Chapter 19
Beyond Classical Biocontrol: New Perspectives on *Trichoderma*

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19.1 Introduction

Bioengineering, a term often used synonymously with "genetic engineering" or "biotechnology," is an emerging field that combines engineering principles with the study of biological systems. The end goal usually is to produce economically viable, biological products that can augment or replace those produced via synthetic chemistry or other "nongreen" technologies. The three most important genera of filamentous fungi used in biotechnology and bioengineering are *Aspergillus*, *Penicillium*, and *Trichoderma*. All three of these major genera encompass species with a wide range of known applications in agricultural, industrial, and medical biotechnology, and each has been the subject of a recent scholarly monograph [for *Aspergillus*, see Gupta (2016); for *Penicillium* see Gupta and Rodriguez-Couto (2017); and for *Trichoderma* see Gupta et al. (2014)].

In this chapter, we will focus on the genus *Trichoderma*, with a particular emphasis on some less known aspects of its bioengineering potential. *Trichoderma* research has generated a voluminous scientific literature that has been reviewed, and reviewed well, by others. We will not attempt to revisit material on the industrial production of cellulases by *Trichoderma reesei* or their use in biofuel production from cellulosic waste. Nor will we cover the traditional use of *Trichoderma* formulations as biological control agents whereby *Trichoderma* species kill pathogens through multiple complementary mechanisms that include mycoparasitism; induction of systemic resistance in plants; competition and rhizosphere competence; secretion of siderophores; production of chitinases and glucanases; and the fungicidal action of toxigenic secondary metabolites and peptides. For excellent and

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comprehensive treatments of this material, see Samuels (1996), Howell (2003), Mukherjee et al. (2013), Gupta et al. (2014), and Samuels and Hebbar (2015).

Our goal here is to highlight understudied and underappreciated aspects of the uses of *Trichoderma* in biotechnology. In the first part of the chapter, we will focus on the use of *Trichoderma* volatiles to enhance plant growth or increase resistance to abiotic stress and the ability of many *Trichoderma* species to function as efficient biological fungicides at a distance through the emission of volatile organic compounds. In the second part of the chapter, we will shift our focus to the unexpected benefits of *Trichoderma* treatments on yields of desirable plant secondary metabolites by certain high-value medicinal, aromatic, and spice plant species. In our summary section, we will recommend promising avenues for future research that will enhance the bioengineering potential of *Trichoderma* as well as raise some important research questions.

19.2 Volatile Organic Compounds

Ecologists recognize that in many habitats, water is in short supply and that chemical signals can best be transmitted through the air (Bitas et al. 2013). It is well-known that *Trichoderma* is a successful rhizosphere inhabitant. The rhizosphere environment is well suited for volatile-mediated communication since the partners are spatially close to one another and because volatiles are more likely to accumulate and reach their threshold of activity in enclosed soil pockets than in above-ground atmosphere (Baily and Weisskopf 2012).

Work in our laboratory and others has shown that *Trichoderma* can have beneficial effects on plant growth without physical contact between plants and the fungus, relying solely on the transmission of signals in the atmosphere (Hung et al. 2013; Kottb et al. 2015; Lee et al. 2015, 2016, 2017). These beneficial effects are mediated by volatile organic compounds (VOCs), which are a large group of carbon-based substances with low molecular weight, low polarity, low boiling point, and high vapor pressure. Many of them are lipophilic (Herrmann 2010). They can be classified according to their boiling points (bp) into three groups: (1) very volatile organic compounds (bp <0 °C to 50–100 °C), (2) ordinary volatile organic compounds (bp 50–100 °C to 240–260 °C), and (3) semi-volatile organic compounds (bp 240–260 °C to 380–400 °C) (WHO 1989). Usually, however, they are classified based on their chemical properties as alkenes, acids, esters, ketones, thiols, and their derivatives (Piechulla and Degenhardt 2014; Hung et al. 2015). Almost all volatiles possess distinct odors, so outside the world of science, they are often described by their aroma properties with adjectives like "aromatic," "earthy," "fruity," "floral," "leathery," "moldy," and so forth (Sell 2006). Certain fungal volatiles are produced commercially for use as natural flavoring agents (Fratz and Zorn 2011). The human olfactory system can detect many VOCs in extremely low concentrations (McGann 2017).

It is well-known that *Trichoderma* spp. generate a diverse array of volatile organic compounds. As with other fungal species, the qualitative nature and quantitative amount of fungal volatile compounds detected vary enormously with the producing species and strain, nutrient source, available moisture, temperature, pH, light-dark status, and other environmental parameters (Zeppa et al. 1990; Korpi et al. 2009). Moreover, the purification, separation, quantification, and identification methods used for experimental analysis also affect the number and kinds of volatiles detected (Zhang and Li 2010; Hung et al. 2015).

A few specific examples, all referring to different published volatile profiles from *T. atroviride*, will illustrate how difficult it is to generalize from studies conducted using diverse strains, culture methods, and purification/detection methods. GC-MS analysis of *T. atroviride* strain IMI 352941 grown on a defined synthetic medium revealed 19 pyrone and dioxolane derivatives after pre-concentration of the fermentation broth on a C18 column, with 5,5-dimethyl-2*H*-pyran-2-one as the predominant pyrone (Keszler et al. 2000). Using strains of *T. atroviride* isolated from Czech soils, different media, and grown with light (conidiated) and dark (nonconidiated) regimes, GC-MS analysis revealed 33 volatile compounds after solid-phase microextraction (SPME). The amounts of 3-octanol, 3-octanone, 1-octen-3-ol, and toluene were higher in the headspaces of sporulating than non-sporulating cultures (Nemcovic et al. 2008). In yet another independently conducted study on *T. atroviride*, 25 volatile compounds were identified in the headspace of solid-grown cultures using SPME-GC-MS analysis. The detected VOCs included alkanes, alcohols, ketones, pyrones (6-pentyl-α-pyrone), furans, monoterpenes, and sesquiterpenes. Thirteen of the 25 identified volatiles were described from *Trichoderma* for the first time: α-phellandrene, β-phellandrene, α-terpinene, γ-terpinene, α-terpinolene, 2-n-heptylfuran, trans-p-menth-2-en-7-ol, α-bergamotene, β-farnesene, α-curcumene, α-farnesene, β-bisabolene, and nerolidol (Stoppacher et al. 2010).

Analyses of VOCs from other *Trichoderma* species reveal an even broader range of volatile profiles. For example, *T. harzianum* cultured in potato dextrose broth yielded 278 volatile compounds when identified by GC-MS using three different capillary columns with different nonpolar, medium polar, and high polar stationary phases. These VOCs encompassed alkanes, ketones, pyrones, furans, alcohols, monoterpenes, and sesquiterpenes (Siddiquee et al. 2012). A separate GC-MS analysis of VOCs produced by *T. viride* and *T. asperellum* revealed alkyl- and alkenyl-2*H*-pyran-2-ones and the new derivatives (*E*)-6-(pent-2-en-1-yl)-2*H*-pyran-2-one, 6-propyl-2*H*-pyran-2-one, and 6-heptyl-2*H*-pyran-2-one. The major compounds in the cultures of *T. viride* and *T. asperellum* were 6-pentyl-2*H*-pyran-2-one and (*E*)-6-(pent-1-en-1-yl)-2*H*-pyran-2-one, respectively (Wickel et al. 2013).

Interactions with other microbes also affect the VOC profile detected. For example, when different species and strains of *Trichoderma* and *Fusarium* are cocultured, the VOCs these fungi produce cause a variety of interspecific effects that vary with the species and strains paired with one another (Li et al. 2018). *T. asperellum*, *T. harzianum*, *T. viride*, and *T. virens* all produced volatiles that inhibited growth of *F. oxysporum*. SPME-GC-MS analysis of headspace volatiles produced by *T. harzianum* grown on potato dextrose agar revealed alcohols such as 2-methyl-1-propanol, 3-methyl-1-butanol, 1-pentanol, 1-hexanol, 1-heptanol, 1-octanol, and 2-phenylethyl alcohol (Li et al. 2018). In this analysis, fewer compounds were detected from *T. virens*. Both species produced 1-octen-3-ol, 3-octanone, and acetic acid (Li et al. 2018).

To recapitulate, VOCs are part of the extraordinary range of fungal metabolism. They occur in chemical families, all of which are easily transmitted through the atmosphere, which means they can act at a distance. In recent years, the volatiles produced by *Trichoderma* have become the subject of increased scrutiny due to their biological activities that include roles in both pathogen management and plant growth promotion, summarized in the sections below.

19.2.1 Fungicidal Action of Volatile Organic Compounds and "Trichofumigation"

Application of fungicidal synthetic chemicals is the most common strategy to control plant pathogens. Many of these chemicals have carcinogenic and toxigenic properties which, due to their long degradation periods, have a negative impact on the environment. Moreover, after years of continuous use, pathogens frequently develop genetic resistance necessitating the use of even higher amounts of problematic chemical fungicides (Bautista-Banos et al. 2006). Over the years, much of the interest in *Trichoderma* formulations surrounds their ability to suppress or kill plant pathogenic species through a variety of mechanisms that range from mycoparasitism to secretion of toxic metabolites (Vinale et al. 2008)

Moreover, there is increasing evidence that suites of volatiles emitted by a variety of fungal species can serve as chemical weapons against plant pathogens. One early and definitive demonstration of the fungicidal potential of fungal VOCs emerged from studies of the volatile mixtures emitted by *Muscodor albus* and related endophytes (Strobel 2006). When this fungus was grown in Petri plates in an enclosed incubator with other similarly cultured fungi, many of the other cultures died. The phenomenon was named "mycofumigation" and attributed to the combined action of the VOCs emitted by the growing endophyte (Strobel et al. 2001). We now know that laboratory co-cultivation of numerous isolates of *Trichoderma* with other fungi can lead to the inhibition or death of target strains, in the absence of physical contact between *Trichoderma* and the other species. This phenomenon is entirely comparable to the mycofumigation effects of the endophytic fungi isolated by the Strobel group. Thus, the exploitation of the fungicidal properties of certain *Trichoderma* volatile compounds promises an innovative and environmentally friendly approach for the reduction of unwanted fungal growth by what might be dubbed "Trichofumigation."

For instance, Bruce et al. (2000) investigated the inhibitory effects of VOCs from *T. aureoviride* against different wood-decay fungi. *T. aureoviride* was grown in low nutrient media-containing amino acids such as phenylalanine, arginine, and glutamine. The VOCs produced by cultures grown on all amino acid combinations, but especially on phenylalanine and arginine, caused reduction in the growth of *Neolentinus lepideus* (also known as *Lentinus lepideus* or "the train wrecker" because of its ability to decompose old railroad ties). The medium with arginine alone or all amino acids together was effective in inhibiting growth of *Gloeophyllum trabeum*, a common brown rot species, while *Trametes versicolor* (also known as *Coriolus versicolor* or turkey tail) was least affected by the VOCs. In the same way, the VOCs produced by *T. aureoviride* and *T. viride* significantly inhibited the growth and protein production of wood-rotting basidiomycete *Serpula lacrymans*, a destructive dry rot, although the VOCs from *T. pseudokoningii* had no effect (Humphris et al. 2002).

In yet another example, the volatile compounds produced by two Egyptian isolates of *T. harzianum*, T23 and T16, when grown in dual culture, reduced mycelial growth of *Fusarium moniliforme* by 51% and 43%, respectively. The fungistatic effect was correlated with the presence of 6-pentyl-α-pyrone, and it was found that the effect was enhanced when the solid medium was supplemented with the compound (El-Hasan et al. 2007). Nevertheless, other studies involving different strains of *T. harzianum*, cultured in different ways, display fungicidal activity despite the absence of detectable 6-pentyl-α-pyrone. In this context, *T. harzianum* VOCs showed significant inhibition against the growth of *Fusarium oxysporum* f. sp. *cucumerinum*. Twelve volatiles were detected in the headspace of this strain, including pentadecane, α-cubebene, hexahydrofarnesol, pristane, verticillol, 2,4-di-tert-butylphenol, β-bisabolene, α-curcumene, lignocerane, nerolidol, biformen (6CI), and 2,6,10-trimethylundeca-5,9-dienal (Zhang et al. 2014).

Table 19.1 provides a list of published "Trichofumigation" studies in which it has been shown that exposure to volatile mixtures emitted by growing dual cultures of *Trichoderma* and selected plant pathogenic fungi inhibits or prevents growth of the pathogen. In most cases, it is not known which of the specific chemical compound or compounds from the *Trichoderma* VOC mixture is responsible for the fungicidal and fungistatic activity. However, as mentioned above, in several cases the "Trichofumigation effect" has been associated with strains that generate a single compound, namely, 6-pentyl-α-pyrone (also known as 6-n-pentyl-2H-pyran-2-one; IUPAC = 6-pentylpyran-2-one) (see Fig. 19.1) (Hanson 2005). This lactone has been known since the 1970s and has a distinct coconut odor (Collins and Halim 1972; Moss et al. 1975). In plate tests against *Rhizoctonia solani* and *Fusarium oxysporum* f. sp. *lycopersici*, a strong correlation was found between strains of *T. harzianum* that inhibited growth of the pathogens and those that produced 6-pentyl-α-pyrone (Scarselletti and Faull 1994). Furthermore, this compound not only inhibited growth of *Fusarium*, but it lowered production of the mycotoxin deoxynivalenol by up to 80% (Cooney et al. 2001). Another interesting effect of 6-pentyl-α-pyrone is its nematocidal activity (Yang et al. 2012).

As a generally regarded as safe (GRAS) natural product, 6-pentyl-α-pyrone is used as a natural aroma/flavoring compound in the food and cosmetics industry, where it is also useful for its antimicrobial properties. Scaled-up fermentation

Table 19.1 Antifungal activity by *Trichoderma* volatile organic compounds ("Trichofumigation")

Trichoderma species	Target organism(s)	Reference
T. asperellum	*Fusarium solani* *Rhizoctonia solani*	Qualhato et al. (2013)
T. aureoviride	*Neolentinus lepideus* *Gloeophyllum trabeum*	Bruce et al. (2000)
T. aureoviride *T. viride*	*Serpula lacrymans*	Humphris et al. (2002)
T. brevicompactum *T. longibrachiatum* *T. virens*	*Fusarium oxysporum*	Anees et al. (2018)
T. ghanense *T. tomentosum*	*Rhizoctonia solani*	Qualhato et al. (2013)
T. gamsii	*Phoma herbarum* *Fusarium flocciferum* *Scytalidium lignicola* *Epicoccum nigrum*	Chen et al. (2016b)
T. harzianum	*Fusarium moniliforme* *Sclerotinia sclerotiorum*	El-Hasan et al. (2007)
T. harzianum	*Fusarium oxysporum*	Zhang et al. (2014)
T. harzianum *T. viride*	*Amylostereum areolatum*	Wang et al. (2019)
T. harzianum *T. viride*	*Fusarium oxysporum* f. sp. *radicis-lycopersici* f. sp. *lycopersici*	Li et al. (2018)
T. koningiopsis	*Phoma herbarum* *Fusarium flocciferum* *Scytalidium lignicola* *Epicoccum nigrum*	Chen et al. (2016a)
Trichoderma spp.	*Pyrenophora teres*	Moya et al. (2018)
T. spirale	*Corynespora cassiicola* *Curvularia aeria*	Baiyee et al. (2019)
T. viride	*Colletotrichum truncatum*	Bankole and Adebanjo (1996)

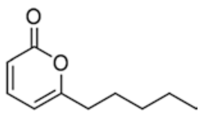


Fig. 19.1 6-amyl-α-pyrone, also known as 6-pentyl-2-pyrone and a number of other chemical names, is a volatile produced by many *Trichoderma* strains. It has a distinct coconut odor

methods have been developed for its commercial production by *T. harzianum*. The yield of these fermentations is limited because when the level of 6-pentyl-α-pyrone increases in the fermentation broth, it has a negative effect on *T. harzianum* growth, thereby limiting yields (Serrano-Carreón et al. 2002). Because the market price of natural aroma compounds is much higher than that of synthetic volatiles, various bioengineering strategies have been developed to circumvent this feedback constraint (Serrano-Carreón et al. 1993).

19.2.2 Enhancement of Plant Growth by Volatile Organic Compounds

Trichoderma VOCs can improve plant growth and enhance plant defenses against both biotic and abiotic stresses. In one study, using *Arabidopsis thaliana* as a model system, plants were grown in a shared atmosphere with *Trichoderma viride* in the absence of physical contact. After 4 weeks of exposure, *A. thaliana* increased total biomass by 45% and total chlorophyll concentration by 58%. A GC-MS analysis of the VOCs produced by *T. viride* found that isobutyl alcohol, isopentyl alcohol, and 3-methylbutanal were the most abundant compounds (Hung et al. 2013). In another study in which VOCs from *T. virens* stimulated *A. thaliana* growth and root development, it was shown that VOC-exposed plants accumulated jasmonic acid and hydrogen peroxide, with an enhanced expression of the jasmonic acid-responsive marker *pLox2:uidA* (Contreras-Cornejo et al. 2014).

The ability of *Trichoderma* to stimulate plant growth in the absence of physical contact has also been shown for *T. asperellum* and *T. atroviride.* In this context, when 7-day-old seedlings of *A. thaliana* were exposed to VOCs from 5-day-old culture of *T. atroviride*, after 2 weeks, plant size, biomass, and chlorophyll content were all increased. VOC profiles were composed of aromatics, alcohols, ketones, aldehydes, and alkenes (Lee et al. 2015). Similarly, *A. thaliana* plants exposed to VOCs of *T. asperellum*, without physical contact, showed a significant increase in trichome numbers; accumulation of defense-related compounds such as H_2O_2, anthocyanin, and camalexin; and increased expression of defense-related genes; 6-pentyl-α-pyrone was the main volatile detected in the headspace (Kottb et al. 2015).

The mixtures of VOCs produced by six different species of *Trichoderma*, including *T. aggressivum*, *T. asperellum*, *T. harzianum*, *T. longibrachiatum*, *T. pseudokoningii*, and *T. viride*, increased the growth of *A. thaliana* and total chlorophyll content. *T. viride* VOCs also enhanced tomato seedlings growth and significant development of lateral roots. More than 141 unique volatile compounds were identified from these *Trichoderma* strains, including hydrocarbons, alcohols, ketones, aldehydes, alkanes, alkenes, esters, aromatic compounds, heterocyclic compounds, and various terpenes (Lee et al. 2016). Moreover, when *A. thaliana* was co-cultivated, in the absence of physical contact, with *T. viride*, *T. atroviride*, *T. longibrachiatum*, *T. citrinoviride*, *T. harzianum*, *T. koningii*, *T. koningiopsis*, *H. orientalis*, and *T. viridescence*, the fresh weights of shoot and roots, as well as the chlorophyll content of plants, were increased (Jalali et al. 2017). Control plants grown with salt stress (100 mM NaCl) had decreased size and chlorophyll content; however, when cocultivated with *T. koningii*, *T. viridescence*, and *H. orientalis*, the VOCs emitted by these three *Trichoderma* species aided the plants in overcoming the stress (Jalali et al. 2017).

As with the fungicidal capacity of *Trichoderma* VOCs with respect to pathogens, most of the published research concerns volatile blends, and less is known about the individual bioactive compounds. It is of interest that 6-amyl-α-pyrone, depending on the concentration, will inhibit or promote seedling growth (Harman et al. 2004; Vinale et al. 2008).

19.3 The Impact of *Trichoderma* on Medicinal, Aromatic, and Spice Plants

Aromatic, medicinal, and spice plants have been used from prehistoric times to the present as botanical raw materials for cosmetic, culinary, perfumery, and/or therapeutic purposes (Sumner 2000; Yaldız et al. 2018). Currently, they are components of natural health foods and traditional medicinal products, as well as the starting materials for value-added ingredients such as essential oils, liquid extracts, powders, and resins (The International Trade Centre 2016). In recent years, there has been a growing demand for natural products, which in turn has led to increasing commercial production of aromatic and medicinal plants for mass-market consumption (Chandra and Sharma 2018). The global market is currently estimated to be around US$ 62 billion and is expected to grow at 15% annually, with Canada, China, Germany, India, and the United States as the largest exporters (Chandra and Sharma 2018; Gahukar 2018). To meet the increased market demand for these high-value natural plant products while not endangering their status as "organic" products, farmers are seeking methods to increase crop productivity without the use of synthetic pesticides and fertilizers. Commercial biofungicides containing *Trichoderma* preparations are well suited for this purpose. They provide a nature-friendly approach that can easily be introduced into organic systems of plant production (Kowalska et al. 2014).

The desirable plant products produced by aromatic, medicinal, and spice plants generally fall under the category of secondary metabolites, an enormous, chemically complex group that can be categorized into structurally similar families such as alkaloids, glycosides, polyphenols, and terpenes. Many of these natural products have been employed for centuries for their pharmacological properties in traditional Chinese medicine, Ayurvedic medicine, and other folk traditions (Chevallier 2001). There are often overlapping uses for crude preparations from a single plant species in food flavoring, aromatherapy, and medicinal purposes (Seidemann 2005). To give two of many possible examples, turmeric, a plant in the ginger family (Zingiberaceae), is used as a dye, a cosmetic, a spice, and a medicinal ingredient (Nair 2013). Similarly, species in mint family (Lamiaceae) are widely employed as flavorings, aromatherapy agents, and therapeutic agents (Lawrence 2006). Plants that produce perfumes, medicinals, and spices are higher in economic value than bulk agricultural crops such as soybean and corn, and plant strains that produce high concentrations of their desirable constituents are sought by specialty farmers.

The earliest and most numerous studies on the beneficial effects of the application of *Trichoderma* spp. on aromatic, medicinal, and spice plants are related to their protective effects against plant pathogens. One such example concerns ginseng, a mainstay of traditional Asian medicine, which produces steroid saponins known as ginsenosides. Ginseng is said to be an adaptogen, a term used in the alternative medicine community to describe a variety of tonics and folk medicine said to build up "vitality" and enhance general physical and mental health. Although modern medicine has provided insufficient research to determine if ginseng extracts actually have the health effects attributed to them, the demand for ginseng root remains high in Korea and China as well as the western natural products market, and the plant has come increasingly under cultivation. A study of *Trichoderma koningiopsis* (strain YIM PH30002) showed that application of the fungus yielded significant growth inhibition of four root-rot pathogens of Chinese ginseng (*Panax notoginseng*): *Phoma herbarum*, *Fusarium flocciferum*, *Scytalidium lignicola*, and *Epicoccum nigrum*. This *Trichoderma* strain grew over each of phytopathogenic fungi with a coiling and twisting mycelium characteristic of mycoparasitism. In addition, this *Trichoderma* strain also produced at blend of at least ten kinds of volatile substances including alkanes, monoterpenes and arenes, heterocycles, and aldehydes, which likely contributed to the fungicidal effect (Chen et al. 2016a). Another strain of *Trichoderma* (*T. citrinoviride* PG87) isolated from Korean or Asian ginseng, *Panax ginseng*, exhibited antagonistic activity against six major ginseng pathogens. The pathogen control was attributed to high activity of the lytic enzyme endo-1, 4-β-D-glucanase (Park et al. 2019).

Several other studies attest to the ability of *Trichoderma* to control pathogens in aromatic medicinal and spice plants. Effective anti-pathogen effects have been shown against diseases caused by *Fusarium* spp. in *Hibiscus sabdariffa* (Parizi et al. 2012), *Zingiber officinale* (Zhang et al. 2017), *Withania somnifera* (Sharma and Trivedi 2010), and *Vanilla planifolia* (Sandheep et al. 2012); *Alternaria* spp. in *Cassia angustifolia* (Tagaram et al. 2015) and *Cuminum cyminum* (Jadeja and Pipliya 2008); *Puccinia thwaitesii* in *Justicia gendarussa* (Ragi et al. 2013); *Pythium aphanidermatum* in *Capsicum annuum* (Muthukumar et al. 2010); and even the hard-to-control nematode *Meloidogyne incognita* in *Mentha arvensis* (Pandey et al. 2011), *Platycodon grandiflorum* (Zhang and Zhang 2009), *Ocimum basilicum* (Tiwari et al. 2017), and *Withania somnifera* (Saikia et al. 2013).

19.3.1 *Trichoderma* Species as Elicitors of Secondary Metabolites in Medicinal, Aromatic, and Spice Plants

Although much of the published research focuses on the plant growth-enhancing properties of *Trichoderma*, increases in size and vigor, anti-pathogenic action, or improved ability to survive in the presence of abiotic stress, *Trichoderma* spp. can provide an additional, less well-understood benefit, namely, the upregulation of gene expression and subsequent yields of high-value secondary metabolites by aromatic, medical, and spice plants. Thus, the application of *Trichoderma* provides multivalent improvements, not just to the physiological pathways involved in shoot growth, root development, and flowering but also to expression of the gene pathways involved in the biosynthesis of economically valuable secondary metabolites. In this section, we review some studies that have measured the increases in the yield of such compounds.

Trichoderma and *Trichoderma* bioproduct applications are able to boost the production of specific biomolecules, such as colchicine in *Gloriosa superba* L. (Alice and Sundravadana 2012), menthol in *Mentha arvensis* L. (Ratnakumari et al. 2014), and tanshinone in *Salvia miltiorrhiza* Bunge (Ming et al. 2013). In basil, for example, *Trichoderma* treatments not only yielded an efficient control of the root-knot nematode *Meloidogyne incognita* but also enriched the essential oil production (Tiwari et al. 2017). In fact, in some medicinal species, *Trichoderma* spp. can be applied solely to improve secondary metabolites yield. In the case of the Chinese Sage (*Salvia miltiorrhiza*) treated with *T. atroviride*, more tanshinones (a class of anti-inflammatory and cytotoxic compounds used in traditional Chinese medicine) were produced in hairy roots due to the increased transcriptional activity of genes involved in the tanshinone biosynthetic pathway (Ming et al. 2013). Most of the overexpressed genes are common for all mono-, sesqui-, and diterpenes products. Table 19.2 provides a summary of the recently published studies on *Trichoderma* spp. effects on secondary metabolite production by medicinal, aromatic, and spice plants.

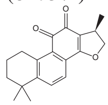
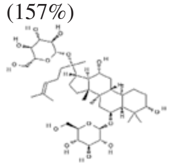
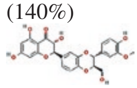
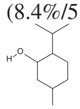
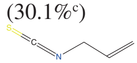
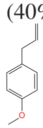
In summary, because of their multiple known mechanisms for enhancing plant growth, biocontrol strains of *Trichoderma* should be considered as a primary component of any integrated pest management program (Verma et al. 2007). With the high premium placed on "organic" methods for the production of natural products for the burgeoning health food and nutraceutical market, the application of *Trichoderma* formulations on high-value plants deserves increased research scrutiny. The specific use of *Trichoderma* spp. as biological elicitors for high-value natural products such as essential oils and pharmacologically active secondary metabolites represents a realistic target for future agronomic research in such specialty crops.

19.4 Conclusions and Future Prospects

There is current widespread use of *Trichoderma* spp. in agriculture, especially in organic farming. The use of *Trichoderma*, however, needs not be restricted to this production system since these biocontrol agents can be equally, or even more, effective than some chemical products. For example, the ability of *Trichoderma* application to suppress plant nematodes is remarkable. Given that chemical nematocidal agents are expensive, leave toxic residues in plants and soils, and are often ineffective in long-term suppression of nematodes (Zhang and Zhang 2009), *Trichoderma* treatments may be one of the few alternatives left for both organic and nonorganic farmers.

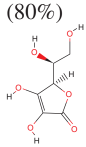
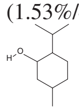
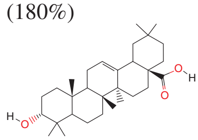
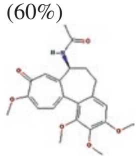
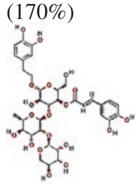
In this review, we focused on two understudied aspects of *Trichoderma* action. The section on VOC-mediated fungal plant interactions highlights the way that the presence of *Trichoderma* can have beneficial actions on plants without physical contact between the fungus and the plant. Biochemists and biologists perform most of their experiments in the aqueous phase, and therefore our general knowledge is

Table 19.2 Enhancement of secondary metabolites in selected aromatic, medicinal, and spice plants by exposure to *Trichoderma*

Trichoderma species (strain)	Plant species Latin name (common name)	Mode of application	Metabolites induced (increase[a, f])	Reference
T. atroviride (D16)	*Salvia Miltiorrhiza* Bunge (red sage or Danshen)	Extract of mycelium added into liquid half-strength B5 medium in hairy roots culture	Cryptotanshinone (8173%)[b] 	Ming et al. (2013)
		Extract of mycelium polysaccharide fraction added into liquid half-strength B5 medium in hairy roots culture	(6496%)	
T. citrinoviride (PG87)	*Panax ginseng* C.A. Mey (Asian or Korean ginseng)	Roots inoculated with 1 mL (1×10^6 spores/mL) in potato dextrose broth by dipping for 3 h	Ginsenoside (157%) 	Park et al. (2019)
T. harzianum (ATCC no. PTA-3701)	*Pogostemon cablin* Benth. (Patchouli)	Basal soil application of vermicompost containing the fungus	Essential oil (4.1%)	Singh et al. (2013)
T. harzianum (KHB)	*Silybum marianum* (L.) Gaertn (milk thistle)	50 mm plugs of medium with mycelium placed on the roots of each plantlet	Silymarin (140%) 	Hasanloo et al. (2010)
T. harzianum (NFCCI 2241)	*Mentha arvensis* L. (field mint)	Basal soil application (20 g per pot) of sorghum-sand mixture containing 2×10^7 CFU/g	Essential oil/menthol (8.4%/5.8%)[c] 	Ratnakumari et al. (2014)
T. harzianum (T22)	*Brassica juncea* (L.) Czern. (Chinese mustard)	Basal soil application (10 g/kg soil) of talc-based formulation containing 2×10^9 CFU/g	Seed oil (30.1%[c]) 	Ahmad et al. (2015)
T. harzianum (ThU)	*Mentha arvensis* L. (field mint)	Basal soil application of 2 g of mint biomass waste materials containing 2.0×10^8 CFU/g	Essential oil (23.8%)	Pandey et al. (2011)
T. harzianum (ThU)	*Ocimum basilicum* L. (Basil)	Basal soil application (2×10^6 spores/g soil) of mycelial material suspended in phosphate buffer	Essential oil/methyl chavicol[d] (40%/41.5%[e]) 	Tiwari et al. (2017)

(continued)

Table 19.2 (continued)

Trichoderma species (strain)	Plant species Latin name (common name)	Mode of application	Metabolites induced (increase[a, f])	Reference
T. longibrachiatum (T1)	*Allium cepa* L. (common onion)	Basal soil application (250 ml per pot) of conidial spore suspension (1×10^7)	Vitamin C (80%) 	Abdelrahman et al. (2016)
T. ovalisporum (NFCCI 2689)	*Mentha arvensis* L. (field mint)	Basal soil application (20 g per pot) of sorghum-sand mixture containing 2×10^7 CFU/g	Essential oil/ menthol (1.53%/4.24%[c]) 	Ratnakumari et al. (2014)
T. viride	*Calendula officinalis* L. (pot marigold)	Dried mycelia powder suspended in plant cell culture medium at a concentration of 0.5 mg/L	Oleanolic acid (180%) 	Wiktorowska et al. (2010)
T. viride	*Gloriosa superba* L. (flame lily, tiger claw)	Basal soil application (2.5 kg/ha) followed by spray (0.2%) of talc-based formulation	Colchicine (60%) 	Alice and Sundravadana (2012)
T. viride	*Teucrium chamaedrys* L. (wall germander)	Mycelial extract supplied at 0.05 mg/ml into cell suspension culture	Teucrioside (170%) 	Antognoni et al. (2012)

[a]Percentage of increase in relation to the control treatment
[b]Only the most significant increase is shown in table. Other compounds were also stimulated by the fungal extract
[c]Mean of two harvests
[d]Under salt stress conditions
[e]*T. harzianum* co-inoculation with *Bacillus tequilensis*
[f]Images of compounds retrieved from PubChem (https://pubchem.ncbi.nlm.nih.gov)

biased toward liquid-phase molecular interactions. Yet in soils, water is frequently limited. Aboveground, organisms need to communicate through the air. Volatile signal moves easily through the atmosphere and can accumulate to appropriate concentrations in soil pockets. There are numerous reports of demonstrable fungicidal effects of *Trichoderma* grown at a distance from a variety of economically destructive fungi, including a wide range of fungal plant pathogens. At low concentrations, VOCs emitted by *Trichoderma* are known to function in interspecific signaling and defense. There is abundant descriptive evidence that *Trichoderma* can not only enhance plant growth but also stimulate an increase in the production of valuable secondary metabolites. While these are valuable phenotypic observations, these findings are correlative, not causal.

A great deal of fundamental work remains to be done. How do plants perceive *Trichoderma* VOCs? Are there different receptor systems in roots and aboveground parts of plants? What kinds of molecular signaling pathways are triggered by volatile signals? How often, and how, do volatiles and other *Trichoderma* metabolites act cooperatively? What mechanistic effects are triggered by *Trichoderma* to increase plant secondary metabolite pathways? How closely do the effects we observe in protected, controlled laboratory environments reflect what occurs in the field? Until we have a better idea of which single compounds are the most active molecules in inducing observed phenotypic changes, it will be difficult to access the wealth of genomics and transcriptomics data now available in public databases.

A few researchers have started using the power of "omics" technologies to decipher the mechanisms by which *Trichoderma* exerts its many plant growth-promoting effects. For example, Abdelrahman et al. (2016) have used metabolic profiling to demonstrate that *T. longibrachiatum* can improve growth of onion plants and increase resistance to the pathogen *Fusarium oxysporum* by triggering plant production of a number of stress-responsive metabolites. Work in our laboratory, using a transcriptomics approach, studied plants treated with vapors of 1-decene. The expression of 123 genes was differentially affected, encompassing genes involved in cell wall modification, auxin induction, stress, and defense responses (Lee et al. 2019). Similarly, a transcript analysis of *Trichoderma* exposed to *Fusarium* volatiles indicated that the several genes, including those for chitinase- and subtilisin-like protease, as well as certain other genes, were upregulated (Li et al. 2018). The field is ripe for more such studies.

The genes responsible for the biosynthesis of some of the most important plant products used for aromas, spices, and traditional medicine are not well understood. Their respective elucidation remains an obstacle to maximizing our ability to use *Trichoderma* to increase yields of valuable plant secondary metabolites. Future experiments will need to address the specific genetically encoded signals that are involved in plant growth promotion, pathogen suppression, and induction of secondary metabolite biosynthetic pathways.

The genus *Trichoderma* has been called the "Swiss army knife" of agricultural biocontrol products (Lorito and Woo 2015). When all the species, strains, formulations, application systems, and beneficial outcomes are added up, the genus deserves this name. *Trichoderma* is a superstar among the beneficial microbes used in agriculture. We believe that the time is right for more basic research on *Trichoderma* molecular biology so as to reach the full potential of this remarkable group of soil-inhabiting fungi in bioengineering.

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