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Volatile Metabolites Produced by Six Fungal Species Compared with Other Indicators of Fungal Growth on Cereal Grains

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Six fungal species, *Penicillium brevicompactum*, *P. glabrum*, *P. roqueforti*, *Aspergillus flavus*, *A. versicolor*, and *A. candidus*, were inoculated on moistened and autoclaved wheat and oat grains. They were cultivated in glass vessels provided with an inlet and outlet for air. Air was passed through the vessels to collect volatile fungal metabolites on porous polymer adsorbents attached to the outlet. Samples were collected at two fungal growth stages. Adsorbed compounds were thermally desorbed, separated by gas chromatography, and identified by mass spectrometry. Differences in the production of volatile metabolites depended more on the fungal species than on the grain type. The fungal growth stage was not an important factor determining the composition of volatiles produced. 3-Methylfuran was produced in similar amounts regardless of the fungal species and substrate (oat versus wheat). The production of volatile metabolites was compared with the production of ergosterol and CO_2 and the number of CFU. The production of volatile metabolites was more strongly correlated with accumulated CO_2 production than with actual CO_2 production and more strongly correlated with ergosterol contents of the grain than with numbers of CFU.

Fungal growth in stored cereals decreases their nutritional value and can pose health hazards because of the formation of mycotoxins and potentially allergenic spores. There is thus a need for methods that can accurately quantify the degree of fungal infection in grains at an early stage of mold growth.

Earlier studies have shown that volatile fungal metabolites can be used as indicators of fungal growth in stored cereals (14). A number of compounds have been reported as volatile fungal metabolites in grains. The most common of these are 3-octanone, 1-octen-3-ol, and 3-methyl-1-butanol (1, 10, 14). In addition, in our previous studies (2, 3), 3-methylfuran (identified as 2-methyl-furan in 2) was found to be produced by all examined storage molds during growth on grain. The above-mentioned metabolites seem to have potential as indicators of fungal growth in grains. Another way of using volatile fungal metabolites as indicators of mold growth is to measure the total concentration of a group of compounds. Kaminski et al. (9) used carbonyl compounds for this purpose.

Marked differences in the production of volatile metabolites have been reported between closely related species and even between strains of the same fungus (4, 5, 13). This was also confirmed for molds occurring on wheat (2). Before volatile fungal metabolites can be used to quantify fungal growth in cereals, a closer look at the range of metabolites produced by the most common grain-deteriorating fungi is essential. In an earlier study, the choice of cereal-based agar medium did not markedly influence the volatile metabolites produced by *Penicillium aurantiogriseum* (3). These results agree with those of Abramson et al. (1), who found the same odorous, volatile fungal metabolites regardless of cereal type. Similarly, the volatile metabolites produced during growth of *Phlebia radiata* on a range of substrates were generally the same (6). However, 1-octen-3-ol has been reported to be produced during the enzymatic breakdown of lipids (15). 1-Octen-3-ol levels could therefore be expected to vary with different lipid contents in substrates. When studying the range of volatiles produced on cereals, it is therefore advisable to include oats, which have a substantially higher lipid content than other cereals.

Besides being influenced by species and substrate composition, the production of volatile metabolites can be influenced by the duration of fungal growth. For instance, Hubbal and Collins (8) found that volatile terpenes occurred in different ratios depending on the stage of growth of *Ceratocystis variospora*. It was suggested that this variation was due to the metabolic turnover of the terpenes. The relative proportions of different yeast metabolites were dependent on the amount of residual glucose in the medium and thus on the stage of growth of the yeast (11). We also found that the stage of fungal growth can influence the amounts of volatile metabolites produced from molds on cereals (2). It therefore seemed essential to also consider the stage of growth in the present study.

In a previous investigation, we found a high positive correlation between production of CO_2 and that of volatile metabolites (3). Furthermore, volatile metabolites were more strongly correlated with levels of ergosterol than with numbers of CFU. If volatile metabolites are to be used as indicators of fungal growth, the following criteria based on earlier findings should be met. (i) It should be possible to use a single volatile compound or a set of compounds as general indicators of fungal growth. (ii) Differences between fungal species should be greater than differences caused by growth on different cereals. (iii) The time of sampling should not influence the composition of volatile metabolites produced, although quantitative differences may occur. (iv) There should be a strong correlation between the production of CO_2 and that of volatile metabolites. (v) The production of

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volatile metabolites should be more strongly correlated with the production of ergosterol than with the numbers of CFU.

These criteria were tested by cultivating six different molds that commonly occur on cereals on moist, autoclaved wheat and moist, autoclaved oats. Volatile metabolite and CO_2 levels, ergosterol concentrations, and numbers of CFU were measured at two stages of growth.

MATERIALS AND METHODS

Fungal cultures. The following strains were used: *Penicillium brevicompactum* Dierckx CBS 257.29, *Penicillium glabrum* (Wehmer) Westling SLU J3, *Penicillium roqueforti* Thom SIK 5.18.98, *Aspergillus candidus* Link CBS 102.13, *Aspergillus flavus* Link CBS 569.65, and *Aspergillus versicolor* (Vuill.) Tiraboschi CBS 111.32.

Cereal grain. The wheat used (cultivar Drabant; harvested in 1989; moisture content, 8.2%) had the following microbiological status: exogenous flora, 8.0×10^4 CFU; endogenous infection level, 88%; ergosterol content, 0.70 μg/g (dry weight). Species of *Alternaria* and *Cladosporium* dominated the exogenous microflora and were also prominent endogenously together with *Fusarium* species.

The oats (cultivar Vital; harvested in 1989; moisture content, 10.9%) used had the following microbiological status: exogenous flora, 1.2×10^6 CFU; endogenous infection level, 75%; ergosterol content, 4.0 μg/g (dry weight). All the genera in wheat were also found in oats; in addition, *Acremonium* species were found both exogenously and endogenously.

The grain was stored in the laboratory in steel containers at 18°C in a dry place before use.

Equipment used for cultivation, sampling, and analysis. The equipment used for cultivating fungi and sampling volatile metabolites was as described by Börjesson et al. (2). We used a Varian 3700 gas chromatograph coupled to an Incos 50 Quadropole mass spectrometer (Finnigan MAT). The column used was a J & W DB 1701, which was described previously (3). The temperature was programmed to remain at 30°C for the initial 2 min and then to increase by 4°C per min to 200°C.

The data collection system, library of mass spectra, and equipment for measuring CO_2 were as described by Börjesson et al. (3). For measuring ergosterol, we used a Novapak C_{18} column (3.9 by 150 mm; Waters) and a HP series 1050 UV spectrophotometer (Hewlett-Packard). For statistical evaluation of the data, software from SYSTAT, Inc., was used.

Preparation of grains. The water content of the grains was raised to about 25% by adding distilled water. The grains were then shaken vigorously in an Erlenmeyer flask once every hour for 6 h. Then 400 g of wheat or oats was added to each cultivation container, and the containers were autoclaved at 121°C for 15 min. To prevent contamination of the containers, they were provided with spore filters and protective adsorbents (Tenax GC, which allows water to pass through). Air was then passed through the containers at a flow rate of 30 ml/min for 4 days, which reduced the background of volatile metabolites emanating from the grains by about 50%. To keep the moisture content of the grains constant, the ingoing air was passed through a flask filled with deionized charcoal-filtered water.

To make sure that the grains were sterile, CO_2 levels were continuously measured. No increase was detected; thus, the containers could be regarded as sterile.

Preparation of spore suspensions and inoculation of fungi. The fungi were cultivated in petri dishes with 2% malt extract agar containing 50 U of penicillin and 50 μg of streptomycin per ml. After about 1 week, spores were liberated by adding 10 ml of sterile tap water containing 0.02% Tween 80 to the petri dishes and rubbing the agar surface with a glass rod. Each spore suspension was filtered through a cotton cloth and diluted to 10^4 spores per ml. Ten milliliters of the appropriate spore suspension was added to each cultivation container.

Cultivation and sampling. During the sampling of volatile metabolites, the protective adsorbent and the sampling adsorbent were filled with Chromosorb 102. This change was made to prevent low-molecular-weight compounds, which can pass through the Tenax GC adsorbent, from contaminating the sample. In addition, the moisturizing flask for the ingoing air was removed, and the flow was lowered to 20 ml/min to diminish the risk of volatile metabolites breaking through the sampling adsorbent. The level of CO_2 was measured every day, starting on day 2 after inoculation.

Samples of volatile metabolites were collected at CO_2 production rates of ca. 7 and 21 mg/h for all species except *P. roqueforti*. Because the production rate of CO_2 for this species seldom reached 21 mg/h, the second sample was collected 10 days after inoculation (Fig. 1). Each combination of fungus, cereal grain, and sampling occasion was replicated three times, resulting in a total of $6 \times 2 \times 2 \times 3 = 72$ treatment samples + 6 uninoculated controls = 78 samples. The first sampling was made about 4 days after inoculation, and the second sampling was made about 7 days after inoculation. Samples were collected for 8 h, giving a total sampled air volume of 9.6 liters. In a preliminary experiment, considerable breakthrough of the most volatile metabolites occurred when the whole sampling volume was collected on a single adsorbent. Breakthrough was measured by determining the contents of volatile metabolites in a second adsorbent attached in series after the first one. Therefore, the sampling adsorbents were changed after 4 h, which resulted in a negligible breakthrough (4%) of acetone, the most volatile metabolite studied. No breakthrough of the less volatile metabolite 2-methyl-1-propanol was detected.

After the volatile metabolites were sampled, two 10-g samples were removed from each cultivation vessel for ergosterol analysis and CFU count. The samples for ergosterol analysis were kept in 50 ml of methanol at −18°C until analysis.

Analytical procedures. The procedures for desorption, concentration, and injection of volatile metabolites were identical to those used in our previous study (3). For analysis of CO_2, the procedure of Börjesson et al. (2) was used. The procedure for measuring ergosterol was modified from that of Newell et al. (12) as described by Börjesson et al. (3). For counting of CFU, the samples were mixed with 90 ml of sterile tap water containing 0.02% Tween 80. After 15 min the mixture was agitated for 2 min in a Stomacher blender, diluted, and spread on 2% malt extract plates without antibiotics so that any possible bacterial contaminants could be detected.

Statistical evaluation. Correlation coefficients were used to evaluate correlations between different measures of fungal growth. To determine the degree to which fungal species and grain type influenced the production of volatile metabolites, the total variation in the material was condensed by constructing principal components. The principal components were then subjected to an analysis of variance. To avoid skewed distribution, the square root of each metabolite yield was used.

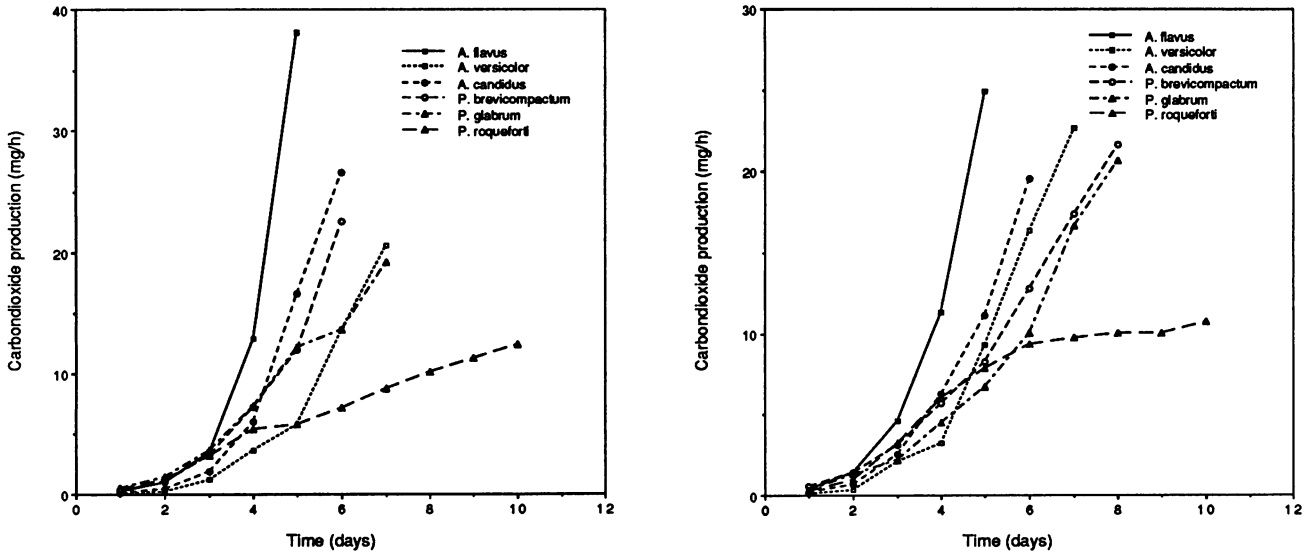

FIG. 1. Production of CO_2 during fungal growth on wheat (left) and oats (right).

RESULTS

Volatile metabolites and CO_2. With the exception of *P. brevicompactum*, the total amount of volatile metabolites produced on a given sampling occasion was in the same range for the different fungi. *P. brevicompactum* deviated primarily in that it produced large amounts of acetone. 3-Methylfuran was detected during the early stage of growth of all fungi examined (Table 1) but was absent in uninoculated controls. Otherwise, the volatile profiles of the examined fungal species were highly variable (Table 1). Coefficients of variation were generally about 50%. For 3-methylfuran, however, the coefficient of variation was about 30%.

Each *Penicillium* species produced at least one compound in an amount much larger than that of any of the other species. The high production of acetone by *P. brevicompactum* was especially noteworthy, as was the production of 3-octanone by *P. glabrum*. *P. roqueforti* produced some sesquiterpenes that were unique for this species. All three *Aspergillus* species produced thujopsene, which was not found in any of the *Penicillium* cultures. Furthermore, *A. candidus* produced a monoterpene that none of the other five species released. On the first sampling occasion, the volatile profiles of the *Aspergillus* species were hard to distinguish from one another. Uninoculated controls contained high amounts of acetone, ethanol, 3-methyl-1-butanol, and acetic acid as well as a number of aldehydes and hydrocarbons.

Differences related to grain type were generally small (Table 1). For example, 1-octen-3-ol, a metabolite that may be produced during the breakdown of lipids, was produced in similar amounts by *P. glabrum* regardless of the type of grain used.

The above-mentioned observations indicate that variations related to fungal species were greater than those related to grain type. To verify this, an analysis of variance was performed on principal components constructed from the raw data without subtracting uninoculated control values. The analysis included data for all 19 volatile metabolites presented in Table 1 as well as 3-methyl-1-butanol, acetic acid, and ethanol. These compounds were included because they have previously been reported as fungal metabolites. Their abundance in uninoculated controls could have obscured the production by fungi in this investigation. The first six components constituted 80% of the total variation in the material, as follows: component 1, 21%; component 2, 20%; component 3, 15%; component 4, 11%; component 5, 7%; and component 6, 6%. The remaining components each constituted less than 5% of the total variance, and altogether they only accounted for 20% of the variance. Thus, their importance was considered to be minor. When the first six components were subjected to an analysis of variance, F values (and thus the degree of variation) were generally higher for the fungal species than for the grain type (Table 2).

For all fungal species, the total amount of volatile metabolites produced (summed from Table 1) was higher on the late sampling occasion than on the early one (Table 3).

Some variation in CO_2 production between species was found (Fig. 1). *P. roqueforti* was notable in showing an exceptionally slow increase in CO_2 production.

A low positive correlation between total volatile metabolites and CO_2 (0.22, $n = 72$, $P < 0.05$) was found. However, for the compound whose production showed the least variation among species of fungi, i.e., 3-methylfuran, the correlation coefficient with CO_2 was higher (0.65; $n = 72$, $P < 0.05$). Similarly, when the total production of volatile metabolites was compared with that of CO_2 for each fungus individually, the correlations were increased; the correlations were even higher for accumulated production of CO_2 and volatile metabolite production (Table 4).

CFU and ergosterol. No contamination was found during the counting of CFU in either treatment or control vessels. Almost without exception, the CFU counts and ergosterol contents of the grain increased between the two sampling occasions (Table 3).

The mean coefficients of variation for each combination of sampling occasion and kind of grain were 94% for CFU and 32% for ergosterol. This reflects the fact that, at a given growth stage, the variation between the species of fungi in terms of CFU tended to exceed the corresponding variation in ergosterol content.

TABLE 1. Production of volatile metabolites[a]

Species and metabolite	Production of the indicated metabolite (ng/h) in fungi grown on:			
	Wheat		Oats	
	Early	Late	Early	Late
P. brevicompactum				
Acetone	7,300[b]	24,000[b]	2,300[b]	15,000
2-Propanol	44[b]	298[c]	5.8[c]	15[c]
3-Methylfuran	2.7[b]	4.8	2.7	6.9
2-Methyl-1-propanol	6.2[c]	10[b]	4.8[c]	2.3
3-Pentanone	7.4	1.7[b]	12[b]	
2-Methyl-1-butanol	0.8	12[c]	17	3.9
2-Butanone	9.8	28	10[c]	25
P. glabrum				
3-Methylfuran	8.7	26	11	35
2-Methyl-1-propanol	2.2[c]	0.67[c]	3.8[b]	
3-Pentanon	20[b]	32[b]	32[b]	13
2-Methyl-1-butanol	2.8[b]	1.4[c]	16[b]	11
Octadiene[d]	2.5[b]	6.1	4.6[b]	17
2-Butanone	11	22	20[b]	27
Dimethylbenzene			0.43[b]	0.27[c]
Limonene	0.40[b]	0.20[b]	1.2	0.6
3-Octanone	6.5	12	11[b]	26
1-Octen-3-ol	4.3[b]	3.2[b]	2.7[c]	4.5[c]
P. roqueforti				
3-Methylfuran	5.4	12	4.3	13
2-Methyl-1-propanol	10[b]	83	14[c]	81[b]
2-Methyl-1-butanol	10[c]	8	11	6
Octadiene[d]	3.7	2.3[b]	7.7	7.7
Dimethylbenzene				0.17[b]
Limonene	0.17[b]	0.47[b]	0.73[c]	3.3[b]
3-Octanone	0.57[b]		0.37[b]	
Sesquiterpene 1	4	7	1.6[b]	5.5
Sesquiterpene 2	8.2	15	3.3[b]	21[b]
A. flavus				
3-Methylfuran	4.7	30	3.9	23
Nitrometan	1.7[b]	9.4		5.8
2-Methyl-1-propanol		13		2.4[b]
1-Penten-3-ol	1.7[c]	2.6	4.3[b]	1.8[c]
Octadiene[d]			1.0[b]	1.8[b]
Dimethylbenzene	0.17[b]	7.5[b]		19
Ethylbenzene	0.1	1.5		4.5[b]
Limonene		2.1		1.4
Thujopsene		0.96		0.82[b]
A. versicolor				
3-Methylfuran	4.7	27	5.7	30
2-Methyl-1-propanol		2.9[c]	11[b]	5.8
1-Penten-3-ol			6.5[b]	
2-Methyl-1-butanol				7
Octadiene[d]	1.9	3.8[b]	3.0[b]	7.2
Dimethylbenzene		2.9	0.27[b]	8.7
Ethylbenzene		0.83[b]		2.1[b]
Limonene		1.7		1.0
Thujopsene		1.5		0.67
A. candidus				
3-Methylfuran	8.4[b]	32	5	34
2-Methyl-1-propanol	2.9[c]	9.3	3.4[c]	7.6[c]
1-Penten-3-ol	1.2[c]	4.3[b]	2.2[c]	3.5[c]
2-Methyl-1-butanol		3.6	5.4[b]	10
Dimethylbenzene		1.3[b]		4.3[b]
Ethylbenzene		0.6[b]		1.3[b]
Monoterpene 1		14		13
Thujopsene		0.62		0.40[b]

[a] The volatile metabolite contents in uninoculated control vessels were subtracted from the measured values to give the results shown (n = 3). Samples were taken about 4 days (early) and 7 days (late) after inoculation.
[b] Coefficient of variation, >50%.
[c] Coefficient of variation, >100%.
[d] 1,3-Octadiene and an unidentified isomere.

TABLE 2. *F* values (explained variance/residual variance) for fungal species and grain types for the first six principal components[a]

Factor	*F* value for component:					
	1	2	3	4	5	6
Species (df 5, 66)	51	43	58	20	3.4	11
Grain type (df 1, 70)	4.7	8.6	35	11	37	1.7

[a] Data from 22 volatile metabolites were used.

A low (0.35, $n = 72$) but statistically significant ($P < 0.05$) correlation between volatile metabolites and ergosterol was found, whereas no significant correlation was found between CFU and volatile metabolite production. A higher correlation (0.65, $n = 72$, $P < 0.05$) was found between accumulated CO_2 and ergosterol. The correlation between CFU and accumulated CO_2 was 0.57 ($n = 72$, $P < 0.05$). For each fungus individually, the correlation between CFU and accumulated CO_2 was generally higher. Thus, the correlation coefficients for the six fungal species were 0.65, 0.79, 0.82, 0.83, 0.96, and 0.88 ($n = 12$, $P < 0.05$). However, this trend was not found when ergosterol and accumulated CO_2 were compared; in this case, the correlation coefficients for the six species were 0.7, 0.7, 0.28, 0.6, 0.63, and 0.45 ($n = 12$, $P >$ 0.05 for two species).

The ergosterol contents, calculated on the basis of CO_2 production were, on average, only 11% lower than measured values. The calculation was based on the assumptions that CO_2 and biomass are produced in equal amounts (on a weight basis) during fungal growth and that the biomass contains 0.2% ergosterol.

DISCUSSION

This investigation indicates that 3-methylfuran can be used as an indicator of mold growth in cereals. Production levels showed little variation related to the fungal species cultured or the grain type used as a substrate. With regard to production of the other volatile metabolites, large differences among fungal species were found, indicating that these compounds are less suitable as indicators of general mold growth. However, in combination, these metabolites can be used as indicators of fungal growth. Terpenes were the most suitable compounds for use in differentiating between fungal species. It was easier to distinguish between *Penicillium* species than between *Aspergillus* species (Table 1). Thujopsene was produced by all *Aspergillus* species but not by any of the *Penicillium* species, suggesting that it can be used to discriminate between the two genera. However, Halim et al. (7) reported that *Penicillium decumbens* produces thujopsene. Therefore, it seems more reasonable to try and use thujopsene production as an additional diagnostic character in classification at the species level. The often-cited metab-

TABLE 3. Total production of volatile metabolites, CO_2, and ergosterol and levels of CFU

Substrate	Species	Sampling time	Production of:			Accumulated CO_2 (mg)	No. of CFU (10^5/g [dry wt])
			Metabolites (ng/h)	CO_2 (mg/h)	Ergosterol (μg/g [dry wt])		
Wheat	*P. brevicompactum*	Early	7,400[a]	9.4	5.1[a]	330	11.6[a]
	P. brevicompactum	Late	24,000[a]	33	7.9[a]	1,600	79[b]
	P. glabrum	Early	58[a]	8	2.8[a]	330	56
	P. glabrum	Late	100	27	10.3[a]	1,700	390
	P. roqueforti	Early	42[a]	7.8	4.0[a]	390	40[a]
	P. roqueforti	Late	130	13	4.8	1,600	290
	A. flavus	Early	8.4[a]	12	4.0[a]	320	1.9[b]
	A. flavus	Late	67	60	8.3	1,800	6.3
	A. versicolor	Early	6.6[a]	8.3	4.3[a]	240	25[a]
	A. versicolor	Late	41	20	7.2	900	280
	A. candidus	Early	12[a]	9.6	3.6[a]	290	15[b]
	A. candidus	Late	66	30	7.6	1,100	250
Oats							
	P. brevicompactum	Early	2,400[a]	7.0	3.3[a]	300	4.4
	P. brevicompactum	Late	15,000	24	15	1,900	100[a]
	P. glabrum	Early	100[a]	6.6	4.9[a]	350	120[a]
	P. glabrum	Late	130	23	16	1,800	500[a]
	P. roqueforti	Early	43[a]	8.1	3.3[a]	300	21
	P. roqueforti	Late	140[a]	11	6.1	1,500	210[a]
	A. flavus	Early	9.2[b]	8.8	4.1[a]	260	0.64
	A. flavus	Late	60	28	3.7[a]	1,000	2.2
	A. versicolor	Early	26[a]	7.9	7.3	200	20[b]
	A. versicolor	Late	62	24	13	1,300	610
	A. candidus	Early	16	6.6	4.0[a]	210	16[a]
	A. candidus	Late	74	23	6.5	1,100	250[a]

[a] Coefficient of variation >50%.
[b] Coefficient of variation, >100%.

TABLE 4. Correlation coefficients (r) between total production of volatile metabolites and actual and accumulated production of CO_2[a]

Species	r between production of metabolites and:	
	Actual CO_2	Accumulated CO_2
P. brevicompactum	0.78	0.79
P. glabrum	0.31 ($P > 0.05$)	0.35 ($P > 0.05$)
P. roqueforti	0.63	0.77
A. flavus	0.48 ($P > 0.05$)	0.58
A. versicolor	0.78	0.83
A. candidus	0.59	0.70

[a] $n = 12$ and $P < 0.05$ if not stated otherwise.

olite 3-methyl-1-butanol showed little potential value as an indicator in this investigation. It was present in large amounts in uninoculated controls, possibly as a result of past fungal activity, which may have obscured the production by the inoculated fungi. Furthermore, the autoclaving in itself might have introduced volatiles such as carbonyls. Interestingly, most of the examined species produced 2-methyl-1-butanol but not 3-methyl-1-butanol. These compounds are difficult to separate by gas chromatography and have similar mass spectra. Thus, in previous studies where 3-methyl-1-butanol was reported, both may actually have been present.

Compared with the results from our earlier study (3), the correlation obtained here between volatile metabolites and CO_2 production was poor. Interspecies differences in production rates of volatile metabolites were probably responsible for the low correlation. The correlation between production of volatile metabolites and accumulated production of CO_2 was higher, indicating that the ratio of volatile metabolite production to CO_2 production increased over time. At least some volatile metabolites, i.e., terpenes, could have been produced through secondary metabolic pathways. It is not surprising that their production was higher in the late sampling period, by which time a shortage of growth factors had probably started to develop. This phenomenon was especially pronounced for *P. roqueforti*, for which the ratio of terpene production to CO_2 production increased substantially between the early and the late sampling occasions (Tables 1 and 3). A similar phenomenon was found in our previous investigation (3), in which terpenes production during fungal growth was higher on artificial substrates than on substrates based on cereals. Similarly, a shortage of growth factors would be more likely to develop in the artificial substrates than in the cereal-based ones. The presence of volatile secondary metabolites suggests that elevated levels of mycotoxins can occur.

Only minor differences between oats and wheat were found, indicating that volatile metabolites can be used to distinguish between different fungi, regardless of the cereal grain used as a substrate. CFU counts and ergosterol levels increased continuously during fungal growth with the exception of *A. flavus* on oats, where ergosterol levels decreased slightly over time. For CFU counts, large differences between species were found, resulting in a low correlation with metabolic activity measured as CO_2 production. The correlation was much higher when each fungus was analyzed separately because of the pronounced interspecies variation in the degree of sporulation. Species-related differences in the stage at which extensive sporulation commences were probably attributable to interspecies differences in adaptation to the growth medium. For ergosterol, the differences between fungal species were smaller and the correlation with fungal metabolism was higher. Thus, for a sample consisting of a mixed fungal flora, ergosterol production should be a more reliable measure of past metabolic activity than CFU.

In the introduction, five criteria to be tested in this investigation were given. The results indicate the following. (i) 3-Methylfuran can be used as a general indicator of fungal growth. (ii) Differences between fungal species are larger than differences related to the type of grain used as a substrate. (iii) The duration of fungal growth does not influence the profile of the volatile metabolites produced. (iv) There was a weak correlation between CO_2 production and total production of volatile metabolites. The production of volatile metabolites was more strongly correlated with accumulated production of CO_2 than with actual CO_2 production. (v) The production of volatile metabolites was more strongly correlated with the ergosterol contents of the grains than with the numbers of CFU.

We conclude that volatile fungal metabolites can be used to detect and quantify fungal growth in cereals. However, before this technique can be introduced on a large scale, the effects of mixed bacterial and fungal flora on volatile production will have to be determined. We also need information about the capacity of grains to act as adsorbents for these metabolites. If this capacity is high, revolatilization of adsorbed metabolites could be used to detect earlier fungal growth in cereals. Such a method could be employed for quality control.

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