OENO One
VINE AND WINE OPEN ACCESS JOURNAL
Volume 51 > Number 3 > 2017

Stilbenes in the different organs of *Vitis vinifera* cv. Merlot grafted on TK5BB rootstock

Gyöngyi Németh[1], Orsolya Hegyi[2], Attila Dunai[3], László Kocsis[1*]

[1]University of Pannonia, Georgikon Faculty, Department of Horticulture, Deák F. street 16, 8360 Keszthely, Hungary
[2]University of Pannonia, Georgikon Faculty, Department of Animal Sciences and Animal Husbandry, Deák F. street 16, 8360 Keszthely, Hungary
[3]University of Pannonia, Georgikon Faculty, Department of Crop Production and Soil Science, Deák F. street 16, 8360 Keszthely, Hungary

Abstract

Aim: To determine which of the grapevine organs serving as viticultural by-products are the richest in biological active stilbenes, like t-piceid, t-resveratrol and ε-viniferin.

Methods and results: Nine organs, namely roots, canes, buds, shoot tips, inflorescences, clusters at veraison, mature berry skins, seeds and cluster stems, of *Vitis vinifera* cv. Merlot grafted on TK5BB rootstock were collected during one year and their ethanolic extracts were analysed via HPLC-FLD. The stilbene content varied widely among the different organs, the lowest being 3.15 mg/kg dry weight (dw) in the seeds and the highest 2265 mg/kg dw in the buds.

Conclusion: The present research demonstrated that different grapevine organs contain different amounts of stilbene in the vineyard. The winter-buds, the roots and the mature cane internodes of Merlot are significantly richer in t-resveratrol and ε-viniferin than the green vegetative and generative parts that we examined over one year.

Significance and impact of the study: To our knowledge, there has been no assessment of stilbene content in several plant organs of a vine cultivar in a field study. Our research pointed out that pruned canes could be a primary source of stilbene for the health industry. Roots, which are a by-product of grapevine nurseries, are also rich in stilbene.

Key words: grape, resveratrol, piceid, viniferin, plant organs, grafting

manuscript received 26th July 2016 - accepted 3rd April 2017
doi:10.20870/oeno-one.2017.51.4.1861

*Corresponding author: kocsis-l@georgikon.hu

OENO One, 2017, **51**, 3, 323-328
©Université de Bordeaux (Bordeaux, France)

Introduction

Stilbenes are natural phenolic compounds occurring in a number of plant families including *Vitaceae*. Resveratrol is a low molecular weight stilbene molecule, its 3-O-glucoside is piceid and one of the resveratrol oligomers is ε-viniferin, a dehydrodimer. The putative medical benefits of stilbenes were intensively studied during the last decade. Resveratrol is an antioxidant agent that is thought to fend off cardiovascular diseases and cancer; influence Alzheimer's disease and ageing; and prevent bone loss and diabetes (Tosun and Inkaya, 2009). The biological activities of piceid have not been thoroughly studied, but it seems it may also have beneficial effects on health (Chong *et al.*, 2009). ε-Viniferin has promising actions in vitro, like decreasing glucose absorption (Guschlbauer *et al.*, 2013) and inhibiting vascular muscle cell proliferation (Zghonda *et al.*, 2011) and amyloid-beta peptide aggregation (Rivière *et al.*, 2010). Resveratrol and its derivatives are naturally produced by plants as self-defence agents either in response to biotic or abiotic stress or constitutively without elicitors (Jeandet *et al.*, 2010), and thus can be considered as phytoalexins and phytoanticipins. Stilbenes were detected or quantified in different vine organs, such as berries, seeds, berry skin, cluster stems, leaves, canes, roots (Waffo-Teguo *et al.*, 2008), flowers (Keller *et al.*, 2003; Timperio *et al.*, 2012) and buds (Wang *et al.*, 2010; Qsaib *et al.*, 2014). To our knowledge, no measurement of stilbene content in several plant organs of a vine cultivar in a field study has been reported, and only limited data are available on grape root resveratrol and ε-viniferin content.

Materials and methods

1. Plant material and field conditions

Vitis vinifera cv. Merlot red wine grape cultivar was established on Teleki Kober 5BB (TK5BB) rootstock in 2004 in Szekszárd, Hungary (46°19'30''N/ 18°41'09''E, 130 m altitude). The effective temperature was 3538°C and rainfall was 798 mm in 2013.

2. Sample collection

Visually healthy plant organs were collected from 10 randomly chosen vines from the vineyard. Samples were pooled replicates and we made three technical repeats. The shoot tips and inflorescences were collected at full bloom in 2013. Clusters were collected at veraison and at harvest in 2013. The internodes of the cane, the bud and the root samples were collected in the dormant season in February 2014 (Figure 1).

3. Sample processing

Sample preparation and extraction was performed with minor modifications according to the method of Rayne *et al.* (2008) within a day after collection. The ripe cluster was destemmed, and the skin and seeds were separated from the berries. The samples of plant material were treated with liquid nitrogen, then ground into powder (Moulinex AR100) and kept at -70°C in the dark until further processing. 1 g of fresh sample powder (particle size <1 mm) was extracted in 80% ethanol with 10 minutes ultrasound treatment at 38 kHz (Realsonic RS-26), and 10 minutes mixing (VWR-IKA VMS-C4) at room temperature. The extraction was performed three times with the samples. The mixture was centrifuged at 5500 g at



Figure 1. investigated organs of the grape plant. Winter-buds (1), cane internodes (2), surface roots of the rootstock (3), shoot tip with a young leaf and a tendril at the beginning of the leaf expansion (4), inflorescence at full bloom (5), cluster at veraison (6), and cluster stem (7a), berry skins (7b) and seeds (7c) of a ripe grape cluster.

©Université de Bordeaux (Bordeaux, France)

2 °C for 10 minutes and filtered. The combined supernatants were evaporated under a nitrogen stream at 35 °C (IKA-WERKE RV06-ML, HB4 basic). The dried residue was re-dissolved in 1 mL of extracting solution and kept at -20 °C in the dark. The solution was filtered (pore size 0.45 µm cellulose filter) prior to HPLC injection.

4. Chemicals and reagents

All chemicals and t-resveratrol (>99% purity), t-piceid (>95% purity) and ε-viniferin (>95% purity) standards were obtained from VWR International.

5. Chromatographic methods

HPLC-FLD analyses were conducted on Perkin Elmer Flexar chromatograph system and Chromera-Flexar FL data collection software. The analytical column Zorbax Eclipse XDB-C8 4.6×150 mm was thermostatically controlled at 30 °C. Mobile phase "A" was water with 0.5% formic acid and mobile phase "B" was acetonitrile with 10% water and 0.5% formic acid. The flow rate was 1 mL/min and the injection volume was 20 µL. Gradient elution was performed from 10% to 55% eluent "B" in 45 minutes with the following gradient program: 0 –> 1.0 min 10% B; 1.0 –> 45.0 min 10% –> 55% B; 46.0 –> 51.0 min 80% B; 51.0 –> 53.0 min 80% B; 53.0 –> 58.0 min 80% –> 10% B; 58.0 –> 61.0 min 10% B. The fluorimetric detector was set at 330 nm excitation and 374 nm emission wavelength for stilbenes according to Pezet *et al.* (1994) and Timperio *et al.* (2012).

Quantification was performed using external 5-point calibration curve (0.15, 0.3, 3.0, 9.0, 30.0 mg/L for t-piceid and ε-viniferin; 0.075, 0.15, 1.5, 4.5, 15.0 mg/L for t-resveratrol). The assay for stilbene standards was linear with high correlation coefficient (r=0.996 for t-piceid, 0.998 for t-resveratrol and 0.991 for ε-viniferin). The slope of the calibration curve was $4x10^{-6}$ (t-piceid and ε-viniferin) and $2x10^{-6}$ ppm/mFLUs (t-resveratrol). The limit of detection was 0.03 mg/L for t-piceid and ε-viniferin and 0.015 mg/L for t-resveratrol. The limit of quantification was the lowest concentrations of the calibration curves.

6. Statistical analysis

Data were analysed by two-way analysis of variance (ANOVA), and Duncan Multiple Range Post Hoc test was performed with SPSS 15. $P<0.05$ was considered statistically significant.

Results and discussion

In this study, biologically valuable stilbene molecules of viticultural by-products were measured from different organs of the plant. Mature canes with winter-buds are waste products of pruning and grafting; roots are available from the nurseries or sell off vineyards; shoot tips, inflorescences and immature clusters are removed from the vines during summer pruning; and cluster stems and berry skin and seeds of mature clusters are by-products of the wine making process. These plant organs are available each year from vine cultivation. The examined organs contained a wide range of stilbene contents. The sum of the three examined molecules was lowest in seeds (3.15 mg/kg dry weight (dw)) and highest in buds (2265 mg/kg dw) (Table 1). In the inflorescences of Merlot at full bloom, stilbenes were not detectable. Timperio *et al.* (2012) reported that Merlot flowers without *Botrytis cinerea* inoculation showed low concentrations of t-resveratrol (pg/µL magnitude in samples) and no δ-viniferin, which is a different resveratrol dimer than what we have investigated. Keller *et al.* (2003) in healthy Gamay flowers detected no ε-viniferin and resveratrol but 3.69 mg/kg fresh weight (fw) piceid. The winter-pruned canes contain nodes with buds and internodes. Most studies on cane stilbene content do not specify whether the canes were measured with or without buds. Lambert *et al.* (2013) reported 1181 mg/kg dw t-resveratrol and 2263 mg/kg dw ε-viniferin in Merlot canes. Our results are higher (2081 ± 170 mg/kg dw and 385.1 ± 33.4 mg/kg dw ε-viniferin in buds and internodes, respectively) but in line with Qsaib *et al.* (2014), who measured 90 mg/kg dw ε-viniferin in buds and 60 mg/kg dw ε-viniferin in internodes of Merlot. Buds were significantly richer in ε-viniferin than internodes and other grape organs. Cane internodes were significantly the richest organs in t-resveratrol and t-piceid (Table 1).

The rootstock of the investigated grafting was *Vitis berlandieri x Vitis riparia* TK5BB. The t-resveratrol content in roots was remarkable (113.0 ± 6.2 mg/kg dw) and that of ε-viniferin was the second highest among all organs (1476 ± 181 mg/kg dw). Wang *et al.* (2010) in roots of young *Vitis vinifera* cv. Cabernet-Sauvignon raised in pot measured approx. 4.8 mg/kg fw t-resveratrol, and Ji *et al.* (2014) in roots of wild *Vitis amurensis* reported 61.2-123.4 mg/kg dw over a year. Bavaresco *et al.* (2003) measured 15.5 mg/kg fw t-resveratrol and 32.3 mg/kg fw t-piceid in roots of Chardonnay TK5BB grafts. Bavaresco *et al.* (2000) reported

OENO One, 2017, **51**, 3, 323-328
©Université de Bordeaux (Bordeaux, France)

Table 1. Stilbene content of Merlot plant organs

Plant organs	t-piceid	t-resveratrol	ε-viniferin
bud	14.65 ± 1.57 c	168.3 ± 10.4 b	2081 ± 170 a
cane internode	125.9 ± 10.5 a	344.5 ± 32.2 a	385.1 ± 33.4 c
root	26.14 ± 1.26 b	113.0 ± 6.2 c	1476 ± 181 b
shoot tip	4.97 ± 0.08 d	2.96 ± 0.29 e	6.48 ± 0.29 d
inflorescence	nd*	nd*	nd*
cluster at veraison	16.08 ± 5.64 c	2.26 ± 0.16 e	18.46 ± 4.81 d
cluster stem	14.50 ± 2.26 c	17.43 ± 2.34 de	98.51 ± 6.13 d
berry skin	nd*	31.24 ± 1.37 d	121.54 ± 3.82 d
seed	nd*	0.78 ± 0.02 e	2.37 ± 0.38 d

Results are expressed as mg/kg dry weight and represent average values of triplicate determinations (n=3) ± standard deviation. *not detectable.
Values in the same column with different letters are statistically different ($p<0.05$) based on Duncan Multiple Range Post-Hoc test.

640 mg/kg fw t-resveratrol and 980 mg/kg fw ε-viniferin in roots of ungrafted TK5BB rootstocks.

Berries of other varieties were investigated by Bavaresco *et al.* (1997) at different developmental stages. They measured a few mg/kg fw stilbenes in berries at veraison. Our results contain berry and cluster stem stilbenes together at veraison, which are similarly low, as previously mentioned.

Bavaresco *et al.* (1997) measured 38 mg/kg fw t-resveratrol and 54 mg/kg fw ε-viniferin in cluster stems of Merlot and an average of 113 mg/kg dw and 288 mg/kg dw in other cultivars, respectively (Anastasiadi *et al.*, 2012). Our data (17.43 ± 2.43 mg/kg dw and 98.51 ± 6.13 mg/kg dw, respectively) are comparable with these obtained from the literature.

The skin of mature grapes contains t-piceid besides the cis isomer (Romero-Pérez *et al.*, 2001; Liu *et al.*, 2013; Vincenzi *et al.*, 2013), although it was not measured in this work. Our results for t-resveratrol contents in mature Merlot grape skin (31.24 ± 1.37 mg/kg dw) are similar to the values measured by Romero-Pérez *et al.* (2001) (38.26 mg/kg dw). The ε-viniferin values measured in Merlot grape skin (121.54 ± 3.82 mg/kg dw) were higher than in other blue grape cultivars measured by Anastasiadi *et al.* (2010) (5.1-65.3 mg/kg dw).

In Merlot grape seeds, t-piceid was not detectable and a low concentration of t-resveratrol (0.78 ± 0.02 mg/kg dw) was measured, as reported by Sun *et al.* (2006). In grape seeds, ε-viniferin was detected and low amount was measured (2.37 ± 0.38 mg/kg dw), contrary to Anastasiadi *et al.* (2010).

The variation of stilbene concentrations measured in grape organs could be high between studies. Ji *et al.* (2014) showed that the t-resveratrol concentration in *V. amurensis* organs varied significantly between seasons. The time between pruning and the analysis of the cane samples has an effect on the measurement results (Vergara *et al.*, 2012). t-Resveratrol concentration in berries is significantly affected by variety and elevation (Bavaresco *et al.*, 2007) and stilbene concentration by genotype, ripening (Gatto *et al.*, 2008) and viticultural factors (Bavaresco, 2003). The sample preparation and the extraction method may cause differences as well (Romero-Pérez *et al.*, 2001; Aaviksaar *et al.*, 2003; Liu *et al.*, 2013; Soural *et al.*, 2015). Studies including concentrations expressed in fresh weight of plant material are difficult to compare with others using dry weight.

Conclusion

As a consequence of increasing interest in natural bioactive compounds to meet the demands of the health industry, the present research provides an overall characterization of stilbene content in grapevine organs originating from the vineyard. The winter-buds, the roots and the mature cane internodes of Merlot are significantly richer in t-resveratrol and ε-viniferin than the green vegetative and generative parts we examined over one year. Canes may be an adequate, stable (Zhang *et al.*, 2011) and economic (Rayne *et al.*, 2008) source of resveratrol. In addition, roots and buds may be good source of ε-viniferin-rich ethanolic extracts. Our research pointed out that roots could be an easily accessible

©Université de Bordeaux (Bordeaux, France)

source of stilbenes, as they are by-products of the grafting preparation for storage or planting.

Acknowledgements : We are grateful to László Wágner and Károly Dublecz for the opportunity to work with the laboratory of the Department of Animal Sciences and Animal Husbandry. We thank Takler Winery, with special thanks to Ferenc Takler Jr. for the plant material, and the OMSz for the meteorological data.

References

Aaviksaar A., Haga M., Pussa T., Roasto M. and Tsoupras G., 2003. Purification of resveratrol from vine stems. *Proceedings of the Estonian Academy of Sciences, Chemistry* **52**, 155-164.

Anastasiadi M., Pratsinis H., Kletsas D., Skaltsounis A.L. and Haroutounian S.A., 2010. Bioactive non-coloured polyphenols content of grapes, wines and vinification by-products: evaluation of the antioxidant activities of their extracts. *Food Research International* **43**, 805-813. doi:10.1016/j.foodres.2009.11.017

Anastasiadi M., Pratsinis H., Kletsas D., Skaltsounis A.L. and Haroutounian S.A., 2012. Grape stem extracts: polyphenolic content and assessment of their in vitro antioxidant properties. *LWT - Food Science and Technology* **48**, 316-322. doi:10.1016/j.lwt.2012.04.006

Bavaresco L., 2003. Role of viticultural factors on stilbene concentrations of grapes and wine. *Drugs under Experimental and Clinical Research* **29**, 181-187.

Bavaresco L., Cantù E., Fregoni M. and Trevisan M., 1997. Constitutive stilbene contents of grapevine cluster stems as potential source of resveratrol in wine. *Vitis* **36**, 115-118.

Bavaresco L., Cantù E. and Trevisan M., 2000. Chlorosis occurrence, natural arbuscular-mycorrhizal infection and stilbene root concentration of ungrafted grapevine rootstocks growing on calcareous soil. *Journal of Plant Nutrition* **23**, 1685-1697. doi:10.1080/01904160009382133

Bavaresco L., Pezzutto S., Fornaroli A. and Ferrari F., 2003. Grapevine iron-chlorosis occurrence and stilbene root concentration as affected by the rootstock and arbuscular mycorrhizal infection. *Acta Horticulturae* **603**, 401-410. doi:10.17660/ActaHortic.2003.603.51

Bavaresco L., Pezzutto S., Gatti M. and Mattivi F., 2007. Role of the variety and some environmental factors on grape stilbenes. *Vitis* **46**, 57-61.

Chong J., Poutaraud A. and Hugueney P., 2009. Metabolism and roles of stilbenes in plants. *Plant Science* **177**, 143-155. doi:10.1016/j.plantsci.2009.05.012

Gatto P., Vrhovsek U., Muth J., Segala C., Romualdi C., Fontana P., Pruefer D., Stefanini M., Moser C., Mattivi F. and Velasco R., 2008. Ripening and genotype control stilbene accumulation in healthy grapes. *Journal of Agricultural and Food Chemistry* **56**, 11773-11785. doi:10.1021/jf8017707

Guschlbauer M., Klinger S., Burmester M., Horn J., Kulling S.E. and Breves G., 2013. trans-Resveratrol and ε-viniferin decrease glucose absorption in porcine jejunum and ileum in vitro. *Comparative Biochemistry and Physiology Part A: Molecular & Integrative Physiology* **165**, 313-318. doi:10.1016/j.cbpa.2013.03.040

Jeandet P., Delaunois B., Conreux A., Donnez D., Nuzzo V., Cordelier S., Clément C. and Courot E., 2010. Biosynthesis, metabolism, molecular engineering A.nd biological functions of stilbene phytoalexins in plants. *Biofactors* **36**, 331-341. doi:10.1002/biof.108

Ji M., Li Q., Ji H. and Lou H., 2014. Investigation of the distribution and season regularity of resveratrol in *Vitis amurensis* via HPLC–DAD–MS/MS. *Food Chemistry* **142**, 61-65. doi:10.1016/j.foodchem.2013.06.131

Keller M., Viret O. and Cole F.M., 2003. *Botrytis cinerea* infection in grape flowers: defense reaction, latency, and disease expression. *Phytopathology* **93**, 316-322. doi:10.1094/PHYTO.2003.93.3.316

Lambert C., Richard T., Renouf E., Bisson J., Waffo-Téguo P., Bordenave L., Ollat N., Mérillon J. M. and Cluzet S., 2013. Comparative analyses of stilbenoids in canes of major *Vitis vinifera* L. cultivars. *Journal of Agricultural and Food Chemistry* **61**, 11392-11399.

Liu C., Wang L., Wang J., Wu B., Liu W., Fan P., Liang Z. and Li S., 2013. Resveratrols in *Vitis* berry skins and leaves: their extraction and analysis by HPLC. *Food Chemistry* **136**, 643-649. doi:10.1016/j.foodchem.2012.08.017

Pezet R., Pont V. and Cuenat P., 1994. Method to determine resveratrol and pterostilbene in grape berries and wines using high-performance liquid chroma-tography and highly sensitive fluorimetric detection. *Journal of Chroma-tography A* **663**, 191-197. doi:10.1016/0021-9673(94)85245-6

Qsaib S., Mateus N., Ikbal F., Rifai L.A., De Freitas V. and Koussa T., 2014. Direct identification and characterization of phenolic compounds from crude extracts of buds and internodes of grapevine (*Vitis vinifera* cv Merlot). *Natural Product Communi-cations* **9**, 1569-1572.

Rayne S., Karacabey E. and Mazza G., 2008. Grape cane waste as a source of trans-resveratrol and trans-viniferin: high-value phytochemicals with medicinal and anti-phytopathogenic applications. *Industrial Crops and Products* **27**, 335-340. doi:10.1016/j.indcrop.2007.11.009

Rivière C., Papastamoulis Y., Fortin P.-Y., Delchier N., Andriamanarivo S., Waffo-Teguo P., Kapche G.D.,

©Université de Bordeaux (Bordeaux, France)

Amira-Guebalia H., Delaunay J.-C., Mérillon J.-M., Richard T. and Monti J.-P., 2010. New stilbene dimers against amyloid fibril formation. *Bioorganic & Medicinal Chemistry Letters* **20**, 3441-3443. doi:10.1016/j.bmcl.2009.09.074

Romero-Pérez A.I., Lamuela-Raventós R.M., Andrés-Lacueva C. and de la Torre-Boronat M.C., 2001. Method for the quantitative extraction of resveratrol and piceid isomers in grape berry skins. Effect of powdery mildew on the stilbene content. *Journal of Agricultural and Food Chemistry* **49**, 210-215. doi:10.1021/jf000745o

Soural I., Vrchotová N., Tříska J., Balík J., Horník Š., Cuřínová P. and Sýkora J., 2015. Various extraction methods for obtaining stilbenes from grape cane of *Vitis vinifera* L. *Molecules* **20**, 6093-6112. doi:10.3390/molecules20046093

Sun B., Ribes A.M., Leandro M.C., Belchior A.P. and Spranger M.I., 2006. Stilbenes: quantitative extraction from grape skins, contribution of grape solids to wine and variation during wine maturation. *Analytica Chimica Acta* **563**, 382-390. doi:10.1016/j.aca.2005.12.002

Timperio A.M., d'Alessandro A., Fagioni M., Magro P. and Zolla L., 2012. Production of the phytoalexins *trans-resveratrol* and delta-viniferin in two economy-relevant grape cultivars upon infection with *Botrytis cinerea* in field conditions. *Plant Physiology and Biochemistry* **50**, 65-71. doi:10.1016/j.plaphy.2011.07.008

Tosun I. and Inkaya A.N., 2009. Resveratrol as a health and disease benefit agent. *Food Reviews International* **26**, 85-101. doi:10.1080/87559120802525459

Vergara C., von Baer D., Mardones C., Wilkens A., Wernekinck K., Damm A., Macke S., Gorena T. and Winterhalter P., 2012. Stilbene levels in grape cane of different cultivars in southern Chile: determination by HPLC-DAD-MS/MS method. *Journal of Agricultural and Food Chemistry* **60**, 929-933. doi:10.1021/jf204482c

Vincenzi S., Tomasi D., Gaiotti F., Lovat L., Giacosa S., Torchio F., Segade S.R. and Rolle L., 2013. Comparative study of the resveratrol content of twenty-one Italian red grape varieties. *South African Journal of Enology and Viticulture* **34**, 30-35.

Waffo-Teguo P., Krisa S., Richard T. and Mérillon J. M., 2008. Grapevine stilbenes and their biological effects. *In: Bioactive molecules and medicinal plants*, pp 25-54. Ramawat K.G., Mérillon J.-M. (eds.), Springer, Berlin, Germany. doi:10.1007/978-3-540-74603-4_2

Wang W., Tang K., Yang H.-R., Wen P.-F., Zhang P., Wang H.-L. and Huang W.-D., 2010. Distribution of resveratrol and stilbene synthase in young grape plants (*Vitis vinifera* L. cv. Cabernet Sauvignon) and the effect of UV-C on its accumulation. *Plant Physiology and Biochemistry* **48**, 142-152. doi:10.1016/j.plaphy.2009.12.002

Zghonda N., Yoshida S. Araki M., Kusunoki M., Mliki A., Ghorbel A. and Miyazaki H., 2011. Greater effectiveness of ε-viniferin in red wine than its monomer resveratrol for inhibiting vascular smooth muscle cell proliferation and migration. *Bioscience, Biotechnology, and Biochemistry* **75**, 1259-1267. doi:10.1271/bbb.110022

Zhang A., Fang Y., Li X., Meng J., Wang H., Li H., Zhang Z. and Guo Z., 2011. Occurrence and estimation of trans-resveratrol in one-year-old canes from seven major Chinese grape producing regions. *Molecules* **16**, 2846-2861. doi:10.3390/molecules16042846

©Université de Bordeaux (Bordeaux, France)