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1 White fungal covering on the wall paintings of the Saint-Savin-sur-Gartempe
2 Abbey church crypt: a case study

3

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16

17 Abstract

18 The crypt of Saint-Savin-sur-Gartempe Abbey church contains a notable example of
19 Romanesque wall paintings. A recurrent white fungal covering has affected these wall paintings
20 since the late 1970s. Its composition and changes were studied from 2005 to 2015 through
21 microbial isolations, ATP activity measurements and antimicrobial susceptibility testing. ATP
22 monitoring showed that the fungal activity level remained high throughout the ten years, with
23 spatial and temporal variations over time. *Engyodontium album* was the main agent isolated
24 from the fungal covering, showing change in fungal strains compared to those isolated before
25 2005. This change in the fungal community could result from the previous application of
26 econazole nitrate as a biocidal product. However, multiple susceptibility tests showed a possible
27 variation of active ingredients that could lead to the successful elimination of the fungal
28 contamination. Alternative solutions for the preservation of the wall paintings against fungal
29 covering are discussed.

30

31 Keywords

32 Cultural heritage, Fungal contamination, Wall painting, ATP activity, Biocidal products,
33 *Engyodontium album*

34

35

36

37 1. Introduction

38 The role played by microorganisms in the deterioration of cultural heritage has been recognized
39 for some time now (Sterflinger and Piñar, 2013). Microorganisms such as bacteria, fungi,
40 cyanobacteria and algae can be involved in the degradations of wall paintings (Ciferri, 1999),
41 resulting in material losses and/or aesthetical deteriorations through the discoloration of the
42 paintings. This discoloration can be caused by the production of colour pigments by the
43 microorganisms, most frequently green, pink or black (Imperi et al., 2007; Martin-Sanchez et
44 al., 2011; Nugari et al., 2009), or by the alteration of the painting pigments by microorganisms
45 (Abdel-Haliem et al., 2013; Giustetto et al., 2015). Further damage can be caused by the
46 penetration of fungal hyphae in the microstructure of paintings, leading the wall surface to
47 crumble and resulting in material losses (Rosado et al., 2013). The microorganisms use the wall
48 paintings as a substrate (Garg et al., 1995), and condensation moisture forming on the surface
49 of wall paintings provides ample water for their growth. Moreover, microorganisms can also
50 use the organic substances present in the binding medium of paints, the restoration adjunction
51 materials, the plaster and even the dust layer covering the paintings surface as nutrients
52 (Karpovich-Tate and Rebrikova, 1991).

53 This study focuses on wall paintings that are affected by fungal contamination at Saint-Savin-
54 sur-Gartempe Abbey church. The abbey is located in the Western France (N46 33 52.992 E0
55 51 57.996), and was designated as a World Heritage Site by UNESCO in 1983. It is a notable
56 demonstration of eleventh and twelfth century Romanesque art, and was in all probability
57 founded at the beginning of the ninth century (Deschamps, 1949). The abbey church that we
58 know today was rebuilt during the eleventh century. Each part of the church, i.e., porch, nave,
59 gallery, shrine and crypt, is decorated with remarkable wall paintings. The paintings on the
60 crypt vault are dedicated to the martyrs Saint Savin and Saint Cyprien (Fig. 1). These paintings
61 are the topic of our paper.

62 The crypt is underground and is located 40 m from the water-course of the Gartempe river, with
63 the floor of the crypt located 1.80 m above the average water level of the river. Originally, the
64 entire crypt was painted. Successive floods over time have removed the paintings from floor
65 level to a height of 1.5 m (Stefanaggi et al., 1986). The crypt walls are composed of ashlar and
66 hearting. They are covered with two plasters composed of sand and lime (50:50): *arricio* and
67 *intonaco* (Brochard, 1998). The sand found in the *arricio* plaster is coarse, yellow in colour,
68 and mixed with small-sized gravel ranging from reddish-coloured to black. *Intonaco* contains
69 sand that is also yellow, but is thinner and highly micaceous. Both sands are slightly
70 argillaceous. The wall paintings were created as a *fresco*: the pigments are mixed with water
71 then applied on the freshly applied *intonaco*, where they bind with the plaster and become part
72 of the paint layer. At least two different white pigments, several ochres (yellow and red), wood-
73 ash black, green earths and traces of lapis-lazuli have been used for the paintings.

74 The first report of a white covering (Fig. 2) in the crypt, and particularly on wall paintings, was
75 recorded in the archives of the *Laboratoire de Recherche des Monuments Historiques* in 1979,
76 when the issue was mentioned in a letter following a control visit to the abbey church by the
77 National Department of Cultural Heritage (Inspection des Monuments Historiques). The letter
78 explained that the priest responsible for the church had noted the presence of this white covering
79 a few years previously, after the very long drought of 1976, and explained that the affected area
80 had increased in size over the years. This means that the problem has been known about for at
81 least the last forty years and indeed much longer, since the regional archives periodically
82 mention the presence of mold in the crypt from 1943 onwards. The crypt was the only part of
83 the church that was regularly affected by the development of fungi. In 1979, the covering was

84 first thought to be of chemical origin, but it soon became obvious that a large part of the
85 covering was of microbial nature, specifically molds (microscopic fungi). Several campaigns
86 of sampling and cleaning were consequently carried out from 1980 to 2000, but the covering
87 always returned after periods of varying lengths (Brochard, 1998). Several different fungal
88 genera were identified during these former campaigns, including *Penicillium*, *Aspergillus*,
89 *Doratomyces*, *Fusarium*, *Acremonium* and most recently *Beauveria*. All of these fungal species
90 have previously been recorded on wall paintings (Berner et al., 1997; Gorbushina and Petersen,
91 2000; Sugiyama et al., 2009). Initial attempts to remove the white cover from the wall paintings
92 by cleaning with moist compresses and dry gum failed, with the fungi rapidly reappearing. The
93 susceptibility of isolated strains against biocide products was then tested and econazole nitrate
94 (Firooz et al., 2015) was used to treat the paintings in 1987 and again at the end of 1999. The
95 reoccurrence of the white fungal covering was reported in 2005.

96 This paper studies the characteristics of the white fungal covering since its last reappearance in
97 2005, through: i) the identification of the microorganisms responsible for this phenomenon; ii)
98 the monitoring of its spatial and temporal evolution, and iii) the susceptibility testing of these
99 microorganisms to four commonly used biocide products. Microbiological samplings were
100 carried out to identify involved microorganisms, then the evolution of the white covering was
101 monitored over time using adenosine triphosphate (ATP) measurements. ATP is the primary
102 energy carrier for all living organisms. It is in this form that cells expend the energy acquired
103 for all energy-dependent processes including biosynthesis, mobility and various maintenance
104 functions (Shama and Malik, 2012). The presence of ATP on the substrate therefore indicates
105 the presence of living organisms. Measurements are based on the reaction of luciferin-luciferase
106 bioluminescent in the presence of ATP. The intensity of the bioluminescence is proportional to
107 the ATP content. This method is routinely used in domains such as food processing. It is not
108 commonly used in the domain of cultural heritage (Rakotonirainy and Arnold, 2008; Ranalli et
109 al., 2005; Roig et al., 2013), despite its ability to provide immediate results for monument
110 preservation teams (Ranalli et al., 2003). Finally, the susceptibility of isolated strains to several
111 biocide products was tested *in vitro* on Petri dishes.

112

113 2. Material and Methods

114 2.1 Sampling

115 The study was performed between September 2005 and November 2015. Classic
116 microbiological sampling was carried out at the beginning and at the end of the study in order
117 to check that the same microorganisms were involved in the development of the white fungal
118 covering throughout the study. ATP monitoring was performed in April 2008, July 2012 and
119 November 2015.

120

121 2.2 Isolation, cultivation and identification of microbial strains

122 Samples were taken from locations across the entire vault surface. Twenty-one different spots
123 were tested in 2005. Six more were tested in 2015. Each sample was taken with a sterile swab
124 then inoculated in Petri dishes (diameter = 8 cm) containing sterilized Mal Extract Agar medium
125 (Thorn and Raper, 1945) at 25 °C for seven days. If needed, strains were sub-cultured on the
126 same medium and separated. The isolated strains were identified by cross-checking their

127 macroscopic and microscopic characteristics with those listed in reference books (Domsch et
128 al., 1980; Seifert et al., 2011).

129 Morphological identifications were compared to molecular identifications. DNA was extracted
130 as described by Edel et al. (2001). ITS1F/ITS4 primers (Gardes and Bruns, 1993; White et al.,
131 1990) were used to amplify the nuclear ribosomal internal transcribed spacer (ITS) region. PCR
132 was performed in 25 µl reactions, with 1 µl of template DNA, 1 U of Taq DNA polymerase
133 (Invitrogen, Carlsbad, USA), 2.5 µl of 10X Taq DNA polymerase buffer, 1 µl of 2 mmol.l⁻¹
134 dNTPs (Thermo scientific, Waltham, USA) and 1.5 µl of each 10 µmol.l⁻¹ primer (Eurogentec,
135 Seraing, Belgium). Amplifications were performed on a PrimeG thermocycler (Bibby
136 Scientific, Stone, UK) with the following parameters: a 4 min step at 94 °C, followed by 30
137 cycles at 94 °C for 30 s, at 55 °C for 30 s, and at 72 °C for 40 s, and a final 10 min extension at
138 72 °C. Isolates were identified by BLAST comparison to the NCBI GenBank database (Altschul
139 et al., 1990).

140

141 2.3 ATP assay

142 Samples were taken from points across the entire vault surface. Sampling points remain visible
143 for years, making it necessary to take subsequent samples from new areas. Twenty-seven
144 locations were tested in 2008. Thirty-three more points were tested in 2012 and 2015. As the
145 white veil covered almost the whole of the vault surface, control samples were taken from three
146 small, previously cleaned areas. ATP samples were taken with sterile swab from 10 cm² surface
147 areas. The procedure was carried out according to the manufacturer's instructions. The
148 bioluminescence was measured with HY-LITE®2 instrument (Merck KGaA, Darmstadt,
149 Germany). The result is expressed in Relative Light Unit (RLU). The manufacturer's guidelines
150 consider a sample to be highly contaminated when the result is three times that of the control
151 sample.

152

153 2.4 *In vitro* antimicrobial susceptibility testing

154 The susceptibility of isolated strains to four different biocide products (Table 1) was tested by
155 disk diffusion method on Malt Extract Agar medium. The first biocide contained econazole
156 nitrate (Sigma Aldrich, Saint-Louis, USA), when the three others were commercial products
157 combining quaternary ammonium (Tezel and Pavlostathis, 2015) and isothiazolinone (Gaylarde
158 et al., 2011). The plates (144 cm²) were inoculated with a spore suspension of the tested strain,
159 then cellulose discs (diameter = 6 mm, BioMerieux, Marcy-l'Étoile, France) soaked in biocide
160 were laid on the plates and incubated at 25 °C for seven days. The diameter of the inhibition
161 zones excluding the disk was measured in millimeters. Duplicate tests were carried out for all
162 biocide products.

163

164 2.5 Statistical analysis

165 The results of ATP assays and susceptibility tests were analyzed using analysis of variance
166 (ANOVA). This statistical test checks if the means of several samples are equal or not (Cohen,
167 1977), and is useful to compare three or more means. This test is practical when comparing the
168 effect of one or more qualitative variables on a quantitative response variable.

169 Model 1: ATP assays.

170 The wall paintings were divided into two parts from the north wall to the south wall and into
171 three parts from the west wall to the east wall in order to check the spatial heterogeneity of the
172 fungal development (Fig. 3). The temporal heterogeneity was also tested, i.e. the spatial North-
173 South position of the assay, the spatial West-East position of the assay and the year of testing
174 were used as qualitative independent variables. The quantitative variable was the measured
175 quantity of ATP. Data were power transformed to ensure normality and variance equality. Only
176 second level analysis was carried out.

$$177 \text{ATP assay}_{ijk} = \mu + \text{Year}_i + \text{North-South position}_j + \text{West-East position}_k + \text{Year*North-South} \\ 178 \text{position}_{ij} + \text{Year*West-East position}_{ik} + \text{North-South position*West-East position}_{jk} + \varepsilon_{ijk} \quad (1)$$

179 where μ is a constant, *Year* is the effect of the year of the assay ($i = 1, 2, 3$), *North-South position*
180 is the effect of the North-South position of the assay ($j = 1, 2$), *West-East* is the effect of the
181 West-East position of the assay ($k = 1, 2, 3$), *Year*North-South position*, *Year*West-East*
182 *position*, *North-South position*West-East position* are the interactions effects and ε is the
183 residual error.

184

185 Model 2: Effect of the biocide products on the isolated species.

186 For this test, the individual fungal species and type of biocide product tested were used as
187 qualitative independent variables. The size of the inhibition halo was the quantitative variable.

188

$$189 \text{Inhibition halo size} = \mu + \text{Fungal species}_i + \text{Biocide product}_j + \text{Fungal species*Biocide} \\ 190 \text{product}_{ij} + \varepsilon_{ijk} \quad (2)$$

191 where μ is a constant, *Fungal species* is the effect of the different isolated species ($i = 1, 2, 3$),
192 *Biocide product* is the effect of the different tested biocide products ($j = 1, \dots, 4$), *Fungal*
193 *species*Biocide product* is the interaction effect and ε is the residual error.

194 When significant, the ANOVA tests were completed by *post hoc* analysis to rank the different
195 means. Thus, the significance of differences between means was assessed using the Newman-
196 Keuls method (Keuls, 1952). The ANOVA models and the Newman-Keuls tests were carried
197 out using R with $\alpha = 5\%$.

198

199 3. Results and Discussion

200 3.1 Identification of the species responsible for the white fungal covering

201 In 2005, one sample did not produce any microbial growth, while the twenty others permitted
202 the isolation of just one fungal strain. Examination of its morphological features led to the
203 identification of *Engyodontium sp.* (Fig. 3), and subsequent molecular identification showed it
204 to be *Engyodontium album* (Genbank accession number KX821728). In 2015, one sample did
205 not produce any microbial growth. One fungal strain, namely *E. album*, was isolated on four
206 samples. On the remaining sample, *E. album* was isolated as well as two more fungal strains
207 that were morphologically related to *Verticillium spp.* but which were matched with undefined
208 Hypocreales species by molecular identification. We named these strains Hypocreales sp. 2

209 (Genbank accession number KX821729) and Hypocreales sp. 3 (Genbank accession number
210 KX821730). The presence of the white fungal covering can therefore be mainly attributed to *E.*
211 *album*. None of the studies of these wall paintings prior to 2005 recorded the presence of this
212 species. The strains isolated at the time were not conserved, but the only one among them which
213 could be morphologically mistaken for *E. album* was *Beauveria* sp. Although both genera are
214 characterized by conidial sympodial growth (Seifert et al., 2011), *Engyodontium* produces
215 unbranched or verticillate conidiophore while *Beauveria* produces branched, penicillate or
216 trichodermoid conidiophores. Consequently, it can be assumed that these species were not
217 confused and that the main agent responsible for the development of the white fungal covering
218 has changed between the former studies and the present study.

219 The development of *E. album* on wall paintings has already been recorded (Berner et al., 1997;
220 Saarela et al., 2004; Sáiz-Jiménez and Samson, 1981). More generally, *E. album* seems grow
221 well in locations with damp conditions and cool but stable temperatures such as churches,
222 catacombs, crypts and even caves (Bastian et al., 2010). This species is known as an
223 entomogenous fungus (Humber et al., 2005). Arthropods play a role in their dissemination by
224 transporting the spores on their bodies and in fecal pellets. They are also a nutrient source for
225 entomogenous fungi, which feed on dead arthropods (Jurado et al., 2008). The occurrence and
226 the extent of such dissemination mechanism remain to be tested in the Saint-Savin-sur-
227 Gartempe crypt.

228

229 3.2 Development of the white fungal covering over time, monitored through ATP assay

230 Microbial contamination on the crypt vault wall painting at Saint-Savin-sur-Gartempe Abbey
231 church appeared to be high for all the locations and years considered. Control sample values
232 ranged from 22 RLU (± 3) in 2012 to 3600 RLU (± 458) in 2015 (Table 2). The highest and
233 lowest fungal sample values were recorded in 2012, ranging from 17 RLU (± 2) to 86400 RLU
234 (± 12779). The sample medians were 31600 RLU in 2008, 9414 RLU in 2012 and 19333 RLU
235 in 2015. Examples of tolerable threshold values are given by the ATP meter manufacturer.
236 Although these examples relate to the food processing industry, for which this instrument was
237 originally designed, they nevertheless provide a reliable idea of the contamination level in the
238 crypt. 1500 RLU is considered to be a high value in a vegetable processing environment, which
239 is considered to be the least sensitive to microbial activity. This value is much lower than the
240 values recorded in this study. However, similar ATP levels to those recorded on wall paintings
241 have already been measured with the same instrument, for example in liquid samples of air
242 humidifier water tanks (Liebers et al., 2014) or on wooden vegetable crates (Lehto et al., 2011).
243 The ATP meter manufacturer advises users to class a measurement as highly contaminated
244 when the value is three times that of the control sample. According to this guideline, 100 % of
245 the samples were highly contaminated in 2008, 94 % in 2012 and 73 % in 2015. The relatively
246 lower level of contamination in 2015 in comparison with 2008 and 2012 may be explained by
247 a slight contamination of the control sample, for which the ATP value was relatively high (3600
248 RLU); although the 2015 control point had been cleaned during the preceding monitoring
249 campaign (2012) and appeared to be free of contamination, it may have been re-colonized
250 between the two sampling periods.

251 Figure 4 shows a map of the ATP values for samples taken from the vault during the three years
252 of monitoring. The eastern side of the paintings appears to be less contaminated than the western
253 side and the central section. This spatial heterogeneity in fungal growth, as well as temporal
254 heterogeneity, were statistically supported by ANOVA results, which showed a significant

255 effect of the year and of the west/east spatial positioning on the model, while north/south spatial
256 positioning and interactions had no effect (Table 3). Average ATP values were significantly
257 higher on the central part of the painting than on the western side, and were higher on the
258 western side than on the eastern side (Table 4). Furthermore, average ATP values were
259 significantly higher in 2008 and 2015 than in 2012. Thus, nine years after the last cleaning by
260 biocide products (1999), the white covering had returned to the same maximum level and did
261 not show any real decrease over the next seven years, although some temporal variations
262 occurred. Renewed microbial colonization after cleaning has already been observed (Martin-
263 Sanchez et al., 2012). Furthermore, the survival of microbial covering at a high activity level
264 over several years involved particularly favorable conditions in the crypt, both in terms of the
265 atmosphere and nutrient availability. The relative humidity level is a particularly critical factor
266 in microbial contaminations. A period of several weeks with a relative humidity of 70 % is
267 enough to cause fungal contamination (Garg et al., 1995; Sterflinger, 2010). Two short climatic
268 condition studies of the crypt, from April to June 2012 and from March to May 2013, showed
269 that relative humidity is in general above 90 %, which creates a continually favorable
270 environment for microbial development (Merckx, 2013). The available nutrients on wall
271 paintings have not been tested, but several possible sources are known. As the wall painting is
272 a *fresco*, pigments are embedded in a superficial carbonated layer and therefore the nutrients
273 cannot be supplied by the potential presence of organic binders without the modification of this
274 substrate. However, organic matter such as pollen, carbonaceous particles, products used in
275 previous restoration and insect fecal pellets carried by wind, moisture or capillary spread can
276 also be nutrient sources (Giustetto et al., 2015). Finally, saprotrophic microorganisms can also
277 feed on the dead cells of autotrophic colonizers (Ciferri, 1999).

278 The results proved that the presence of white fungal covering was not uniform across time and
279 space. These variations could correspond to seasonal climatic variations, since measurements
280 were taken in April and November in 2008 and 2015 and in July in 2012 (D'Agostino, 2013).
281 Spatial microclimatic variations and spatial differences in nutrient availability in the crypt could
282 also explain the differences observed (Cataldo et al., 2009). Stefanaggi and Callède
283 (unpublished) used the contact resistivity method to measure the moisture at the surface of the
284 painted layer every two months in 1980, at the beginning of the white veil study. Their results
285 clearly proved the existence of a spatial and temporal microclimatic heterogeneity (Fig. 5). This
286 heterogeneity should still exist today, as the conservation conditions have not changed.

287

288 3.3 Efficiency of biocide products against targeted strains and possible solutions for the non-
289 chemical treatment of this contamination

290 Isolated *Hypocreales* sp. 2 grew too slowly on solid medium to be tested for its susceptibility
291 to biocide products, unlike *E. album* (Fig. 6) and *Hypocreales* sp. 3. The tested species, the
292 biocide product and the interaction between these two factors all had significant effects on the
293 inhibition halo size (Table 5). These results were expected, as different fungal species vary in
294 their susceptibility to any given active ingredient (Sandle et al., 2014). For this reason, at least
295 three or four different products were tested in this study.

296 Biocide products were more effective against *Hypocreales* sp. 3 than against *E. album*. While
297 almost no inhibition halo was obtained for the use of the econazole nitrate-based product against
298 *E. album* (Table. 6), the three products combining quaternary ammonium and isothiazolinone
299 were effective against this strain. This information is essential because econazole nitrate was

300 used on the crypt wall paintings in the past. Econazole salts are widely used for human health
301 requirements (Firooz et al., 2015), but are also used in the field of cultural heritage (Sequeira et
302 al., 2012). They have the advantage of drying fast due to their preparation in alcoholic solution.
303 Econazole nitrate was found to be effective when tested against strains isolated in the crypt until
304 1999. Since 2005, econazole nitrate has been an ineffective means to eliminate the new isolated
305 species.

306 Biocide product N°2, based on didecyldimethylammonium chloride and a strong concentration
307 of ochtilinone, was the most effective against *E. album*. The two other products, based on the
308 same combination of active ingredients, were ranked according to their concentrations. Biocide
309 product N°2 was also the most effective treatment against *Hypocreales* sp. 3. The three other
310 products were ranked from most efficient to least efficient, as follows: biocide product N°4,
311 econazole nitrate, and biocide product N°3. The biocide product based on
312 didecyldimethylammonium chloride, which was effective against all isolated fungal species,
313 could therefore be used to treat the white covering. This may be a necessary alternative to the
314 previously used product, which is now inefficient. In general, a rotation of different active
315 ingredients can prevent the appearance of microbial resistances (Langsrud and Sundheim, 1997;
316 Langsrud et al., 2003). The scenario of a naturally resistant population developing through
317 biocide pressure whilst the sensitive population disappears has already been observed in the
318 field of cultural heritage (Martin-Sanchez et al., 2012).

319 The chemical cleaning method was chosen in this study because it had already been used in the
320 past to clean the wall paintings of the crypt. Indeed, the use of liquid biocide products remains
321 the main remedial method generally used by the restorers. This method removes fungal
322 coverings with minimum damage to the paintings (Sterflinger, 2010; Stupar et al., 2014).
323 Physical methods such as irradiation by rays required specific equipment and are not technically
324 suitable to clean wall paintings on a vault, whilst the use of other chemical methods, such as
325 fumigation, is highly toxic (Fernandes, 2006). This does not mean, however, that the use of
326 biocide products should be considered as a long-term solution for the preservation of wall
327 paintings in the crypt. The white fungal covering has always returned to the crypt walls after
328 previous treatments because of the particularly favorable climatic conditions for fungal growth.
329 Moreover, current legislation encourages a reduction in the use of biocide products due to health
330 and environmental concerns (The European Parliament and the Council of the European Union,
331 2012). Further studies should therefore focus on preventing fungal growth. The most long-
332 lasting solution for the crypt lies in controlling its climatic conditions. The combination of high
333 relative humidity and poor ventilation is the key factor in fungal growth (Cataldo et al., 2005).
334 However, it seems unlikely that the control of climatic conditions will suffice to preserve wall
335 paintings from fungal contamination: technically speaking, the necessary decrease in relative
336 humidity from around 90 % to 60 % is practically attainable in the crypt. Moreover, a strong
337 and rapid decrease in humidity could cause salt precipitation, which is as damaging to wall
338 paintings as fungal growth (Iordanidis et al., 2011; Sawdy and Price, 2005). Computational
339 fluid dynamics (CFD) tools are increasingly used in the field of cultural heritage to study
340 climatic preservation conditions (Albero et al., 2004; Balocco and Grazzini, 2009). In
341 particular, CFD has been used to evaluate several scenarios for the preservation of the crypt of
342 Lecce Cathedral (D'Agostino and Congedo, 2014; D' Agostino et al., 2013). These tools could
343 be useful in the case of Saint-Savin-sur-Gartempe Abbey church crypt. Finally, since fungal
344 strains recovered from the crypt wall paintings are entomogenous species, it could be interesting
345 to determine whether some arthropod species play a role in fungal dissemination in the present
346 case. Several authors have already suggested focusing on these species control as a means to
347 control fungal dissemination (Jurado et al., 2008; Trovão et al., 2013). The presence of

348 arthropod species in the crypt has not been verified to date. The first step would be to trap
349 individuals with the use of sticky blunder traps, bait traps or UV traps (Querner, 2015), then
350 check for the presence of fungal species on their bodies.

351

352 5. Conclusions

353 The use of ATP measurements to monitor the white fungal cover on the wall paintings of Saint-
354 Savin-sur-Gartempe Abbey church crypt showed that this fungal cover retained a high activity
355 level during the ten-year experimental period. Spatial and temporal variations in fungal activity
356 occurred on the shelf during the study. The comparison of ATP measurements with a previous
357 climatic study showed that these variations could be linked with variations of moisture at the
358 surface of wall paintings. The entomogenous species *Engyodontium album* was identified as
359 the main agent of the fungal covering, indicating a different fungal species compared to the
360 species that were isolated during former studies in the crypt. The biocide product used to treat
361 previous fungal coverings was ineffective against *E. album*. Although alternative biocide
362 products were effective against *E. album*, a more long-term solution needs to be found in the
363 future to avoid fungal development on the wall paintings. Our next investigations will focus on
364 the management of climatic conditions in the crypt and on the possible involvement of certain
365 arthropod species in the dissemination of fungal strains, which could facilitate the control of
366 fungal development on wall paintings.

367

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557 Table 1: Active ingredients of the four biocidal products tested

Biocide number	Active ingredient	Concentration (%)
1	Econazole nitrate (CAS 68797-31-9)	0.2
2	Didecyldimethylammonium chloride (CAS 7173-51-5) Ochtilinone (CAS 26530-20-1)	< 1.2 < 0.3
3	Benzalkonium chloride (CAS 63449-41-2) Ochtilinone (CAS 26530-20-1)	< 1.65 < 0.02
4	Benzalkonium chloride (CAS 63449-41-2) Ochtilinone (CAS 26530-20-1)	<2.5 <0.05

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559 Table 2: ATP values in RLU measured for each location and each sampling date

Location of the measure	Year of the measure		
	2008	2012	2015
Control	1 118 (13)	22 (3)	3 600 (458)
1	42 800 (3 347)	79 400 (5 413)	34 750 (1 708)
2	31 600 (894)	469 (178)	31 667 (1 155)
3	49 800 (2 168)	10 500 (577)	15 667 (577)
4	35 400 (1 140)	33 200 (1 789)	33 500 (2 082)
5	42 600 (2 302)	86 400 (12 779)	47 333 (1 528)
6	48 800 (2 387)	65 200 (1 643)	73 200 (7 050)
7	8 040 (371)	17 (2)	41 667 (1 528)
8	56 600 (1 949)	23 600 (548)	17 750 (957)
9	38 880 (2 280)	53 200 (2 683)	29 000 (3 559)
10	8 580 (409)	9 414 (1 277)	3 600 (458)
11	18 200 (837)	16 067 (14 052)	7 167 (58)
12	5 740 (230)	30 (3)	7 533 (404)
13	10 080 (576)	6 414 (302)	5 433 (289)
14	3 360 (182)	17 200 (837)	9 067 (306)
15	3 620 (217)	1 533 (175)	13 667 (1 528)
16	20 800 (1 483)	27 833 (983)	34 000 (1 000)
17	42 600 (1 817)	55 560 (1 342)	84 200 (9 149)
18	37 800 (2 683)	3 040 (498)	53 000 (0)
19	10 920 (1 110)	336 (74)	3 467 (666)
20	17 800 (1 095)	1 048 (105)	1 433 (58)
21	9 900 (1 140)	8 667 (532)	9 800 (346)
22	16 600 (1 517)	22 000 (1 414)	29 667 (577)
23	39 200 (1 789)	1 620 (130)	12 667 (577)
24	71 200 (5 070)	1 520 (295)	11 000 (0)
25	36 200 (1 643)	8 080 (1 474)	19 333 (577)
26	28 400 (894)	2 767 (579)	20 000 (1 732)
27	74 200 (2 387)	42 400 (2 966)	32 333 (2 082)
28		13 600 (548)	9 067 (551)
29		4 343 (879)	12 333 (577)
30		840 (116)	18 333 (577)
31		28 600 (894)	19 333 (2 517)
32		65 000 (6 055)	69 000 (7 000)
33		4 967 (898)	30 500 (6 658)
Median	31 600	9 141	19 333
Mean	29 987 (20 128)	21 058 (25 160)	25 469 (20 649)

560 The values in brackets show the technical standard errors obtained through three consecutive measurements of the
561 same biological sample.

562 Table 3: ANOVA table for ATP monitoring

	Degrees of Freedom	Mean Square	Fisher's F
Year	2	1977	5.02**
Positioning North/South	1	1066	0.02
Positioning East/West	2	3264	16.6***
Year x North/South	2	21	0.11
Year x East/West	4	74	0.38
North/South x East/West	2	73	0.37

563 * *p*-value < 0.05; ** *p*-value < 0.01; *** *p*-value < 0.001

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566 Table 4: ATP averages (RLU) for significant factors

2008	Year 2012	2015	West side	Positioning Central section	East side
29987a	21058b	25469a	27283b	39761a	11543c

567 Different letters in the columns indicate significant differences.

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570 Table 5: ANOVA table for the study of inhibition halo size of the different biocidal products

	Degrees of Freedom	Mean Square	Fisher's F
Species	1	487	688***
Biocidal product	3	785	363***
Repetition	1	4	2
Species*Biocidal product	3	151	70***

571 * *p*-value < 0.05; ** *p*-value < 0.01; *** *p*-value < 0.001

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574 Table 6: Inhibition halo size averages (mm) for the interaction Species*Biocidal product

	Inhibition halo size
Hypocreales sp. 3* Product N°2	59,59a
<i>Engyodontium sp.</i> * Product N°2	47,63b
Hypocreales sp. 3*Product N°4	43,53c
Hypocreales sp. 3*Product N°1	39,63d
Hypocreales sp. 3*Product N°3	34,99e
<i>Engyodontium sp.</i> *Product N°4	28,67f
<i>Engyodontium sp.</i> *Product N°3	22,34g
<i>Engyodontium sp.</i> * Product N°1	1,98h

575 Different letters in the columns indicate significant differences

576 **Blue:** Most efficient product

577 **Red:** Inefficient product

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Figure 1: Crypt vault of Saint-Savin-sur-Gartempe Abbey church.

The picture is taken from the east of the crypt towards the west. The wall paintings on the vault illustrate the martyrs Saint Savin and Saint Cyrien. Photograph: J.P. Bozellec

(online color)

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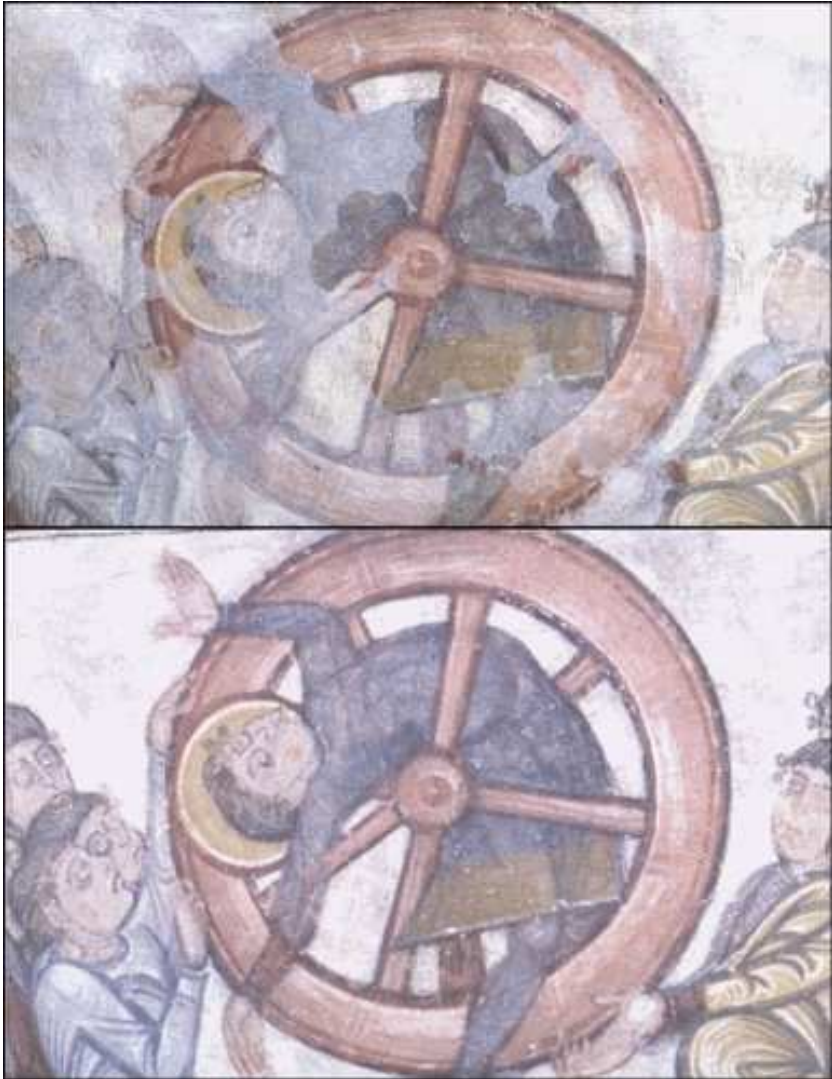
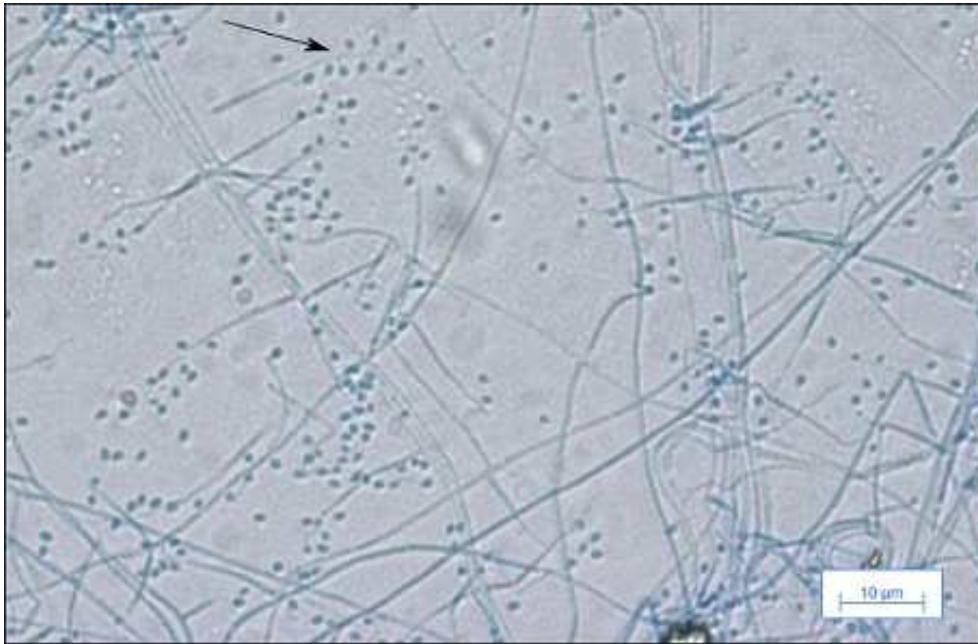


Figure 2: Contamination of "Breaking on the wheel"

The picture illustrates the state of the wall painting before (upper picture) and after (lower picture) the removal of the white fungal covering. Photograph: J.P. Bozellec

(online color)

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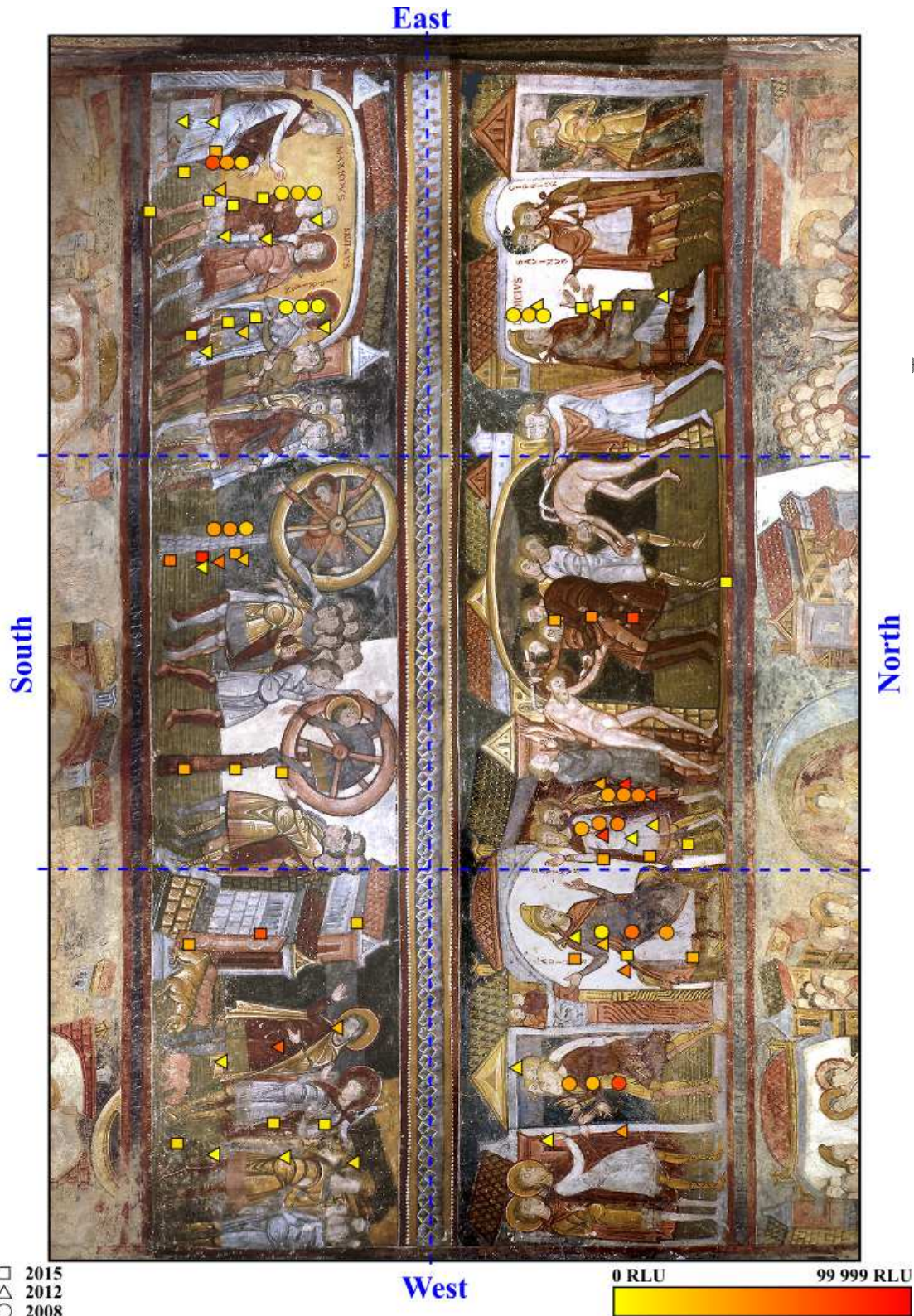
652 Figure 3: Microscopic observation of *Engyodontium album*.

653 The microscopic preparation was carried out using lactophenol blue coloration. The picture was
654 taken with a bright field microscope (x400). The arrow shows the typical elongate
655 conidiogenous cell and the typical sympodial growth of conidia.

656 (online color)

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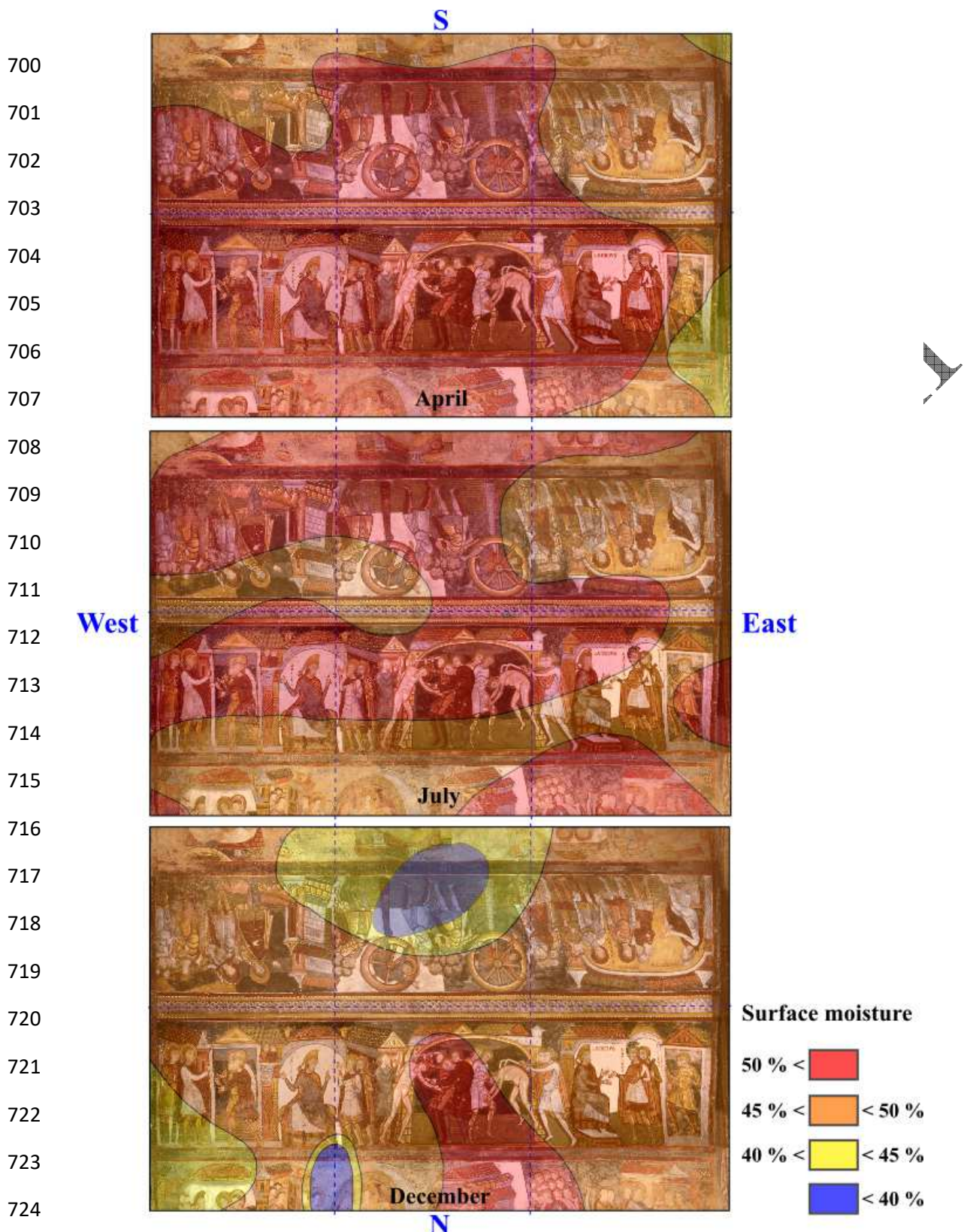


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695 Figure 4: Map of white fungal covering monitoring by ATP assay on vault wall paintings.

696 Each sample point is depicted according to the sampling year (circles for 2008, triangles for
697 2012 and rectangles for 2015) and the level of ATP activity. Yellow points are the least active
698 and red points are the most active. Photograph: D. Bouchardon

699 (online color)



725 Figure 5: Comparison of the surface moisture map at the surface of the wall paintings. Assessed
 726 using the contact resistivity method in April, July and December 1980.

727 Each color corresponds to a level of relative humidity at the surface of the paintings from blue
 728 (the lowest relative humidity) to red (the highest relative humidity). The pictures illustrate the
 729 spatial and temporal changes in relative humidity.

730 (online color)

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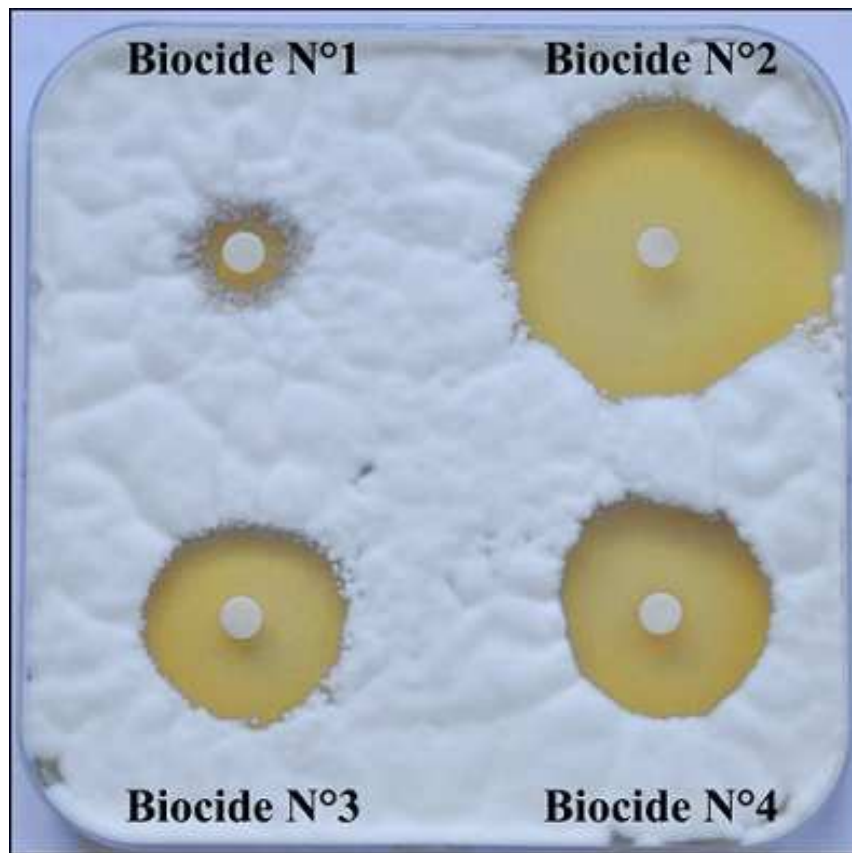
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747 Figure 6: Susceptibility test of *Engyodontium album* to the four selected biocidal products.

748 *Engyodontium album* was inoculated across the entire Petri dish. Cellulosic disks soaked in
749 biocide products were then placed in the dish. After seven days of growth, the inhibition halo
750 around each disk shows the effectiveness of the product against *Engyodontium album*.

751 (online color)

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