his review noting the need for new and different techniques,
376 as well as for new concepts and skills, for the breakthroughs needed for harnessing
377 suppressive soils for the biocontrol of soilborne pathogens. Today we can state that
378 these new techniques are available and are already applied to conceive suppressive soils

379 in a different way. New technologies, such as high-throughput sequencing of PCR-
380 amplified products have revolutionized microbial ecology. NGS, particularly
381 metagenomics, provides new opportunities to investigate more deeply the diversity and
382 function of soil microbial communities at genus and species levels. As an example,
383 metagenomics - where the whole DNA is extracted from a single sample and sequenced
384 in parallel - provides a deeper analysis concerning the diversity and the functional
385 potential of microbial communities, also including those isolates that are commonly
386 defined as non-culturable (Kuske et al., 2015; Jiang et al., 2016). While accuracy and
387 accessibility of these tools are improving, their cost is declining, thus allowing them to
388 become routine in both laboratory and in the field (Schlatter et al., 2017). In addition,
389 proteomics and metabolomics analysis can furnish useful insights into the key markers
390 responsible for imparting antagonism against soilborne plant pathogens in these natural
391 microbe-based plant defense examples, where the disease suppressive potential of a soil
392 still remains dependent on several factors such as soil pH or soil type (Kathri et al.,
393 2021).

394

395 **From “silver bullet” to SynCom**

396 The lessons learned from suppressive soils, including the role microbial community
397 plays in the suppressive features of these soils, suggest that microbial consortia may
398 work better than a single BCA isolate. For many years, especially in the first decades
399 of the biological control era, the exploitation of a well-selected and developed single
400 genetic individual as a biocontrol agent for a specific pathogen/disease has been
401 considered a “silver bullet”, *i.e.* a magic solution to finally win the battle (Vannacci and
402 Sarrocco, 2018). Such approaches missed one of the core principles of microbial
403 ecology, where the ecology of the pathogens, the plants and the beneficial individuals

404 was overshadowed. The awareness of the impact of microbial communities on this tri-
405 partite interactions has been strengthened during the last 10 years and a deeper
406 understanding of these four-actor relationships are providing unexpected opportunities
407 to develop innovative biocontrol methods (Sebastien et al., 2015); this can be
408 considered a real breakthrough in biological control.

409 The plant innate immune system is important for eliminating plant pathogens and for
410 controlling accommodation of beneficial microbes. The balancing act by the plant host
411 of these two activities, apparently in conflict, implies a primary role of the innate
412 immune system in maintaining microbial homeostasis for plant health, thus explaining
413 why all healthy plants establish an intimate association with microbes (Hacquard et al.,
414 2017). The observation that diseases affecting monoculture of crops can modulate
415 microbial community changes to favor disease-suppressive soils (the “cry for help”
416 hypothesis) gave rise to a new theory, supported by an increasing number of studies
417 that identified the role of plant genetic components in reshaping the microbiota in
418 response to stresses. The composition of microbiota can be temporally and spatially
419 controlled by plants in response to environmental perturbations, thus highlighting the
420 importance of a precise make-up of the microbiota in environment adaptation, much
421 more powerful than a single inoculation of “beneficial microbes” (Wang and Yi, 2022).
422 This new perspective on the role played by microbiota opened the possibility to
423 artificially set up tailor-made Synthetic Microbial Communities (SynCom) conceived
424 to assure a durable and flexible protective activity toward plants (Vannier et al., 2019),
425 potentially more efficient than individual strains (Duran et al., 2018). Probably because
426 functional redundancy can confer higher robustness and adaptability, it is frequently
427 observed that multi-isolate consortia can outperform single isolates, thus suggesting
428 how SynComs can offer a unique opportunity for improving upon existing community

429 functions by acting as better inoculants (Eng and Borenstein, 2019). However, it is not
430 fully understood whether artificial microbial communities can outcompete the natural
431 communities already interacting with the plant (Agoussar and Yergeau, 2021).

432 A number of microbes - usually varying from 30 to 200 isolates - can be selected to
433 assemble an artificial SynCom (Wang and Yi, 2022), characterized by a taxonomical
434 composition similar to that of the natural soil communities, aimed to best mimic the
435 function of the original one (Liu et al., 2019). However, one of the main conditions in
436 the design of a SynCom is that microorganisms must be culturable (Marin et al., 2021).

437 In other fields, such as human microbiology, metagenomics revealed that the majority
438 of bacteria remain uncultured, while culturomics - consisting of multiple culture
439 conditions combined with the rapid identification of bacteria – allowed the culture and
440 identification of unknown bacteria, thus enabling the culture of hundreds of new
441 microorganisms and providing exciting new perspectives on host–bacteria relationships
442 (Lagier et al., 2018; Diakite et al., 2020)

443 Recently, machine learning methods allowed researchers to mathematically predict key
444 strains related to community functions (Herrera et al., 2018), thus reinforcing the
445 “tailor-made” concept of SynCom. But it is clear that further investigations are needed
446 to deeply understand the genetic and biochemical mechanisms modulating
447 host/microbiota interactions (especially under stress conditions) in order to support the
448 potential of paving the way toward the design of highly performing SynComs.

449

450 **Perspectives and final remarks**

451 The use of beneficial (micro)organisms is becoming critical in the management of plant
452 diseases in several parts of the world, mainly due to their sustainable features (Food
453 Safety, EU Commission, <https://food.ec.europa.eu/plants/pesticides/micro->

454 organisms_en). Concomitantly, we can see a shift in the direction of research. From the
455 “silver bullet” approach, inherited from the use of synthetic chemistry in crop
456 protection, through the use of Synthetic Communities, both following an inundative
457 and industrial strategy, we will hopefully arrive to manage the resident natural
458 (micro)organisms thanks to the knowledge we will acquire around the “cry for help”
459 phenomenon. This will be a change in the perspective: less dependence on inundative
460 treatments but greater reliance on resident (micro)biota to increase resilience of crops,
461 thus approaching one of the ten elements of agroecology, where resilience is key to
462 sustainable food and agricultural systems (FAO, 2018).

463 Undoubtedly, the knowledge of the impact of the plant microbiome on improving plant
464 growth and survival in responses to biotic and abiotic stresses enhances the availability
465 of tools aimed to enhance crop productivity (Trivedi et al., 2022). For example, plant
466 microbiomes could be included within breeding processes to introduce additional
467 genetic variation, such as propagating plants in the presence of the microbiome in order
468 to maintain plant-microbe interactions when introduced into new environments (Wand
469 and Haney, 2020). Similarly, better understanding of how the assembly of stress-
470 alleviating microbiota is governed would support the design of crops able to
471 dynamically enlist beneficial microbiota under stress conditions (Wang and Song,
472 2022).

473 During the 1970s Lester R. Brown - author and co-author of over 50 books on global
474 environmental issues - helped pioneer the concept of sustainable development.
475 Presently, after more than 50 years, we can still appreciate the modernity of Brown’s
476 thinking, and, in the context of plant pathology and plant protection, we cannot escape
477 from the specter of climate change and its role as one of the main driving forces for the
478 emergence and re-emergence of pathogens and diseases (El-Sayed and Kamel, 2020).

479 Climate change, together with intensive agricultural practices, is the main factor
480 responsible for biodiversity loss, new global distribution of plant pathogens,
481 contamination of soil, air and water resources with negative impact on agroecosystem
482 and human health (De Clerck et al, 2021). In view of the need for sustainable
483 development and considering the efforts made to improve the knowledge on biological
484 control and render it a reality for the management of plant diseases, the use of beneficial
485 (micro)organisms must now more than ever be a driver of agricultural research in order
486 to improve the actual social situation where access to food is a prerogative.

487

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495

496 **References**

- 497 Agoussar, A., and Yergeau, E. 2021. Engineering the plant microbiota in the context of
498 the theory of ecological communities. *Current Opinion in Biotechnology*. 70:220-
499 225. <https://doi.org/10.1016/j.copbio.2021.06.009>.
- 500 Alabouvette, C., Rouxel, F., and Louvet, J. 1979. Characteristics of fusarium-wilt
501 suppressive soils and prospects for their utilization in biological control, 165-182. In:
502 B. Schippers, W. Gams: "Soil-Borne Plant Pathogens";. Academic Press, 686 pp.

- 503 Anderson, P.J. and Anderson, H.W. 1912. The chestnut blight fungus and a related
504 saprophyte. *Phytopathology*. 2:204–210.
- 505 Anné, J. 1983. Protoplasts of filamentous fungi in genetics and metabolite production.
506 *Experientia Supplementum*. 46:167-78. doi: 10.1007/978-3-0348-6776-4_21.
- 507 Baker, K.F., and Cook, R.J., 1974. Biological control of plant pathogens. American
508 Phytopathological Society, St. Paul, MN.
- 509 Bakker, P.A.H.M., Pieterse, C.M.J., de Jonge, R., and Berendsen, R.L. 2018. The soil-
510 borne legacy. *Cell*. 172:1178–1180.
- 511 Berg, G., and Koskella, B. 2018. Nutrient- and Dose-Dependent Microbiome-Mediated
512 Protection against a Plant Pathogen. *Current Biology*. 28(15):2487–92 e3.
- 513 Bocos-Asenjo, I.T., Niño-Sánchez, J., Ginésy, M., and Diez, J.J. 2022. New Insights on
514 the Integrated Management of Plant Diseases by RNA Strategies: Mycoviruses and
515 RNA Interference. *International J. Molecular Sciences*. 23:9236.
516 <https://doi.org/10.3390/ijms23169236>
- 517 Boland, G.J. 2004. Fungal viruses, hypovirulence, and biological control of *Sclerotinia*
518 species. *Canadian J. Plant Pathology*. 26(1):16-18. DOI:
519 [10.1080/07060660409507107](https://doi.org/10.1080/07060660409507107).
- 520 Burdon, J.J., and Zhan, J. 2020. Climate change and disease in plant communities. *PLoS*
521 *Biology*. 18(11): e3000949. <https://doi.org/10.1371/journal.pbio.3000949>.
- 522 Chandrashekara, C., Kumar, R., Bhatt, J.C., and Chandrashekara, K. 2012. Suppressive
523 soils in plant disease management. In: *Eco-Friendly Innovative Approaches in Plant*
524 *Disease Management*; Singh, V.K., Singh, Y., Singh, A., Eds.; International Book
525 Distributors and Publisher: New Delhi, India; pp. 241–256.
- 526 Codex Alimentarius. 2022. [https://www.fao.org/food-safety/food-control-](https://www.fao.org/food-safety/food-control-systems/policy-and-legal-frameworks/codex-alimentarius/en/)
527 [systems/policy-and-legal-frameworks/codex-alimentarius/en/](https://www.fao.org/food-safety/food-control-systems/policy-and-legal-frameworks/codex-alimentarius/en/).

- 528 Collinge, D.B., and Sarrocco, S. 2022. Transgenic approaches for plant disease control:
529 status and prospects 2022. *Plant Pathology*. 71:207-225.
530 <https://doi.org/10.1111/ppa.13443>.
- 531 Collinge, D.B., Jensen, D.F., Rabiey, M., Sarrocco, S., Shaw, M.W., and Shaw, R. 2022.
532 Biological control of plant diseases –What has been achieved and what is the
533 direction? *Plant Pathology*. 71(5):1–241024-1047.
534 <https://doi.org/10.1111/ppa.13555>.
- 535 Conrath, U., Beckers, G.J., Langenbach, C.J., and Jaskiewicz, M.R. 2015. Priming for
536 enhanced defense. *Annual Review of Phytopathology*. 53:97-119.
- 537 Chun, J., Ko, Y.H., and Kim, D.H. 2020. Transcriptome Analysis of *Cryphonectria*
538 *parasitica* Infected with *Cryphonectria Hypovirus 1* (CHV1) Reveals Distinct Genes
539 Related to Fungal Metabolites, Virulence, Antiviral RNA-Silencing, and Their
540 Regulation. *Frontiers in Microbiology*. 11:1711.
- 541 DeClerck, F.A.J., Koziell, I., Benton, T., Garibaldi, L.A., Kremen, C., Maron, M., et al.
542 2021. A Whole Earth Approach to Nature Positive Food: Biodiversity and
543 Agriculture. Bonn United Nations Food Systems Summit 2021 – Scientific Group 1–
544 26 (CGIAR, 2021). <https://doi.org/10.48565/scfss2021-h174>.
- 545 Diakite, A., Dubourg, G., Dione, N., Afouda, P., Bellali, S., Ngom, I.I., Valles, C., Tall,
546 M.L., Lagier, J.C., and Raoult, D. 2020. Optimization and standardization of the
547 culturomics technique for human microbiome exploration. *Science Reports*.
548 15:10(1):9674. doi: 10.1038/s41598-020-66738-8.
- 549 Druzhinina, I.S., and Kubicek, C.P. 2016. Familiar Stranger: Ecological Genomics of the
550 Model Saprotroph and Industrial Enzyme Producer *Trichoderma reesei* Breaks the
551 Stereotypes. *Advances in Applied Microbiology*. 95:69-147. doi:
552 10.1016/bs.aambs.2016.02.001.

- 553 Druzhinina, I.S., Seidl-Seiboth, V., Herrera-Estrella, A., Horwitz, B.A., Kenerley, C.M.,
554 Monte, E., Mukherjee, P.K., Zeilinger, S., Grigoriev, I.V., and Kubicek, C.P. 2011.
555 *Trichoderma*: the genomics of opportunistic success. *Nature Review Microbiology*.
556 9(10):749-59. doi: 10.1038/nrmicro2637.
- 557 Duran, P., Thiergart, T., Garrido-Oter, R., Agler, M., Kemen, E., Schulze-Lefert, P., et al.
558 2018. Microbial Interkingdom Interactions in Roots Promote Arabidopsis Survival.
559 *Cell*. 175(4):973–83 e14.
- 560 El-Sayed, A., and Kamel, M. 2020. Climatic changes and their role in emergence and re-
561 emergence of diseases. *Environmental Science and Pollution Research*.
562 27(18):22336-22352. doi: 10.1007/s11356-020-08896-w.
- 563 Eng, A. and Borenstein, E. 2019. Microbial community design: methods, applications,
564 and opportunities. *Current Opinion Biotechnology*. 58:117-128.
565 <https://doi.org/10.1016/j.copbio.2019.03.002>
- 566 EPPO 2005. EPPO Standards – Diagnostic protocols for regulated pests - PM7/45
567 *Cryphonectria parasitica*. EPPO Bull. 35:295–298.
- 568 FAO, IFAD, and WFP. 2013. The State of Food Insecurity in the World: The Multiple
569 Dimensions of Food Security 2013 (FAO, 2013).
- 570 FAO. 2018. The 10 elements of Agroecology.
571 <https://www.fao.org/documents/card/en/c/I9037EN/>.
- 572 García-Pedrajas, M.D., Cañizares, M.C., Sarmiento-Villamil, J.L., Jacquat, A.G., and
573 Dambolena, J.S. 2019. Mycoviruses in Biological Control: From Basic Research to
574 Field Implementation. *Phytopathology*. 109:1828-1839.
575 <https://doi.org/10.1094/PHYTO-05-19-0166-RVW>.
- 576 Grente, M.J. 1975. La lutte biologique contre le chancre du chataignier par
577 “hypovirulence contagieuse”. *Annual Review of Phytopathology*. 7:216–218.

578 Hacquard, S., Spaepen, S., Garrido-Oter, R., and Schulze-Lefert, P. 2017. Interplay
579 Between Innate Immunity and the Plant Microbiota. *Annual Review of*
580 *Phytopathology*. 55(1). doi:10.1146/annurev-phyto-080516-035623.

581 Haas, D., and Defago, G. (2005). Biological Control of Soil-Borne Pathogens by
582 Fluorescent Pseudomonads. *Nature Reviews Microbiology*. 3:307–19.
583 <https://doi.org/10.1038/nrmicro1129>.

584 Heiniger, U. and Rigling, D. 2009. Application of the *Cryphonectria Hypovirus (CHV-*
585 *I)* to control the chestnut blight, experience from Switzerland. *Acta Horticulturae*.
586 815:233–245.

587 Herrera Paredes, S., Tianxiang, G.T., Law, T.F., Omri, M., Finkel, O.M, Mucyn, T.,
588 Teixeira, P.J.P.L., Salas González, I., et al. 2018. Design of Synthetic Bacterial
589 Communities for Predictable Plant Phenotypes. *PLoS Biology*. 16: e2003962.
590 <https://doi.org/10.1371/journal.pbio.2003962>.

591 Hoffmann, V., Paul, B., Falade, T. et al. 2022. A one health approach to plant health.
592 *CABI Agric Biosci* 3:, 62 (2022). <https://doi.org/10.1186/s43170-022-00118-2>.

593 Hornby, D. 1983. Suppressive soils. *Annual Review of Phytopathology*. 21(1):65-85.

594 Ježić, M., Schwarz, J.M., Prospero, S., Sotirovski, K., Risteski, M., Ćurković-Perica, M.,
595 Nuskern, L., Krstin, L., Katanić, Z., Maleničić, E., Poljak, I., Idžojtić, M., and
596 Rigling, D. 2021. Temporal and Spatial Genetic Population Structure of
597 *Cryphonectria parasitica* and Its Associated Hypovirus Across an Invasive Range of
598 Chestnut Blight in Europe. *Phytopathology*. 111(8):1327-1337.
599 <https://doi.org/10.1094/PHYTO-09-20-0405-R>.

600 Jiang, Y., Xiong, X., Danska, J., and Parkinson, J. 2016. Metatranscriptomic analysis of
601 diverse microbial communities reveals core metabolic pathways and microbiome-
602 specific functionality. *Microbiome*. 4:2. <https://doi.org/10.1186/s40168-015-0146-x>

- 603 Khatri, S., Bhattacharjee, A., and Sharma, S. 2021. “Omics” Approaches for
604 Understanding Soil Suppressiveness in Agriculture. In: Pudake, R.N., Sahu, B.B.,
605 Kumari, M., Sharma, A.K. (eds) Omics Science for Rhizosphere Biology.
606 Rhizosphere Biology. Springer, Singapore. [https://doi.org/10.1007/978-981-16-](https://doi.org/10.1007/978-981-16-0889-6_4)
607 [0889-6_4](https://doi.org/10.1007/978-981-16-0889-6_4).
- 608 Kubicek, C.P., Herrera-Estrella, A., Seidl-Seiboth, V., Martinez, D.A., Druzhinina, I.S.,
609 Thon, M., Zeilinger, S., Casas-Flores, S., Horwitz, B.A., and Mukherjee, P.K. 2011.
610 Comparative genome sequence analysis underscores mycoparasitism as the ancestral
611 life style of *Trichoderma*. Genome Biology. 12(4):R40. doi.org/10.1186/gb-2011-12-
612 4-r40.
- 613 Kubicek, C.P., Steindorff, A.S., Chenthamara, K. et al. 2019. Evolution and comparative
614 genomics of the most common *Trichoderma* species. BMC Genomics. 20:485.
615 <https://doi.org/10.1186/s12864-019-5680-7>.
- 616 Kuske, C.R., Hesse, C.N., Challacombe, J.F., Cullen, D., Herr, J.R., Mueller, R.C., Tsang,
617 A., and Vilgalys, R. 2015. Prospects and challenges for fungal metatranscriptomics
618 of complex communities. Fungal Ecology. 14:133-137.
- 619 Landa, B.B., Mavrodi, O.V., Schroeder, K.L., Allende-Molar, R., and Weller, D.M. 2006.
620 Enrichment and genotypic diversity of pHID-containing fluorescent *Pseudomonas*
621 spp. in two soils after a century of wheat and flax monoculture. FEMS Microbiology
622 Ecology. 55:351-368. <https://doi.org/10.1111/j.1574-6941.2005.00038.x>.
- 623 Lagier, J.C., Dubourg, G., Million, M. et al. 2018. Culturing the human microbiota and
624 culturomics. Nature Review Microbiology. 16:540–550.
625 <https://doi.org/10.1038/s41579-018-0041-0>.
- 626 Lee, S.-Y., Nelson, B.D., Ajayi-Oyetunde, O., Bradley, C.A., Hughes, T.J., and Hartman,
627 G.L., Eastburn, D.M., and Domier, L.L. 2016. Identification of Diverse Mycoviruses

628 through Metatranscriptomics Characterization of the Viromes of Five Major Fungal
629 Plant Pathogens. *Journal of Virology*. 90:6846–6863.

630 Lee, K.M., Yu, J., Son, M., Lee, Y., and Kim, K.H. 2011. Transmission of *Fusarium*
631 *boothii* mycovirus via protoplast fusion causes hypovirulence in other
632 phytopathogenic fungi. *PLoS One*. 6:e21629.
633 <https://doi.org/10.1371/journal.pone.0021629>.

634 Li, H., Bian, R., Liu, Q., Yang, L., Pang, T., Salaipeh, L., Andika, I.B., Kondo, H. and
635 Sun, L. 2019. Identification of a novel hypovirulence-inducing hypovirus from
636 *Alternaria alternata*. *Frontiers in Microbiology*. 10:1076. doi:
637 10.3389/fmicb.2019.01076.

638 Lorito, M., Woo S.L., Harman G.E., and Monte E. 2010. Translational Research on
639 *Trichoderma*: From 'Omics to the Field. *Annual Review of Phytopathology*.
640 48(1):395–417. doi:10.1146/annurev-phyto-073009-114314.

641 Loper, J.E., Hassan, K.A., Mavrodi, D.V., Davis, E.W., II, Lim, C.K., and Shaffer, B.T.
642 2012. Comparative genomics of plant-associated *Pseudomonas* spp.: Insights into
643 diversity and inheritance of traits involved in multitrophic interactions. *PLoS*
644 *Genetics*. 8:e1002784. <https://doi.org/10.1371/journal.pgen.1002784>.

645 Maloi, O.C. and Lang, K.J. 2003. CARL FREIHERR VON TUBEUF: Pioneer in
646 Biological Control of Plant Diseases. *Annual Review of Phytopathology*. 41:41–52.
647 doi: 10.1146/annurev.phyto.41.052002.095444

648 Mangenot, F. and Diem, H. G. 1979. Fundamentals of biological control. In: *Ecology of*
649 *Root Pathogens*, ed. S.V. Krupa, Y.R. Dommergues, pp. 207-66. Amsterdam:
650 Elsevier. 281 pp.

651 Marín, O., González, B., and Poupin, M.J. 2021. From Microbial Dynamics to
652 Functionality in the Rhizosphere: A Systematic Review of the Opportunities With

- 653 Synthetic Microbial Communities. *Frontiers in Plant Science*. 12:650609. doi:
654 10.3389/fpls.2021.650609.
- 655 Massart, S., Martinez-Medina M., and Jijakli M.H. 2015. Biological control in the
656 microbiome era: Challenges and opportunities. *Biological Control*. 89:98-108.
657 <https://doi.org/10.1016/j.biocontrol.2015.06.003>.
- 658 Mendoza-Mendoza, A., Zaid, R., Lawry, R., Hermosa, R., Monte, E., Horwitz, B.A., and
659 Mukherjee, P.K. 2019. Molecular dialogues between *Trichoderma* and roots: Role of
660 the fungal secretome, *Fungal Biology Reviews*. 32(2):62-85.
661 <https://doi.org/10.1016/j.fbr.2017.12.001>.
- 662 Morán-Diez, M.E., Martínez de Alba, Á.E., Rubio, M.B., Hermosa, R., and Monte, E.
663 2021. *Trichoderma* and the plant heritable priming responses. *Journal of Fungi*. 7:318.
- 664 Mukherjee, P.K., Horwitz, B.A., Herrera-Estrella, A., Schmoll, M., and Kenerley, C.M.
665 2013. *Trichoderma* Research in the Genome Era. *Annual Review of Phytopathology*.
666 51(1):105–129. doi:10.1146/annurev-phyto-082712-102353.
- 667 Muñoz, I.V., Sarrocco, S., Malfatti, L., Baroncelli, R., and Vannacci, G. 2019. CRISPR-
668 Cas for Fungal Genome Editing: A New Tool for the Management of Plant Diseases.
669 *Frontiers in Plant Science*. 10:135. doi: 10.3389/fpls.2019.00135.
- 670 Myers, J.M., Bonds, A.E., Clemons, R.A., Thapa, N.A., Simmons, D.R., Carter-House,
671 D., Ortanez, J., et al. 2020. Survey of Early-Diverging Lineages of Fungi Reveals
672 Abundant and Diverse Mycoviruses. *mBio*. 11:e02027-20.
- 673 Nuss, D.L. 2005. Hypovirulence: Mycoviruses at the Fungal–Plant Interface. *Nature*
674 *Review Microbiology*. 3:632–642.
- 675 Papavizas, G.C. 1985. *Trichoderma* and *Gliocladium*: Biology, Ecology, and Potential for
676 Biocontrol. *Annual Review of Phytopathology*. 23:23-54

- 677 Papavizas, G.C., and Lumsden, R.D. 1980. Biological control of soil-borne fungal
678 propagules. *Annual Review of Phytopathology*. 18:389-413.
- 679 Prospero, S., Conedera, M., Heiniger, U. and Rigling, D. 2006. Saprophytic activity and
680 sporulation of *Cryphonectria parasitica* on dead chestnut wood in forests with
681 naturally established hypovirulence. *Phytopathology*. 96:1337–1344.
- 682 Rigling D., and Prospero, S. 2018. *Cryphonectria parasitica*, the causal agent of chestnut
683 blight: invasion history, population biology and disease control. *Molecular Plant
684 Pathology*. 19(1):7-20. doi: 10.1111/mpp.12542.
- 685 Risoli, S., Cotrozzi, L., Sarrocco, S., Nuzzaci, M., Pellegrini, E., and Vitti, A. 2022.
686 *Trichoderma*-Induced Resistance to *Botrytis cinerea* in *Solanum* Species: A Meta-
687 Analysis. *Plants*. 11:180. <https://doi.org/10.3390/plants11020180>.
- 688 Roberts, M.R., and Taylor, J.E. 2016. Exploiting plant induced resistance as a route to
689 sustainable crop production. In: Collinge, D.B. (Ed.) *Biotechnology for plant
690 disease control*. Wiley Blackwell: New York and London, pp. 319–339.
- 691 Ruiz-Padilla, A., Rodríguez-Romero, J., Gómez-Cid, I., Pacifico, D., and Ayllón, M.A.
692 2021. Novel Mycoviruses Discovered in the Mycovirome of a Necrotrophic Fungus.
693 *MBio*. 12:e03705-20.
- 694 Sagova-Mareckova, M., Omelka, M., and Kopecky, J. 2023. The golden goal of soil
695 management – disease-suppressive soils. *Phytopathology* (in press).
- 696 Sarrocco, S., Esteban, P., Vicente, I., Bernardi, R., Plainchamp, T., Domenichini, S.,
697 Vicente Munoz, I., Puntoni, G., Baroncelli, R., Vannacci, G., and Dufresne, M. 2021.
698 Straw competition and wheat root endophytism of *Trichoderma gamsii* T6085 as
699 useful traits in the biocontrol of Fusarium Head Blight. *Phytopathology*. 111:7.
700 <https://doi.org/10.1094/PHYTO-09-20-0441-R>.

- 701 Sarrocco, S., Matarese, F., Baroncelli, R., Seidl-Seiboth, V., Kubicek, C.P., Vannacci, G.,
702 and Vergara, M. 2017. The constitutive endopolygalacturonase TvPG2 regulates the
703 induction of plant systemic resistance by *Trichoderma virens*. *Phytopathology*.
704 107:537-544. <https://doi.org/10.1094/PHYTO-03-16-0139-R>
- 705 Sarrocco S., and Vannacci G. 2018. Preharvest application of beneficial fungi as a strategy
706 to prevent postharvest mycotoxin contamination: A review. *Crop Protection*. 110:
707 160-170. <https://doi.org/10.1016/j.cropro.2017.11.013>.
- 708 Schlatter, D., Kinkel, L., Thomashow, L., Weller, D., and Paulitz T. 2017. Disease
709 Suppressive Soils: New Insights from the Soil Microbiome. *Phytopathology*.
710 107(11):1284-1297. doi: 10.1094/PHYTO-03-17-0111-RVW.
- 711 Shores, M., Harman, G.E., and Mastouri, F. 2010. Induced Systemic Resistance and
712 Plant Responses to Fungal Biocontrol Agents. *Phytopathology*. 48(1):21–43.
713 doi:10.1146/annurev-phyto-073009-114450
- 714 Son, M., Yu, J., and Kim, K.H. 2015. Five Questions about Mycoviruses. *PLOS*
715 *Pathogens*. 11(11): e1005172. <https://doi.org/10.1371/journal.ppat.1005172>.
- 716 Springer, J.C., Davelos Baines, A.L., Fulbright, D.W., Chansler, M.T. and Jarosz, A.M.
717 2013. Hyperparasites influence population structure of the chestnut blight pathogen,
718 *Cryphonectria parasitica*. *Phytopathology*. 103:1280–1286.
- 719 Stasz, T.E., Harman, G.E., and Weeden, N.F. 1988. Protoplast preparation and fusion in
720 two biocontrol strains of *Trichoderma harzianum*. *Mycologia*. 80:141-150.
- 721 Strom, N.B., and Bushley, K.E. 2016. Two genomes are better than one: history, genetics,
722 and biotechnological applications of fungal heterokaryons. *Fungal Biology and*
723 *Biotechnology*. 3(1): 4.

- 724 Suzuki, N., Ghabrial, S.A., Kim, K.H., Pearson, M., Lee, Marzano, S.Y., Yaegashi, H.,
725 Xie, J., Guo, L., Kondo, H., Koloniuk, I., Hillman, B.I., and Consortium, I.R. 2018.
726 ICTV virus taxonomy profile: Hypoviridae. *J. General Virology*. 99:615–616.
- 727 Trivedi, P., Batista, B.D., Bazany, K.E., and Singh, B.K. 2022. Plant-microbiome
728 interactions under a changing world: responses, consequences and perspectives. *New*
729 *Phytologist*. 234(6):1951-1959. doi: 10.1111/nph.18016.
- 730 Tronsmo, A., Djurle, A., Munk, L., Collinge, D.B., and Yuen J. 2020. Biological control
731 of plant diseases. In: *Plant Pathology and Plant Diseases*. CABI Publishing, UK, pp.
732 464.
- 733 Vainio, E. J., Jurvansuu, J., Hyder, R., Kashif, M., Piri, T., Tuomivirta, T., Poimala, A.,
734 Xu, P., Mäkelä, S., Nitisa, D., and Hantula, J. 2018. *Heterobasidion partitivirus* 13
735 mediates severe growth debilitation and major alterations in the gene expression of a
736 fungal forest pathogen. *J. Virology*. 92:e01744-17.
- 737 van Dijk, M., Morley, T., Rau, M.L. et al. 2021. A meta-analysis of projected global food
738 demand and population at risk of hunger for the period 2010–2050. *Nat Food* 2:494-
739 -501. <https://doi.org/10.1038/s43016-021-00322-9>.
- 740 Vannier N., Agler M., and Hacquard S. 2019. Microbiota-mediated disease resistance in
741 plants. *PLoS Pathogen*. 15(6):e1007740
742 <https://doi.org/10.1371/journal.ppat.1007740>.
- 743 Vicente, I., Baroncelli, R., Hermosa, R., Monte, E., Vannacci, G., and Sarrocco S. 2022.
744 Role and genetic basis of specialised secondary metabolites in *Trichoderma*
745 ecophysiology. *Fungal Biology Reviews*. 39:83-99.
746 <https://doi.org/10.1016/j.fbr.2021.12.004>.
- 747 Wang, Q., Cheng, S., Xiao, X., Cheng, J., Fu, Y., Chen, T., Jiang, D., and Xie, J. 2019.
748 Discovery of Two Mycoviruses by High-Throughput Sequencing and Assembly of

- 749 Mycovirus-Derived Small Silencing RNAs from a Hypovirulent Strain of *Sclerotinia*
750 *sclerotiorum*. *Frontiers in Microbiology*. 10:1415.
- 751 Wang, N.R., and Haney, C.H. 2020. Harnessing the genetic potential of the plant
752 microbiome. *Biochem (Lond)*. 42: 20–25. doi: <https://doi.org/10.1042/BIO20200042>.
- 753 Wang, Z., and Yi, S. 2022. Toward Understanding the Genetic Bases Underlying Plant-
754 Mediated “Cry for Help” to the Microbiota. *iMeta*. 1:e8.
755 <https://doi.org/10.1002/imt2.8>
- 756 Weindling, R. 1932. *Trichoderma lignorum* as a parasite of other soil fungi.
757 *Phytopathology*. 22:837–845.
- 758 Weller, D.M., Raaijmakers, J.M., McSpadden Gardener, B.B., and Thomashow, L.S.
759 2002. Microbial populations responsible for specific suppressiveness to plant
760 pathogens. *Annual Review of Phytopathology*. 40:309-348.
761 <https://doi.org/10.1146/annurev.phyto.40.030402.110010>.
- 762 Woo, S.L., Hermosa, R., Lorito, M. et al. 2022. *Trichoderma*: a multipurpose, plant-
763 beneficial microorganism for eco-sustainable agriculture. *Nature Review*
764 *Microbiology*. <https://doi.org/10.1038/s41579-022-00819-5>.
- 765 Woo, S.L., Ruocco, M., Vinale, F., Nigro, M., Marra, R., Lombardi, N., Pascale, A.,
766 Lanzuise, S., Manganiello, M., and Lorito, M. 2014. *Trichoderma*-based Products and
767 their Widespread Use in Agriculture. *The Open Mycology Journal*. 8:71-126. DOI:
768 10.2174/1874437001408010071.
- 769 World Health Organization (WHO). 2021. Tripartite and UNEP support OHHLEP’s
770 definition of “One Health”. December 1 2021, News Release.
771 [https://www.who.int/news/item/01-12-2021-tripartite-and-unep-support-ohhlep-s-](https://www.who.int/news/item/01-12-2021-tripartite-and-unep-support-ohhlep-s-definition-of-one-health)
772 [definition-of-one-health](https://www.who.int/news/item/01-12-2021-tripartite-and-unep-support-ohhlep-s-definition-of-one-health). Accessed 8 May 2022.

773 Xu, D., Pan, L., Zhao, H., Zhao, M., Sun, J., and Liu, D. 2011. Breeding and identification
774 of novel koji molds with high activity of acid protease by genome recombination
775 between *Aspergillus oryzae* and *Aspergillus niger*. *J. of Industrial Microbiological*
776 *Biotechnology*. 38(9):1255–65.

777 Yong, L., XinQin, Y. and Bai, Y. 2019. “Reductionist Synthetic Community Approaches
778 in Root Microbiome Research.” *Current Opinion in Microbiology*. 49:97-102.
779 <https://doi.org/10.1016/j.mib.2019.10.010>.

780 Zhang, H., Xie, J., Fu, Y., Cheng, J., Qu, Z., Zhao, Z., Cheng, S., Chen, T., Li, B., Wang,
781 Q., et al. 2020. A 2-Kb Mycovirus Converts a Pathogenic Fungus into a Beneficial
782 Endophyte for *Brassica* Protection and Yield Enhancement. *Molecular Plant*
783 *Pathology*. 13:1420–1433.

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First International Workshop on *Trichoderma* and *Gliocladium*



Participants at the First International Workshop on *Trichoderma* and *Gliocladium* were (L-R): First row: G. C. Papavizas, D. Fravel, W. L. Chao, B. Dubos, K. E. Conway, J. Beagle-Ristalno, M. Christensen, Y. Elad, Y. Henis; second row: G. R. Goss, I. Chet, M. Windham, G. E. Harman, R. Lifshitz, G. Vannaccl, E. Nelson, M. T. Dunn, J. A. Lewis, J. J. Marois, G. A. Kuter, W. Chen, R. D. Lumsden, R. Baker; third row: C. R. Howell, L. F. Johnson, J. C. Locke, G. Brandes, B. Pons, R. B. Bahme, J. L. Ricard, J. M. Kraft, P. Davel, T. H. Abd-El Molty, S. B. Maul, E. E. Nelson.

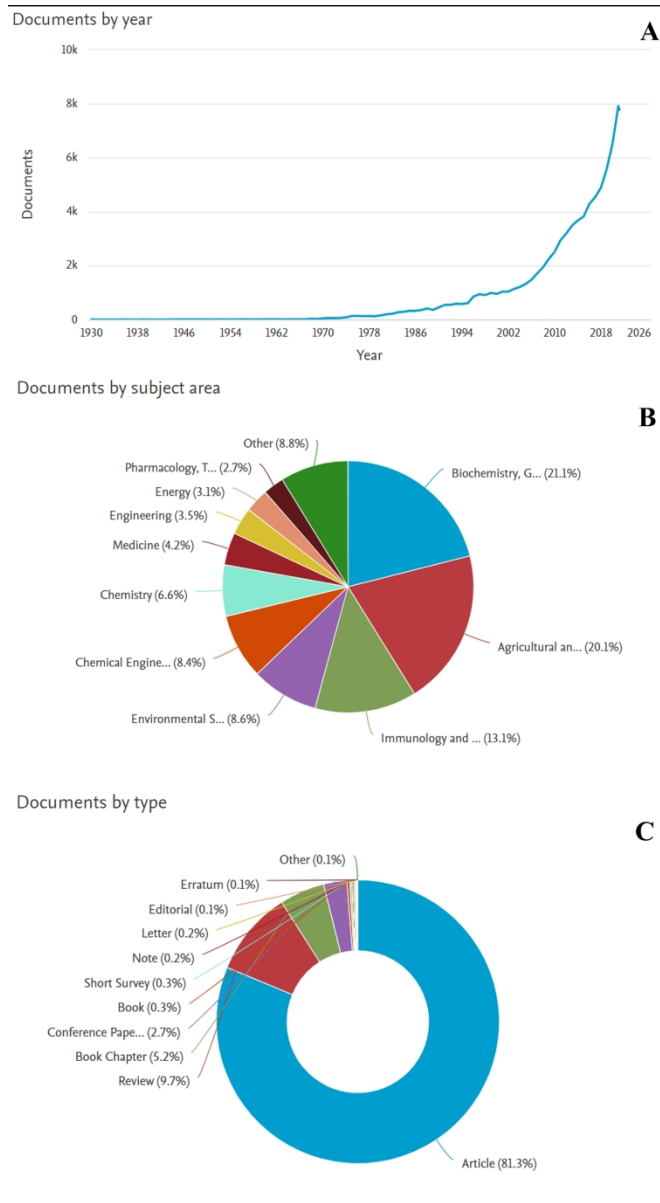
. Participants at the First International Workshop on *Trichoderma* and *Gliocladium*, USDA Beltsville Agricultural Research Center, 3-5 April 1984 (Phytopathology News, 18(7), July 1984).

146x103mm (330 x 330 DPI)



Castanea sativa shoot showing symptoms of chestnut blight (A): chestnut shoot showing a healing canker (B) as result of hypovirulence (kindly provided by Giovanni Vannacci).

146x109mm (330 x 330 DPI)



Statistics concerning available literature focusing on *Trichoderma* spp. (Source: www.scopus.com).

155x278mm (300 x 300 DPI)