

Trichoderma: a multipurpose, plant-beneficial microorganism for eco-sustainable agriculture

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Abstract

Trichoderma is a cosmopolitan and opportunistic ascomycete fungal genus including species that are of interest to agriculture as direct biological control agents of phytopathogens. *Trichoderma* utilizes direct antagonism and competition, particularly in the rhizosphere, where it modulates the composition of and interactions with other microorganisms. In its colonization of plants, on the roots or as an endophyte, *Trichoderma* has evolved the capacity to communicate with the plant and produce numerous multifaceted benefits to its host. The intricacy of this plant–microorganism association has stimulated a marked interest in research on *Trichoderma*, ranging from its capacity as a plant growth promoter to its ability to prime local and systemic defence responses against biotic and abiotic stresses and to activate transcriptional memory affecting plant responses to future stresses. This Review discusses the ecophysiology and diversity of *Trichoderma* and the complexity of its relationships in the agroecosystem, highlighting its potential as a direct and indirect biological control agent, biostimulant and biofertilizer, which are useful multipurpose properties for agricultural applications. We also highlight how the present legislative framework might accommodate the demonstrated evidence of *Trichoderma* proficiency as a plant-beneficial microorganism contributing towards eco-sustainable agriculture.

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Introduction

Trichoderma (teleomorph *Hypocrea*) is a genus of filamentous fungi that is capable of feeding on other fungi (mycotrophism) and is a ubiquitous colonizer in almost all environments (including agricultural, forestry, mountain, grassland and desert ecosystems, and fresh and marine waters); it prevails in any biotope and has an extensive geographical distribution worldwide¹. *Trichoderma* species grow rapidly on various substrates and are prolific spore producers, easily recognized by the presence of abundant green conidia.

A review in 2004 by Harman et al.² presented *Trichoderma* species as opportunistic, avirulent plant symbiont fungi, and discussed the diverse mechanisms of action employed by the fungus that contribute to its positive impact on plants. Of particular interest were processes involved in the biological control of plant diseases, with direct action on phytopathogens and indirect mechanisms through induction of local and systemic defences in plants. Also noted was the stimulation of root development and plant growth, producing benefits to both the host plant and the invading fungus that resulted in favourable consequences for agriculture. Harman et al.² described the plant–microorganism interaction as intricate, involving multifaceted crosstalk modulated by root colonization and the plethora of compounds produced by *Trichoderma* that activate biochemical and genetic pathways determining plant defence responses to biotic and abiotic stresses.

The advancement of omics investigations^{3,4} has resulted in increased understanding of the ecological events involved in the evolutionary progression of *Trichoderma* fungi from common soil dwellers growing on decaying organic matter (noted for their outstanding saprotrophic action on dead fungi and oomycetes), to mycoparasitism of other fungi (including those of taxonomically close species) and to interactions with plants that involve colonization of the rhizosphere and endophytism⁵. Omics approaches have also served to unveil the processes and regulation dynamics of the beneficial effects of *Trichoderma* to plants^{6,7} that are of agricultural interest. The application of molecular methods for species identification and classification has resulted in the exponential expansion of *Trichoderma* taxonomy – starting 50 years ago with only 9 species aggregates described for the genus to >400 species recognized today⁸ (Box 1).

Research interests have since expanded towards an integrated analysis of the multipurpose properties of *Trichoderma* as fungi beneficial to plants for applications and improvement of agricultural production. This variation in research objectives can be attributed to ongoing changes in agricultural policies and management over time, with an increasing focus on sustainability for the future. *Trichoderma* contributes positive effects to the agroecosystem; thus, considerations are being given to the role that this fungus has in innovative agricultural strategies as an established and accepted biotechnological tool.

In this Review, we present the latest advances in *Trichoderma* research, including ecophysiology and lifestyle changes that result in species diversification; opportunism and competition (discussing the complex relationships between plants and microbiota); use of the fungus as a direct biological control agent (BCA) in crop protection; indirect BCA effects stimulating plant immunity; and the capacity of *Trichoderma* as a plant biostimulant, both in promoting plant growth and activating defence against abiotic stress. Considerations are also presented on how to advance *Trichoderma* applications in real-world scenarios, bioformulation improvements and policy deliberations. We conclude by discussing how the use of *Trichoderma* could maximize opportunities for reducing chemical inputs, thus providing cleaner resources and healthier prospects for a more environmentally sustainable agriculture system.

Ecophysiology and lifestyle changes

The genus *Trichoderma* demonstrates enormous morphological uniformity and nutritional diversity and has a high number of species members, greater than that found in other fungal groups with similar lifestyles^{1,8}. These traits might be attributed to at least four major shifts over the course of evolution in nutritional habits and ecological roles that could have impacted the *Trichoderma* lifestyle, in which each transition has led to notable bursts in species diversification^{9,10} (Fig. 1). These shifts were first, from being a parasite of plant-decomposing fungi to being a feeder on decaying plant matter (saprotroph); second, to living in the soil as a saprotroph, with mycotrophic (obtaining nutrients from live or dead fungi) and phytophagic (obtaining nutrients from plants) abilities; third, to feeding on living fungi (mycoparasite); and fourth, to establishing interactions with living plants. Genetic and comparative genomics studies^{5,9–11} have demonstrated that *Trichoderma* are fungi that have constantly reshaped their genome to improve their ability to rapidly colonize and successfully compete in novel habitats. Mycotrophy is a very ancient trait of the *Trichoderma* genus and is a major lifestyle for many of its species, which has facilitated the evolution of its positive interactions with plants^{4,5}.

Phylogenomic analysis has shown that the genus *Trichoderma* shares at least one common ancestor with entomoparasitic hypocrealean fungi¹⁰, and the most ancient species of the genus evolved around the time of the Cretaceous–Paleogene extinction event (66 million years ago)¹¹. These fungi were mycoparasitic on Basidiomycota hosts, from which they acquired genes by horizontal transfer that subsequently conferred to *Trichoderma* the ability to grow on dead wood substrates, a lifestyle typical of their targeted fungal prey^{5,9,10}. *Trichoderma* might have made a successive leap from this habitat as a participant in the strong burst of fungal populations subsequently found as feeders on the decaying biomass of plants killed by the Cretaceous–Paleogene extinction¹². Most of the carbohydrate hydrolysing genes required for saprotrophic growth as mycotrophs and phytophages were probably acquired 20–30 million years ago, resulting in the diversification and establishment of noted infrageneric sections and/or clades (such as section *Trichoderma* (ST), section *Longibrachiatum*, and clades *Harzianum* and *Virens* (HV))^{10,11}.

Many soil-living species of *Trichoderma* developed a distinctive genus characteristic for their capacity to produce a plethora of hydrolytic enzymes (exochitinase and endochitinase) that enabled them to mycoparasitize Ascomycota fungi or other phylogenetically close species (adelphoparasitism), a trait that was rare or absent in the ancestors of *Trichoderma*^{9,10}. Approximately 40% of the vast number of hydrolytic enzymes secreted by *Trichoderma* have originated by lateral gene transfer from taxonomically close plant-associated ascomycetes¹⁰. *Trichoderma* spp. also expanded their ability to parasitize or hyperparasitize and to obtain nutrients from diverse soil-borne organisms such as *Phytophthora*, *Pythium*, *Rhizoctonia* and nematodes⁹. In addition, they developed mutualistic relationships with insects as demonstrated by the protection of termites from infection by entomopathogenic fungi (*Metarhizium*)¹³. Species diversity in *Trichoderma* was favoured by gene gains in the taxonomic groups of HV and ST and losses in section *Longibrachiatum*, coupled with a rare frequency of sexual reproduction and a high rate of conidiation (asexual reproduction) that increased the adaptive variation input by mutation⁹.

Subsequently, the presence of fungal prey and root-derived nutrients probably attracted the most opportunistic *Trichoderma* species to colonize the rhizosphere, whereby other species became internal plant colonizers or endophytes as the most recent major

evolutionary event of the genus^{5,9,11} (Fig. 1). Three key features have contributed to *Trichoderma* becoming an endophyte⁹ as a result of its progressive ‘intimacy’ in plant–microbiota interactions: as a saprotroph, it initiated the decay–degradation process after plant host death; as a mycoparasite, it was capable of parasitizing the primary fungal decomposers that colonized the vegetative tissues; and as a mutualistic non-pathogenic symbiont, it was able to interact and communicate with the living plant host, providing benefits such as growth promotion and protection against biotic and abiotic stresses. Over time, *Trichoderma* became an opportunistic plant colonizer, developing mechanisms that enabled it to overcome plant defences and not be recognized as a foe.

Opportunism in the rhizosphere

Trichoderma opportunism is evidenced by the ability of this fungus to colonize a wide range of habitats, employing a combination of traits to compete for space and nutritional resources, resist environmental stresses, repair cell damage, and modify the ecological living conditions to its advantage (for example, by detoxifying noxious compounds or changing the substrate pH). The substantial increase in *Trichoderma* growth in the presence of pectin, xylan or other mucigel-released substances supports the notion that root-derived nutrients are attractors for *Trichoderma*, providing incentives for this microorganism to physically colonize roots^{14,15}. The production of reactive oxygen species (ROS) in *Trichoderma* has been linked to antagonism against phytopathogens containing cellulose in their cell wall, such as *Pythium ultimum*¹⁶, as well as the fine-tuning of molecular crosstalk communication with plants that establishes beneficial effects¹⁷. Furthermore, tomato root exudates obtained from plants subjected to various biotic and abiotic stresses (such as pathogen attack, wounding or salt) were enriched with ROS and oxylipins, which were capable of stimulating growth and acting as selective chemo-attractants to *Trichoderma*¹⁸. *Trichoderma* can also enhance antioxidant defence in plants subjected to abiotic stresses, resulting in a decrease of ROS levels in the plant and thus limiting tissue damage^{19,20}. Compared to other filamentous fungi, *Trichoderma* has its own robust antioxidant system with a potential role in protecting genome stability by elimination of ROS⁴. Evidence suggests that H₂O₂ and oxylipins produced by *Trichoderma atroviride* might act as signal molecules in response to injury and cell damage (as in plants and animals)²¹.

The photoreactivation system that repairs DNA damage caused by UV radiation and is involved in the regulation of carbon and nitrogen metabolism in response to light has been studied extensively in *Trichoderma*; the ENVOY photoreceptor of *T. atroviride*, a repressor of blue light-induced genes, modulates the expression of genes involved in DNA repair, acting as a growth and conidiation checkpoint²². The high opportunistic ability of *Trichoderma* might also be a consequence of the activation of a complete range of heat shock proteins that confers tolerance to cold, heat, oxidative, osmotic or saline stresses²³. *Trichoderma* genomes exhibit a high number of genes that encode for ATP-binding cassette (ABC) transporters⁴, which could bestow increased tolerance to toxic compounds present in the rhizosphere²⁴. *Trichoderma* also secrete siderophores^{25,26}, which may help them to compete in the rhizosphere and to solubilize phosphates²⁷. The application of selected *Trichoderma* strains able to solubilize diverse phosphate sources enhanced phosphate uptake by plants, resulting in increased growth promotion²⁸. Volatile organic compounds (VOCs), such as 6-pentyl-2H-pyran-2-one (6-PP), have known antibiotic activity, and, at low doses (similar to those expected to be released by *Trichoderma*

Box 1

Trichoderma taxonomic scenario

Persoon (1794) proposed the genus name *Trichoderma* (teleomorph *Hypocrea*) for a group of wood-decaying filamentous fungi that were producers of green asexual conidia masses at hyphal termini. These fungi are now classified as Division Ascomycota, Order Hypocreales and Family Hypocreaceae. The pleomorphism of this fungus led to the use of a dual nomenclature¹; however, the sexual form (*Hypocrea*) is rarely found in nature, barely growing on plant debris and on specific basidiomycete fungi present in decaying bark, whereas the asexual form (*Trichoderma*) is abundant. Following the principle of ‘one name, one species’¹⁵⁹, after a vote by the International Subcommittee on *Trichoderma* and *Hypocrea* taxonomy (www.trichoderma.info), *Trichoderma* was preferentially chosen by 54 votes to 22 over *Hypocrea* as the name to be adopted, and this genus name has been officially in use since 2013 (ref.¹⁶⁰).

Today, the genus *Trichoderma* consists of >400 species (375 with valid nomenclature as of 2020) characterized by in vitro cultures, DNA barcoding and integration of publicly available whole-genome sequences⁸. DNA-based analysis has resulted in both the simplification and the complication of species classification and nomenclature for *Trichoderma*⁸. A species identification system has been elaborated by sequencing the ribosomal RNA locus internal transcribed spacers ITS1 and ITS2, different fragments of the *tef1* gene (encoding the translation elongation factor 1 α), together with a species confirmation marker based on a fragment of the *rpb2* gene (encoding the second largest subunit of RNA polymerase II)⁸. In the present *Trichoderma* taxonomic framework, some species names have become obsolete, and it might be misleading to assign biological activities, such as the production of certain metabolites, to species described with an old name. For example, DNA barcoding identification of the active substances in four commercial biocontrol products determined that none were *Trichoderma harzianum* as indicated on the label⁶³. Indeed, the worldwide marketed strain *T. harzianum* T-22 (ref.²) was renamed as *Trichoderma afroharzianum* after DNA barcoding analysis⁶³. Therefore, the systematic reform of *Trichoderma* taxonomy might produce technical complications as some species names indicated in patents and commercial registration dossiers might need nomenclature revisions.

in nature), they may act as signalling molecules to modulate seed germination, plant growth, root architecture and immune responses in the absence of direct physical contact^{29,30}.

Trichoderma genomes harbour phytohormone genes³¹ involved in the production of auxins, gibberellins, abscisic acid, salicylic acid or cytokinins that, in a strain-dependent and/or culture medium-dependent manner, have been linked to hyphal growth, root colonization, activation of the plant antioxidant machinery and promotion of plant performance under abiotic stress^{20,32}. The role of auxins produced by *Trichoderma* has not been established beyond doubt, but these compounds have been linked to root hair initiation and development effects

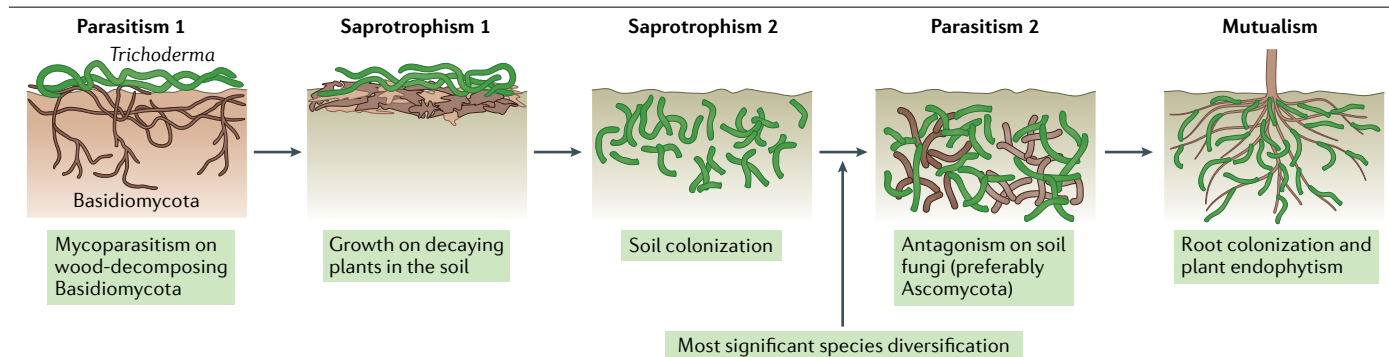


Fig. 1 | Evolutionary shifts in *Trichoderma* ecophysiology. Parasitism 1: evidence suggests that mycoparasitism of the wood-decomposing Basidiomycota is the most ancient trait of the genus *Trichoderma* (in green throughout). Saprotrophism 1: *Trichoderma* shifted to the development of saprotrophism and grew on plants killed during the Cretaceous–Paleogene extinction event (and acquired genes encoding a wide range of carbohydrate hydrolytic enzymes). Saprotrophism 2: many species remained as

saprophytes, colonizing the soil. Parasitism 2: species of the genus made major ecophysiological changes to parasitize taxonomically close Ascomycota as well as oomycetes, basidiomycetes and nematodes. Mutualism: the presence of potential fungal prey and plant root-derived nutrients in the rhizosphere facilitated a new leap towards a mutualistic relationship with living plants. The most recent ecophysiological change by some *Trichoderma* species is the internal colonization of plants (endophytism).

in plants³³. *Trichoderma* might also stimulate plant auxin transport and signalling, resulting in plant growth promotion³³. Notably, excessive accumulation of plant auxins can have the opposite effect, as rhizosphere acidification induced by *Trichoderma* can lead to inhibition of root growth through an auxin-dependent mechanism³⁴.

Particularly interesting is the role of non-secreted molecules located in the cell wall of *Trichoderma* as well as the relative proportions of components in the cell membrane. For instance, the cysteine-rich cell wall protein QID74 of *Trichoderma harzianum* enhances the formation and elongation of root hairs, thus increasing the absorptive surface area plus translocation efficiency of nutrients into the shoots, resulting in more plant biomass³⁵. The balance of structural squalene and ergosterol is necessary to maintain cellular membrane stability in fungi and is important for the ability of *Trichoderma* to colonize the roots as well as having a critical role in the regulation of plant defence mechanisms and biocontrol action³⁶. The prolific endophytic colonization by some *Trichoderma* is not only a direct biocontrol mechanism that prevents pathogen colonization in the host but it can also produce plant-beneficial effects, such as increased photosynthetic capacity and growth promotion, accompanied by an increased tolerance to biotic and abiotic stresses^{37–39}. For example, colonization of the apoplast of olive tree roots by *Trichoderma* prevents the pathogen *Verticillium dahliae* from accessing the vascular bundles, thus providing effective disease control of Verticillium wilt⁴⁰. However, endophytic colonization by *Trichoderma* does not guarantee a corresponding beneficial effect for the plant²⁰.

Microbiomes and non-target microorganisms

The evolutionary leap to colonize the rhizosphere and plant roots, coupled with antagonism or biocontrol action, suggests that *Trichoderma* is an intrepid conqueror of ecological niches, a determined competitor and an aggressive antagonist. Therefore, it is important to investigate and evaluate the impact that *Trichoderma* strains might have on non-target organisms and plants as well as the effects in given soil and rhizosphere environments. *Trichoderma* has been proposed as a marker of healthy soils⁴¹. A core *Trichoderma* biome seems to be present in both endemic and cosmopolitan plants from different continents, in which endemic plant populations harbour a substantially higher proportion of antagonistic *Trichoderma* species⁴². The cultivation systems used and

the crops cultivated can have diverse effects on soil properties, which in turn influences fungal diversity (for both pathogenic and beneficial fungi)^{43–45}. Microbiome diversity is highest in bulk soil and decreases in rhizosphere and endosphere samples^{44,45}. However, with inoculations of *Trichoderma*, prokaryote and eukaryote populations are modified in bulk soil and in the two root-associated compartments. The use of *Trichoderma* strains alone or in combination with organic compost in crop plants has been reported to maintain plant growth and cause changes in the structure and function of microbial communities in the rhizosphere in terms of microbial community composition and effect on phosphorus solubilization (resulting in total rhizosphere soil microbial community changes and stimulation of potentially beneficial microbial consortia)^{46,47}. Diverse organic amendments added to soil differentially influence the growth and disease suppression capacity of microorganisms, including beneficial fungi such as *Trichoderma*, and increase plant root proliferation⁴⁸. *Trichoderma* can aid in the maintenance of microbiome diversity when growth conditions are compromised as was observed when the application of *Trichoderma* alone increased the number and diversity of many genera of beneficial plant bacteria in the wheat rhizosphere after the microbiome had been negatively affected by high doses of inorganic nitrogen fertilizers⁴⁵ or, similarly, when dual inoculations with *Trichoderma* and endophytes were found to enrich the microbiome of plants subjected to drought stress⁴⁹.

The compatibility of *Trichoderma* with mycorrhizal fungi has been frequently questioned for three reasons: first, the high mycoparasitic potential of *Trichoderma* and its attack on arbuscular mycorrhizal fungi (AMF) in vitro⁵⁰; second, the distinct and *Trichoderma* species-dependent VOC emission profiles against ectomycorrhizal fungi⁵¹; and third, the ability of AMF to compete for nutrients and colonization sites and boost the systemic defences of plants⁵², which might potentially hinder *Trichoderma* colonization in the rhizosphere. The behaviour of AMF reflects their ability to induce the systemic defences of plants and means that they can be effectively considered as indirect BCA. However, despite assumptions that *Trichoderma* and mycorrhizal fungi could not be applied simultaneously, their combined use has been noted to increase crop yields⁵³, and greenhouse studies have proven their compatibility when applied together to tomato seedlings^{54,55}. Furthermore, the application of *Trichoderma* aided AMF interactions with a

non-mycorrhizal *Brassica* host that improved rapeseed productivity⁵⁶, and a single application of *Trichoderma* increased the levels of AMF in the wheat rhizosphere⁴⁵. Importantly, compatibility studies need to be conducted for these two beneficial fungi and evaluated on a case-by-case basis, bearing in mind that root colonization by *Trichoderma* is much faster than that of mycorrhizal fungi.

Some *Trichoderma* spp. have been noted to produce harmful effects, including *T. aggressivum*, *T. pleuroti* and *T. pleurotica*, which have been reported as pathogens of edible mushrooms^{8,57}. The production of the trichothecene toxin trichodermin by *Trichoderma brevicompactum* resulted in phytotoxic effects on tomato plants, thus invalidating the mycotoxin-producing strains as BCAs⁵⁸.

With the ability to grow at high temperatures (37 °C), *Trichoderma longibrachiatum* has been noted as a human opportunistic pathogen in patients who are immunocompromised⁵⁹. Fortunately, the undesirable activities observed with some *Trichoderma* do not correspond to those species commonly used in agriculture and commercially marketed. In addition, as a prerequisite for product registration, the microorganism must be tested and certified as not having any potential negative health effects.

Interestingly, *Trichoderma* can have deleterious effects on leaf-cutting ants (on the fungal gardens they grow for feeding as well as on their survival) owing to antagonism with their fungal symbiont. However, the endophytic colonization by *Trichoderma* of the plant material transported to the ant nests can act as an effective 'Trojan-horse' strategy that results in beneficial effects to the plant as it provides protection from these damaging agricultural and forestry pests⁶⁰.

Direct biocontrol in crop protection

Trichoderma can be considered as a multipurpose BCA owing to its combined potential actions, with direct antagonism to the target organism (Supplementary Table 1) and indirect activities through the plant host to stimulate a defence response to a multitude of biotic stress factors. The direct action of *Trichoderma* as a BCA (Fig. 2) has been extensively addressed in many reviews, including that by Harman et al.². However, it is important to note that not all *Trichoderma* species or strains have the same capacity for pathogen or pest control, respond equally to diverse crops or cultivars^{61,62}, function effectively in different geographic locations, or are able to maintain a consistent standard level of protection in all field conditions or over extended timeframes. In particular, the strains of *Trichoderma* that are of interest to agriculture⁶³ are principally distributed among species of the infrageneric groups ST (*T. atroviride*, *T. gamsii*, *T. viride*, *T. asperellum* and *T. asperelloides*) and HV (*T. harzianum sensu lato*, *T. afroharzianum*, *T. guizhouense* and *T. virens*)⁸ (Box 1). In general, efficacy as a BCA depends on the biological characteristics of the *Trichoderma* strain, including rapid growth, prolific sporulation and opportunistic colonization of the environment, as well as on the biochemical arsenal of host cell wall-degrading enzymes (CWDEs)^{2,4,64}, cumulative secondary metabolites^{65,66} and released VOCs⁶⁷ (all of which affect interactions with the host plant⁶⁸, influence the soil microbiome^{51,69} and subsequently affect the biocontrol of plant attackers⁷⁰).

The antagonism or direct biocontrol activity by *Trichoderma* of plant-damaging organisms can be attributed to the following five principal mechanisms^{2,71}: parasitism, whereby *Trichoderma* is a predator that obtains nutrients from the target prey (that is, if the prey is a fungal phytopathogen, then *Trichoderma* is a mycoparasite feeding on a fungal disease agent); antibiosis, by production of secondary metabolites that inhibit competitors, limiting and impeding microorganism

proliferation or plant pathogen attack; enzymatic activity (for example, chitinases) and production of secondary metabolites with biological activity against nematodes and insect pests; competition for ecological niches and resources (such as soil and mucigel nutrient uptake, ROS tolerance, growth on roots) that contribute to *Trichoderma* colonization of the soil, rhizosphere and endosphere (endophytism); and the production and release of VOCs can attract parasitoids and predators of insect pests. Moreover, a sixth mode of action for biocontrol (which is indirect rather than direct) involves the induction of immunity in the plant host, whereby *Trichoderma* activates plant defence responses and mechanisms that provide protection against biotic and abiotic stress (discussed below).

Trichoderma can directly control some economically important agricultural phytopathogen ascomycetes (*Botrytis*, *Colletotrichum*, *Erysiphe*, *Fusarium*, *Magnaporthe*, *Sclerotinia*, *Verticillium*), basidiomycetes (*Rhizoctonia*, *Athelia*, *Armillaria*, *Ustilago*, *Puccinia*) and oomycetes (*Pythium*, *Phytophthora*)⁷². Furthermore, different secondary metabolites can inhibit certain bacteria (*Clavibacter*, *Pseudomonas*, *Xanthomonas*)^{66,69,73}. Genes associated with mycoparasitism, demonstrating transcriptional response to the prey^{4,74}, and CWDEs produced by *Trichoderma* in interactions with phytopathogens have been extensively identified and have been proven to effectively inhibit spore germination, hyphal growth and the development of dormant fungal vegetative structures (such as sclerotia and chlamydospores) in a wide range of pathogens⁷⁵. CWDEs, such as chitinases⁷⁶, glucanases^{77–80} and proteases⁷⁴, alone or in combination^{81,82}, have demonstrated their antagonistic role against plant pathogenic fungi and oomycetes (Supplementary Table 1).

Trichoderma produces >120 different types of secondary metabolite, with the most relevant chemical structures being terpenes, pyrones, polyketides and non-ribosomal peptides. Some of these secondary metabolites possess antibiotic activity, inhibiting the growth and multiplication of fungi^{66,83}, oomycetes^{84,85} and bacteria^{66,73}. The application of purified secondary metabolites has biocontrol effects on target pathogens comparable to those obtained by using the living *Trichoderma* producer⁸⁶. Secondary metabolites that can permeabilize cell membranes might work synergistically with CWDEs to promote cell disruption⁸⁷. Although *Trichoderma* might be considered a necrotrophic mycoparasite that destroys its prey, microscopic evidence⁸⁸ demonstrates that the fungus might instead penetrate through open holes in the cell wall and not extensively damage the prey, thus using what might be called a hemi-biotrophic parasitic mode of action⁷⁵.

Trichoderma has long been noted to have suppressive effects on *Meloidogyne* root-knot nematodes (RKN)⁸⁹. Similar findings have been confirmed for other nematodes (*Heterodera*, *Haemonchus*, *Pratylenchus* or *Globodera*), whereby inhibition occurs via *Trichoderma* parasitism⁹⁰, egg lysis by proteases⁹¹ and chitinases⁹², or suppression of egg hatching by secondary metabolites⁷³. Furthermore, *Trichoderma* has demonstrated direct biocontrol of insects through enzymatic activity on the midgut peritrophic matrix⁹³ and inhibition of cuticle formation⁹⁴. In addition, extracts of secondary metabolites can have inhibitory effects on insect larvae⁹⁵.

As competitors in the plant environment, *Trichoderma* spp. can interfere with or counteract the attack strategies used by phytopathogens to invade plants. For example, proteases secreted by *Trichoderma* can inhibit enzymes produced by pathogens to disrupt plant tissues for penetration⁹⁶. Secondary metabolites produced by *Trichoderma* can downregulate the expression of pathogen genes involved in the pathogenicity process; for example, polyketides released by *Trichoderma arundinaceum* can modulate the phytotoxic sesquiterpenes

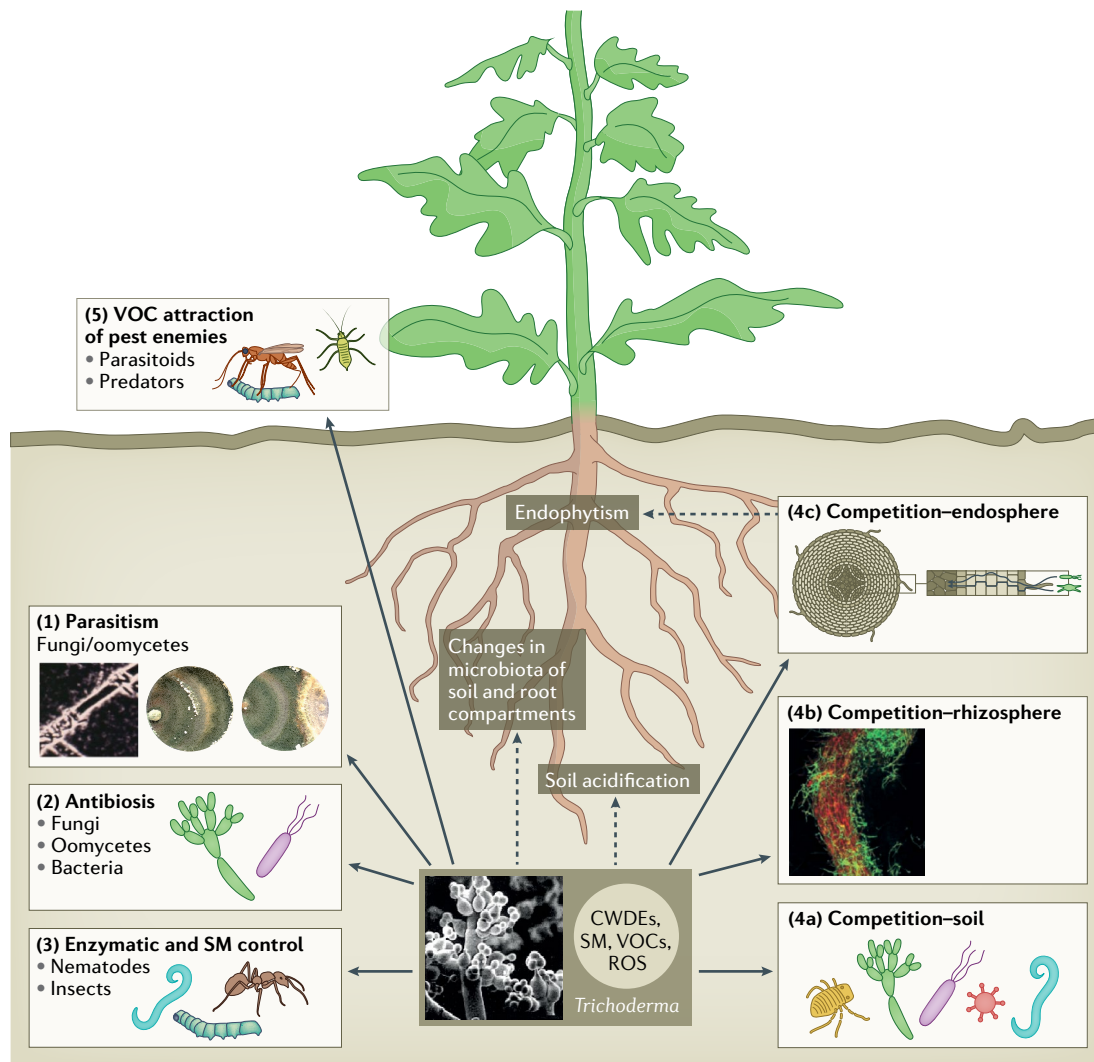


Fig. 2 | *Trichoderma* as a direct biological control agent. *Trichoderma* is found in the soil and plant root zone (rhizosphere), where it produces cell wall-degrading enzymes (CWDEs), secondary metabolites (SM), volatile organic compounds (VOCs) and reactive oxygen species (ROS), all of which permit the fungus to have diverse roles in crop protection. (1) Parasitism of phytopathogenic fungi and oomycetes. (2) Antibiosis by secondary metabolites inhibits growth of fungi, oomycetes and bacteria. (3) Control of nematodes and insects in the soil via enzymes (proteases, chitinases) and secondary metabolites. (4) *Trichoderma*

acidifies the soil and affects the structure and abundance of microbiota in soil and root compartments; in addition, its ability to take up nutrients, tolerate ROS and grow on roots, and its compatibility with other plant-beneficial microorganisms, enable it to compete successfully in the soil (4a), rhizosphere (4b) and endosphere (4c). *Trichoderma* colonization of the rhizosphere and root tissues (endophytism) inhibits the occupation of these spaces by potentially pathogenic microorganisms and nematodes. (5) VOCs produced by *Trichoderma* can attract parasitoids and predators of insect pests.

of *Botrytis cinerea* that are involved in virulence and growth⁹⁷. A final biocontrol mechanism that is considered a direct effect by *Trichoderma* and not via the plant is the production of VOCs such as 6-PP, which are released in the environment and are able to attract parasitoids and predators of insect pests⁹⁸.

Indirect biocontrol and priming of defences

Trichoderma acts as an indirect BCA by activating plant immune responses (Fig. 3), resulting in a faster and stronger induction of defence mechanisms upon perception of a subsequent triggering stimulus (Box 2); this form of defence is known as priming⁹⁹. By inducing priming, *Trichoderma* can provide the plant with long-lasting

defence through the balance of different phytohormone-dependent pathways⁶. Priming is not exclusively associated with indirect biocontrol as reinforcement of plant responses to biotic and abiotic stresses are very similar in their genesis and establishment, although they are activated by stimuli of very different nature. The molecular interaction between *Trichoderma* and plants, and the manner in which signals are activated and systemically transmitted, have been the subject of several reviews^{6,7,100}. Structural components of the *Trichoderma* cell wall and membrane (for example, chitin, β -glucans and sterols) act as microorganism-associated molecular patterns (MAMPs)^{36,101,102}. Plant cell wall oligomers released by the hydrolytic activity of *Trichoderma* on plant tissues¹⁰³ or derivatives from its antagonistic biocontrol action of

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other microorganisms¹⁰⁴ might function as damage-associated molecular patterns, which, once recognized by pattern-recognition receptors (PRRs), activate MAMP-triggered immunity, which is stronger than pathogen-triggered immunity, thus conferring plant resistance³.

The plant reacts to the arrival of *Trichoderma* at the roots with an increase in levels of salicylic acid (a key phytohormone that controls early root colonization) to limit *Trichoderma* to the apoplastic space of the epidermis and cortex^{40,105}. Next, *Trichoderma* increases the level of a second layer of plant immunity by means of an array of apoplastic effector proteins and metabolites such as xylanase EIX¹⁰⁶, LysM protein Tal6 (ref.¹⁰⁷), cerato-platanin Sm1 (ref.¹⁰⁸), the peptaibol alamethicin¹⁰⁹, and the terpenes trichodiene¹¹⁰ and harzianum A¹¹¹, among others¹¹². The apoplastic effector-triggered defence is considered effector-triggered immunity regardless of whether it is activated at the PRR level.

The secreted effectors¹¹³, together with ROS tolerance, might enable endophytic colonization and allow *Trichoderma* to establish an avirulent relationship with the plant and long-lasting priming that keeps plant response at or just below the threshold for effective resistance³ (Box 2). How the cytoplasmic nucleotide-binding site leucine-rich repeat (NLR) receptors interact with the effectors released by *Trichoderma* is not well understood. In tomato plants, NLR receptors are over-represented in the leaf proteome of plant roots inoculated with *T. atroviride* and *Rhizoctonia solani*¹¹⁴, and NLR genes have also been induced by harzianic acid released by *T. harzianum*⁸⁶. NLR-like proteins are upregulated in the leaf maize proteome after the inoculation of roots with *T. afroharzianum*¹¹⁵. *Trichoderma* effectors are not known to be ligands for NLR, although the signal transduction and events that follow receptor activation by *Trichoderma* and their effects on plants

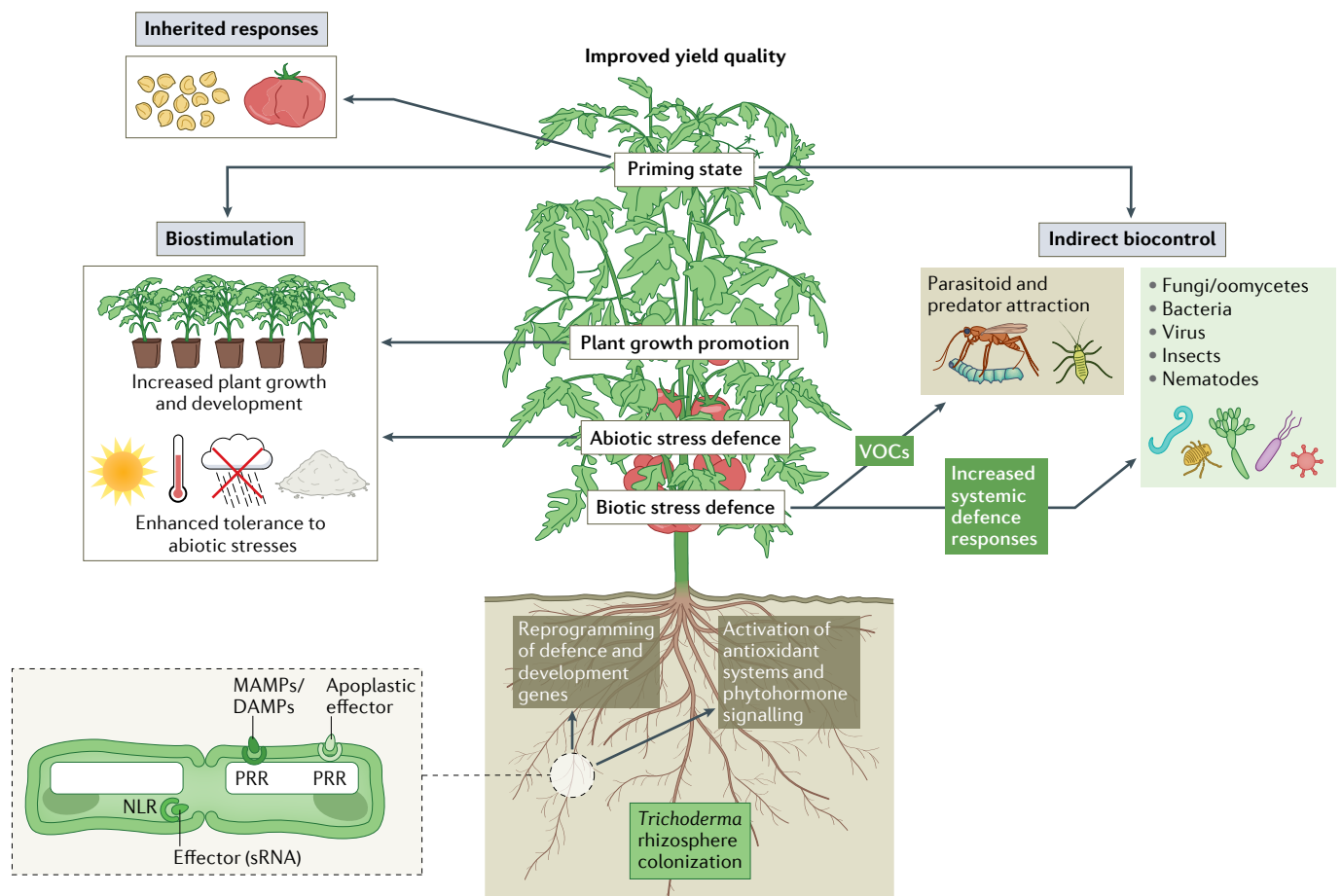


Fig. 3 | *Trichoderma* as an indirect biological control agent, biostimulant and priming inducer. Colonization of the rhizosphere by *Trichoderma* induces local and systemic defence responses in the plant. *Trichoderma* microorganism-associated molecular patterns (MAMPs) and damage-associated molecular patterns (DAMPs) are recognized by pattern-recognition receptors (PRRs) on the plant cell membrane, which also recognize apoplastic effectors secreted by *Trichoderma*. In addition, the release of small RNA (sRNA) by the fungus leads to upregulation of plant cytoplasmic nucleotide-binding site leucine-rich repeat (NLR) receptors. After *Trichoderma* recognition, the plant reprogrammes genes involved in defence and development, and activates antioxidant systems and phytohormone signalling that lead to biotic stress defence (indirect biocontrol) by increasing systemic immune responses to attack by fungi, oomycetes,

bacteria, viruses, insect herbivores and nematodes, as well as by releasing plant volatile organic compounds (VOCs) that are able to attract parasitoids and predators of insect pests; abiotic stress defence (biostimulation) that results in enhanced tolerance and adaptation to abiotic stresses (such as drought, salinity and extreme temperatures); plant growth promotion (biostimulation), whereby *Trichoderma* stimulates plant growth and development when the plant does not need the activation of the defence-immunity response; priming state, which activates a condition that provides long-lasting indirect biocontrol and biostimulation effects throughout the life of the plant; and inherited responses, in terms of defence and growth traits that can be passed on to offspring by transmission of genetic and epigenetic marks in the seeds. The overall positive results of *Trichoderma* in the soil are noted in improved crop yields and quality.

Box 2

Plant defence layers and the role of *Trichoderma* in priming defence

Plant cells individually auto-defend from invaders and then forward the defence signal to neighbouring cells, resulting in systemic transmission to the entire plant. The first layer of innate defence is regulated by pattern-recognition receptors (PRRs) situated on the plant cell surface that perceive structural components of the invaders known as pathogen-associated molecular patterns, microorganism-associated molecular patterns (MAMPs) and damage-associated molecular patterns (DAMPs; which are small molecules resulting from the hydrolytic action of the attackers on the plant or released by the action of biological control agents on their prey). MAMP-triggered immunity (MTI) or DAMP-triggered immunity is transmitted and amplified through cascades of mitogen-activated protein kinases, which convert external stimuli into intracellular responses, resulting in transcriptional reprogramming that leads to plant cell wall fortification; increases in levels of intracellular calcium; production of reactive oxygen species, antimicrobial secondary metabolites and pathogenesis-related proteins; and accumulation of defence phytohormones such as salicylic acid, jasmonic acid and ethylene. Attackers can overcome and suppress MTI by deploying specific effector proteins into the host cytoplasm. Plants activate a second specific defence layer, known as effector-triggered immunity (ETI), following cytoplasmic effector recognition by nucleotide-binding site leucine-rich repeat protein (NLR) receptors. ETI is quicker

and more intense than MTI and is associated with early oxidative burst and hypersensitive response cell death to prevent the invasion of pathogens.

Priming activated in the plant by *Trichoderma* (see figure) follows a different defence dynamic (green arrows) to the untreated control plant (red arrows). PRRs recognize *Trichoderma* MAMPs and host plant or *Trichoderma* prey DAMPs, which increases the level of MTI. *Trichoderma* induces a stronger and more intense defence than MTI via apoplastic effectors, which are also recognized by PRRs (ETI). The plant enters a state of priming in which defence responses are not activated but remain 'alert' around the threshold for effective resistance. With a stress challenge, there is a faster and stronger induction of plant defence from a level of resistance greater than that of the control plant. When the stress ceases, the *Trichoderma*-treated plant enters a post-challenge priming state in which the defence level is once again maintained around the threshold for effective resistance. The response events are stored in the 'transcriptional memory' of the plant, which discriminates between single and repeated stresses, and can modulate transcription of response genes to future stress during the current lifetime of the plant (to the dotted line). Subsequently, offspring from the *Trichoderma*-treated plant acquire an inherited memory, whereby, when exposed to stress, they can activate heritable priming also at a level of effective resistance.

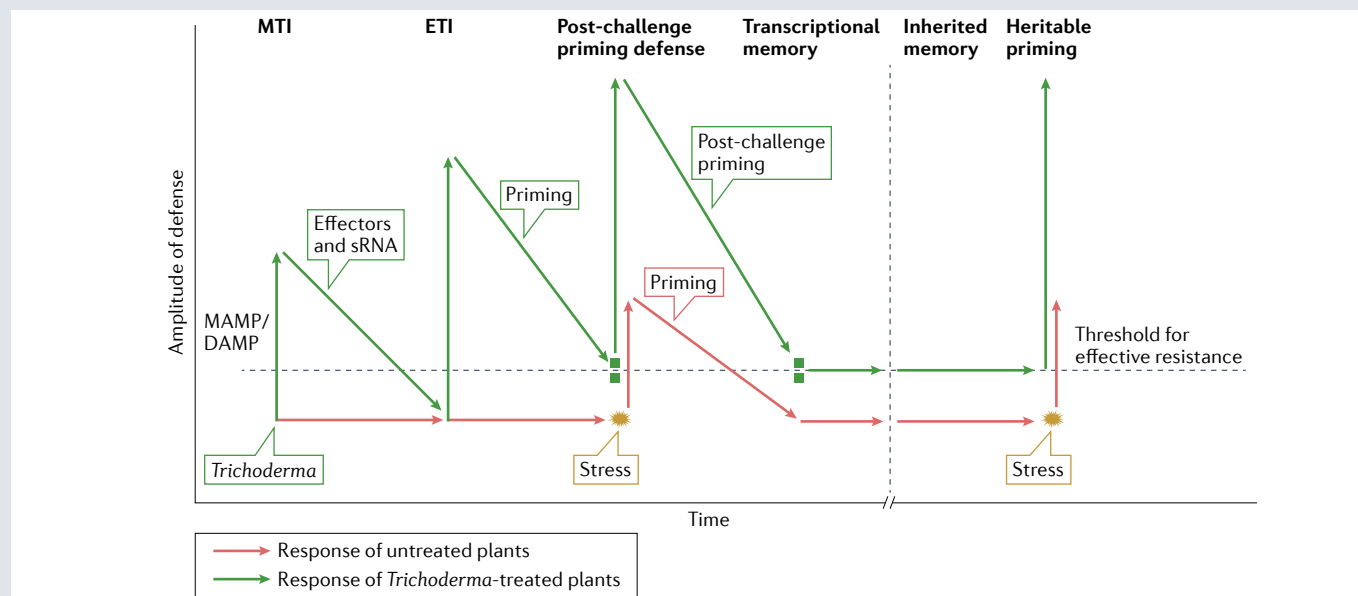


Figure adapted from ref.¹⁶¹, Springer Nature Limited; and based on knowledge of previous studies^{3121,132}. sRNA, small RNA.

are increasingly being studied and understood, including the potential role of bidirectional cross-kingdom small RNA trafficking^{7,112}.

The salicylic acid-dependent defences that limit early growth of *Trichoderma* can spread throughout the plant, constituting a defence

model known as systemic-acquired resistance, which has been found to be effective against biotrophic pathogens¹¹⁶. *Trichoderma* suppresses salicylic acid defences and induces jasmonic acid biosynthesis and jasmonic acid-responsive genes, which are expressed in the root cells, thus

generating a signal that spreads systemically¹⁰². This defence model is known as jasmonic acid–ethylene-dependent induced systemic resistance and is especially effective against necrotrophic pathogens and herbivore attack¹¹⁶. To colonize the roots, *Trichoderma* takes advantage of the antagonism between salicylic acid and jasmonic acid¹⁰². Pioneering work demonstrated that cucumber roots colonized by *T. asperellum* accumulated substantial levels of jasmonic acid and ethylene in 24 h (ref.¹¹⁷), supporting the notion that priming activated by beneficial microorganisms conforms to an induced systemic resistance response¹¹⁶. However, evidence suggests that *Trichoderma*-triggered defences against pathogenic fungi and even viruses are modulated by both jasmonic acid–ethylene and salicylic acid^{111,118,119}.

Improved plant fitness, as promoted by *Trichoderma*, also prevents nematode access to the roots¹²⁰. In tomato roots in which RKN complete their life cycle, *Trichoderma* reprogrammes plant immunity by adapting salicylic acid-dependent and jasmonic acid-dependent defences according to the nematode infection stage^{121,122}. As a general mechanism, *Trichoderma* also primes defences by different means and at diverse frequencies in leaves and roots via small RNA-mediated gene silencing and by inducing the transcription of core components of the RNA-dependent DNA methylation machinery that sharpens the expression of both salicylic acid and jasmonic acid–ethylene defence-related genes¹²³.

Another indirect biocontrol mechanism of *Trichoderma* is the activation of plant systemic defences by VOCs, in which their release results in an oxidative burst that is effective against aphids¹²⁴. *Trichoderma* can also enhance the expression of genes encoding for protective enzymes against moths¹²⁵. Furthermore, *Trichoderma* is able to alter plant metabolic pathways leading to the induction of various plant systemic defences¹²⁶ such as the production of phytochemicals that act as anti-feed deterrents^{127,128} or negatively affect the insect gut proteome equilibrium¹²⁹, activate the release of plant VOCs with high attractivity to parasitoids and predators of aphids^{124,130}, or decrease feeding by herbivorous insects¹³¹.

The speed and efficiency at which plants adapt to their surroundings might also be facilitated by *Trichoderma* given that it has a role in balancing defences and growth as well as alleviating the effects of unfavourable environmental conditions. Abiotic stress-mediated phytohormones share common regulators with MAMPs and/or damage-associated molecular patterns. However, in natural settings in which plants are exposed to a mix of stimuli, PRR pathways display a substantial divergence in sensitivity to biotic or abiotic perturbations and signal transduction. Given that abiotic stresses modify water fluxes, solute concentrations and ion homeostasis, the Ca²⁺ pumps or channels required for PRR-dependent defences have an important role in regulating potential gradients across membranes and conditioning plant immunity¹³². Abiotic stress sensing induces cytoplasmic Ca²⁺ accumulation, leading to an extracellular ROS burst and activation of Ca²⁺-dependent protein kinase cascades; these mechanisms enable the plant to cope with the variation in ambient conditions by prioritizing functions involved with plant growth regulation and responses to environmental stresses¹³². A hydrophobin secreted by *Trichoderma* triggers the plant Ca²⁺ signalling pathway¹³³, which opens up an interesting line of research on G protein recognition and signalling activation and/or deactivation that is compatible with the effects that *Trichoderma* has on plants⁷.

Early studies described how *Trichoderma* favours the production of plant metabolites associated with increased drought tolerance^{37,134} and how its enhancement of antioxidant defence delays the onset of a water-deficit response¹⁹. Numerous reports illustrated that *Trichoderma* could affect ROS scavenging and has 1-amino-1-cyclopropane-carboxylic

acid deaminase (ACCD) activity leading, respectively, to activation of plant antioxidant machinery and regulation of plant ethylene levels under drought, waterlogging, and osmotic, salinity, chilling, or heat stress^{135–138}. *Trichoderma* also increased plant growth and salt tolerance by direct contact¹⁰² or through VOCs¹³⁹. However, the promotion of growth and development triggered by the combination of *Trichoderma* and inorganic fertilizers to salt-stressed plants led to a dysregulation of the phytohormone network as overstimulated plants in suboptimal conditions were unable to adapt to the contradictory signalling¹⁴⁰. Without a doubt, plant–*Trichoderma* crosstalk is dynamic and the expression of salicylic acid and jasmonic acid–ethylene-dependent defence genes might overlap in an undulating pattern that responds to both biotic and abiotic stresses^{14,141}. This plant effect disappears over time, becoming imperceptible several weeks after the plant has been in contact with *Trichoderma*¹⁴². Given that the plant–*Trichoderma* interaction varies with timing and the plant and/or fungal species-strain involved, time course studies are needed to determine which transduced signal for each specific plant response is prevalent at a given time.

Once the *Trichoderma*-activated priming signals gradually disappear, the plant activates a ‘transcriptional memory’, in which cells previously primed by a particular stimulus show increased rates of gene expression upon subsequent re-stimulation¹⁴³. The capacity for defence priming can be inherited, generating a second level of memory known as ‘heritable priming’, that can be passed-on to the offspring (Box 2). These next-generation plants express a stronger defence response than offspring of un-primed plants¹⁴⁴. The beneficial action that *Trichoderma* has on plants is modulated by molecular networks that condition the immediate and long-lasting systemic responses, orchestrating the metabolic trade-offs between plant growth and defence⁷; for example, in the *Trichoderma*–tomato–RKN interaction, the tomato progeny inherit both resistance to RKN as well as growth-promotion effects without compromising the level of defence in the plant offspring in responding to the nematode attack¹²¹.

Applications in agriculture

Modern agriculture policies have been radically changed by the Sustainable Development Goals of the 2015 UN General Assembly, which were later focused on food and agriculture as the key factors to address concerns regarding fertilizer handling, pesticide use and management practices¹⁴⁵. Climatic changes and intensive agricultural practices have created biodiversity loss, changes in the geographic distribution of plant-damaging pests and pathogens, and contamination of soil, air and water resources by chemicals that negatively impact not only the agroecosystem but also human health¹⁴⁶. Progressive modifications in agricultural policies are aimed at reducing the use of synthetic chemical products; thus, the growing importance of plant-beneficial *Trichoderma* in this process is apparent from its increased use as a biological alternative to agrochemicals and the intensified research linking the fungus to ‘sustainable agriculture’ as noted in the recent literature searches conducted for this review^{38,44,48,71,72}. *Trichoderma* has become a popular protagonist as the key component of plant biostimulants, bioprotectants, biofertilizers, soil amendments, soil integrators, biodegraders and bioremediators¹⁴⁷ (Fig. 4; Supplementary Table 2).

Trichoderma has remained as a renowned BCA of phytopathogens and as a mycoparasite that uses direct antagonism and other mechanisms in the biocontrol of important plant diseases. Therefore, it is of no surprise that *Trichoderma* is the active BCA substance in many commercial preparations registered as plant protection products (PPPs). In general, authorization as a microbial BCA can only be

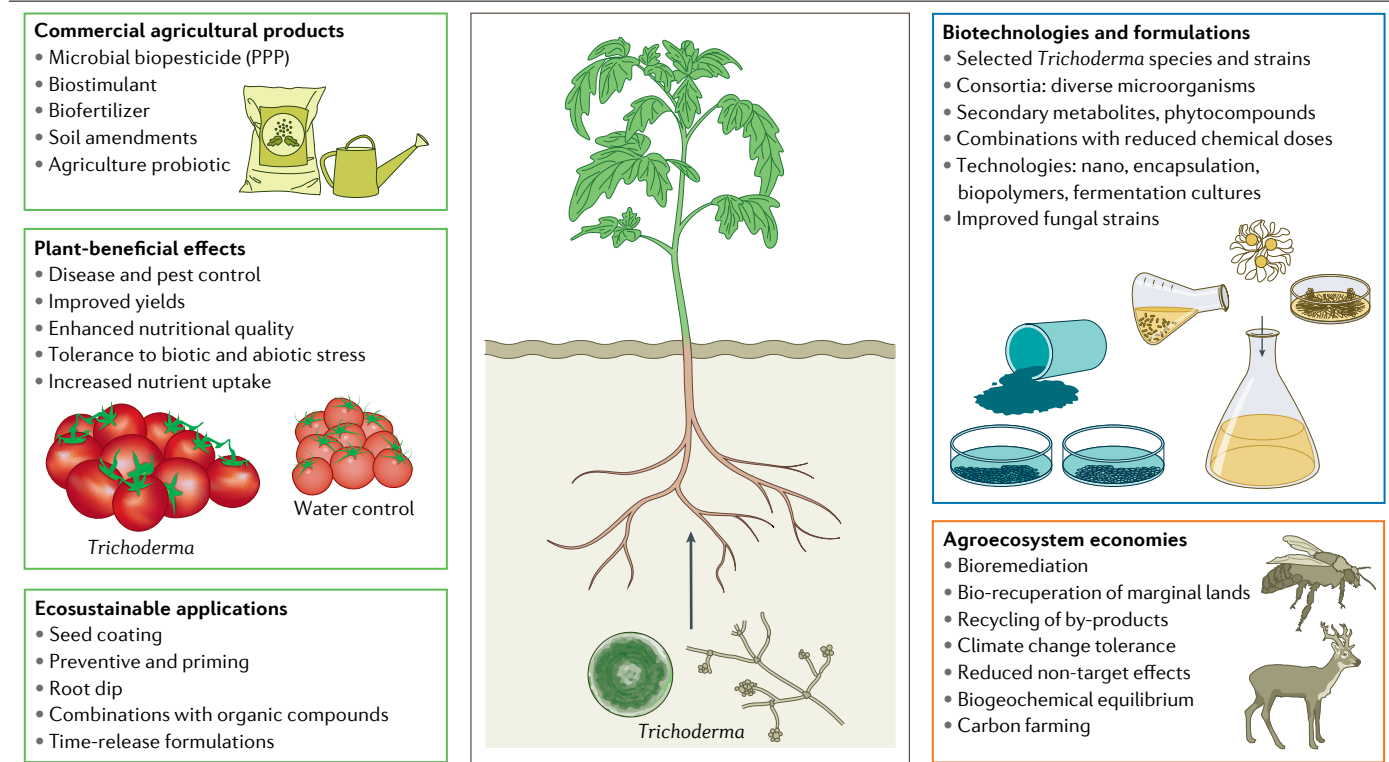


Fig. 4 | Overview of the (potential) contribution of *Trichoderma* to eco-sustainable agriculture. Green border: available commercial products containing *Trichoderma*, their plant-beneficial effects and different application approaches for efficient and sustainable use of resources. Blue border: potential strategies for producing more efficient *Trichoderma* products by using biotechnologies based

on industrial fermentation processes, or manipulation of different living plant-beneficial microorganisms and bioactive components in formulation preparations. Orange border: prospective uses of *Trichoderma* applications to enhance agricultural sustainability, improve agroecosystem equilibrium, and contribute to green and circular economies. PPP, plant protection product.

provided by the appropriate designated institutions after passing a rigorous evaluation process (for efficacy and safety) to support its claims as a PPP, a procedure following that used for chemical phytosanitary products. The usefulness of *Trichoderma* is demonstrated by the increase from 21 BCA registrations, with strains of 8 *Trichoderma* species, worldwide in 2014 (ref.⁷²) to 144 global registrations in 40 countries, comprised of 11 *Trichoderma* species and 44 strains, that are available in 10 diverse product formulations (as noted in an internet search conducted for this Review (2022); Supplementary Table 2). This survey indicated that Brazil has the most active market (28% of total registrations), followed by Colombia (18%), then consolidated European Union (15% in 22 countries). The information provided about the manufactured products from India and China, the biggest Asian consumers of *Trichoderma*-based products, as well as the developing markets in Central and South America are not fully complete owing to the diverse registration procedures used by the regulatory authorities in comparison to the European and North American counterparts. For instance, in India, many governmental research institutions are funded to isolate and test 'agriculturally important microbes' for bioefficacy and to then develop the dossier for registration by the Central Insecticides Board and Registration Committee. Any company can buy the dossier, strain, technology transfer and training, submit for registration, and then manufacture their own product. Currently, in India, this process is only possible for the species *T. viride* and *T. harzianum*. The product claims for the *Trichoderma* microbial biofungicide correspond

to the control of phytopathogens as previously mentioned for direct BCAs and for use in a large variety of crops, including vegetables, field crops (such as wheat, rice, bean and soya), soft fruits, ornamentals and flowers, herbs and aromatics, golf course turf, arboriculture, coffee, orchards and grapevines (Supplementary Table 2).

Although many *Trichoderma* species and strains have been registered for use as PPPs and have recognized plant growth-promotion effects, by definition, they cannot be registered or commercially distributed as plant biostimulants in Europe¹⁴⁷. The regulatory framework varies by country and, in some nations, *Trichoderma* strains are allowed to be marketed with claims as plant inoculants, strengtheners or biostimulants, irrespective of whether the active substances exert direct or indirect biocontrol and without an evaluation process verifying efficacy. Policy-makers are still investigating whether the multipurpose use of *Trichoderma* species, as both PPPs and biostimulants, is possible and how this can be regulated given that the capacity of many *Trichoderma* strains to exert indirect biocontrol and to act as biostimulants is determined by the plant host, depending upon which process the plant exploits (defence versus growth), and by various stimuli in the agroecosystem. This dilemma with *Trichoderma* is an important issue to be faced for future regulation revisions. Given that scientific evidence supports the validity of *Trichoderma* applications both for biocontrol and biostimulation effects together (and just singly), it is time to unify the legal framework regarding these non-harmful and agriculturally useful fungi, defining them as 'plant-beneficial microorganisms'.

Eco-sustainable agriculture

To increase the general success and implementation of biological products in eco-sustainable agriculture, the focus needs to be on improving their shelf life, efficacy and standards to a level similar to that of chemical products used to date⁷¹. Strategies aimed at improving the yield of conidia and chlamydospores and their stress tolerance are of great importance for the development of cost-effective and durable agricultural applications of *Trichoderma*. Technologies to produce an ideal *Trichoderma* product for agriculture should exploit its multipurpose assets by selecting species or strains that have potential biocontrol, rhizosphere competence, endophytic colonization characteristics, and can induce disease resistance and/or promote plant growth (Box 3 and Fig. 4). Components of microbial consortia can include *Trichoderma* species or strains alone with different capabilities, or compatible combinations with other plant-beneficial microorganisms such as biocontrol bacteria (*Bacillus*, *Pseudomonas*) and/or other fungi (*Coniothyrium*, non-pathogenic *Fusarium* or *Rhizoctonia*), entomopathogenic fungi (*Beauveria*, *Metarhizium*), nematode trapping fungi (*Arthrobotrys*, *Dactylellina*, *Drechlerella*), mycorrhizal fungi (AMF, ectomycorrhizal fungi, *Serendipita*), and/or plant growth-promoting rhizobacteria (*Azotobacter*, *Azospirillum*, *Bacillus*, *Pseudomonas*)⁴⁷ (Supplementary Table 2). Bioformulations could contain living *Trichoderma* plus bioactive compounds from other microbial and/or botanical sources (such as algae or phyto-extracts) or with natural carriers to improve application efficacy⁷². For example, *Trichoderma*, its secondary metabolites (such as 6-PP), phytohormones, plant extracts and polymers (such as cellulose, galactomannan or chitosan) have been tested successfully for biocontrol and/or plant growth-promotion effects^{148–150}. To this end, the search, selection and practical use of synthetic communities generated from *Trichoderma*-based root microbiomes²⁰ or synthetic communities from *Trichoderma*-fostered microbiomes⁴⁷ will be important in developing new generation biofertilizers and agricultural probiotics to aid microbiota recruitment or restoration, particularly where intensively cultivated lands suffer from soil fatigue.

With regard to future agricultural mandates aimed at reducing the use of chemical products in farm management, the selection of *Trichoderma* strains tolerant to agrochemicals and compatible with inorganic fertilizers will be a useful strategy¹⁵¹. Industrial processes can use technologies to improve *Trichoderma* qualities by inducing the production of bioactive compounds responsible for the beneficial effects in agricultural production or protecting these qualities during fermentation^{152,153}. Other innovations include encapsulation and nanoparticle technologies¹⁵⁴ for the delivery and dissemination of *Trichoderma* spore inoculum and/or the compounds produced, which could be important during the preparation of fermentation culture supernatants and application over extensive cultivated areas. *Trichoderma* has a role in the present transitions in agriculture towards a green economy (to reduce environmental impacts and ensure food safety) and a circular economy (to recycle agri-food waste to produce value-added products, such as substrate sources to cultivate plant-beneficial microorganisms, including *Trichoderma*, or organic matter formulations, with/without these microorganisms, for direct application as soil amendments). Biotechnological advances might permit the safe and widespread use of *Trichoderma* gene expression in plants to confer increased resistance to pathogens¹⁵⁵ and tolerance to abiotic stresses¹⁵⁶, thus reducing the use of agrochemicals and improving the ability of crops to overcome adverse environmental conditions.

The ongoing findings in *Trichoderma* research prompt a reflection on the effective use of this fungus in agriculture and the development of practical uses with selected potential strains (Fig. 4). New applications

include crop cultivation in marginal lands, improved crop resilience to unfavourable climate changes¹⁵⁷, bioremediation for the reduction of pollutants in contaminated sites¹⁵⁸, and a general contribution to the reduction of methane and carbon dioxide emissions in the atmosphere³⁸.

Conclusions

This article has provided an overview of the advances in *Trichoderma* research that support its applications as a successful BCA and plant biostimulant for improved crop protection and production. Notable changes have occurred in the systematics of the genus-species complex that will affect the nomenclature and how the fungal group is recognized and nominated, subsequently influencing species or strain selection for use in biotechnological development. Progress is also noted in scientific investigations regarding the multitrophic, interkingdom relationships that *Trichoderma* establishes in the agroecosystem; the evolutionary events that have given rise to rhizosphere and endophytic colonization of the host plant; and interactions with the plant microbiota and other non-target organisms that affect the surrounding soil ecology, influence plant growth, and contribute to environmental and human well-being. However, the most exciting scientific discoveries – made possible with modern omics techniques – are those offering insights into the plant

Box 3

Ideal characteristics of a *Trichoderma*-based product for future sustainable agriculture

The ideal commercial product based on *Trichoderma* will be multifunctional and capable of the following diverse beneficial effects for agriculture:

1. Direct biocontrol of plant pathogens and pests, thus reducing the need for chemical pesticides
2. Multiple capabilities for crop protection in a single product, exhibiting a broad spectrum of biocontrol activity against pathogenic microorganisms, nematodes and insects
3. Activation of plant defence mechanisms providing indirect biocontrol of plant pathogens and pests
4. Activation of plant defence mechanisms that increase tolerance to abiotic stress
5. Activation of plant defence priming against biotic and abiotic stresses at the time of attack or damage, which can activate over time and has a long-term duration
6. Provision of heritable beneficial traits in seedbed and nursery plants
7. Stimulation of plant growth to increase crop productivity and yields
8. Improvement of soil nutrient availability and fertilization, leading to increased plant uptake and assimilation
9. Improvement of quality of harvested products by increasing nutritional values and storage attributes
10. Decreased use of chemical products in agriculture, thus reducing risks to the environment and consumer health

Glossary

Agroecosystem

An ecosystem subjected to anthropological activities that are finalized in agricultural production involving the continuous manipulation of biotic and abiotic components to obtain maximum yields and quality of the produce such as food, textile and biofuel plant products, and animal goods.

Biofertilizers

Products that contain living organisms that promote plant growth by increasing the supply or availability of primary nutrients to the host plant.

Biological control agent

(BCA). A natural enemy or antagonistic organism used in plant protection that can inhibit or eliminate harmful organisms and their negative effects through direct or indirect mechanisms of parasitism, antibiosis, competition or induced plant defence.

Bioprotectants

Biological tools providing the protection of plants or the environment from biotic and/or abiotic stress by methods of biocontrol or bioremediation.

Biostimulant

A biological product used to improve plant nutrient use efficiency, tolerance to abiotic stress, quality traits or availability of confined nutrients in the soil by using components of microbial or non-microbial origins.

responses to this fungus guest, including induced defence responses that provide indirect biocontrol to a variety of phytopathogens, the effects on priming and plant memory, and increased tolerance to a diverse range of biotic and abiotic stresses. *Trichoderma* is a model system for studying and deciphering the beneficial microorganism–plant and microorganism–microorganism interactions that these fungi establish among themselves and their surroundings.

Without a doubt, *Trichoderma* is a fascinating microorganism – an opportunist that is versatile and in continuous evolution, a true survivor of the multitude of ecological changes over the millennia. The main question that arises is, how can we harness *Trichoderma* diversity to develop long-term efficient strategies to improve agricultural production and protection? Can adverse climatic conditions be counteracted with *Trichoderma*? Can *Trichoderma* help assure global food security? Can agriculture truly become auto-sufficient by using alternative biological solutions such as *Trichoderma* without the implementation of synthetic

Eco-sustainable agriculture

A system that generates increasing prosperity by reducing chemical inputs and implementing alternative methods to minimize negative impacts to the environment, biodiversity, and human and animal health, thus permitting the renewal of natural resources.

Plant protection products

(PPPs). Products consisting of an approved active substance (chemical or biological) with the capacity to protect plants or plant products against harmful organisms, that positively influence the life processes of plants, preserve plant products, destroy undesired plants or parts of plants, or control or prevent undesired growth of plants.

Priming

An adaptive strategy improving plant defence capacity whereby an initial stimulus activates the physiological, transcriptional, metabolic and epigenetic mechanisms that enable the plant to respond more rapidly and/or efficiently to subsequent exposure to biotic or abiotic stress.

chemicals? How can optimal *Trichoderma* species or strains be selected, formulated and applied to obtain consistent efficacy? At present, the major bottleneck to the development of innovative *Trichoderma*-based products is how to overcome the restrictions imposed by registration and authorization procedures, including inflexible terminology and definitions; inadequate consideration of the variable characteristics of living biological organisms (they are not single purified elements) and the differing effects depending upon interactions between organisms; slow dossier evaluation; and limited communication among researchers, policy-makers, stakeholders and end users. These restrictions are in direct contrast to the legislative policies for agriculture of the future, as mandated by many governments globally, aiming to find solutions in the short-term that provide alternatives to synthetic chemicals, minimize negative impacts to the environment, develop green and circular economies, and implement a One Health approach (a concept that aims to optimize the health of people, animals and the environment). Multidisciplinary investigations are needed to understand the multipurpose properties of *Trichoderma* to maximize the benefits from this green fungus, thus leading to improved quality of life and safe, eco-sustainable agriculture.

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Author contributions

S.L.W. and M.L. conceptualized the idea of this manuscript. S.L.W. and R.H. collected data, designed the content for the article, designed figures and drafted the tables. S.L.W., R.H., M.L. and E.M. contributed substantially to the discussion of the content. S.L.W. and E.M. wrote the article. All authors reviewed and edited the final version of the draft and agreed to the published version of the manuscript.

Competing interests

The authors declare no competing interests.

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