Mitteilungen Klosterneuburg 58 (2008): 17-22

Airborne fungal microflora of selected types of wine-cellars in Austria

ANDREAS CLEMENZ[1], KATJA STERFLINGER[2], WOLFGANG KNEIFEL[3] und KARIN MANDL[1]

[1] Höhere Bundeslehranstalt für Wein- und Obstbau
A-3400 Klosterneuburg, Wiener Straße 74
E-mail: Karin.Mandl@hblawo.bmlfuw.gv.at

[2] Universität für Bodenkultur, Department für Biotechnologie, Institut für angewandte Mikrobiologie
A-1190 Wien, Muthgasse 18

[3] Universität für Bodenkultur, Department für Lebensmittelwissenschaften und technologie, Abteilung Lebensmittelqualitätssicherung
A-1190 Wien, Muthgasse 18

Fungi are well-known as dominating components of the airborne microflora in wine cellars. In this study, different types of Austrian wine-cellars were examined for their environmental conditions as well as for their aerial fungal spectrum. Air samples were taken under controlled conditions, and data loggers were used to monitor temperature as well as humidity over a period of nine months. The fungal densities ranged from 55 to 12,050 CFU/m³ air, using different culture media. The fungal samples were identified microscopically. In general, members of the genera Penicillium, Aspergillus, Cladosporium, Exophilia, Phialophora, Phoma, Trichoderma *and* Ulocladium *were found. In three cellars, the species* Aspergillus fumigatus *and* A. niger *could be isolated. In addition, other species with allergy-provoking potential like* Aspergillus versicolor, Cladosporium sphaerospermum *and* Cl. herbarum *were identified.*
Keywords: wine-cellar, airborne microflora, fungi, Austria

Luft-Mikroflora ausgewählter Kellertypen in Österreich. *Schimmelpilze gelten als Hauptbestandteil der Mikroflora von Weinkellern. In dieser Studie wurden die Umgebungsbedingungen sowie das Schimmelpilzspektrum mehrerer österreichischer Kellertypen erfasst. Mit Hilfe eines Luftkeimsammlers wurden Proben genommen; Datenlogger dienten zur Erfassung der lokalen Temperaturprofile sowie der Feuchtigkeitsentwicklung über einen Zeitraum von neun Monaten. Die registrierten Keimzahlen bewegten sich zwischen 55 und 12.050 KBE/m³ Luft, wobei verschiedene Kultivierungsmedien zum Einsatz kamen. Die Identifizierung einzelner Isolate erfolgte mikroskopisch. Generell konnten die Gattungen* Penicillium, Aspergillus, Cladosporium, Exophilia, Phialophora, Phoma, Trichoderma *und* Ulocladium *nachgewiesen werden. In drei Kellern wurden* Aspergillus fumigatus *und* A. niger *isoliert. Daneben wurden auch andere potenziell allergieauslösende Pilze, wie* Aspergillus versicolor, Cladosporium sphaerospermum *und* Cl. herbarum *identifiziert.*
Schlagwörter: Weinkeller, Luftkeimspektrum, Pilze, Österreich

La microflore de types de caves sélectionnés en Autriche. *Les champignons de moisissure sont considérés comme élément principal de la microflore des caves à vin. Les conditions environnementales et le spectre des champignons de moisissure dans plusieurs types de caves autrichiennes ont été saisis dans le cadre de la présente étude. Des échantillons ont été recueillis à l'aide d'un collecteur de germes aériens, des enregistreurs de données ont servi à saisir les profils de température locaux et du développement de l'humidité sur une période de neuf mois. Les nombres de germes enregistrés se situaient entre 55 et 12.050 KBE/m³ d'air dans les différents milieux de culture utilisés. Les isolats ont été identifiés par voie microscopique. En général, on a décélé les genres* Penicillium, Aspergillus, Cladosporium, Exophilia, Phialophora, Phoma, Trichoderma *et* Ulocladium. Aspergillus fumigatus *et* A. niger *ont été isolés dans trois caves. En outre, on a également identifié d'autres champignons pouvant déclencher des réactions allergéniques,*

tels que Aspergillus versicolor, Cladosporium sphaerospermum *et* Cl. herbarum.
Mots clés : cave, spectre des germes aériens, champignons, Autriche

In recent years, increased attention has been given to the presence of fungi in indoor air for different reasons. Moulds are known as destroyers of organic compounds and also may have negative health effects on humans. There are several reports on the role of moisture and mould in homes and different working locations, but there is only limited information about the environment of wine production (Picco et al., 2004; Goto et al., 1989; Simeray et al., 2001).
Moulds and bacteria occasionally influence the quality of wine and also the health status of the people working in a contaminated environment. In principle, bioaerosols can cause four different types of illness: allergy, infection, irritation and toxic diseases, whereas the individual susceptibility is variable (Fung et al., 2003; Samson et al., 2002). Several studies have shown that fungal contamination of the indoor air is associated with irritative and non-specific respiratory symptoms, as well as the development of respiratory diseases such as asthma (Miller et al., 2004).
Besides this negative impact on human health, fungal contaminants may also affect the quality of the wine. Chatonnet et al. (1994) were able to demonstrate that moulds and bacteria transform chlorophenols, which are used in wood preservatives, into chloroanisols. Moreover, chloroanisols can migrate into the wine from the atmosphere of the cellar. Probably, substances like 2,3,6-trichloranisol (TCA) may induce a typically mouldy taste in wine. In analogy, the development of 2,4,6-tribromoanisole has been shown to cause an earthy taste (Chatonnet et al., 2004). Even other volatile compounds may result from the metabolism of moulds and bacteria and possess solubility in a mixture of water and ethanol. A substance named geosmine can be enriched by *Penicillium expansum* and has been identified as one of the causes of earthy taste and smell (Darriet et al., 2000; Mattheis et al., 1992).The purpose of this study was to screen the airborne fungal microflora of a selection of different types of wine cellars in Austria.

Material and methods

Samples were collected in eight different wine cellars in Lower Austria and Burgenland.

Wine cellar types

Depending on their construction, the wine cellars were categorized in three different groups. A protocol proposed in the "Schimmelpilz-Leitfaden" (Seifert, 2002) was adapted and used for categorization and registration of visible mould growth and stains on the wall:

- "Old subterranean cellar" with brick or limestone walls and packed sod floors
- "House cellars" of small family-run wineries, with brick or stone walls and concrete or flagstone floors
- "Modern air-conditioned cellars", non-subterranean, built with concrete and air conditioning, with the possibility to regulate the temperature

Registration of climate data

In all cellars temperature and relative humidity were registered over a period of nine months in one-hour intervals with two different types of data loggers, four of the type ilog (Escort, VWR ,Wien) and four of the type TFM (ELV Elektronik AG, http://www.elv.de). The data loggers were applied at about the half ceiling height at the sampling locations.

Microbial sampling and culturing

For the sampling and culturing of the microbiota, three different culture media were used (Thorne et al., 1992). Malt extract agar (MEA) was used as collective medium for the fungi; CASO-agar was used for the total viable count of all culturable microorganisms and Dichloran Glycerol agar (DG18, Roth Nr. AE26.1), which detects xerophilic fungi.
The samples were collected with the MAS-100 Eco air sampler (VWR, Wien). Measurements were carried out in the middle of the room at a height of approximately 1.5 meter, aspiring three different volumes (20, 50 and 100 l) of air. Air plates were produced in duplicates. The same was done outdoors as a reference in order to be able to analyze how much the indoor air is influenced by an air flow from the exterior. The MEA and DG18 plates were incubated for 6 days at room temperature, before colonies were enumerated. The CASO agar plates were evaluated first after incubating for two days at 28 °C. Incubation was continued at room temperature and the second count for the total viable count was performed after 6 days. The colony counts

expressed in colony forming units (CFU) were related to the air volume aspired and calculated as CFU/m^3.
For the identification of the fungi, pure cultures were grown from morphologically different colonies. Then the isolates were conserved under sterile water using the method of BURDSALL et al. (1994). For identification, the fungi were inoculated on MEA, DG18, Czapek Dox Agar (CZA) and, in some cases, also on Corn Meal Infusion Agar (CMA). The identification was done according to their micro- and macroscopical morphological properties, based on different literature (DOMSCH et al., 1993; KLICH et al., 1988; PITT et al., 1991; ROBERT et al., 2004; ELLIS et al., 1971)

Results and discussion

Temperature and relative humidity profiles

The 'old subterranean cellars' generally showed a very high humidity throughout the year. In two of three cellars the average relative humidity exceeded 90%, but the temperature was at a very constant level in all three cellars investigated. This is an important pre-requisite for a good development of wine. The 'house cellars' are characterized by high relative humidity values and little seasonal fluctuations in the temperature as well. None of the three 'air conditioned modern cellars' had an annual average of more than 75 % relative humidity. This can be helpful to keep the microbial load as low as possible. These results were enabled by air-conditioning and are also supported by the modern construction of these halls, which were above ground level in most cases. In the older cellar types, the humidity often permeated through the cellar walls as could be seen in typical stains. Sometimes water even penetrated the walls crevices trickling off from the walls. These patterns offer a perfect ground for the growth of moulds. Exemplarily, in Figures 1 and 2 climate diagrams of an old subterranean cellar and an air-conditioned cellar, respectively, are shown.

Microbial load

Because of the high dampness of the walls and indoor air growth conditions were optimal for fungi in the old subterranean cellars. Moreover, the walls of these cellars are often covered with layers of organic substances from earth, insects, algae and bacteria thus providing a wide range of nutrients and minerals. In this category the mean value of the colony forming units on MEA-media was 4,769 CFU/m^3. The variations between different cellars and samplings, however, were high. The lowest fungal count in this category was 848 CFU/m^3 and the highest 12,050 CFU/m^3. In all three cellars the fungal contamination of the walls was clearly visible and the values of the indoor air always were significantly higher than outdoors. The values observed in the house cellars were very similar to those of the old subterranean cellars. On MEA-media the average fungal count was 4,293 KBE/m^3.
As concluded from the climatical data of the wine cellars, the microbial load of the internal air of the air-conditioned cellars was relatively low. The average fungal

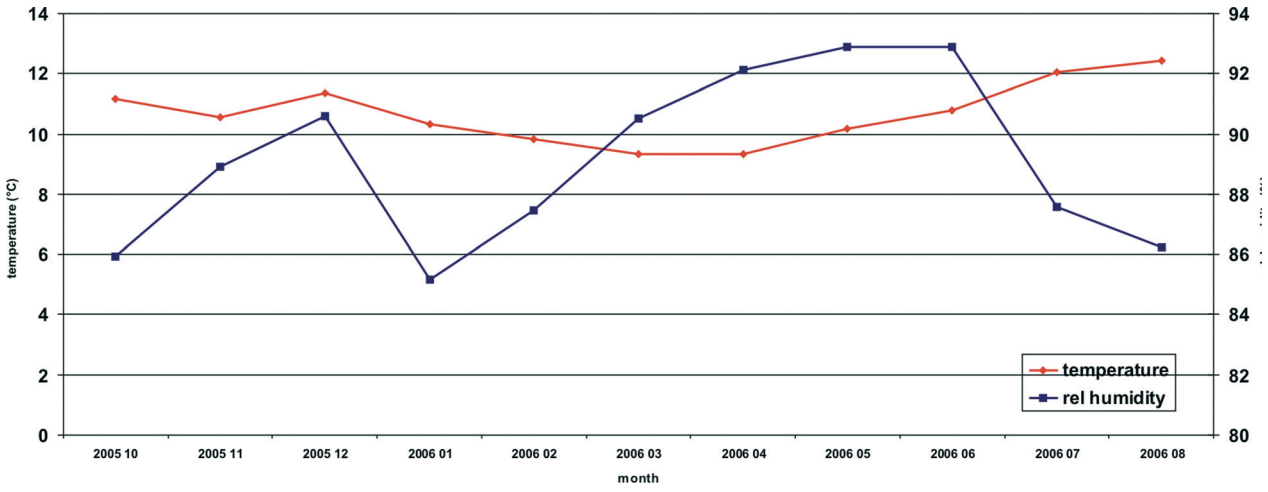

Fig. 1: Climatical development of a typical subterranean cellar

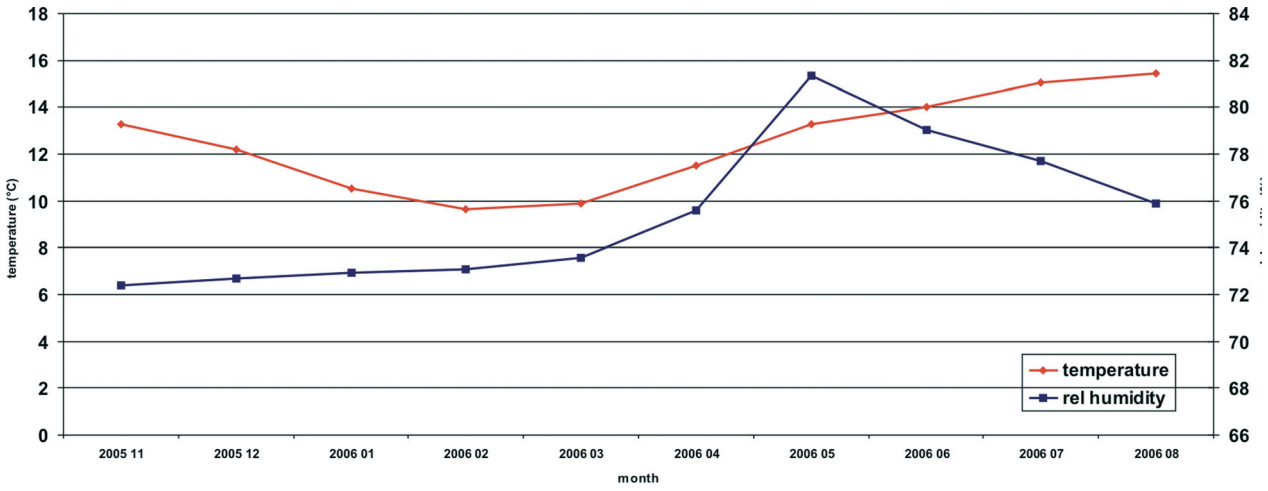

Fig. 2: Climatical development of a typical air-conditioned cellar

count on MEA-media was only 357 CFU/m^3. Interestingly, higher counts were observed on DG18-media with an average of 868 CFU/m^3. This is due to the fact that DG18 agar is particularly suitable for xerophilic fungi, germinating and growing at RH values of above 65 to 70%. The average values of all three categories are summarized in Table 1. The reason for the much smaller spore contamination in the modern cellars is on one hand the low humidity and on the other hand the modern construction of these cellars which makes cleaning more easy and thus avoids accumulation of organic pollutions on walls and water intrusion through the cellar walls can be avoided. To limit the growth of the moulds the relative humidity should not exceed 75 % over longer periods (Rowan et al., 1999). But to avoid fungal growth also the effect of condensation and hygroscopic materials like wood or paper should be taken into account. Whereas condensation of water from warm air on cold walls can be one of the most important sources of humidity in an old cellar, these effects do much less occur in modern buildings with appropriate thermic isolation.

In particular, in the air of old subterranean cellars and in the house cellars rather high fungal counts have been observed, which can be classified as not being normal. In each case, the values of the indoor air were higher than those measured outdoors. This is a clear sign for some indoor source of contamination. Fungal counts like, e.g., 12,050 CFU/m^3 as measured in one cellar, can constitute some big disadvantage for the people working in this area, especially for allergic persons. Lavoie et al. (1997) has concluded from his findings related to a sorting and composting plant of household waste that bacterial loads exceeding 10,000 CFU/m^3 and the presence of A*spergillus fumigatus*, necessarily preclude the use of effective respiratory protection. With culture methods only 1 to 50 % of the actual spore concentration in the air can be measured (Miller et al., 2004). This inaccuracy, the variations between measurements and the lack of validated standard procedures for the measurements make it difficult to evaluate and to interpret the results. In particular, in connection with possible detrimental health effects, there is some increasing need for having available threshold levels, as no dose-response data are available so far (Jensen et al., 1998).

Table 1: Mean microbial counts in three cellar categories

Culture medium	Detection of:	Cellar category	CFU/m³ (arith. mean)
CASO	Total aerobic microbial count	Old subterranean cellar type	3.456
DG18	Fungal count		4.088
MEA	Fungal count		4.769
CASO	Total aerobic microbial count	House cellar type	4.643
DG18	Fungal count		4.624
MEA	Fungal count		4.293
CASO	Total aerobic microbial count	Air-conditioned cellar type	717
DG18	Fungal count		868
MEA	Fungal count		357

Microbial diversity

A reliable analysis of the indoor air quality is always based on quantitative and on qualitative analysis of the fungal community. In this study fungi were identified based on their morphology alone. Because of this, species identification was not always possible and a molecular approach based on DNA-sequences would have been necessary. But we could compare the air quality of the different cellars and relate these findings to the results obtained from outside. The old subterranean cellars and the house cellars were clearly dominated by the genus *Penicillium* (77% in old subterranean cellars 78% in house cellars) and *Aspergillus* (15% in house cellars, 16 % in old subterranean cellars). A survey of French wine cellars was performed by SIMERAY et al. (2001) and revealed similar results. Figure 3 demonstrates that the quality of the aeromycological flora of the old subterranean and the house cellars is rather similar, and also much less diverse than the outside air. Obviously, a distinct microflora has developed in older cellars. The aeromycological diversity of the air-conditioned cellars, however, is considerably bigger and much more agrees with that of the outside air. This means that the aeromycological flora of the air-conditioned cellars is mainly influenced by the outside air and not by an indoor source.

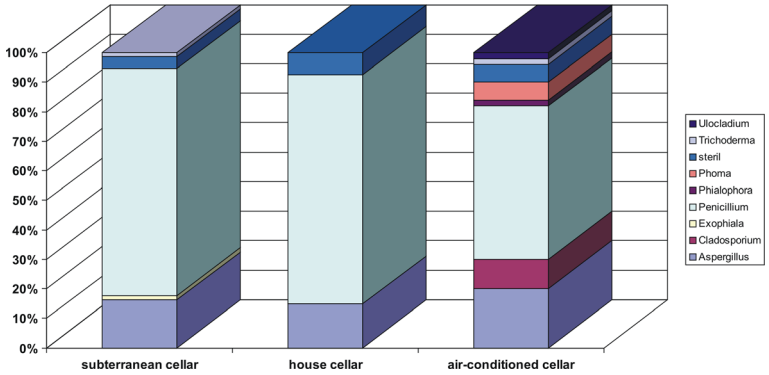

Fig. 3: Aeromycological composition depending on the type of cellar

In three cellars, the thermophilic species *Aspergillus fumigatus* and *A. niger* could be isolated. These representatives are known to be able to produce mycotoxins and also are related with allergies and infections. Other potentially allergy-provoking species like *Aspergillus versicolor*, *Cladosporium sphaerospermum* and *Cl. herbarum* have also been isolated.

During recent years, hygienic design of wine production has undergone some considerable modification with special regard to cellar construction. This development has led to some improvements in the controllability of the climate as well as of the microbial load of the cellars and can therefore be seen as some improvement of the microbial load at such locations. This is definitely of advantage for the health status of employees at their working places. On the other hand, improved status of the level of airborne contamination may also allow a better tailor-made control and more detailed monitoring of the conditions of wine production. But traditional settings of wine production may not be considered as some competitive disadvantage, since products of high quality may be also obtained if proper microecological conditions exist. This study has shown, that each wine cellar category possesses some resident airborne microflora, which can be influenced by external contaminants, in particular if classical buildings are considered. Unfortunately there is only limited information available, which of the predominating fungal colonization is of higher, which of lower value for the development of wine. Hence studies like this can serve as a contribution to complete the heterogeneous puzzle of environmental conditions of wine production.

Conclusion

In the old cellars and in the house cellars very high mould concentrations were measured. Only viable spores could be registered with the methods used in this project. Therefore it is very likely that the actual spore levels are much higher. There are limits to the interpretation of aeromycological data, because no dose-response data is available so far, but it is obvious that the amounts of spores measured in the air of some of the monitored cellars is not healthy, especially in the cellars of large wineries, where people work eight hours a day. Also to secure a high level of quality in the production of wine, it is very important to keep the level of microorganisms in the air as low as possible. Bacteria and moulds produce different volatile metabolites, which can contaminate the atmosphere and other materials, which get into contact with the wine.

The results of this research show clearly, that the control of the cellar climate and a modern construction are the keys to a high quality product and to secure the workers' health. For further investigations it would be

very interesting to use molecular biology techniques for the identification of moulds and bacteria. Sampling in shorter intervals and also in the summer months could give a better view on the development of the microflora in the wine cellars.

References

BURDSALL, J. H.H. and DORWOTH, E.B.1994: Preserving cultures of wood decaying Basidiomycotina using sterile distilled water in cryovials. Mycologia 86(2): 275-280

CHATONNET, P., GUIMBERTAU, G., DUBOURDIEU, D. et BOIDRON, J. 1994: Nature et origine des odeurs de 'mois' dans les caves. Incidences sur la contamination des vins. J. Int. Sci. Vigne et du Vin 28: 131-151, 183

CHATONNET, P., BONNET, S., BOUTOU, S. and LABADIE, M.D. 2004: Identification and responsibility of 2,4,6-tribromoanisole in musty, corked odors in wine. J. Agric. Food Chem. 52(5): 1255-1262

DARRIET, P., PONS, M., LAMY, S. and DUBOURDIEU, D. 2000: Identification and quantification of geosmin, an earthy odorant contaminating wines. J. Agric. Food Chem. 48(10): 4835-4838

DOMSCH, K.H., GAMS, W. and ANDERSON, T.-H. (1993): Compendium of soil fungi, volume1. - D-85386 Eching: IHW-Verlag, 1993

ELLIS, M.B. (1971): Dematiaceous hyphomycetes. - Wallingford, Oxon: CAB International, 1971

FUNG, F. and HUGHSON, W.G. 2003: Health effects of indoor fungal bioaerosol exposure. Appl. Occup. Environ. Hyg. 18: 535-544

GOTO, S., TAKAYAMA, K. and SHINOHARA, T. 1989: Occurrence of moulds in wine storage cellars. J. Ferment. Bioeng. 68(4): 230-232

JENSEN, P.A. and SCHAFER, M.P. (1998): Sampling and characterization of bioaerosols. In: NIOSH manual of analytical methods, method 0800, issue 1: 82-112. - Atlanta, 1998

KLICH, M.A. and PITT, J.J. (1988): A laboratory guide to common Aspergillus species and their telemorphs. - North Ryde (AUS): CSIRO Division of Food Processing, 1988

LAVOIE, J. and ALIE, R. 1997: Determining the characteristic to be considered from a worker health and safety standpoint in household waste sorting and composting plants. Ann. Agric. Environ. Med. 4: 123-128

MATTHEIS, J.P. and ROBERTS, R.G.1992: Identification of geosmin as a volatile metabolite of Penicillium expansum. Appl. Environ. Microbiol. 58(9): 3170-3172

MILLER, J.D., GILBERT, N.L. and DALES, R.E. (2004): Fungal contamination in public buildings: health effects and investigation methods. - Ottawa: Health Canada, 2004

PICCO, A.M. and RODOLFI, M 2004: Assessments of indoor fungi in selected wineries of Oltrepo Pavese (Northern Italy) and Sottoceneri (Switzerland). Am. J. Enol. Vitic. 55(4): 355-362

PITT, J.I. (1991): A laboratory guide to common Penicillium species. - North Ryde (AUS): CSIRO Division of Food Processing, 1991

ROBERT, A., SAMSON, E.S.H. and FRISVAD, J.C. (2004): Introduction to food and airborne fungi. Number7. - Utrecht: Centraalbureau voor Schimmelcultures, 2004

ROWAN, N.J., JOHNSTONE, C.M., MCLEAN, R.C., ANDERSON, J.G. and CLARKE, J.A. 1999: Prediction of toxigenic fungal growth in buildings by using a novel modelling system. Appl. Environ. Microbiol. 65(11): 4814-4821

SAMSON, R.A., HOEKSTRA, E.S., FRISVAD, J.C. and FILTENBORG, O. (2002): Introduction to food- and airborne fungi. - Utrecht: Centraalbureau voor Schimmelcultures, 2002

SEIFERT, B. (2002): Leitfaden zur Vorbeugung, Untersuchung, Bewertung und Sanierung von Schimmelpilzwachstum in Innenräumen. - Berlin: Umweltbundesamt, 2002

SIMERAY, J., MANDIN, D., MERCIER, M. and CHAUMONT, J.-P. 2001: Survey of viable airborne fungal propagules in French wine cellars. Aerobiologia 17(1): 19-24

THORNE, P.S., KIEKHAEFER, M.S., WHITTEN, P. and DONHAM, K.J. 1992: Comparison of bioaerosol sampling methods in barns housing swine. Appl. Environ. Microbiol. 58(8): 2543-2551

Received October 18, 2007