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Citrantifidiene and Citrantifidiol: Bioactive Metabolites Produced by *Trichoderma citrinoviride* with Potential Antifeedant Activity toward Aphids

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Two novel metabolites with potential antifeedant activity were isolated from cultures of the fungus *Trichoderma citrinoviride* strain ITEM 4484 grown in solid-state fermentation on sterile rice kernels. The producing strain was identified at species level by sequence analysis of the internal transcribed spacer regions ITS-1 and ITS-2 of the nuclear rDNA and a fragment of the translation elongation factor gene TEF-1α. Fractionation by column chromatography and TLC of the culture organic extract, followed by feeding preference tests on the aphid pest *Schizaphis graminum* (Rondani), allowed the purification of 5.8 and 8.9 mg/kg of culture of two bioactive metabolites, which were named citrantifidiene and citrantifidiol (**1** and **2**). Citrantifidiene and citrantifidiol, whose structures were determined by spectroscopic methods (NMR and MS) are a symmetrical disubstituted hexa-1,3-dienyl ester of acetic acid and a tetrasubstituted cyclohexane-1,3-diol, respectively. The pure metabolites influenced the feeding preference of *S. graminum* restraining individuals from feeding on wheat leaves dipped in 5% aqueous methanol solution containing 0.57 mg/mL of citrantifidiene or 0.91 mg/mL of citrantifidiol.

KEYWORDS: Aphids; *Schizaphis graminum*; *Trichoderma citrinoviride*; disubstituted hexa-1,3-dienyl ester of the acetic acid; tetrasubstituted cyclohexane-1,3-diol; citrantifidiene; citrantifidiol; antifeedant activity; biocontrol

INTRODUCTION

Aphids are a major cause of loss of agricultural production and reduction of its quality. They induce a direct noxious effect on crop health, caused by subtraction of sap, and an indirect effect related to the spread of insect-transmitted virus diseases. Aphid management relies mainly on the use of synthetic insecticides, and almost all biological control options available are intended for greenhouse aphid pests. However, the extensive use of synthetic pesticides has caused major drawbacks, such as environmental pollution, development of resistant populations of pests, killing of beneficial insects, and contamination of food. Therefore, there is a demand for alternative means of aphid control with low environmental impact.

In recent years there has been a growing interest in the potential use of microbial metabolites as agrochemicals. Microbial metabolites are expected to overcome the problems associated with resistance of pests and are generally more biodegradable and more environment-friendly than their synthetic counterparts (*1*). In particular, fungi are an endless source of secondary metabolites with different biological activities. It is known that some fungi and fungal metabolites can affect several traits of insect biology, such as survival, development, fecundity, and feeding activity (*2*). In the context of new pest control strategies with low environmental impact, a promising field is the search for natural compounds capable of interfering with the pest insect's processes of host plant selection. Nevertheless, data on the antifeedant effect of fungal metabolites toward insect pests are still sparse (*3*–*8*), and very few fungal metabolites with antifeedant activity have been isolated and characterized so far. Ganassi et al. (*8*) found that fungal isolates of genus *Trichoderma* influenced the feeding preference of the aphid *Schizaphis graminum*, one of the most important pests

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of cereal crops, and a potential antifeedant activity of these fungi toward aphids was conceived.

This paper describes the isolation and structural elucidation of two new fungal metabolites with antifeedant activity toward aphids that were produced in solid culture by *Trichoderma citrinoviride* ITEM 4484. The structures of these compounds, named citrantifidiene and citrantifidiol (**1** and **2**), were determined by extensive use of spectroscopic (essentially NMR and MS) techniques. In addition, since misidentification of producing strains is a major problem in the field of bioactive fungal metabolites (*9*), we herein report the molecular characterization of *T. citrinoviride* ITEM 4484 on the basis of sequence analysis of the internal transcribed spacer regions ITS-1 and ITS-2 of the nuclear rDNA and a fragment of the translation elongation factor gene TEF-1α.

MATERIALS AND METHODS

Fungal Strain. The producing strain used in this study was isolated from soil under *Abies* sp. in Tyrol (Austria). After reisolation from a single germinated conidium, the strain was maintained in purity in the culture collection of the Institute of Sciences of Food Production with the accession number ITEM 4484. The strain was identified as belonging to the species *Trichoderma citrinoviride* Bissett on the basis of both morphological characters according to Gams and Bissett (*10*) and molecular techniques. For the molecular characterization, genomic DNA of the strain ITEM 4484 was extracted from lyophilized mycelium as described by Gallo et al. (*11*). A region of the nuclear rDNA comprising the two diagnostic regions ITS-1 and ITS-2 and a fragment of the translation elongation factor gene (TEF-1α) were amplified by specific primers. The primers used for polymerase chain reaction (PCR) amplification were ITS1 for (5′-TCC GTA GGT GAA CCT GCG G-3′) and ITS4 rev (5′-TCC TCC GCT TAT TGA TAT GC-3′) (*12*), and TEF728 for (5′-CAT CGA GAA GTT CGA GAA GG-3′) (*13*) and TEF1 rev (5′-GCC ATC CTT GGA GAT ACC AGC-3′) (*14*). PCR amplification conditions for ITS1/ITS4 and TEF-1α consisted of a denaturation step at 94 °C for 5 min, 35 cycles of 30 s at 94 °C, 50 s at 55 °C, and 50 s at 72 °C followed by a final extension step of 5 min at 72 °C. PCR products were sequenced directly. All sequence data were obtained using the ABI Prism Big Dye Deoxy Terminator Cycle Sequencing kit (Applied Biosystem, Foster City, CA), and reactions were analyzed using a model 310 Genetic Analyzer (Applied Biosystem). Sequence data were then analyzed by *Trich*OKEY v2.0, a program for the quick molecular identification of *Trichoderma* species that has been developed by the International Subcommission on *Trichoderma* and *Hypocrea* taxonomy (ISTH) of the International Commission for the Taxonomy of Fungi (ICTF), accessed through the ISTH Web site (http://www.isth.info/index.php).

Aphids. The aphids used belonged to the species *Schizaphis graminum* (Rondani) (Homoptera: Aphididae). The original *S. graminum* stock was collected in a cereal field located near Modena (Italy). Aphids were reared for several generations on wheat plants (*Triticum durum* Desf.) in a thermostatic chamber at 20 °C under a L16:D8 light: dark cycle to induce parthenogenesis. Trials were carried out using alate and apterous adult morphs. Alate offspring were obtained in the laboratory by crowding.

General Experimental Procedures. Optical rotation was measured in $CHCl_3$ solution on a JASCO (Tokyo, Japan) P-1010 digital polarimeter. IR and UV spectra were determined as neat and in CH_3CN solution, respectively, on a Perkin-Elmer Fourier transform infrared (FT-IR) spectrometer and a Perkin-Elmer Lambda 25 UV−vis spectrophotometer (Norwalk, CT). ^1H and ^{13}C NMR spectra were recorded at 600 and 500 and at 150 MHz, respectively, in $CDCl_3$, on Bruker (Karlsruhe, Germany) spectrometers. The solvent was used as internal standard. Carbon multiplicities were determined by DEPT spectra (*15*). DEPT, COSY-45, HSQC, HMBC, and NOESY (*15*) experiments (see the Supporting Information) were performed using Bruker microprograms. EI were taken at 70 eV on a Shimadzu (Kyoto, Yapan) Qp5050. Electrospray (ESI) MS were recorded on a Waters micromass Q-Tof model (Milford, MS). Analytical and preparative thin-layer chromatography (TLC) were performed on silica gel (Kieselgel 60 F_{254}, 0.25 and 0.50 mm, respectively, Merck, Darmstadt, Germany) or reverse phase (KC18 F_{254}, 0.20 mm, Whatman, Maidstone, United Kingdom) plates; the spots were visualized by exposure to UV radiation and/or by spraying with 10% H_2SO_4 in methanol and then with 5% phosphomolybdic acid in methanol followed by heating at 110 °C for 10 min. Column chromatography: silica gel (Kieselgel 60, 0.063−0.20 mm, Merck, Darmstadt, Germany).

Table 1. ^1H and ^{13}C NMR Data of Citrantifidiene (**1**)[a,b]

C	δ[c]	^1H	J (Hz)	HMBC
1,4	157.5 (s)			4.38, 2.38, 1.99
2,3	117.1 (d)	5.81 (br s)		1.99
5,7	29.2 (t)	2.38 (td)	6.4, 0.6	1.99
6,8	66.0 (t)	4.38 (t)	6.4	
2 × MeCO	171.0 (s)			1.99
2 × MeCO	23.0 (q)	1.99 (s)		

[a] The chemical shifts are in δ values (ppm) from TMS. [b] 2D ^1H, ^1H (COSY) ^{13}C, and ^1H (HSQC) NMR experiments delineated the correlations of all protons and the corresponding carbons. [c] Multiplicities determined by DEPT spectrum.

Production, Extraction, and Purification of Citrantifidiene and Citrantifidiol (1 and 2). *Trichoderma citrinoviride* ITEM 4484 was cultured in 500 mL Erlenmeyer flasks containing 200 g of autoclaved rice kernels previously adjusted to approximately 45% moisture and inoculated with 10−15 small pieces (2 × 2 mm) of a fresh culture (5−7 days old) of the fungus on potato-dextrose-agar. The cultures were incubated at 25 °C under a photoperiod of 12 h for 4 weeks. The harvested culture material was dried in a forced draft oven at 35 °C for 48 h, was finely ground in a laboratory mill (Molino Cyclone LMLF; PBI International, Milan, Italy) to a particle size ≤0.2 mm, and was stored at 4 °C until use. One kilogram of dried material was extracted with a MeOH−H_2O (1% NaCl) mixture (55:45 v/v), was defatted by *n*-hexane, and then was extracted with CH_2Cl_2 as previously reported (*16*). The organic extracts were combined, were dried (Na_2SO_4), and were evaporated under reduced pressure yielding a brown oil (15 g) showing a significant antifeedant activity. The organic extract was fractionated by column chromatography (CC) using as eluent $CHCl_3$-*i*-PrOH 85:15 yielding 10 groups (A−L) of homogeneous fractions. The last fraction was eluted with methanol. The residues of the B (1.58 g) and C (1.28 g) fraction groups showed high antifeedant activity. Purification of fraction C (1.28 g) of the initial column by CC eluted with petroleum ether-Me_2CO 7:3 gave nine groups of homogeneous fractions (C1−C9). The residues of fractions C2 (80.1 mg), C3 (431.6 mg), and C7 (54.6 mg) showed good antifeedant activity. Purification of fraction C2 by preparative TLC (eluent petroleum ether-Me_2CO 7:3) gave seven groups of homogeneous fractions (A1−G1), and the residues of the fractions D1 (15 mg) and G1 (8.8 mg) showed a high antifeedant activity. The fraction D1 was purified by reversed-phase TLC (eluent EtOH−H_2O 6:4) yielding a pure metabolite as an amorphous solid (R_f 0.74 eluent EtOH-H_2O 6:4, 5.8 mg) that has been named citrantifidiene (**1**). Purification of fraction C3 by CC (eluent petroleum ether-Me_2CO 7:3) gave 11 groups of homogeneous fractions (A2−M2), and only the residue of the fraction B2 (73.2 mg) showed a good antifeedant activity. This fraction was purified by two preparative TLC steps on silica gel (eluent EtOAc-*n*-hexane 6:4) and reversed-phase (eluent EtOH−H_2O 6:4) plates yielding another metabolite as an amorphous solid (R_f 0.58, eluent EtOH-H_2O 6:4, 8.9 mg) that has been named citrantifidiol (**2**).

Citrantifidiene (1). IR ν_{max} cm^{-1} 3423, 1725, 1676; UV λ_{max} nm (log ϵ): 213 (4.43); ^1H and ^{13}C NMR spectra: see **Table 1**; EI MS (rel. int) *m/z*: 257 $[M-H]^+$ (2), 239 $[M-H-H_2O]^+$ (2), 209 $[M-H-H_2O-H_2CO]^+$ (2), 167 $[M-H-H_2O-H_2CO-CH_2CO]^+$ (28), 149 $[M-H-H_2O-H_2CO-CH_2CO-H_2O]^+$ (64), 130 $[M/2+H]^+$ (4), 129 $[M/2]^+$ (4), 113 $[M/2+H-OH]^+$ (11), 99 $[M/2-H_2CO]^+$ (9), 71 $[M/2+H-OH-CH_2CO]^+$ (68), 57 $[M/2-H_2CO-CH_2CO]^+$ (100), 43 $[CH_3CO]^+$ (93). HR ESI MS *m/z*: 281.1112 [calcd for $C_{12}H_{18}NaO_6$, 281.1103, M + Na]$^+$.

Citrantifidiol (2). $[\alpha]^{25}D$: −9.7 (*c* 1.5); IR ν_{max} cm^{-1} 3432, 1641; UV λ_{max} nm: <200; ^1H and ^{13}C NMR: see **Table 2**; HR ESI MS *m/z*: 279 $[M+K]^+$, 263.1998 [calcd for $C_{15}H_{28}NaO_2$, 263.1987, M + Na]$^+$.

Table 2. ^{1}H and ^{13}C NMR Data of Citrantifidiol (**2**)[a,b]

C	δ[c]	^{1}H	J (Hz)	HMBC
1	81.3 (s)			1.69, 1.26, 1.04
2	44.2 (d)	1.60 (m)	6.8	1.26, 1.04
3	74.9 (s)			1.49, 1.17
4	54.3 (s)	1.86 (m)		1.69, 1.49, 1.17, 1.04
5	24.4 (t)	1.86 (m)		1.60
		1.69 (m)		
6	40.4 (t)	1.86 (m)		1.26
		1.56 (m)		
7	40.4 (t)	1.49 (t)	8.3	2.05, 1.69, 1.17
8	22.7 (t)	2.05 (dt)	7.0, 8.3	1.49
9	124.5 (d)	5.12 (br t)	7.0	2.05, 1.69, 1.62, 1.49
10	131.8 (s)			2.05, 1.69, 1.62
11[d]	25.1 (q)	1.69 (s)		
12	26.1 (q)	1.26 (s)		1.86, 1.60
13	14.5 (q)	1.04 (d)	6.8	1.17
14	25.7 (q)	1.17 (s)		1.04
15[d]	17.7 (q)	1.62 (s)		1.69
OH		1.11 (br s)		
		1.21 (br s)		

[a] The chemical shifts are in δ values (ppm) from TMS. [b] 2D ^{1}H, ^{1}H (COSY, TOCSY) ^{13}C, and ^{1}H (HSQC) NMR experiments delineated the correlations of all protons and the corresponding carbons. [c] Multiplicities determined by DEPT spectrum. [d] Assigned on the basis of NOESY effects (**Table 3**).

Table 3. 2D ^{1}H NOE (NOESY) Data Obtained for Citrantifidiol (**2**)

considered	effects
5.12 (H-9)	2.05 (H_2-8), 1.69 (Me-15)
2.05 (H_2-8)	5.12 (H-9), 1.69 (Me-15), 1.49 (H_2-7), 1.17 (Me-14)
1.86 (H-5)	1.56 (H-6′), 1.26 (Me-12), 1.17 (Me-14)
1.69 (Me-15)	5.12 (H-9), 2.05 (H_2-8)
1.26 (Me-12)	1.86 (H-5)
1.17 (Me-14)	1.86 (H-5)
1.04 (Me-13)	1.86 (H-6), 1.86 (H-4)

Bioassays. The biological activity of the crude organic extract of *T. citrinoviride*, of the fractions obtained in the purification process and of pure citrantifidiene and citrantifidiol, was assessed by a feeding preference test on the aphid *S. graminum*. Extract, fractions and pure compounds were solubilized in 5% (v/v) aqueous methanol to perform feeding preference tests as previously described (*8*). The crude organic extract was tested at a concentration corresponding to 500 mg of rice culture per mL of 5% aqueous methanol. Samples of pure citrantifidiene and citrantifidiol were dissolved in 5% aqueous methanol to obtain approximately the content of 100 g of rice culture per mL of solution. Final concentrations were 0.57 mg/mL for citrantifidiene and 0.91 mg/mL for citrantifidiol. A 5% aqueous methanol solution was used as the control. For the tests, excised wheat leaves of about 2 cm in length, obtained from seedlings grown under controlled environment until growth stage 11 (first leaf unfolded) according to Zadoks et al. (*17*), were used. The excised leaves were dipped in the different solutions for 10 s and then were placed on wet filter paper in 12 cm Petri dishes. In each dish, two leaves, one treated with the test solution and one dipped in the control solution, were arranged in parallel with ventral side up at a distance of 4 cm. Aphids were placed between the two leaves with a fine brush, and their position was recorded every hour for 8 h, starting from the initial access that began less than 1 h after leaf excision. For each treatment, 30 alate morphs were tested separately and 6 replicates per treatment were run. The test was performed three times with the organic extract and twice with chromatographic fractions (data not shown) and pure metabolites. The test on apterous morphs was performed only with citrantifidiol because of shortage of pure citrantifidiene. This latter experiment was performed twice. The raw data obtained from feeding preference tests were analyzed by the generalized linear models (GLM) repeated measures procedure to separate treatment effects and the treatment∗time interaction effects and were compared using a test of within subject effects with SPSS 14.0 for Windows software (SPSS Inc., Chicago, IL). The differences of the means between the number of aphids per leaf in each of the experimental treatments and the number of aphids on related controls over time were analyzed and adjusted with Bonferroni test for the number of comparisons.

RESULTS AND DISCUSSION

Amplification of the target regions of the genomic DNA resulted in a 554 bp sequence of the ITS1/ITS4 region that comprised the two diagnostic regions ITS-1 (195 bp) and ITS-2 (168 bp) and the internal 5.8S rDNA gene and a 580 bp fragment of TEF-1α (sequences not shown). Analyses of these sequences by *Trich*OKEY v2.0 allowed the univocal identification of the producing strain as *T. citrinoviride*.

The solid culture of *T. citrinoviride* (1 kg) was exhaustively extracted as reported in the experimental section. The organic extract, showing a high antifeedant activity, was purified by a combination of column chromatography and TLC on direct and reverse phase as described in the experimental section. We obtained two bioactive metabolites as amorphous solids, which were named citrantifidiene and citrantifidiol (**1** and **2 Figure 1** 5.8 and 8.9 mg/kg). In addition, we isolated four more pure compounds (data not shown), which on the basis of preliminary spectroscopic investigation appear closely related to trichodimerol, a metabolite which belongs to the complex structural group of bisorbicillinoids (*18*).

Citrantifidiene (**1**) has a molecular weight of 258 accounting for a molecular formula of $C_{12}H_{18}O_6$ as deduced from HR ESI mass spectrum. This formula is consistent with four unsaturations associated with the double bond and the ester carbonyl groups as deduced from the IR spectrum and preliminary ^{1}H and ^{13}C NMR spectra. The IR spectrum also showed bands attributable to hydroxy group (*19*) while the UV spectrum exhibited the presence of extended conjugated chromophores (*20*).

The ^{1}H NMR spectrum (**Table 1**) showed a broad singlet, typical of a proton of trisubstituted olefinic group at δ 5.81 (*21*), as well as a triplet ($J = 6.4$) and a triple doublet ($J = 6.4$ and 0.6 Hz) at δ 4.38 and 2.38 of a hydroxyethyl side chain bonded to a quaternary olefinic carbon, which coupled themselves in the COSY spectrum (S1). The methyl singlet of an acetoxy group was also observed at δ 1.99 (*21*). These structural features were confirmed by the analysis of the ^{13}C NMR spectrum (**Table 1**), which was assigned in agreement with DEPT and HSQC (S1) data. The signal of the ester carbonyl group and those of the quaternary and secondary olefinic carbons resonated at the typical chemical shifts values of δ 171.0, 157.5, and 117.1, respectively, as well as those of the two methylene carbons of the hydroxyl ethyl side chain at δ 66.0 and 29.2. Finally, the methyl of the acetoxy group appeared at δ 23.0 (*22*). On the basis of these results, the molecular formula of $C_{12}H_{18}O_6$ and the lack of optical activity suggest that citrantifidiene is a symmetrical compound formulated as acetic acid 4-acetoxy-6-hydroxy-1-(2-hydroxy-ethyl)-hexa-1,3-dienyl ester of acetic acid (**1**).

The structure assigned to citrantifidiene was confirmed by couplings observed in the HMBC spectrum (S1) (**Table 1**) and is supported by data from EI mass spectrum. The pseudomolecular ion $[M - H]^+$ at *m/z* 257 showed ions produced by fragmentation mechanisms typical of the functionalities present in **1** (*21*). In fact, the pseudomolecular ion $[M - H]^+$, losing in succession H_2O, H_2CO, CH_2CO, and H_2O residues, produced the ions at *m/z* 239, 209, 167, and 149, respectively. Significant was the presence of ions accounting for half and the corresponding protonated molecules $[M/2]^+$ and $[M/2 + H]^+$ at *m/z* 129 and 130 as well as those yielded by fragmentation pattern similar to those observed for the whole compounds. In fact, the ion $[M/2 + H]^+$, losing in

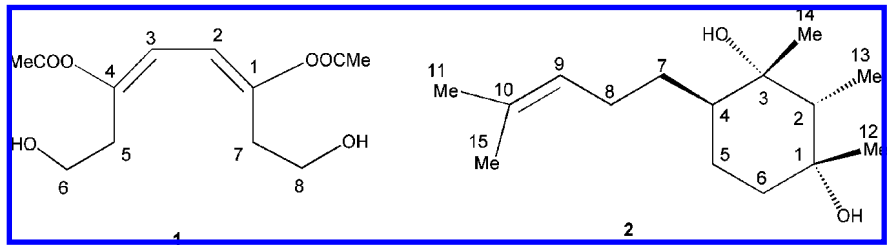

Figure 1. Structures of citrantifidiene and citrantifidiol (**1** and **2**).

succession HO and H_2CO residues, yielded the ions at *m/z* 113 and 71. Alternatively, the ion $[M/2]^+$ by successive loss of H_2CO and CH_2CO residues produced the ions at *m/z* 99 and 57. Finally, the significant acetyl ion $[CH_3CO]^+$ was present at *m/z* 43. The HR ESI mass spectrum showed the sodium cluster $[M + Na]^+$ at *m/z* 281.1112.

Furthermore, the *E*-stereochemistry of the double bond could be assigned because of the clear effect observed in a NOESY spectrum between the olefinic proton at δ 5.81 and the methyl of acetoxy group at δ 1.99. This was consistent with the typical allylic coupling ($J = 0.6$ Hz) observed between the olefinic proton and the methylene group at δ 2.38 (*23*).

Citrantifidiol (**2**) has a molecular formula of $C_{15}H_{28}O_2$ as deduced from HR ESI mass spectrum. It is consistent with two unsaturations, one of which was attributed to a trisubstituted olefinic group in agreement with the typical bands observed in the IR spectrum, which also showed bands typical of hydroxy groups (*19*). The absence of absorption maxima in the UV spectrum (*20*) suggested that the other unsaturation could be attributed to a cyclohexane ring. These structural features were confirmed by the investigation of the ^1H and ^{13}C NMR spectrum.

The ^1H spectrum (**Table 2**) showed the broad triplet ($J =$ 7.0 Hz) of the olefinic proton (H-9) at δ 5.12 (*21*), which is in the COSY spectrum (S2) coupled with the protons of the adjacent methylene group (CH_2-8). This appeared as a double triplet ($J = 7.0$ and 8.3 Hz) at δ 2.05 being also coupled with the protons of another methylene group (CH_2-7), which resonated as a triplet ($J = 8.3$ Hz) at δ 1.49. The olefinic proton was also long-range coupled ($J < 1$ Hz) with the two singlets at δ 1.69 and 1.62, which were assigned to the two methyl groups (Me-11 and Me-15) bonded to quaternary olefinic carbon (C-10) (*21*). These results suggested the presence in **2** of a 4-methylpent-3-enyl residue which was bonded to the penta-substituted cyclohexane ring. In fact, the signal of a complex multiplet at δ 1.86, accounting for three overlapping protons, was assigned, also on the basis of the correlations observed in the HSQC spectrum (S2), to the proton of a methyne (HC-4) group and one proton for each of the two adjacent methylene (CH_2-5 and CH_2-6) groups of the cyclohexane ring. The other protons of these latter two groups resonated as complex multiplets at δ 1.69 and 1.56 (H-5′ and H-6′). They coupled with the geminal protons (H-5 and H-6), probably between themselves, and H-5′ also coupled with H-4. Finally, we observed two singlets at δ 1.17 and 1.26, assigned to tertiary methyl groups (Me-14 and Me-12), and a doublet ($J = 6.8$) due to secondary methyl group (Me-13). The latter coupled with the proton (H-2) of the adjacent methyne carbon, which appeared in part overlapped with the multiplet of H-6′ at δ 1.60 (*21*). The presence of a 4-methyl-penten-3-enyl and a hexas-ubstituted cyclohexane ring in **2** was confirmed by the investigation of the ^{13}C NMR spectrum (**Table 2**). Considering also the couplings observed in the HSQC spectrum, the signals observed at the typical δ values of 131.8, 124.5, 40.4, and 22.7 were assigned to the quaternary and secondary olefinic carbons and the two methylene carbons of the 4-methyl-penten-3-enyl side chain (C-10, C-9, C-7, and C-8, respectively) (*22*). The carbons of the two methyl groups of the same residue resonated at δ 25.1 and 17.7 (Me-11 and Me-15). The couplings observed in the HSQC spectrum also allowed the assignment of the six carbons of the cyclohexane ring, two of which appeared quaternary and hydroxylated, and those of three methyl groups. In fact, the singlet signals (**Table 2**) observed at δ 81.3 and 74.9 were assigned to C-1 and C-3, while those resonating as doublets at δ 54.3 and 44.2 and as triplets at δ 40.4 (overlapped to C-7) and 24.4 were assigned to C4, C-2, C-6, and C-5, respectively. The signals of the three methyl groups bonded to this ring were observed at δ 26.1, 25.7, and 14.5 and were assigned to Me-12, Me-14, and Me-13. They, together with the 4-methyl-penten-3-enyl side chain, were located on C-1, C-3, C-2, and C-4 of the cyclohexane ring on the basis of the several couplings observed in the HMBC spectrum (**Table 2**) (S2). These results allowed the assignment of the structure of citrantifidiol as 1,2,3-trimethyl-4-(4-methyl-pent-3-enyl)-cyclo-hexane-1,3-diol (**2**).

This structure was supported from the results of the HR ESI spectrum that showed the potassium and sodium clusters at *m/z* 279 and 263.1998 and by the NOE couplings observed in the NOESY spectrum (**Table 3**).

Furthermore, the NOESY data, supported by an inspection of a Dreiding model, suggested that the cyclohexane ring assumes a chair conformation and that of the methyl groups Me-12, Me-13, and Me-14, bonded at C-1, C-2, and C-3, are axial while and the 4-methylpenten-3-enyl, located on C-4, is equatorial. This relative configuration is depicted in structure **2**.

The organic extract of *T. citrinoviride* and citrantifidiene and citrantifidiol exhibited a clear ability to influence the feeding preferences of the alate morphs of the aphid *S. graminum* restraining them from visiting treated leaves. In feeding preference tests, GLM analysis revealed a treatment effect of both the organic extract (GLM organic extract: treatment $F_{1,136} =$ 569.717, $P < 0.01$) and the pure metabolites citrantifidiene (GLM: treatment $F_{1,88} = 45.500$, $P < 0.01$) and citrantifidiol (GLM: treatment $F_{1,88} = 151.724$, $P < 0.01$). GLM analysis did not reveal treatment∗time interactions (GLM organic extract: treatment∗time $F_{7,136} = 0.416$ $P > 0.05$, GLM citrantifidiene: treatment∗time $F_{7,88} = 0.854$ $P > 0.05$, and GLM citrantifidiol: treatment∗time $F_{7,88} = 0.405$ $P > 0.05$).

The Bonferroni adjustment for the number of comparisons revealed that the mean (*M*) of the number of aphids counted on control leaves ($M = 3.521$, standard error (SE) = 0.079) was significantly greater than that of the number of aphids on leaves dipped in organic extract ($M = 0.674$, SE = 0.056) over the time. Moreover, the same analysis revealed that the average number of aphids counted on leaves treated with either citrantifidiene ($M = 1.458$, SE = 0.114) or citrantifidiol ($M =$ 0.958, SE = 0.76) was significantly smaller than the number of aphids counted on correspondent control leaves ($M = 2.896$, SE = 0.107 and $M = 3.042$, SE = 0.109, respectively).

In feeding preference tests carried out with apterous morphs, GLM analysis revealed a treatment effect of the pure metabolite citrantifidiol (GLM: treatment $F_{1,88} = 26.286$, $P < 0.01$). GLM analysis did not reveal treatment∗time interactions (GLM citrantifidiol: treatment∗time $F_{7,88} = 0.501$ $P > 0.05$).

The Bonferroni adjustment for the number of comparisons revealed that the mean of the number of apterous morphs counted on control leaves ($M = 2.719$, SE = 0.095) was significantly greater than that of the number of specimens on leaves dipped in citrantifidiol ($M = 1.906$, SE = 0.073) over the time.

Citrantifidiene and citrantifidiol are two new fungal metabolites isolated from *T. citrinoviride* ITEM 4484 and show an interesting antifeedant activity. They appear to have a very original carbon skeleton among the naturally occurring compounds. The compound most closely structurally related to citrantifidiol appears to be magydardienediol, a diterpene previously isolated from the plant *Magydaris panacifolia* (*24*). Citrantifidiene, a hexa-1,3-dienyl ester of acetic acid, is quite different from citrantifidiol, which is a tetrasubstituted derivative of a cycloexane-1,3-diol. They also appear very different from the other four metabolites, produced by the same fungus, that are related to trichodimerol, a metabolite which belongs to the complex structural group of bisorbicillinoids. The antifeedant activity exhibited by citrantifidiene and citrantifidiol opens an interesting perspective of practical use of these metabolites or the producing fungal strain in control of the aphid pest *S. graminum* and warrants further investigations on their mode of action, systemicity, volatility, and performances under field conditions.

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Supporting Information Available: COSY, HSQC, and HMBC spectra of citrantifidiene and citrantifidiol (**1** and **2**). This information is available free of charge via the Internet at http://pubs.acs.org.

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