TRICHODERMA SPECIES — OPPORTUNISTIC, AVIRULENT PLANT SYMBIONTS

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Trichoderma spp. are free-living fungi that are common in soil and root ecosystems. Recent discoveries show that they are opportunistic, avirulent plant symbionts, as well as being parasites of other fungi. At least some strains establish robust and long-lasting colonizations of root surfaces and penetrate into the epidermis and a few cells below this level. They produce or release a variety of compounds that induce localized or systemic resistance responses, and this explains their lack of pathogenicity to plants. These root–microorganism associations cause substantial changes to the plant proteome and metabolism. Plants are protected from numerous classes of plant pathogen by responses that are similar to systemic acquired resistance and rhizobacteria-induced systemic resistance. Root colonization by *Trichoderma* spp. also frequently enhances root growth and development, crop productivity, resistance to abiotic stresses and the uptake and use of nutrients.

AXENIC
An axenic system comprises a single type of microorganism.

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Trichoderma spp. (BOX 1) are free-living fungi that are highly interactive in root, soil and foliar environments. It has been known for many years that they produce a wide range of antibiotic substances[1] and that they parasitize other fungi (BOX 2). They can also compete with other microorganisms; for example, they compete for key exudates from seeds that stimulate the germination of propagules of plant-pathogenic fungi in soil[2] and, more generally, compete with soil microorganisms for nutrients and/or space[3]. Furthermore, they inhibit or degrade pectinases and other enzymes that are essential for plant-pathogenic fungi, such as *Botrytis cinerea*, to penetrate leaf surfaces[4].

These direct effects on other fungi are complex and remarkable and, until recently, were considered to be the bases for how *Trichoderma* spp. exert beneficial effects on plant growth and development. Research on these topics has generated a large body of knowledge, including the isolation and cloning of a range of genes that encode proteins of which some have antimicrobial activity. There are several recent reviews of these materials and their mechanisms of action[5–8], so they will not be covered here in any detail. This research has also produced several useful findings, including that genes that encode fungitoxic cell-wall-degrading enzymes can be used to produce transgenic plants that are resistant to disease[9–11], and the discovery of enzymes that are useful in the bioprocessing of chitin[12].

However, it is becoming increasingly clear that our understanding of the mechanisms of biocontrol has been incomplete. In addition to the ability of *Trichoderma* spp. to attack or inhibit the growth of plant pathogens directly, recent discoveries indicate that they can also induce systemic and localized resistance to a variety of plant pathogens (TABLE 1). Moreover, certain strains also have substantial influence on plant growth and development. Their enhancement of plant growth has been known for many years and can occur in both AXENIC systems[13,14] and natural field soils[15,16]. These new findings are dramatically changing our knowledge of the mechanisms of action and uses of these fungi. We now consider that the direct effects of

Box 1 | *Trichoderma* species

Trichoderma is a genus of asexually reproducing fungi that are often the most frequently isolated soil fungi; nearly all temperate and tropical soils contain 10^1–10^3 culturable propagules per gram. These fungi also colonize woody and herbaceous plant materials, in which the sexual TELEOMORPH (genus *Hypocrea*) has most often been found. However, many strains, including most biocontrol strains, have no known sexual stage. In nature, the asexual forms of the fungi persist as clonal, often HETEROKARYOTIC, individuals and populations that probably evolve independently in the asexual stage[86]. They show a high level of genetic diversity, and can be used to produce a wide range of products of commercial and ecological interest. They are prolific producers of extracellular proteins, and are best known for their ability to produce enzymes that degrade cellulose and chitin — although they also produce other useful enzymes[68]. For example, different strains produce more than 100 different metabolites that have known antibiotic activities[1].

Trichoderma species have long been recognized as agents for the control of plant disease and for their ability to increase plant growth and development. They are becoming widely used in horticulture, and the most useful strains show a property that is known as 'rhizosphere competence' — that is, the ability to colonize and grow in association with plant roots[16]. Much of the known biology and many of the uses of these fungi have been documented recently[68,87]. The taxonomies of these fungi are being revised significantly, and many new species are being recognized. In this article, we have tried to use the current binomials; the older taxonomic names for specific strains can be found in the references that are cited in this review.

these fungi on plant growth and development are crucially important for agricultural uses and for understanding the roles of *Trichoderma* in natural and managed ecosystems.

Recent research has also generated data regarding the direct interactions of *Trichoderma* spp. with other microorganisms, including several molecular studies of the roles of specific genes[17–24], but these data are outside the scope of this paper. It is the purpose of this paper to review and consolidate the rapidly developing knowledge of the mechanisms by which *Trichoderma* spp. provide beneficial effects to plants, and to advance the case that at least some strains of these fungi are opportunistic, avirulent plant symbionts.

Induction of resistance in plants

Localized and systemic induced resistance occurs in all or most plants in response to attack by pathogenic microorganisms, physical damage due to insects or other factors, treatment with various chemical inducers and the presence of non-pathogenic RHIZOBACTERIA[25,26]. Much progress has been made in elucidating the pathways involved in this resistance; in many cases, salicylic acid or jasmonic acid, together with ethylene or nitrous oxide, induce a cascade of events that lead to the production of a variety of metabolites and proteins with diverse functions[27,28] (BOX 3). Different pathways are induced by different challenges, although there seems to be crosstalk or competition between the pathways[29].

Bacterial and fungal inducers of plant resistance. In recent years, substantial advances have been made in identifying the induced systemic-resistance pathway that is activated by rhizobacteria, which is the closest analogue of induced resistance activated by *Trichoderma*. The rhizobacteria-induced systemic resistance (RISR) pathway phenotypically resembles systemic acquired resistance (SAR) systems in plants. However, it differs in the fact that root colonization by rhizobacteria does not result in the detectable expression of pathogenesis-related proteins (BOX 3), and root colonization by at least some bacterial strains does not induce accumulation of salicylic acid in the plant[30]. Instead, plants are potentiated to react rapidly to attack by pathogens. In *Arabidopsis*, RISR requires functional plant responses to jasmonic acid and ethylene, can increase sensitivity to these compounds and, like SAR, is dependent on the transcription factor NPR1 (REF. 31). The ability of rhizobacteria to induce systemic resistance has long been known[31,32].

Certain fungi other than *Trichoderma* spp. enhance plant growth and productivity, and can also induce resistance responses in plants. For example, the genus *Fusarium* includes both plant-pathogenic and non-pathogenic races and strains. The non-pathogenic fusaria are known to have strong biocontrol activities; recently, these fungi were shown to induce resistance to pathogenic strains and races of fusaria or *Pythium ultimum*[33–35]. Like *Trichoderma*, the fusaria have several different mechanisms for the direct antagonism of plant pathogens and the induction of plant resistance, and all of these mechanisms are probably important in biocontrol[33,35,36]. Similarly, the fungal genus *Rhizoctonia* contains both plant-pathogenic and non-pathogenic species and strains, with those that are non-pathogenic frequently acting as biocontrol agents; again, these organisms induce plant resistance[37]. Finally, obligate plant-symbiotic MYCORRHIZAL FUNGI might initially suppress plant resistance during the infection process[38], but enhanced systemic resistance sometimes occurs once mycorrhizal fungi are established in plant roots[39]. Other fungi, including strains of *Penicillium* and *Phoma*, have similar abilities[40]. The effects of the resistance-inducing fungi have many similarities to those of *Trichoderma* spp. — most notably, they infect the outer cells of plant roots, are limited to these layers, and induce resistance responses. So, studies of *Trichoderma* and these other fungal species are likely to provide general knowledge that is relevant to all of these systems.

Induced resistance by Trichoderma species. The induction of resistance in plants by *Trichoderma* spp. has been poorly studied compared with the responses that are induced by rhizobacteria, perhaps because the *Trichoderma* research community has focused on factors that are associated with direct effects on other fungi, especially MYCOPARASITISM and ANTIBIOSIS. What was probably the first clear demonstration of induced resistance by *Trichoderma* was published in 1997 by Bigirimana *et al.*[41] They showed that treating soil with *Trichoderma harzianum* strain T-39 made leaves of bean plants resistant to diseases that are caused by the fungal pathogens *B. cinerea* and *Colletotrichum lindemuthianum*, even though T-39 was present only on the roots and not on the foliage. The same group extended their findings from *B. cinerea* to other DICOTYLEDONOUS PLANTS[42]. Similar studies have now been carried out with a wide range of plants, including both MONOCOTYLEDONS and

TELEOMORPH
The sexual form of a fungus.

HETEROKARYOTIC
A fungus or other organism that contains multiple types of nucleus.

RHIZOBACTERIA
Bacteria that are commonly associated with, and colonize, roots.

MYCORRHIZAL FUNGI
Mycorrhizae are associations (usually mutualistic) between a fungus and the root of a plant, and are found in most plants. The fungi associate with the primary cortex of the root.

MYCOPARASITISM
Parasitism of one fungus by another fungus.

ANTIBIOSIS
Strains acting through antibiosis produce antifungal metabolites.

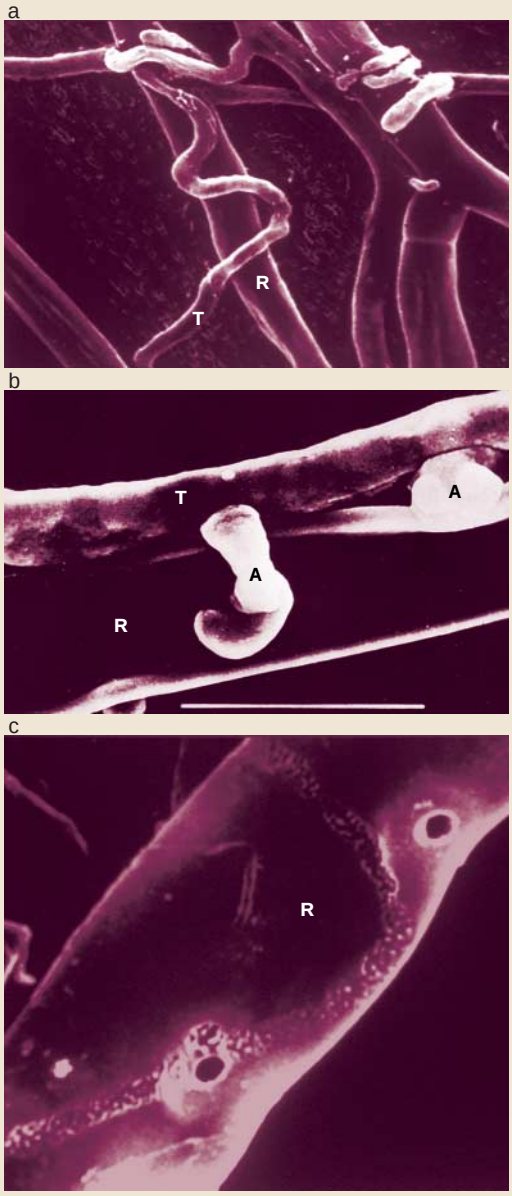
DICOTYLEDONOUS PLANTS
Flowering plants, the seedlings of which have two seed leaves (cotyledons).

MONOCOTYLEDONS
Flowering plants that have only one seed leaf (cotyledon).

Box 2 | Mycoparasitism

Trichoderma spp. parasitize a range of other fungi. The events leading to mycoparasitism are complex, and take place as follows: first, *Trichoderma* strains detect other fungi and grow tropically towards them[88]; remote sensing is at least partially due to the sequential expression of cell-wall-degrading enzymes (BOX 4). Different strains can follow different patterns of induction, but the fungi apparently always produce low levels of an extracellular exochitinase. Diffusion of this enzyme catalyses the release of cell-wall oligomers from target fungi, and this in turn induces the expression of fungitoxic endochitinases[21], which also diffuse and begin the attack on the target fungus before contact is actually made[56,89]. Once the fungi come into contact, *Trichoderma* spp. attach to the host and can coil around it and form APPRESSORIA on the host surface. Attachment is mediated by the binding of carbohydrates in the *Trichoderma* cell wall to lectins on the target fungus[90]. Once in contact, the *Trichoderma* produce several fungitoxic cell-wall-degrading enzymes[6], and probably also peptaibol antibiotics[91]. The combined activities of these compounds result in parasitism of the target fungus and dissolution of the cell walls. At the sites of the appressoria, holes can be produced in the target fungus, and direct entry of *Trichoderma* hyphae into the lumen of the target fungus occurs. There are at least 20–30 known genes, proteins and other metabolites that are directly involved in this interaction, which is typical of the complex systems that are used by these fungi in their interactions with other organisms.

In the figure, 'R' indicates hyphae of the plant pathogen *Rhizoctonia solani*, 'T' indicates hyphae of *Trichoderma* spp., and 'A' indicates appressoria-like structures. In a, the *Trichoderma* strain is in the process of parasitizing a hypha of *R. solani*. The coiling reaction is typical of mycoparasitic interactions. b shows a higher magnification of the *Trichoderma–R. solani* interaction, showing the appressoria-like structures, and c shows an *R. solani* hypha from which the *Trichoderma* has been removed. The scale bar in b represents 10 μm; *R. solani* hyphae are typically 5–6 μm in diameter, and mycoparasitic hyphae of *Trichoderma* spp. are ~3 μm in diameter.



dicotyledons, and with different *Trichoderma* species and strains (TABLE 1; see examples in FIGS 1–3). The ability of *T. harzianum* strain T-22 to induce systemic resistance to pathogens in maize (FIG. 2a) is particularly noteworthy as there are, so far as we are aware, no similar reports of resistance being induced in this crop by any other root-associated commensal or symbiotic microorganism. Therefore, the capacity to induce resistance to a range of diseases — which are caused by various classes of plant pathogen (including fungi, bacteria and viruses) — in a variety of plants seems to be widespread in this fungal genus (TABLE 1). However, much more remains to be understood about the specific systems involved.

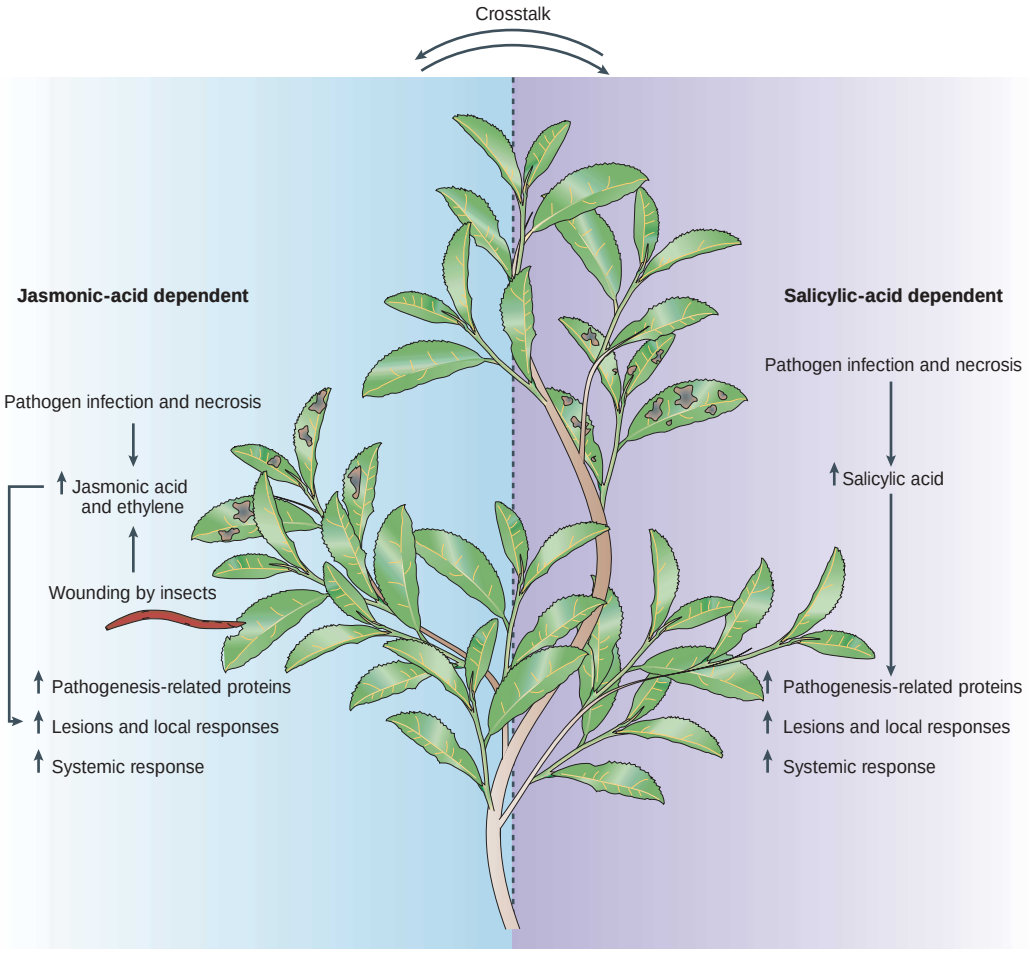
Most of the examples in TABLE 1 show systemic resistance because disease control occurs at a site that is distant from the location of the *Trichoderma*. It is more difficult to examine the role of induced resistance to pathogens that cause seed or root diseases, as both the biocontrol organism and the pathogen are located at the same site. However, strains of *Trichoderma virens* have been obtained that differed in their abilities to produce antibiotics or to be mycoparasitic[43]. Mutant and wild-type strains were tested for their abilities to control the seedling disease of cotton that is caused by *Rhizoctonia solani*, but neither antibiotic production nor mycoparasitism was strongly associated with biocontrol (FIG. 1a). However, the abilities of the strains to induce terpenoid PHYTOALEXIN defence compounds in the seedlings were strongly correlated with disease control (FIG. 1a).

APPRESSORIA
Specialized pressing organs from which a minute infection peg can grow and infect a cell.

PHYTOALEXINS
Low-molecular-weight compounds that have antimicrobial activity and are produced by plants in response to attack by pathogens.

Box 3 | Induced-resistance systems in plants

Induced-resistance systems in plants are complex, but have been partially elucidated in several model plant systems. The figure shows a model of induced resistance in tomato. There are three generally recognized pathways of induced resistance in plants. Two of these pathways involve the direct production of pathogenesis-related (PR) proteins; in one pathway, the production of PR proteins is generally the result of attack by pathogenic microorganisms, and in the other pathway, PR proteins are generally produced as a result of wounding, or necrosis-inducing plant pathogens — for example, herbivory by insects — although both pathways can be induced by other mechanisms. Typically, the pathogen-induced pathway relies on salicylic acid produced by the plant as a signalling molecule, whereas the herbivory-induced pathway relies on jasmonic acid as the signalling molecule. These compounds, and their analogues, induce similar responses when they are applied exogenously, and there is considerable crosstalk between the pathways[29]. The terminology that is associated with these two pathways is confusing, and depends on the tradition of individual researchers[27]; the jasmonate-induced pathway is designated as induced systemic resistance, and this term is also used to refer to the quite different process that is initiated by rhizobacteria[94]. For more information on this subject, see REFS 31,95.



The jasmonate- and salicylate-induced pathways are characterized by the production of a cascade of PR proteins. These include antifungal chitinases, glucanases and thaumatins, and oxidative enzymes, such as peroxidases, polyphenol oxidases and lipoxygenases. Low-molecular-weight compounds with antimicrobial properties (phytoalexins) can also accumulate. The triggering molecules in the *Trichoderma* responses are unknown, and in this paper we refer to any plant-wide processes that result in the direct accumulation of PR proteins or phytoalexins as systemic acquired resistance (SAR).

The third type of induced resistance has been best-described as being induced by non-pathogenic, root-associated bacteria, and is described in this paper as rhizobacteria-induced systemic resistance (RISR). It is phenotypically similar to the jasmonate- and salicylate-induced systems, as it results in systemic resistance to plant diseases. However, it is functionally very different, as the PR proteins and phytoalexins are not induced by root colonization by the rhizobacteria in the absence of attack by plant-pathogenic microorganisms. However, once pathogen attack occurs, the magnitude of the plant response to attack is increased and disease is reduced. Thus, RISR results in a potentiation of plant defence responses in the absence of the cascade of proteins that is typical of the jasmonate- or salicylate-induced systems.

Figure modified with permission from REF. 29 © (2001) Kluwer.

The data indicate that, contrary to our previously held opinions on biocontrol mechanisms, direct effects on plant pathogens are only one mechanism of control, and are perhaps less important than induced resistance. In this case, the *T. virens* strains seem to induce localized, but not systemic, resistance[7]. However, most of the other strains listed in TABLE 1 do induce systemic resistance in a variety of plants. One of the more useful findings is that induced resistance — at least with the rhizosphere-competent strain T-22 — provided control that was both spatially and temporally distant from the point of application. In field trials with tomato, disease caused by natural infection with *Alternaria solani* was substantially reduced on foliage by root application of T-22 more than 100 days earlier (FIG. 1b). It is not yet known how long the induced-resistance response lasts in the absence of the inducing *Trichoderma* strain. However, with rhizosphere-competent strains that grow continuously with the plant, long-term systemic resistance can occur.

So, the data indicate that induced localized or systemic resistance is an important component of plant-disease control by *Trichoderma* spp. However, different mechanisms might be responsible for biocontrol caused by different strains, in different plants, and with different pathogens. For example, targeted mutation of *Trichoderma atroviride* P1 to remove endochitinase activity decreased its biocontrol efficacy towards *B. cinerea* when P1 conidia were applied to foliage[23], which indicates that mycoparasitic abilities are important for the biocontrol of this pathogen. Conversely, the same mutant strain had greater biocontrol ability against *R. solani* than the parental line[23], a result that is consistent with the data shown in FIG. 1, which show the effects of *T. virens* against the same pathogen on cotton.

Research with *T. virens* has generated other interesting results. Terpenoid phytoalexins are generally toxic to most fungi, but the biocontrol strains are much more resistant to these compounds than are most other fungi[43]. In fact, *Trichoderma* spp. in general have been found to be highly resistant to a variety of toxins and XENOBIOTIC compounds, including antibiotics produced by other microorganisms, plant antimicrobial compounds and chemical fungicides[44]. The molecular basis of this resistance — which makes this fungus an active colonizer of toxic environments and a strong competitor — has been partially elucidated with the recent discovery that *Trichoderma* strains produce a set of ATP-binding cassette (ABC) transporters. These ATP-dependent permeases mediate the transport of many different substrates through biological membranes, and overexpression of ABC-transporter genes decreases the accumulation of toxicants in *Trichoderma* cells[45]. In *Trichoderma* spp., ABC transporters have recently been shown to be important in many processes. These include resistance to environmental toxicants that are produced by soil microflora or introduced by human activity (for example, fungicides and heavy-metal pollutants), and secretion of factors (antibiotics and cell-wall-degrading enzymes) that are necessary for the establishment of a compatible interaction with a host fungus, or for the creation of a favourable microenvironment[45]. ABC transporters are probably necessary for the establishment of mycoparasitic interactions with plant pathogenic fungi; knockout mutants of *T. atroviride* that lack specific transporters were inhibited by toxins from *B. cinerea*, *R. solani* and *P. ultimum* and were less effective fungal parasites[45,46].

Biochemical elicitors of disease resistance

Three classes of compound that are produced by *Trichoderma* strains and induce resistance in plants are now known. These are proteins with enzymatic or other functions, homologues of proteins encoded by the avirulence (*Avr*) genes, and oligosaccharides and other low-molecular-weight compounds that are released from fungal or plant cell walls by the activity of *Trichoderma* enzymes.

Proteins with enzymatic or other functions. Even before convincing evidence of induced resistance in plants by *Trichoderma* strains was available, a 22-kDa xylanase that is secreted by several species was shown to induce ethylene production and plant defence responses[47–49]. Interestingly, this small protein is readily translocated through the vascular system of tobacco when introduced through cut PETIOLES. It produced only localized resistance reactions and necrosis[50], so systemic induced resistance by this protein would require translocation within the plant.

More recently, a series of proteins and peptides that are active in inducing terpenoid phytoalexin biosynthesis and peroxidase activity in cotton was found to be produced by strains of *T. virens*. Six separate peptides or proteins that ranged from 6.2 to 42 kDa in size were shown to have elicitor activity[51]. One of these was crossreactive with the ethylene-inducing xylanase described above, and another — of 18 kDa — had amino-terminal sequence similarity to a serine proteinase from *Fusarium sporotichioides*[51].

Avr homologues. The protein products of *Avr* genes have been identified in a variety of fungal and bacterial plant pathogens. They usually function as race- or pathovar-specific elicitors that are capable of inducing hypersensitive responses and other defence-related reactions in plant cultivars that contain the corresponding resistance gene[52,53]. Proteome analysis of T-22 identified proteins that are homologues of Avr4 and Avr9 from *Cladosporium fulvum*; *T. atroviride* strain P1 also produces similar proteins (REF. 54; M.L., unpublished observations; confirmed independently from T-22 by G.H. and J. Chen). Papers that describe the interactions of plants with these compounds will soon be published.

Oligosaccharides and low-molecular-weight compounds. *Trichoderma* mutants that are transformed with reporter systems, which are based on green fluorescent protein or specific enzymatic activities (glucose oxidase) under the control of biocontrol-related promoters[55,56], have been produced. This has permitted the isolation

XENOBIOTIC
A chemical that is present in a natural environment that does not normally occur in nature.

PETIOLE
A slender stem that supports a leaf.

Table1 | Evidence for, and effectiveness of, induced resistance in plants by *Trichoderma* species

Species and strain	Plant	Pathogens	Evidence or effects	Time after application	Efficacy	References
T. virens G-6, G-6-5 and G-11	Cotton	*Rhizoctonia solani*	Protection of plants; induction of fungitoxic terpenoid phytoalexins	4 days	78% reduction in disease; ability to induce phytoalexins required for maximum biocontrol activity	43
T. harzianum T-39	Bean	*Colletotrichum lindemuthianum*; *Botrytis cinerea*	Protection of leaves when T-39 was present only on roots	10 days	42% reduction in lesion area; number of spreading lesions reduced	41
T. harzianum T-39	Tomato, pepper, tobacco, lettuce, bean	*B. cinerea*	Protection of leaves when T-39 was present only on roots	7 days	25–100% reduction in grey-mould symptoms	42
T. asperellum T-203	Cucumber	*Pseudomonas syringae* pv. lachrymans	Protection of leaves when T-203 was present only on roots; production of antifungal compounds in leaves	5 days	Up to 80% reduction in disease on leaves; 100-fold reduction in level of pathogenic bacterial cells in leaves	66
T. harzianum T-22; *T. atroviride* P1	Bean	*B. cinerea* and *Xanthomonas campestris* pv. phaseoli	Protection of leaves when T-22 or P1 was present only on roots; production of antifungal compounds in leaves	7–10 days	69% reduction in grey-mould (*B. cinerea*) symptoms with T22; lower level of control with P1. 54% reduction in bacterial disease symptoms.	M. L., unpublished observations
T. harzianum T-1 & T22; *T. virens* T3	Cucumber	Green-mottle mosaic virus	Protection of leaves when *Trichoderma* strains were present only on roots	7 days	Disease-induced reduction in growth eliminated	99
T. harzianum T-22	Tomato	*Alternaria solani*	Protection of leaves when T-22 was present only on roots	3 months	Up to 80% reduction in early blight symptoms from natural field infection	100
T. harzianum T-22	Maize	*Colletotrichum graminicola*	Protection of leaves when *Trichoderma* strains were present only on roots	14 days	44% reduction of lesion size on wounded leaves; no disease on non-wounded leaves	72
Trichoderma GT3-2	Cucumber	*C. orbiculare*, *P. syringae* pv. lachrymans	Protection of leaves when *Trichoderma* strains were present only on roots; induction of lignification and superoxide generation	1 day	59% and 52% protection from disease caused by *C. orbiculare* or *P. syringae*, respectively	40
T. harzianum	Pepper	*Phytophthora capsici*	Protection of stems when *Trichoderma* strains were present only on roots; enhanced production of the phytoalexin capsidiol	9 days	~40% reduction in lesion length	101
T. harzianum NF-9	Rice	*Magnaporthe grisea*; *Xanthomonas oryzae* pv. oryzae	Protection of leaves when NF-9 was present only on roots	14 days	34–50% reduction in disease	Tong Xu, unpublished observations

and characterization of bioactive molecules that are released by the action of *Trichoderma*-secreted cell-wall-degrading enzymes on the cell walls of fungal pathogens and plants. These molecules — which are produced during the multiple interactions that occur in nature between *Trichoderma*, fungal pathogens and plant roots — function as inducers of the antagonistic gene-expression cascade in *Trichoderma*, and some also function as elicitors of plant defence mechanisms[57,58]. The nature and structure of these compounds are known, and will be published shortly.

Interactions with plants

Trichoderma strains are well known for their ability to colonize roots, but *Trichoderma* conidia have also been applied to fruit, flowers and foliage, and plant diseases can be controlled by their application to any of these sites[16,59,60]. Crucial components of the associations that are considered in this review are microorganism–plant interactions.

*Plant–*Trichoderma *associations.* Some *Trichoderma* strains can colonize only local sites on roots[61], but rhizosphere-competent strains colonize entire root surfaces for several weeks[62] or months[16]. In the few cases that have been examined thoroughly, *Trichoderma* strains colonize root surfaces — sometimes with morphological features reminiscent of those seen during mycoparasitism (FIG. 3a,b) — and hyphae invade the root epidermis (FIG. 3c). Penetration of the root tissue is usually limited to the first or second layers of cells[61,63,64] (FIG. 3d); however, a strain of *Trichoderma stromaticum* that is ENDOPHYTIC in the vascular system in cocoa has been described (REF. 65;

ENDOPHYTE
A non-pathogenic organism living within a plant.

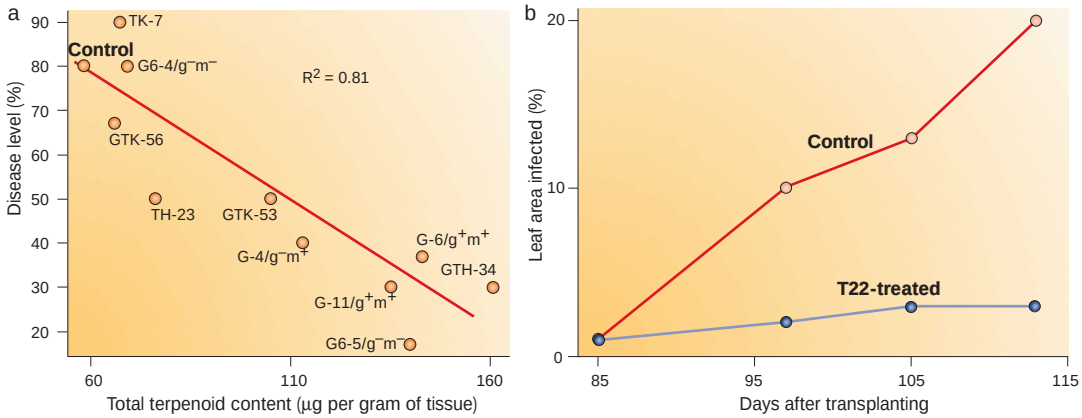


Figure 1 | **Features of induced resistance caused by *Trichoderma* species. a** | Regression analysis of the abilities of several strains of *Trichoderma koningii* (TK-7), *Trichoderma harzianum* (TH-23) and *Trichoderma virens* to control seedling disease caused by *Rhizoctonia solani*, compared to their induction of phytoalexin terpenoids in cotton (graphs produced from data in REF. 43). *T. virens* is known to produce antibiotics, including gliotoxin. These antibiotics have been linked to the ability of *T. virens* to control *R. solani*[96–98], and *T. virens* strains can also be mycoparasitic. In the work described in REF. 43, a number of *T. virens* mutants, with or without the ability to produce gliotoxin (g^+ or g^-, respectively), or with or without the ability to be mycoparasitic (m^+ or m^-, respectively), were studied. These data indicate that the control of *R. solani* disease on cotton seedlings is not due to antibiotic production or mycoparasitism; instead, the biocontrol capability of *Trichoderma* spp. is strongly correlated to the induction of terpenoid phytoalexins — including hemigossypol, deoxyhemigossypol and gossypol — by the seedlings. The original paper contains data on the levels of each phytoalexin[43]. These results indicate that, contrary to previous views, induced resistance is the primary mechanism of the control of this disease on this plant. **b** | The ability of the rhizosphere-competent *T. harzianum* strain T-22 to confer long-term disease resistance in field-grown tomatoes. The data shown are from a study carried out in 2001 (REF. 100). The organism was applied to roots by a drench treatment at the time of transplanting and, when natural infection by *Alternaria solani* occurred — resulting in early blight symptoms on leaves — disease levels were rated. The values shown are significantly different at $p = 0.05$ at 113 days post-transplant[100]. T-22 has been shown to grow as vegetative hyphae and to colonize entire root systems for several months after application[16]; these data indicate that it induces resistance for several months. This test was repeated in 2002, with similar results.

G. Samuels, personal communication). Invasion of the outer root cells by *Trichoderma* strains can result in systemic induced resistance[66], and a systemic vascular-colonizing strain might be even more effective.

Perhaps one of the most intriguing factors is that, although *Trichoderma* spp. and other root-colonizing fungi infect roots, they are usually not plant pathogens. However, in rare cases, particular strains of *Trichoderma* spp. are pathogenic to plants and have been reported to cause diseases of crops such as apples, maize and alfalfa, and some strains can also produce highly phytotoxic metabolites[67]. Moreover, they are prolific producers of enzymes, including those that are necessary to degrade plant cell walls[68]. Thus, the fact that *Trichoderma* spp. (and probably other organisms, such as non-pathogenic *Fusarium* and *Rhizoctonia* strains, mycorrhizae, and other fungi) infect roots and have the intrinsic ability to be plant pathogens, but limit their infection to superficial cells in plant roots, is a remarkable phenomenon.

Some *Trichoderma* strains also grow on leaf surfaces. For example, root or soil application of a transgenic mutant of T-22 that expresses β-glucuronidase did not result in leaf colonization. However, after foliar spray applications of conidia, the spores germinated and numerous T-22 hyphae were seen, including some that parasitized *R. solani* that was resident in the PHYLLOSPHERE[69]. Thus, some *Trichoderma* strains can colonize leaf surfaces under some conditions. However, biocontrol might not be dependent on the growth of *Trichoderma* on leaf surfaces, as the presence of the organism can induce systemic resistance or negatively affect growth or penetration of the pathogen into plants[70,71].

Effects of root colonization on plant metabolism. Several studies have shown that root colonization by *Trichoderma* strains results in increased levels of defence-related plant enzymes, including various peroxidases, chitinases, β-1,3-glucanases, and the lipoxygenase-pathway hydroperoxide lyase[43,63,66,72] (FIG. 3f). In cucumber, the addition of *Trichoderma asperellum* T-203 led to a transient increase in the production of phenylalanine ammonia lyase in both shoots and roots[66], but within 2 days this effect decreased to background levels in both organs. However, if leaves were subsequently inoculated with the bacterial pathogen *Pseudomonas syringae* pv. lachrymans, the expression of many defence-related genes increased several times over (FIG. 3f).

Changes in plant metabolism can lead to the accumulation of antimicrobial compounds. The ability of *T. virens* to induce phytoalexin production and localized resistance in cotton has already been discussed. In cucumber, root colonization by strain T-203 causes an increase in phenolic glucoside levels in leaves; their aglycones (which are phenolic glucosides with the carbohydrate moieties removed) are strongly inhibitory to a range of bacteria and fungi (FIG. 3e).

PHYLLOSPHERE
The area immediately adjacent to a root surface.

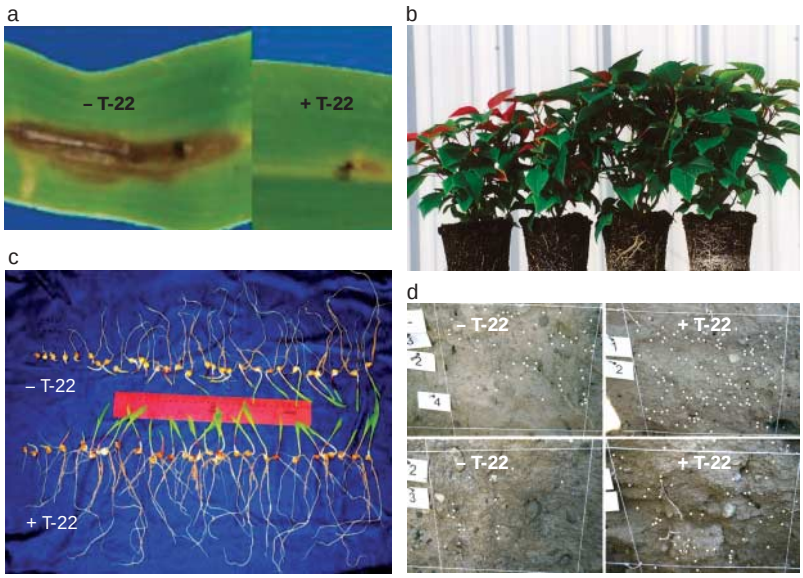


Figure 2 | **Effects of root colonization by *Trichoderma* T-22 on induced systemic resistance and plant growth.** T-22 treatments of seed or soil result in the colonization of the developing root surfaces of plants in a wide variety of soils. This colonization persists throughout the lifetime of annual crops, frequently results in larger roots and shoots, and can also enhance resistance to foliar pathogens. **a** | Lesions of anthracnose caused by *Colletotrichum graminicola* on maize seedlings of line Mo17 that were grown from untreated or T-22-treated seeds, after inoculation at the points marked by the black dots on the leaves. A similar photograph, and supporting data, are published in REF. 72. **b** | Poinsettia plants from a commercial greenhouse. The plant on the far left received no soil fungicide treatment; the second from the left received a single treatment of etridiazole plus thiophanate methyl; the third received a single treatment of etridiazole and monthly applications of thiophanate methyl; the fourth received an early single application of T-22 to the root zone. **c** | Ten-day-old seedlings of the inbred maize line Mo17 grown from untreated or T-22-treated seeds. The differences in size that are seen in the seedlings persist in the mature plant. **d** | Increased deep rooting of maize induced by seed treatment with T-22 — either 25–50 cm (upper panels) or 50–75 cm (lower panels) below the soil surface — ~80 days after planting. The white pins mark root intercepts on the surface of a trench that was dug adjacent to rows of maize grown from T-22-treated or untreated seeds. The areas shown were selected because they represented the mean values of root intercepts across the entire trial. Photograph in **b** provided by Mark Arena, Clemson University, and reproduced by permission of Bioworks Inc., Fairport, USA. Photograph in **c** reproduced, with permission, from REF. 72 © American Phytopathological Society. Photograph in **d** reproduced with permission from REF. 16 © (2000) American Phytopathological Society.

This augmentation of levels of inhibitory compounds occurs only in plants in which the roots are inoculated first with T-203, and whose leaves are then challenged with *P. syringae* pv. lachrymans. This effect was not seen when either organism was applied alone. This response is not restricted to interactions with T-203; leaf extracts from other plants that are inoculated with strains P1 or T-22 have been found to be more antimicrobial than non-treated controls[73] (TABLE 1). So, *Trichoderma* strains do not only produce antibiotic substances directly, they also strongly stimulate plants to produce their own antimicrobial compounds.

Root colonization by these fungi therefore induces significant changes in the plant metabolic machinery. Proteomes from 5-day-old maize seedlings grown from seeds that were either treated or not treated with T-22 were fractionated by two-dimensional gel electrophoresis. Approximately 40% of the proteins that were seen in the presence of T-22 were not visible in gels that contained proteins from untreated plants[72]. Similar results have been obtained by M.L. (unpublished observations) using bean and T-22. Together, the data indicate that these fungi strongly modify plant metabolism, which in most cases benefits the plant.

Summary of induced resistance elicited by Trichoderma spp. The results presented above allow us to formulate a model of the mechanisms by which *Trichoderma* spp. control or reduce plant disease (BOX 4). A variety of strains of *T. virens*, *T. asperellum*, *T. atroviride* and *T. harzianum* induce metabolic changes in plants that increase resistance to a wide range of plant-pathogenic microorganisms and viruses (TABLE 1). Moreover, this response seems to be broadly effective for many plants; for example, T-22 induces resistance in plants as diverse as tomatoes and maize (TABLE 1), which indicates that there is little or no plant specificity. When spores or other propagative structures are added to soil and come into contact with plant roots, they germinate and grow on root surfaces, and at least some infect the outer few root cells. They produce at least three classes of substance that elicit plant defence responses that prevent further infection of roots by plant pathogens. These elicitors include peptides, proteins and low-molecular-weight compounds. In some cases, the resistance is localized — as seems to be the case with *T. virens* on cotton — but in most plant–*Trichoderma* systems, the resistance is systemic. At least for a short period of time, increased expression of defence-related genes occurs throughout the plant. This process can be transitory, but strongly potentiates the expression of defence-related proteins when plants are challenged by pathogens at sites distant from the location of the *Trichoderma* strain. These results indicate that the initial reactions, which include production of pathogenesis-related proteins, have features in common with SAR. At least for the interaction between *T. asperellum* and cucumber, a longer-term response includes low levels of expression of pathogenesis-related proteins before pathogen infection. This response therefore has elements in common with RISR. *Trichoderma* spp. also directly attack other fungi, produce antibiotics, and directly kill or prevent infection by plant pathogenic fungi through other mechanisms.

Improved root and plant growth

Trichoderma spp., and other beneficial root-colonizing microorganisms, also enhance plant growth and productivity. Intuitively, this might seem counterproductive, as most of these species also induce resistance in plants, and switching on resistance pathways must be energetically expensive to the plant. However, many resistance-inducing fungi and bacteria do increase both shoot and root growth. The specific examples that follow are from research on *Trichoderma*, but many other organisms also have similar effects; in fact, resistance-inducing rhizobacteria are widely known as plant-growth-promoting rhizobacteria[32]. At least some non-pathogenic root-colonizing fungi also have similar abilities[13,39].

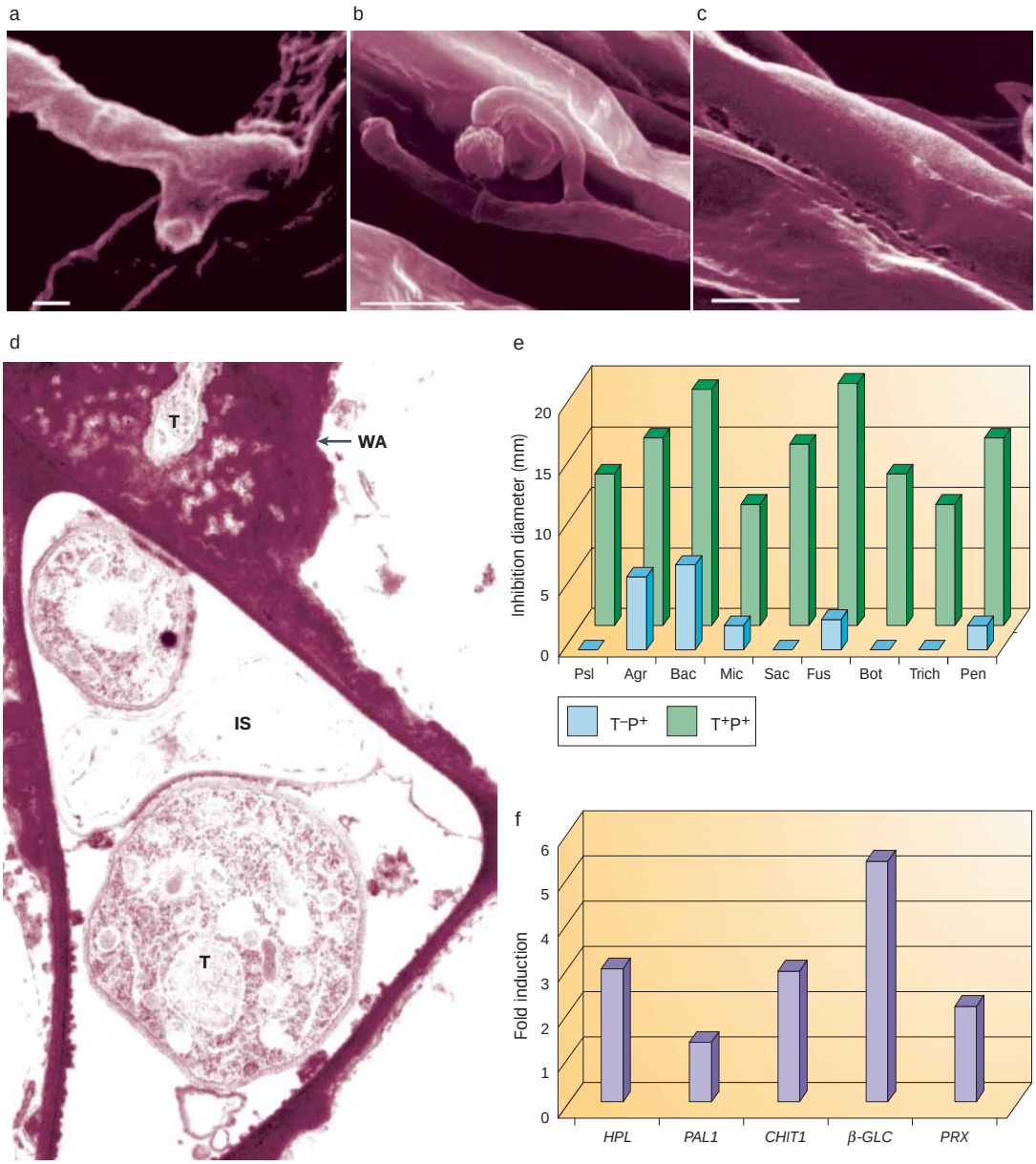


Figure 3 | **Interactions of *Trichoderma asperellum* T-203 with cucumber roots, and effects of these interactions.** *Trichoderma asperellum* was added to cucumber roots in modified hydroponic systems. **a** Shows appresoria-like structures[63], and **b** shows coils around root hairs, which are similar to the interactions of *Trichoderma* with other fungi[63] (BOX 2). **c** | *T. asperellum* also directly penetrates root cortical cells, but is restricted to the outer layer of roots[63]. **d** | Cucumber roots react to infection with the fungus by forming wall appositions (WA) that hyphae of strain T-203 (T) in intracellular spaces (IS) unsuccessfully attempt to penetrate. The increased electron density and osmophilic nature of host cell walls in response to T-203 indicate the production of phenolic compounds, which stain densely on reaction of *O*-hydroxy groups[63]. **e** | Antimicrobial activity of aglycones in the phenolic fraction from leaves of cucumber seedlings in which the roots were either not colonized or colonized by T-203 and then challenged with *Pseudomonas syringae* pv. lachrymans (Psl) (T^-P^+ and T^+P^+, respectively). The seedlings were of the same age and were extracted two days after challenge with Psl. These aglycones have antimicrobial activity against Psl, *Agrobacterium tumefaciens* (Agr), *Bacillus megaterium* (Bac), *Micrococcus lutens* (Mic), *Saccharomyces cerevisiae* (Sac), *Fusarium oxysporum* f. sp. melonis (Fus), *Botrytis cinerea* (Bot), *T. asperellum* (Trich) and *Penicillium italicum* (Pen). Similar data are published in REF. 66 for Psl, Agr, Bac and Mic; this paper extends those findings. **f** | The change (increase over the control, expressed as a proportion) in the expression of *HPL*, *PAL1*, *CHIT1*, *β-GLC* and *PRX*, which encode the putative plant defence-related proteins hydroperoxide lyase, phenylalanine ammonia lyase, chitinase 1, β-1,3-glucanase and peroxidase, respectively. Similar data have been published in REF. 66 for *PAL1* and *HPL*; this paper extends those findings. Differences were measured, using real-time RT–PCR (polymerase chain reaction after reverse transcription of messenger RNA), 48 h after inoculation with Psl on plants in which the roots had been inoculated with T-203 96 h before the assays were carried out. At this time, there was no significant difference in gene expression between untreated plants and plants that were treated with either T-203 or the pathogen alone. Photographs in **a**–**c** are reproduced with permission from REF. 64 © (2000) Elsevier Science.

Although many *Trichoderma* strains are likely to have this ability[13,15], the greatest long-term effects probably occur with rhizosphere-competent strains. In the greenhouse industry, *T. harzianum* strain T-22 is widely used for disease control instead of chemical fungicides because it is safer to use for growers, its disease-control effects last longer than those of synthetic chemical pesticides (FIG. 2b) — so it is less costly than chemical fungicides — and root growth can be as good, or better, than that achieved using pesticides[16] (FIG. 2b).

Increased root development and plant growth. In both academic research and commercial practice, strain T-22 has been shown to increase root development in maize and numerous other plants[16,72] (FIG. 2b–d). This effect lasts for the entire life of annual plants and can be induced by the addition of small amounts of fungus (less than 1 g ha^{-1}) applied as a seed treatment. For example, maize plants were grown in the field from seeds that were either untreated or treated with T-22. Several months later, when the plants were about 2 m tall, trenches were dug adjacent to the rows and the frequencies of root intercepts on the side of the furrow were determined. The presence of root-colonizing T-22 induced about twice as many deep-root intercepts 25–75 cm below the soil surface (FIG. 2d). This resulted in increased drought tolerance, and probably resistance to compacted soils[16]. The ability of T-22 and other strains of *Trichoderma* to induce increased root formation is not restricted to maize or greenhouse crops, and the growth of these plants can be enhanced by the presence of other beneficial root-colonizing microorganisms. Synergy between mycorrhizal fungi and T-22 has been shown[74,75], as well as synergy between *Trichoderma* enzymes and bacterial antibiotics[76]. Moreover, mixtures of different root-colonizing biocontrol agents can provide better results than any one agent used on its own[77]. However, the abilities of combinations of beneficial root-colonizing microorganisms to improve plant performance have been inadequately examined in either managed or natural ecosystems.

Such improvements in root development are frequently associated with increases in yield and biomass. There have been more than 500 commercial and academic trials on the effects of T-22 on maize, and the average increase in yield over those obtained using typical agricultural practices was ~5% (REF. 72). Increases in plant growth were also frequently seen in other field crops, as well as in greenhouse crops[16] (FIG. 2b). *Trichoderma* treatments therefore have the potential to improve overall crop yields and might be particularly important in suboptimal field conditions. Generally, the increased yield of plants is more evident under stressful conditions; when modern crop plants are grown under near-optimal conditions there is little opportunity for yield improvement. However, even under near-optimal conditions, seed treatments of maize sometimes give improved yields[72].

At least in maize, there is a strong genetic component to the yield and plant-growth enhancement that is elicited by T-22. FIGURE 2c shows the response of the inbred line Mo17 to T-22. This line is one of the most positive responders; other lines respond only weakly, and a few actually show a reduction in growth and yield[72]. These differential responses provide an outstanding opportunity to study at the transcriptome or proteome level the responses of plants to beneficial microorganisms, such as T-22, which can give rise to improved yields and greater resistance to pests. It is likely that proteomic or genomic analyses will identify genes and pathways that are involved in resistance to biotic and abiotic stresses and in growth promotion. These techniques also provide important tools for identifying strains that are particularly suited to interacting symbiotically with plants, and fungal metabolites that cause beneficial effects.

In most of the cases mentioned above, it is impossible to separate direct effects on plant growth from the control of pathogenic or other deleterious microorganisms that reduce root growth. However, in the interaction between T-22 and maize, root and shoot growth were increased in both sterilized and non-sterilized soils and in the presence of soil fungicides[72]. Moreover, *T. asperellum* T-203, and other *Trichoderma* strains, increased root growth in axenic systems[13,14]. In most cases, however, improved root development and concomitant increases in plant growth are probably caused both by biocontrol and related effects on root-associated microflora, and by a direct improvement in plant growth. Deleterious root microflora can reduce plant growth in the absence of obvious plant disease[78]. Some deleterious root-associated microflora produce cyanide — probably to maintain their niche in the face of competition[79]. *Trichoderma* spp. are resistant to cyanide and produce two different enzymes that are capable of degrading it in the root zone[80]. Therefore, these fungi can directly increase root growth, control deleterious non-pathogenic root microflora, destroy toxic metabolites produced by deleterious microflora and directly control root pathogens. So, the enhancement of root growth by these fungi, together with concomitant improvements in plant growth and resistance to stress, is accomplished by several different routes. Each of these probably involves multiple mechanisms, as has already been described for the biological control of plant pathogens on roots and foliage.

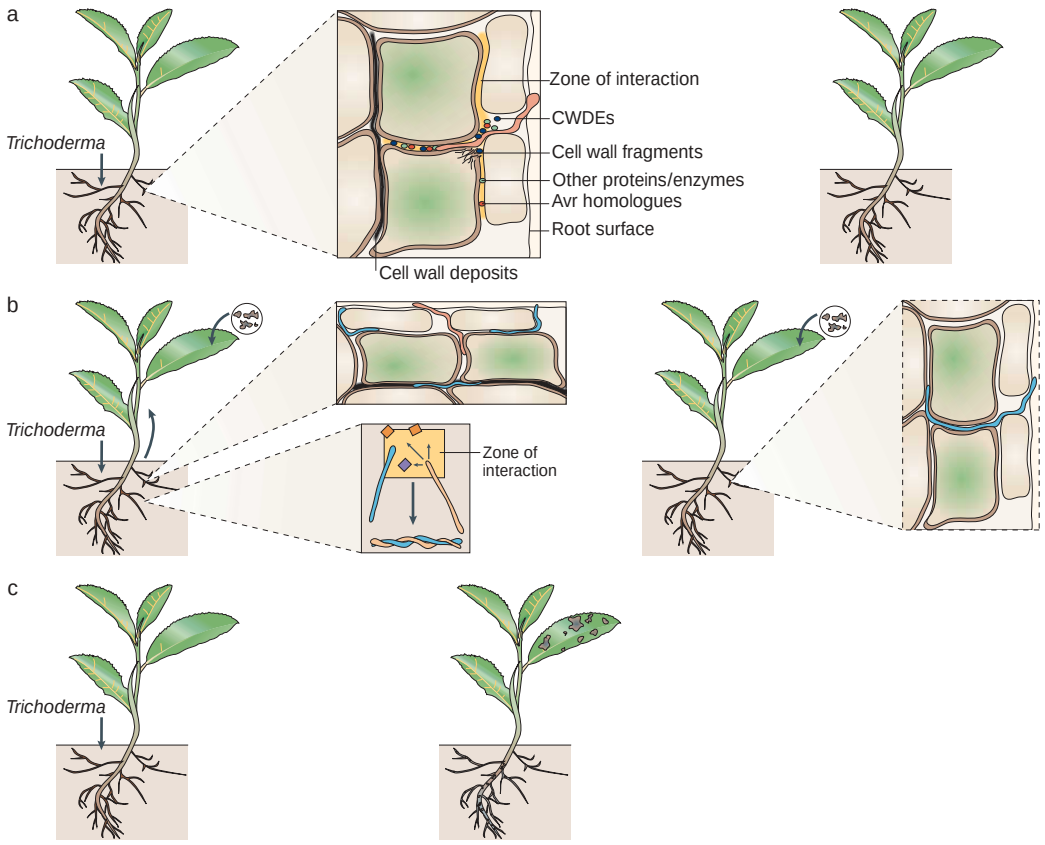
Nutrient-uptake interactions. *Trichoderma* spp. increase the uptake and concentration of a variety of nutrients (copper, phosphorus, iron, manganese and sodium) in roots in hydroponic culture, even under axenic conditions[14]. This increased uptake indicates an improvement in plant active-uptake mechanisms. Moreover, maize generally responds to nitrogen-containing fertilizers by increases in leaf greenness, growth and yield up to a plateau that is generally considered to be the maximum for specific genotypes under the prevalent field conditions. However, plants that are grown from seeds treated with T-22 have been found to give maximum yields with as much as 40% less nitrogen-containing fertilizer than similar plants that were not treated with T-22 (REFS 16,81,82).

Box 4 | Mechanisms of plant-disease control by *Trichoderma*

The results presented and discussed in the review allow us to propose a model of the mechanisms by which *Trichoderma* spp. control or reduce plant disease. The figure shows a schematic overview of the means by which *Trichoderma* spp. control plant pathogens. Other mechanisms also exist, including the inhibition of pathogen enzymes that are necessary for leaf penetration and competition for seed nutrients that are required for pathogen germination in soil.

Trichoderma spores or other biomass can be added to soil by a variety of methods. As shown in a, if the strain (the tan-coloured structure in the magnified section) is rhizosphere competent, it colonizes root surfaces and the outer layers of the cortex. This establishes a zone of interaction into which the *Trichoderma* strain releases bioactive molecules. These include elicitors of resistance, such as homologues of avirulence (Avr) proteins and proteins with enzymatic or other functions. The fungi also produce enzymes that release cell-wall fragments, which also enhance plant resistance responses. The plants produce cell-wall deposits (black layer in the magnified section) and biochemical factors that limit the growth of the *Trichoderma* strain and cause it to be avirulent. A control plant is shown on the right.

Pathogens (shown in blue) can attack roots — as shown in b — but, in the presence of *Trichoderma*, infection is reduced by the same or similar molecules and cell-wall alterations that result in the avirulence of the *Trichoderma* strains (left panel, upper magnified section). Furthermore, several strains induce systemic resistance in plants — even though they are



localized on the roots — probably through the action of a signalling compound (curved arrow going up from the roots). Then, when pathogens attack plant leaves or stems (particles in circles), the plant is potentiated to respond rapidly by producing defence-related enzymes and antimicrobial compounds. In addition, *Trichoderma* strains can attack pathogens in the soil (left panel, lower magnified section) by a variety of mechanisms. The strains respond tropically to the presence of the pathogens, and the interaction begins before the two organisms come into contact (yellow box). The *Trichoderma* produces sensing enzymes (orange rectangles) that release cell-wall fragments from the hyphae of the target pathogen, which increases the release of additional enzymes. Antibiotics can also be produced (purple rectangle). The next step of the interaction is the actual parasitism (which frequently results in the coiling of the *Trichoderma* fungus around the pathogen) and the production of a number of synergistic cell-wall-degrading enzymes and other substances, followed by the infection and death of the target fungus. In the absence of *Trichoderma*, root pathogens (right panel) infect roots and cause disease. Plants are also unprotected against foliar, stem and flower pathogens.

As a consequence of the interactions between *Trichoderma* fungi and plants, a variety of pathogens of roots and the above-ground parts of plants cause less disease in plants in which the roots are colonized by *Trichoderma* (c, left panel). Even in the absence of pathogens or disease, plants frequently have larger roots and higher levels of productivity in the presence of *Trichoderma*. In the absence of *Trichoderma*, either the above-ground or below-ground portions of plants usually have more disease, and are often less robust (right panel).

Moreover, yields can increase above the yield plateau when additional nitrogen-containing fertilizer is used. These data show that T-22 increases the efficiency of nitrogen-containing fertilizer use by maize. Such abilities have the potential to reduce nitrate pollution of ground and surface water, which is a serious adverse consequence of large-scale maize culture. In addition to effects on the efficiency of nitrogen use, analyses indicate that the organism causes a generalized increase in the uptake of many elements, including arsenic, cobalt, cadmium, chromium, nickel, lead, vanadium, magnesium, manganese, copper, boron, zinc, aluminium and sodium. In most cases, however, the increase is small in typical agricultural systems.

Finally, T-22 — and probably other *Trichoderma* spp. — can solubilize various plant nutrients, such as rock phosphate, Fe^{3+}, Cu^{2+}, Mn^{4+} and Zn^{0}, that can be unavailable to plants in certain soils[83]. T-22 reduces oxidized metallic ions to increase their solubility and also produces siderophores that chelate iron[83].

Summary of plant growth effects. *Trichoderma* spp. — with T-22 being one of the most studied examples — increase root growth, and this can increase plant productivity. In many cases, these responses are the result of direct effects on plants, decreased activity of deleterious root microflora, and inactivated toxic compounds in the root zone. The beneficial fungi increase nutrient uptake and the efficiency of nitrogen use, and can solubilize nutrients in the soil. The genetic and molecular bases of these effects are unknown, but there are differences in responses among plant lines and cultivars, at least in maize. Recently, phenotypically similar growth promotion by bacteria was shown to be induced by the release of the volatile compounds acetoin and 2,3-butanediol[84], but the molecular elicitors of plant growth promotion by *Trichoderma* spp. are unknown.

Evolution of the Trichoderma*–plant interaction.* The new information that is summarized and interpreted in this review indicates that *Trichoderma* spp. have evolved as opportunistic plant symbionts. Clearly, they are opportunistic, as they can proliferate, compete and survive in soil and other complex ecosystems. They are also capable of colonizing roots and, in fact, increase in numbers when there are abundant healthy roots in the ecosystem. As they colonize plant roots they invade the superficial layers of the root, but do not penetrate further, at least in part because they elicit plant defence reactions. Therefore, although *Trichoderma* spp. probably have an intrinsic ability to attack plants, they are usually avirulent. The plant defence reactions can become systemic and protect the entire plant from a range of pathogens and diseases, even when *Trichoderma* spp. grow only on the roots. This root colonization also increases the growth of roots and of the entire plant, thereby increasing plant productivity and the yields of reproductive organs. They also help plants to overcome abiotic stresses, and improve nutrient uptake. These findings indicate that *Trichoderma* spp. have developed a symbiotic rather than a parasitic relationship with plants. Similar interactions have occurred with other fungi and bacteria, including avirulent strains of pathogens such as *Fusarium* and *Rhizoctonia*. Thus, the evolution of a symbiotic, as opposed to a pathogenic, relationship with plants has probably occurred many times in different organisms. The fungi themselves (both *Trichoderma* spp. and other beneficial fungi) have many proven abilities to affect plant productivity and health positively; these can be exploited much more efficiently with a better understanding of the mechanisms and systems that operate in interactions between *Trichoderma* spp. and plant pathogens. Moreover, genes such as those encoding the *Trichoderma* Avr proteins might be more useful for engineering plants for disease resistance than their homologues from pathogenic fungi[52,85], because they can be active in many plant species and cultivars.

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Competing interests statement
The authors declare competing financial interests: see Web version for details.

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