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STEROL COMPOSITION OF THE VINE POWDERY MILDEW FUNGUS, *UNCINULA NECATOR*: COMPARISON OF TRIADIMENOL-SENSITIVE AND RESISTANT STRAINS

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Key Word Index—*Uncinula necator*; vine powdery mildew; *Vitis vinifera*; fungicide resistance; fungus; ergosta-5,24(24[1])-dienol; 14α-methylsterols; sterol C-14 demethylase; Δ^5-sterols; triadimenol.

Abstract—Ergosta-5,24(24[1])-dien-3β-ol was the major sterol of conidia of *Uncinula necator*, an obligate pathogenic fungus, which is the causal agent of vine powdery mildew. Other Δ^5-sterols, such as cholesterol, ergost-5-en-3β-ol, stigmasta-5,22-dien-3β-ol and stigmast-5-en-3β-ol found in *U. necator* host plant (*Vitis vinifera*) were detected as minor sterols in conidia of *U. necator*. The triadimenol-sensitive and resistant strains contained similar sterol compositions. Triadimenol treatment led to accumulation of 14α-methylsterols, such as eburicol and obtusifoliol. This suggests that sterol C-14 demethylase is the target for triadimenol in sterol biosynthesis in *U. necator*.

Nomenclature

Trivial names used: amyrin, urs-12-en-3β-ol (**10**); cholesterol, cholest-5-en-3β-ol (**1**); cycloartenol, 4,4,14α-trimethyl-9β,19-cyclo-cholest-24-en-3β-ol; cycloeucalenol, 4α,14α-dimethyl-9β,19-cyclo-ergost-24(24[1])-en-3β-ol (**9**); 4,4-dimethylfecosterol, 4,4-dimethylergosta-8,24(24[1])-dien-3β-ol (**14**); eburicol, 4,4,14α-trimethylergosta-8,24(24[1])-dien-3β-ol (**15**); episterol, ergosta-7,24(24[1])-dien-3β-ol (**12**); ergosterol, ergosta-5,7,22-trien-3β-ol; lanosterol, 4,4,14α-trimethylcholesta-8,24-dien-3β-ol (**16**); 4α-methylfecosterol, 4α-methylergosta-8,24(24[1])-dien-3β-ol (**13**); 24-methylenecycloartanol, 4,4,14α-trimethyl-9β,19-cyclo-ergost-24(24[1])-en-3β-ol (**11**); obtusifoliol, 4α,14α-dimethylergosta-8,24(24[1])-dien-3β-ol (**8**).

INTRODUCTION

On vine (*Vitis vinifera* L.), the causal agent of powdery mildew is *Uncinula necator* (Schw.) Burr., an obligate pathogenic fungus. Sulphur, the oldest multi-site fungicide applied against powdery mildews, was largely used in European vineyards to fight *U. necator*. Dichlofluanid, another multi-site fungicide, dinocap an uncoupler of respiration and antimicrotubular benzimidazoles (e.g. benomyl) and thiophanates (e.g. thiophanate-methyl) were introduced later. Over the last 10 years, these various fungicides have been supplanted by sterol C-14 demethylation inhibitors (DMIs) [1–4]. In France, the DMIs registered against vine powdery mildew are triazoles (cyproconazole, diclobutrazole, difenoconazole, diniconazole, fenbuconazole, flusilazole, hexaconazole, myclobutanil, penconazole, tebuconazole, triadimefon, triadimenol), pyrimidines (fenarimol, nuarimol), pyridines (pyrifenox) and triforine. Because of the specific mode of action and the intensive use of DMIs, field resistance was observed in several countries (France, Italy, Portugal). The first occurrence of resistance was reported in Portugal in 1988, 10 years after the introduction of triadimefon. In France, this phenomenon was initially observed in southern vineyards (Madiran) regularly treated with triadimenol, whereas in Italy, the main DMI was fenarimol [5–7]. The triadimenol-resistant strains were also resistant to some other DMIs, but not to all of them [8, 9]. In order to better understand the biochemistry of the vine powdery mildew and the development of resistance to DMIs (especially triadimenol), sterol compositions of strains sensitive or resistant to triadimenol were studied. The sterol analyses were conducted in conidia and mycelium of various strains cultivated in the absence or presence of triadimenol.

RESULTS

Conidia sterol contents of untreated U. necator *strains*

The total amount and composition of the sterols of conidia of the different *U. necator* strains (Table 1) were similar whatever their sensitivity towards triadimenol

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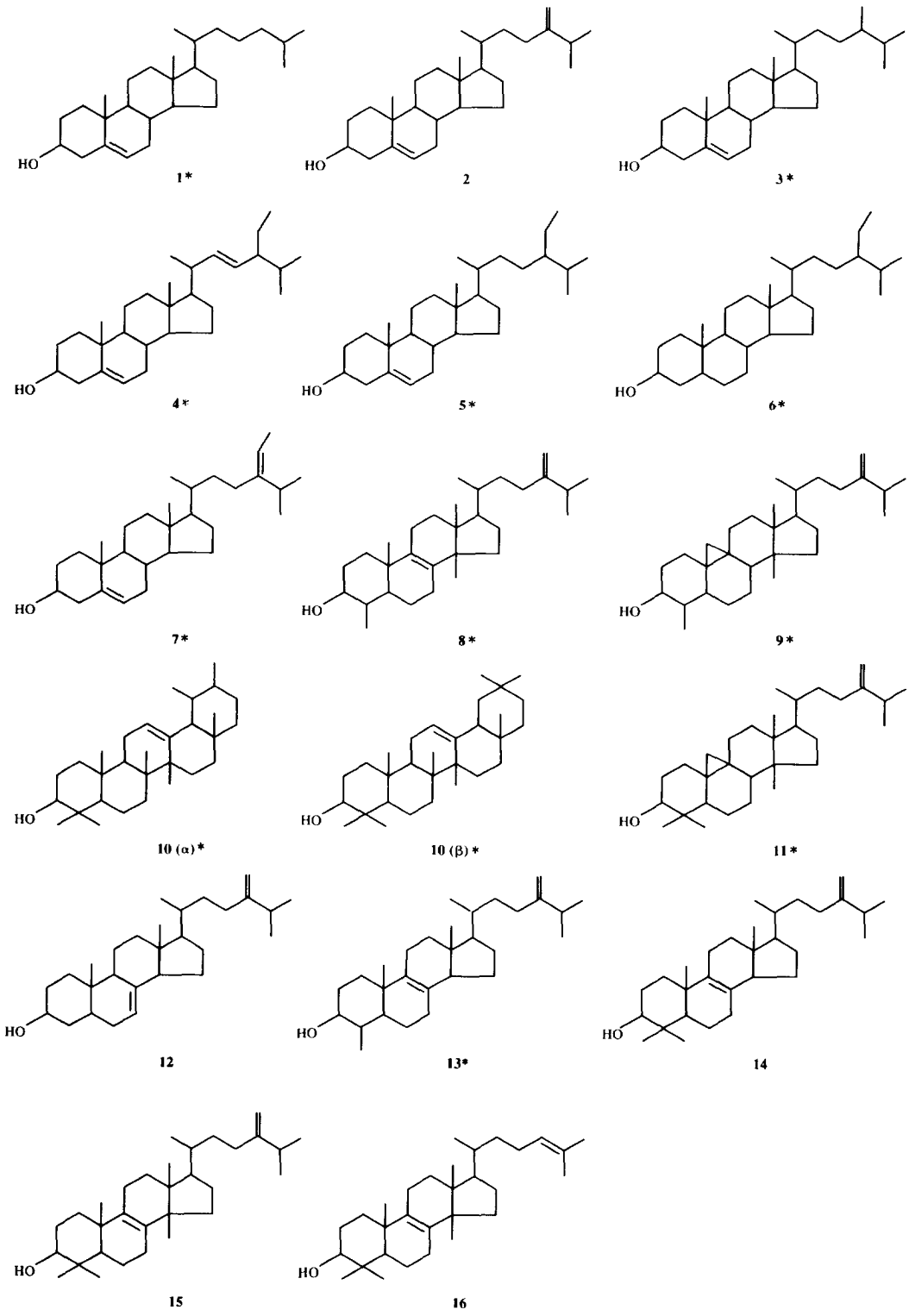

Scheme 1. Structures of sterols detected in *U. necator* conidia. **1**, cholesterol; **2**, ergosta-5,24(24[1])-dien-3β-ol; **3**, ergost-5-en-3β-ol; **4**, stigmasta-5,22-dien-3β-ol; **5**, stigmast-5-en-3β-ol; **6**, stigmastan-3β-ol; **7**, stigmasta-5,24(24[1])-dien-3β-ol; **8**, obtusifoliol; **9**, cycloeucalenol; **10**, amyrins (α and β); **11**, 24-methylenecycloartanol; **12**, episterol; **13**, 4α-methylfecosterol; **14**, 4,4-dimethylfecosterol; **15**, eburicol; **16**, lanosterol. *Sterols also found in the host plant, *V. vinifera*.

Table 1. Characteristics of *U. necator* monoconidial strains

Strains	Triadimenol sensitivity EC^*_{50}	Origin
Sensitive		
CH02	0.23	Bordeaux (France), 1988
MA04	0.13	Madiran (France), 1989
Resistant		
AZ01	8.00	Azambuja (Portugal), 1988
MA02	4.15	Madiran (France), 1989
CA02	3.78	Carregado (Portugal), 1990
FI01	1.42	Firenze (Italy), 1990

*EC_{50}, fungicide concentration ($mg\,l^{-1}$) causing 50% inhibition in fungi cultivated on *V. vinifera* leaf disks [9].

(Table 2). The amount of total sterols varied from 4.2 to 5.5 $\mu g\,mg^{-1}$ dry weight. The major sterol was identified as ergosta-5,24(24[1])-dien-3β-ol (**2**) [Scheme 1]; this 4-desmethylsterol represented 85–95% of total sterols. The mass spectrum of the acetate of ergosta-5,24(24[1])-dien-3β-ol (Table 3) was characterized by a base peak at *m/z* 380 $[M - Ac]^+$, indicating the presence of a 5(6) double bond and peaks at *m/z* 296 $[M - 84 - Ac]^+$ and 281 $[M - 84 - Ac - Me]^+$, due to the 24(24[1]) double bond, as suggested by Rahier and Benveniste [10]. Among the other 4-desmethylsterols, cholesterol (**1**), ergost-5-en-3β-ol (**3**), stigmasta-5,22-dien-3β-ol (**4**), stigmast-5-en-3-ol (**5**), stigmastan-3β-ol (**6**), stigmasta-5,24(24[1])-dien-3β-ol (**7**) and episterol (**12**) represented 2.4–5.4% of total sterols. The most abundant of them was stigmast-5-en-3β-ol (1.3–2.8% of total sterols).

Table 2. Sterol contents in *U. necator* conidia

Sterols	RR_t*	Strains: Sensitive CH02	Sensitive MA04	Resistant AZ01	Resistant MA02	Resistant CA02	Resistant F101
4,4-Dimethylsterols							
Lanosterol	1.47	0.13†	0.14	0.18	0.15	0.19	0.12
α-Amyrin	1.51	0.44	0.37	0.31	0.66	0.21	0.42
Eburicol‡	1.62	1.91	4.29	6.33	3.00	0.37	3.73
4,4-Dimethylfecosterol	1.65	1.95	1.47	1.18	3.41	1.08	1.44
Unknown sterol	1.70	0.38	0.34	0.24	0.19	0.25	0.11
24-Methylenecycloartanol	1.75	0.78	0.73	0.59	1.43	0.40	0.80
Other sterols§		0.13	0.21	0.29	0.29	0.17	0.22
4α-Methylsterols							
Obtusifoliol‖	1.41	0.05	0.13	0.14	0.09	0.03	0.22
4α-Methylfecosterol	1.44	0.06	0.05	0.11	0.14	0.11	0.09
Cycloeucalenol	1.56	—	Tr	Tr	—		
4-Desmethylsterols							
Cholesterol	1.18	0.77	0.73	0.42	1.27	0.28	0.65
Ergosta-5,24(24[1])-dien-3β-ol	1.33¶	90.76	87.24	86.91	85.23	94.79	87.95
Ergost-5-en-3β-ol	1.34						
Stigmasta-5,22-dien-3β-ol	1.39	0.27	0.52	0.23	0.29	0.21	0.42
Episterol	1.41	0.40	0.43	0.37	0.50	0.33	0.42
Stigmast-5-en-3β-ol	1.50	1.68	2.79	1.77	2.20	1.27	2.38
Stigmastan-3β-ol	1.52**	0.09	0.15	0.21	0.29	0.16	0.31
Stigmasta-5,24(24[1])-dien-3ß-ol	1.52						
Other sterols§		0.20	0.42	0.72	0.88	0.14	0.69
4,4-Dimethylsterols		5.72	7.55	9.11	9.12	2.68	6.84
4α-Methylsterols		0.11	0.17	0.25	0.23	0.14	0.31
4-Desmethylsterols		94.17	92.28	90.65	90.65	97.17	92.85
Total sterol amount ($\mu g\,mg^{-1}$ dry wt)		4.32	4.67	5.51	4.18	4.73	4.76

*RR_t, retention time of steryl acetate relative to cholesterol. Oven temps for GC analysis were 300° for 4,4-dimethyl and 4α-methylsterols and 250 to 300° at 2° min^{-1} for 4-desmethylsterols.

†Expressed as percentage of total sterols.

‡Eburicol more or less contaminated by β-amyrin.

§Sum of sterols (4,4-dimethyl or 4-desmethylsterols) in very low amounts (each of them, below 0.1% of total sterols or not identified).

‖Obtusifoliol overestimated because of impurities.

¶Not separately integrated because of the close RR_t and very low level relative to ergosta-5,24(24[1])-dien-3β-ol. The sterol percentage concerns the mixture, but ergost-5-en-3β-ol represented less than 2% of total sterols.

**In mixture, not separately integrated because of the close RR_t, the sterol percentage concerns the mixture.

Tr: trace, not quantified, detected only by GS-MS analysis.

—Not detected.

Table 3. Major ions in the mass spectra of steryl acetates in *U. necator* conidia

Sterol*	4-Desmethylsterols							4α-Methylsterols		4,4-Dimethylsterols	
	1	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**	**11**
Fragmentation†											
$[M]^+$						458 (9)		468 (36)		468 (1)	482 (12)
$[M - Me]^+$						443 (2)		453 (100)			
$[M - 43]^+$	‡										
$[M - Ac]^+$	368 (100)	380 (100)	382 (100)	394 (100)	396 (100)	398 (12)	394 (14)		408 (100)		422 (100)
$[M - Ac - Me]^+$	353 (24)	356 (16)	367 (22)	379 (7)	381 (25)	383 (9)	379 (2)	393 (45)	393 (60)		407 (88)
$[M - 43 - Ac]^+$				351 (10)							379 (65)
$[M - SC]^+$											357 (4)
$[M - 84 - AC]^+$		296 (37)							324 (8)		
$[M - 98 - AC]^+$							296 (100)				
$[M - 84 - Ac - Me]^+$		281 (17)						309 (11)			
$[M - 98 - Ac - Me]^+$							281 (21)				
$[M - SC - 42]^+$						275 (10)		301 (5)			
$[M - SC - Ac]^+$	255 (17)	255 (9)	255 (19)	255 (48)	255 (18)	257 (3)			283 (16)		297 (23)
$[M - SC - Ac - 2H]^+$		253 (31)		253 (3)			253 (13)				
$[M - SC - Ac - Me]^+$								269 (8)			281 (4)
$[M - SC - 26 - Ac]^+$		229 (15)									
$[M - SC - 26 - Ac - H]^+$				228 (14)	228 (5)	230 (13)	228 (9)				
$[M - SC - 42 - Ac]^+$	213 (28)	213 (44)	213 (37)	213 (31)	213 (30)	215 (100)	213 (15)	241 (26)	241 (16)		255 (15)
$[M - SC - 42 - Ac - 2H]^+$							211 (14)				
$[M - SC - 56 - Ac]^+$								227 (33)			
Other prominent	247 (29)		261 (18)		288 (11)			287 (25)	300 (6)	218 (100)	300 (23)
fragments	260 (19)		274 (18)		275 (10)			275 (14)		203 (50)	

*Acetate of: **1**, cholesterol; **2**, ergosta-5,24(24[1])-dien-3β-ol; **3**, ergost-5-en-3β-ol; **4**, stigmasta-5,22-dien-3β-ol; **5**, stigmast-5-en-3β-ol; **6**, stigmastan-3β-ol; **7**, stigmasta-5,24(24[1])-dien-3β-ol; **8**, obtusifoliol; **9**, cycloeucalenol; **10**, α- and β-amyrins; **11**, 24-methylenecycloartanol.

†Ac, acetate (60); Me, methyl (15); 26, C_2H_2 (loss of C-16 and C-17); 42, C_3H_6 (loss of C-15 to C-17); 43, C_3H_7 (loss of C-25 to C-27); 56, C_4H_8 (loss of C-15 to C-17 and C-32 in a 14α-methylsterol); 84, C_6H_{12} (loss of C-23 to C-28 in a 24-methylenesterol); 98, C_7H_{14} (loss of C-23 to C-29 in a 24-ethylidenesterol); SC, sterol side chain.

‡Figures in parentheses are intensities of ions relative to base peak (100) with *m/z* above 200.

The 4α-methyl and 4,4-dimethylsterols represented less than 1% (0.1–0.3) and 10% (2.7–9.1) of total sterols, respectively. The 4α-methylsterols consisted mainly of obtusifoliol (**8**) and 4α-methylfecosterol (**13**). Cycloeucalenol (**9**), a higher plant 4α-methylsterol, was also detected in some strains but only by GS-mass spectrometry analysis and could not be quantified (trace level). The major 4,4-dimethylsterols were eburicol (**15**) and 4,4-dimethylfecosterol (**14**). Lanosterol (**16**), an unknown sterol (RR_t of the acetate: 1.70) and higher plant 4,4-dimethylsterols [24-methylenecycloartanol (**11**), α- and β-amyrins (**10**)] were also found but at lower levels. In the strains MA04, AZ01 and FI01, eburicol (**15**) was the second most abundant sterol after ergosta-5,24(24^1)-dien-3β-ol (**2**). It represented 3.7 to 6.3% of total sterols.

The steryl acetates were identified by comparison of their mass spectra with the spectra published by Rahier and Benveniste [10]. The mass spectra of these sterols are given in Table 3, except for episterol (**12**), 4α-methylfecosterol (**13**), 4,4-dimethylfecosterol (**14**), eburicol (**15**) and lanosterol (**16**) described elsewhere [11]. The mass spectrum of the unknown sterol, as the acetate (RR_t 1.70) contains major ions at *m/z* 467, 407 and 355 (structures of sterols are given in Scheme 1).

Table 4. Sterol contents of *V. vinifera* leaves infected or non-infected by *U. necator*

Sterols	RR_t*	*V. vinifera* leaves Non-infected	Infected by mycelium MA04	CA02
4,4-Dimethylsterols				
α-Amyrin	1.51	2.31†	3.18	2.74
Eburicol	1.62		Tr	Tr
Cycloartenol	1.62‡	8.12	15.36	9.57
β-Amyrin	1.63			
Unknown sterol	1.70	2.04	2.05	2.02
24-Methylenecycloartanol	1.75	3.68	3.10	2.74
Other sterols§		0.07	2.89	3.04
4α-Methylsterols				
Obtusifoliol	1.41	0.19	0.16	0.22
4α-Methylfecosterol	1.44	0.08	0.04	—
Cycloeucalenol	1.56	0.04	0.04	0.06
4α-Methylstigmasta-8,24(24^1)-dien-3β-ol	1.70	0.03	–	—
4α-Methylstigmast-8-en-3β-ol‖	1.75	0.31	0.22	0.39
4α-Methylstigmasta-7,24(24^1)-dien-3β-ol	1.78	0.73	0.78	1.05
Other sterols§		0.12	0.04	0.03
4-Desmethylsterols				
Cholesterol	1.18	0.43	0.28	0.22
Ergosta-5,24(24^1)-dien-3β-ol	1.33	—	0.94	1.88
Ergost-5-en-3β-ol	1.34	1.78	1.61	1.71
Stigmasta-5,22-dien-3β-ol	1.39	9.21	7.55	8.27
Stigmast-5-en-3β-ol	1.50	69.94	57.80	61.98
Stigmastan-3β-ol	1.52‡	2.54	2.38	2.52
Stigmasta-5,24(24^1)-dien-3β-ol	1.52			
Stigmast-8-en-3β-ol	1.56	0.32	0.11	0.29
Stigmasta-7,24(24^1)-dien-3β-ol	1.58	0.31	0.21	0.17
Other sterols§		1.43	1.06	0.95
4,4-Dimethylsterols		12.54	26.58	20.12
4α-Methylsterols		1.51	1.48	1.89
4-Desmethylsterols		85.95	71.94	77.99
Total sterol amount (μg mg^{-1} dry wt)		4.20	4.21	4.00

*RR_t, retention time of steryl acetate relative to cholesterol. Oven temps for GC analysis were 300° for 4,4-dimethyl and 4α-methylsterols and 250 to 300° at 2° min^{-1} for 4-desmethylsterols.

†Expressed as percentage of total sterols.

‡In mixture, not separately integrated because of the close RR_t. The sterol percentage concerns the mixture between β-amyrin and cycloartenol, but cycloartenol represented less than 2% of total sterols.

§Sum of sterols (4,4-dimethyl, 4α-methyl or 4-desmethylsterols) in very low amounts (each of them, below 0.1% of total sterols or not identified).

‖4α-Methylstigmast-8-en-3β-ol overestimated because of contamination by 24-methylenecycloartanol.

Tr: trace, not quantified, detected only by GS–MS analysis.

—Not detected.

Sterol contents of V. vinifera *leaves infected by mycelium of* U. necator

The major 4-desmethylsterols in non-infected *V. vinifera* leaves (control) were stigmast-5-en-3β-ol (**5**) (sitosterol), stigmasta-5,22-dien-3β-ol (**4**) (stigmasterol) and ergost-5-en-3β-ol (**3**) (campesterol). They represented 69.9, 9.2 and 1.8% of total sterols, respectively (Table 4). The other minor 4-desmethylsterols which represented 5.0% of total sterols were mainly cholesterol (**1**), stigmastan-3β-ol (**6**), stigmasta-5,24(24^1)-dien-3β-ol (**7**), stigmast-8-en-3β-ol and stigmasta-7,24(24^1)-dien-3β-ol. The 4α-methyl and 4,4-dimethylsterols represented 1.5 and 12.5% of total sterols, respectively. 4α-Methylstigmasta-7,24(24^1)-dien-3β-ol was the most important 4α-methylsterol (0.7% of total sterols), but other sterols were found, such as 4α-methylstigmast-8-en-3β-ol, 4α-methylstigmasta-8,24(24^1)-dien-3β-ol, obtusifoliol (**8**), 4α-methylfecosterol (**13**) and cycloeucalenol (**9**). The major 4,4-dimethylsterols were an unknown sterol (RR_t of the acetate: 1.70, same mass spectrum as that of the unknown 4,4-dimethylsterol found in conidia), 24-methylenecycloartanol (**11**) and amyrins (α and β) (**10**). They represented 2.0, 3.7 and less than 10.4% of total sterols, respectively. Cycloartenol, another 4,4-dimethylsterol, represented less than 2% of total sterols.

In the sterol composition of the leaves infected by *U. necator* mycelium of strains either sensitive or resistant to triadimenol, ergosta-5,24(24^1)-dien-3β-ol (**2**) and eburicol (**15**) were detected in addition to sterols found in the non-infected leaves (Table 4). Ergosta-5,24(24^1)-dien-3β-ol (**2**) represented 1–2% of infected leaf sterols. Eburicol (**15**) was detected in trace amounts by GC–mass spectrometry analysis of infected leaves. It was not possible to quantify this sterol because of its very low level and RR_t close to that of cycloartenol.

Effect of triadimenol on conidia sterol content of a triadimenol-sensitive strain of U. necator

In the conidia of MA04 strain treated with triadimenol (0.5 mg l^{-1}), the amount of 4-desmethylsterols was slightly lower than in control conidia: 86.0 instead of 91.7% of total sterols. Among 4-desmethylsterols, the major one, ergosta-5,24(24^1)-dien-3β-ol (**2**) decreased from 82.9 to 61.9% of total sterols (Table 5). However, in triadimenol-treated conidia, 4α-methyl and 4,4-dimethylsterols slightly increased: 0.9 and 13.1%, respectively, instead of 0.4 and 7.9% of total sterols in absence of triadimenol. Among 4α-methylsterols, 4α-methylfecosterol (**13**) decreased and obtusifoliol (**8**) (14α-methylsterol) increased. Among 4,4-dimethylsterols, 4,4-dimethylfecosterol (**14**) decreased and eburicol (**15**) (14α-methylsterol) increased (Table 5).

Table 5. Effect of triadimenol on conidia sterol content of a triadimenol-sensitive strain of *U. necator*

Sterols	MA04 Control	MA04 Treated*
4,4-Dimethylsterols		
Lanosterol	0.19†	0.24
α-Amyrin	0.61	0.78
Eburicol‡	3.15	9.46
4,4-Dimethylfecosterol	1.99	0.85
Unknown sterol	0.34	0.61
24-Methylenecycloartanol	0.94	0.69
Other sterols§	0.71	0.45
4α-Methylsterols		
Obtusifoliol‖	0.15	0.81
4α-Methylfecosterol	0.21	0.08
Cycloleucalenol	Tr	—
4-Desmethylsterols		
Cholesterol	1.18	1.99
Ergosta-5,24(24^1)-dien-3β-ol¶ / Ergost-5-en-3β-ol	82.86	61.93
Stigmasta-5,22-dien-3β-ol	0.66	1.33
Episterol	0.32	0.37
Stigmast-5-en-3β-ol	3.38	8.19
Stigmastan-3β-ol** / Stigmasta-5,24(24^1)-dien-3β-ol	0.61	9.02
Other sterols§	1.90	1.84
4,4-Dimethylsterols	7.93	13.09
4α-Methylsterols	0.35	0.89
4-Desmethylsterols	91.71	86.02
Total sterol amount (μg mg^{-1} dry wt)	5.38	6.38

*Triadimenol concentration at 0.5 mg l^{-1}.

†Expressed as percentage of total sterols.

‡Eburicol contaminated with β-amyrin.

§Sum of sterols (4,4-dimethyl or 4-desmethylsterols) in very low amounts (each of them, below 0.1% of total sterol or not identified).

‖Obtusifoliol more or less overestimated because of impurities.

¶Not separately integrated because of the close RR_t and very low level relative to the other, ergost-5-en-3β-ol represented less than 2% of total sterols.

**In mixture, not separately integrated because of the close RR_t. The sterol percentage represents the mixture for the control but in the case of treatment with 0.5 mg l^{-1}, the sterol percentage concerns essentially stigmastan-3β-ol.

Tr: trace, not quantified, detected only by GS–MS analysis.

—Not detected.

DISCUSSION

The major sterol of conidia of *U. necator*, the causal agent of vine powdery mildew, was ergosta-5,24(24^1)-dien-3β-ol (**2**) as found by Loeffler *et al.* [12] for three other species causing powdery mildew (*Podosphaera leucotricha* on apple, *Erysiphe graminis* f. sp. *hordei* on barley and *Sphaerotheca fuligenea* on cucurbit). This sterol was also found in mycelium of *U. necator*. Lanosterol (**16**) and 4,4-dimethylsterols, which are derived from lanosterol, such as eburicol (**15**) and 4,4-dimethylfecosterol (**14**) were present in *U. necator* as found in the other filamentous fungi [11, 13, 14]. This should indicate that *U. necator*, an obligate pathogen, is able, like other species causing powdery mildew [12], to synthesize its own

sterols in the same way as the other types of filamentous fungi [13]. However, the final major sterol was ergosta-5,24(24[1])-dien-3β-ol (**2**), as found for example in the Phycomycete class [15], rather than ergosterol, which is the predominant sterol in most fungi [16]. Ergosta-5,24(24[1])-dien-3β-ol (**2**) might be produced by reduction of the Δ^7-bond of ergosta-5,7,24(24[1])-trien-3β-ol, a precursor of ergosterol found in some fungi [11, 13, 16].

Cholesterol (**1**) and sterols which are commonly present in higher plants [17]; 24-methylenecycloartanol (**11**), cycloeucalenol (**9**), ergost-5-en-3β-ol (**3**), stigmasta-5,22-dien-3β-ol (**4**) and stigmast-5-en-3β-ol (**5**), present in the host plant, *V. vinifera*, were found as minor sterols in *U. necator* conidia. This might be due to passive contamination of conidia by fragments of leaf epidermis during conidia harvest. However, the fact that the proportion of each of these sterols compared to stigmast-5-en-3β-ol (**5**) (major sterol in host plant) was different for some of them, in conidia and in the host plant suggests that passive contamination during the harvest is not the sole explanation for the origin of these sterols. *Uncinula necator*, which is an obligate pathogen, could take up host plant sterols selectively or not, accumulating them differently or not, metabolizing them or not. We can also notice that cholesterol (**1**) (also found in *S. fuliginea* [12]), ergost-5-en-3β-ol (**3**) and stigmasta-5,22-dien-3β-ol (**4**) have been described in some non-obligate pathogenic fungi [16, 18]. So, the minor Δ^5-sterols found in conidia of *U. necator* [cholesterol (**1**), ergost-5-en-3β-ol (**3**), stigmasta-5,22-dien-3β-ol (**4**), stigmast-5-en-3β-ol (**5**)] could either come from host leaves or be synthesized by *U. necator*. Other experiments would be necessary to clarify the origin of these minor sterols in *U. necator*.

Eburicol (**15**) which is the substrate of the sterol C-14 demethylase of filamentous fungi [13] represented less than 6.5% of total sterols for all the strains. In addition, the slight differences in the amounts of this sterol and those of 4-desmethylsterols (90–97% of total sterols) were not correlated to the triadimenol-sensitivity of the strains. These results indicate that sterol C-14 demethylase deficiency could not explain the resistance of the triadimenol-resistant strains originating from different vineyards. Up to now, the only cases of C-14 demethylase deficiency have been described for laboratory resistant mutants such as for example, *Saccharomyces cerevisiae*, *Ustilago maydis* and *Candida albicans*, isolated on media containing polyene antibiotics [19–21].

The conidia sterol content of the sensitive strain, MA04, was slightly affected by triadimenol treatment (at 0.5 mg l^{-1}). However, a slight increase of 14α-methylsterols such as eburicol (**15**), (4,4,14α-trimethylsterol), obtusifoliol (**8**), (4α,14α-dimethylsterol) and a slight decrease of the final 4-desmethylsterol, ergosta-5,24(24[1])-dien-3β-ol (**2**) were observed. Thus, triadimenol inhibits sterol C-14 demethylation in *U. necator*, as described for other fungi [1–4, 14, 22, 23]. Kwok and Loeffler [24] found an effect of DMIs on sterol content which was also slight for conidia of *E. graminis* f. sp. *hordei*. Because of the low quantity of mycelium dry weight compared to that of the host plant tissue, it was not possible to detect any effect of triadimenol treatment (at 0.2 mg l^{-1} for the sensitive strain and 2 mg l^{-1} for the resistant strain, data not shown) on the sterol composition of leaves infected either by triadimenol-sensitive or resistant strains in the absence or presence of triadimenol. Another method of mycelium harvest without the host plant leaf, would be necessary in order to study the effect of triadimenol on the mycelium sterol content of the different strains.

EXPERIMENTAL

Strains. The characteristics of the 6 strains of *Uncinula necator* studied are described in Table 1, their sensitivity to triadimenol was determined by using the method of Steva [9]. Two strains are sensitive to triadimenol with an EC_{50} below 0.5 mg l^{-1} and 4 strains are resistant to triadimenol with an EC_{50} higher than 1 mg l^{-1}. The monoconidial strains are derived from isolates originating from different vineyards.

Host plant. The grapevine cinsaut plants (*Vitis vinifera*) were grown in the greenhouse on a compost- and sand-based substrate. The selected leaves for assay corresponded to the third well-developed leaf of a 2 month-old cutting.

Production of conidia and infected host leaves by mycelium. The strains were cultivated on whole sterile leaves kept alive on agar water (20 g l^{-1}) containing benzimidazole (30 mg l^{-1}), as described by Cartolaro and Steva [25]. The conidia from 12-day cultures produced at 20° were inoculated dry by gravity on whole plants according to a technique adapted from Aslam and Schwarzback [26]. The inoculated plants were placed in a growth chamber (mean temp. 22° with 16 hr light a day) for 14 days. The infected leaves were then taken from plants and the conidia were harvested by filtration after shaking in 1% mannitol soln. The conidia were then frozen. Triadimenol was applied as an emulsifiable formulation (Baytan 5) by spraying in distilled water soln at a concn of 0.5 mg l^{-1}. The treatment was carried out 3 days before *U. necator* inoculation. For studies of host leaves infected by mycelium, the strains were cultivated on leaf disks on agar medium. The infected leaves were harvested 7 days after *U. necator* inoculation in order to avoid production of conidia. A control (non-infected leaves) was carried out. The leaves were then frozen.

Sterol extraction and analysis. The frozen conidia were lyophilized saponified in methanolic KOH (6%) at 70° for 2 hr. The frozen leaves were extracted after lyophilization as described by Costet-Corio and Benveniste [27]. The unsaponifiable lipids from conidia or leaves were subjected to 3 extractions with hexane and then purified on silica gel TLC plates. CH_2Cl_2 was used as the developing solvent (× 2) as described elsewhere [11]. The purified 4,4-dimethyl-, 4α-methyl- and 4-desmethylsterols were acetylated at room temp. for 15 hr using a mixt. of pyridine and Ac_2O and purified on TLC plates (silica gel; CH_2Cl_2; one run). The steryl acetates were then analysed by GC and GC-MS. GC was fitted with a flame ionization detector and an OV-1 glass capillary

column (30 m × 0.32 mm), N_2 0.5 b. The oven temp. was 300° for all analyses except for the host plant 4-desmethylsteryl acetate analyses, when the oven temp. was programmed from 250 to 300° at 2° min^{-1}. Cholesterol was used as a standard for RR_t determination and sterol quantification. GC-MS analyses were performed with a Ribermag R10-10-C spectrometer, the GC used a CPSIL5CB column, temp. programmed from 250° to 320° at 5° min^{-1}, the ionizing potential was 70 eV as described elsewhere [11].

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