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1 **Production and migration of patulin in *Penicillium expansum* molded apples during cold**
2 **and ambient storage**

3

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20 **Running title:** Production and migration of patulin in apples

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Abstract

The ability of three *Penicillium expansum* isolates to produce patulin was first evaluated in YES medium after incubation at 25°C to select a high patulin producer. Then, a spore suspension of the selected *P. expansum* 3.78 strain was inoculated onto the surface of Golden delicious apples and incubated at 8 or 20°C until the mold lesion reached a diameter of 1, 2 or 3 cm. For each lesion size, patulin was quantified from apple samples cut into 1 cm depthwise fractions and widthwise sized cylinders. Maximum patulin concentration, about 80000 ng/g apple, was obtained at 8°C for the center and surface sample of the 3 cm diameter lesion. Patulin was systematically found in highest concentration in the lesions, but still quantified up to one centimeter next to the lesion. Patulin concentrations were not significantly different between 8 and 20°C incubation temperature, except for the 3 cm large lesions. Based on these findings, and for lesions less than or equal to 3 cm in diameter, we recommend to consumers to cut off at least 1 cm around and below the mold spot to limit patulin exposure. Apples should also be stored at cool temperatures, below 8°C, to delay lesion development.

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1. Introduction

44 In the farm to fork approach, the consumer is the final element of the food chain. To
45 ensure food safety, regulations can be enforced at different steps from production to retail.
46 Regarding mycotoxins in food, the European regulation set maximum levels for certain
47 contaminants in foodstuffs for food business operators (Commission regulation (EC) No
48 1881/2006). But at the consumer level, the only action tool is to provide recommendation for
49 good hygiene and practice. These recommendations should be simple and therefore mainly be
50 based on the visual aspect of the moldy food product. This is important as most of the time the
51 consumer will not know what mold species is present, whether it can potentially produce
52 mycotoxins and whether these metabolites are indeed in the food product. Therefore, if
53 mycotoxins can potentially be produced in a mold contaminated food product during storage,
54 especially at the consumer's home, no maximum regulatory levels can be applied but
55 recommendations should be made based on scientific data to limit food waste.

56 Data are limited on the toxicity of **patulin (PAT)** in animals. Signs of acute and chronic
57 intoxication are mainly neurological, but also include weight loss, gastrointestinal disorders
58 and hormonal disturbances. PAT is also cytotoxic, genotoxic and immunotoxic. For the
59 International Agency for Research on Cancer (IARC) there is inadequate evidence for the
60 carcinogenicity of PAT in experimental animals. No evaluation could be made of the
61 carcinogenicity of PAT to humans. PAT is in group 3 (not classifiable as to its carcinogenicity
62 to humans). Based on a no effect level of 43 µg/kg bw/day in a toxicity study in rats and a
63 safety factor of 100, the JECFA (Joint FAO/WHO Expert Committee on Food Additives)
64 derived a provisional maximum tolerable daily intake of 0.4 µg/kg bw (JECFA, 1995).

65 Some fungal species found in food are known to produce mycotoxins and should be
66 considered as biological hazards (Dantigny and Bensoussan, 2013; Pitt, 2013). Related to this,

67 a farm to fork approach was previously used to evaluate strategies to reduce PAT
68 contamination in apple juices using a risk assessment model (Baert et al. 2012). The authors
69 assessed the effects of apple storage conditions on *Penicillium expansum* growth and PAT
70 production as well as apple processing on patulin concentrations found in apple juice. Prior to
71 processing apples for juice production, the European commission N°2003/598/EC
72 recommends that all rotten fruits, even those with only small areas of rot, should be
73 eliminated. To help reduce food losses, it would be useful to only discard the rotten portion of
74 the fruit and any unsafe surrounding areas (i.e. potentially containing mycotoxins). However,
75 this cannot be systematically done in the industry, but trimming recommendations can be
76 provided to the consumer. With this objective in mind, knowledge about the mycotoxin
77 diffusion in molded products is essential (Coton et al. 2019; Rychlik and Schieberle, 2001).
78 Recently, Coton et al. (2019) studied mycotoxin production and migration in a molded cheese
79 model incubated at ambient and refrigerated temperatures and proposed recommendations for
80 consumers to trim the cheese (2 mm) provided only white colored mycelium developed on the
81 surface.

82 In relation to apples, Rychlik and Schieberle (2001) reported that no PAT was detected
83 at a distance greater than 2 cm from the mold infected zone. But the size of the lesion was not
84 stated. In another study, PAT diffusion in apples was assessed for a 20 mm decayed area
85 (Bandoh et al., 2009). However, PAT was not quantified in the surrounding zones around the
86 lesion, but in the apple juices. Recently, PAT migration in Fuji and Golden apples was
87 assessed for 2 and 4 cm lesions but only in depth and not in width (Marín et al., 2006). In
88 these three cited studies, apples were inoculated with a patulin producing *P. expansum* strain
89 and incubated at 20°C. However, the lesion size and PAT production by *P. expansum* on
90 apples also depend on temperature (Baert et al., 2007; Garcia et al., 2011; Welke et al., 2011).
91 In this sense, PAT production by *P. expansum* in apples was also assessed at 1°C and 20°C

92 for 2.5 cm lesions by Morales et al. (2008). Decayed portions, at least 1 cm larger than the
93 lesion, were removed from the apples to quantify PAT, but the mycotoxin was not found in
94 the surrounding tissues.

95 Instead of determining how *P. expansum* develops on apples and its impacts on PAT
96 concentrations in juice and other apple-based products, the objective of the present study was
97 to provide recommendations to consumers concerning moldy apples. Apples were therefore
98 artificially contaminated by a high PAT-producing *P. expansum* strain and stored at 8°C (cold
99 temperature) or 20°C (ambient temperature) until the mold induced lesions reaching 1, 2, or 3
100 cm in diameter. PAT was then quantified within the lesions and in the surrounding tissues
101 both widthwise and depthwise.

102

103

104 **2. Material and methods**

105 *2.1. Isolates and conidial suspensions*

106 Three *P. expansum* strains (UdLTA 3.72, UdLTA 3.78 and UdLTA 3.80) were
107 provided by the University of Lleida (Spain). These strains were isolated from cold stored
108 apples, as previously described by Morales et al. (2007). Glycerol stock cultures were
109 maintained at -80°C.

110 Fungal stock cultures (1 ml) were thawed at ambient temperature for about 1h. Plates
111 that contained Potato Dextrose Agar (PDA, BD Difco, France) were then inoculated with 0.1
112 ml of the stock culture (ca. 1×10^6 conidia.mL⁻¹) and incubated at 25°C for 7d. Fresh conidia
113 were harvested using a sterile loop after flooding the surface of the plates with 2 ml of
114 distilled water containing 0.01% Tween 80 (Sigma Aldrich, Saint Quentin Fallavier, France).
115 After counting conidia using a hemacytometer, conidial suspensions were standardized to

116 1.10⁶ conidia.mL⁻¹. Suspensions were used directly after preparation for the different
117 experiments.

118

119 2.2. *Patulin production on YES medium*

120 Yeast Extract Sucrose (YES) agar medium (Frisvad and Filtenborg, 1983) buffered at
121 pH 4.5 with phosphate-citrate buffer (Chang et al., 1998) was used to evaluate *P. expansum*
122 mycotoxin production *in vitro* as described by Gillot et al. (2017), as this medium is
123 characterized by a high C/N ratio favoring mycotoxin production (Visagie et al., 2014).

124 For PAT determination on agar plates, 1 µL of each spore suspension (1.10⁶ conidia.mL⁻¹)
125 were inoculated in the center of YES plates and incubated for 7 days at 25°C (Coton et al.,
126 2019; Gillot et al., 2017). Each culture was performed in triplicate and, after incubation, the
127 entire contents of the agar plates were stored at -20°C until mycotoxin analyses by Q-TOF
128 LC-MS.

129 Fungal growth was also followed to determine dry weights after 7 days incubation and the
130 obtained values were correlated to the PAT concentrations in YES medium as previously
131 described (Coton et al., 2019; Gillot et al., 2017). Briefly, 1 µL of each spore suspension
132 (1.10⁶ conidia.mL⁻¹) was inoculated in the center of the sterile cellophane disks (Carrefour,
133 France) placed on top of YES agar. Plates were incubated using the same conditions as above.
134 After incubation, cellophane disks containing fungal mycelium were carefully placed into
135 glass Petri dishes and mycelia were removed from the disks by adding sterile distilled water.
136 Mycelia were dried for 48h at 70°C then dry weights were determined. Each culture was
137 performed in triplicate. Results were expressed in ng PAT/g dry mycelium.

138

139 2.3. *Apple inoculation, incubation and sampling*

140

141 In total, 36 “Golden delicious” apples were bought from the local supermarket, and stored
142 at refrigerated temperature until use. Ten μl of freshly prepared conidial suspension (1.10^6
143 conidia. mL^{-1}) were directly inoculated with a sterile pipette cone just under the apple peel.

144 After inoculation, apples were incubated at either 8 or 20°C in hermetically closed plastic
145 containers to maintain water activities at constant values, (c.a 0.99 a_w). For each apple, two
146 perpendicular diameters were measured every day, until 1, 2 and 3 cm diameter lesions were
147 obtained. For each lesion size (in triplicate), 5 cm diameter cylinder plugs, lesions centered,
148 were first removed. The plug was cut into 1 cm sized depths, from D1 (1 cm) to D5 (5 cm),
149 using the device shown on Fig. 1. Then, each 1 cm width disk was cut into 1 cm rings from
150 R1 (1 cm) to R5 (5 cm) as shown on Fig. 1. All samples were stored at -20°C until PAT
151 extraction and quantification.

152

153 2.4. *Patulin extraction from YES medium and apples*

154 For fungal cultures grown on YES medium, PAT was extracted from the homogenized
155 medium containing fungal mycelium using an Ultraturrax T25 digital (IKA, Heidelberg,
156 Germany). Then, PAT was extracted from 4 g aliquots using 25 mL of acetonitrile (ACN)
157 containing 0.1% formic acid (v/v). After adding solvent, samples were vortexed for 30 sec,
158 followed by 15 min sonication, before vortexing again for 30 sec. Samples were centrifuged
159 at 5000 g for 10 min at 4°C. The ACN phase was then collected and stored at -20°C until Q-
160 TOF LC-MS analyses. Before analysis, 1 mL was filtered with a 0.2 μm PTFE membrane
161 syringe 4 mm filter (Phenomenex, Torrance, USA) into an amber vial.

162 For apple samples, PAT was extracted from 2 g homogenized samples using 12.5 mL
163 ethyl acetate. Noteworthy, in a few cases, the cut samples weighed less than 2 g so the volume
164 of solvent was adjusted accordingly. Each sample was then vortexed for 30 sec, sonicated for
165 30 min, again vortexed for 30 sec before centrifuging at 5000 g for 10 min at 4°C. One mL

166 aliquots were filtered through a 0.45 μ m PTFE membrane syringe 4 mm filter (Phenomenex,
167 Torrance, USA) into an amber vial and stored at -20°C until QTOF LC-MS analyses.

168

169 2.5. *Patulin detection and quantification by Q-TOF LC-MS*

170 An Agilent 6530 Accurate-Mass Quadrupole Time-of-Flight (Q-TOF) LC-MS system
171 (Agilent Technologies, Santa Clara, CA, USA) was used for detection and quantification of
172 patulin. The HPLC system included a Binary pump 1260 and degasser, well plate autosampler
173 set to 10°C and a thermostated column compartment. PAT standard used for quantification
174 was obtained from Sigma-Aldrich (St Louis, MO, USA). The stock solution (5 mg/mL) was
175 prepared in dimethyl sulfoxide (DMSO) and stored at -20°C in amber vials. The compound
176 characteristics for PAT identification and quantification are provided in Table 1.

177 To quantify PAT in the different samples, 2 μ L filtered samples (0.45 μ m PTFE
178 membrane) were injected and separated using a ZORBAX Extend-C18 column (2.1 x 50 mm
179 and 1.8 μ m, 600 bar) maintained at 35°C with the flow rate set to 0.2 mL/min. Mobile phases
180 were as follows: solvent A was LC-MS grade water (Carlo Erba Reagents, France) and
181 solvent B was 100% LC-MS grade ACN. Solvent B was maintained at 10% for the first 4
182 min, followed by a gradient of 10-100% of B for 18 min and 5 min at 100% solvent B.
183 Finally, solvent B was maintained at 100% for 5-min post-time. PAT was ionized in ESI-
184 mode (ESI: Electrospray Ionization) and mass spectrometer conditions were: capillary
185 voltage, 4.5 kV; source temperature, 325°C; nebulizer pressure, 50 psig; drying gas, 12 L/min
186 and ion range, 100 to 1000 m/z. PAT detection and identification was performed using the
187 retention time value \pm 1 min and the corresponding quantifier ion listed in Table 1. Standard
188 (using ACN) and matrix matched (using blank apple extracts) calibration curves were
189 obtained by plotting the peak area against mycotoxin concentrations using a 1/x² weighted
190 linear regression model for concentrations ranging from 100 to 250000 ng/ml (Supplementary

191 Figure 1). For YES samples, PAT concentrations were calculated from the same equation
192 using the ACN calibration curve data, then correlated to the calculated fungal biomass
193 (determined dry weight values). PAT concentrations from apple samples were calculated from
194 the matrix-matched calibration curve data using the equation $y = mx+b$, as determined by
195 weighted ($1/x^2$) linear regression. Specific PAT production was expressed as ng per g for
196 apple samples and ng per g dry weight for YES medium.

197 Method performance and validation parameters including linearity (R^2), limit of
198 detection (LOD), limit of quantification (LOQ), recovery rates (from apples spiked with
199 250000 ng/g PAT followed by mycotoxin extraction using ethyl acetate, performed in
200 duplicate) were also assessed according to the ICH guidelines (ICH Harmonized Tripartite
201 Guideline, 2005) and are presented in Table 1. LOD and LOQ values were determined by
202 multiplying the standard deviation of y-intercepts of regression lines divided by the slope, by
203 3.3 and 10, respectively, for the matrix-matched calibration curve (Table 1). Finally, linear
204 range calculations and PAT concentration determinations were carried out using the Agilent
205 MassHunter Workstation Software (Agilent Technologies, Santa Clara, CA, USA).

206

207 **3. Results**

208

209 *3.1. Screening P. expansum isolates for PAT production*

210 PAT production by 3 isolates, after 7 day growth on YES medium, is shown on Fig.2.
211 *P. expansum* 3.72 produced higher PAT concentrations than *P. expansum* 3.80. In contrast to
212 *P. expansum* 3.78, *P. expansum* 3.72 produced relatively constant PAT levels, (c.a. 1000 ng/g
213 dry weight), for all replicates. For this reason, and also because the mean PAT production was
214 about 20% greater than that of isolate 3.78, *P. expansum* 3.72 was selected to study
215 production and migration of PAT in Golden Delicious apples.

216

217 3.2. Patulin production in apples

218 At 8°C, PAT concentrations were determined for 1, 2 and 3 cm diameter sized lesions
219 induced by *P. expansum* (Fig. 3a) in the different widthwise and depthwise cut apple samples.
220 For a 1 cm diameter lesion, PAT was detected at quantifiable concentrations within the lesion,
221 (ring R1-depth D1). However, around the lesion (ring R2-depth D1) and below the lesion
222 (ring R1-depth D2) no PAT was detected above the LOD. For a 2 cm diameter lesion, the
223 highest PAT concentration (c.a. 40000 ng/g of apple) was obtained at the center of the lesion
224 (R1-D1), then decreased sharply in the following apple fractions sampled around (widthwise)
225 and below (depthwise) the lesion. However, PAT was still quantified in the ring up to 3 cm
226 from the lesion at the surface of the apple (R3-D1). In the second centimeter depth, D2, very
227 low PAT concentrations were found compared to the surface of the apple and in the same ring
228 fractions. Levels were below the LOD for R3-D2. Also, only slight variations in PAT
229 concentration in the second centimeter depth were observed among biological triplicates. PAT
230 was not detected (<LOD) at 3 cm depth (R1-D3). For a 3 cm lesion, maximum PAT
231 concentrations, approximately 80000 ng/g of apple, were obtained in the center of the lesion,
232 R1, then decreased with depth. PAT was still detected up to 3 cm in depth (R1-D3) before
233 levels fell below the LOD. In the second ring, R2, PAT concentrations were lower than in the
234 center fraction, R1. As for R1, PAT concentrations in R2 decreased with depth but no PAT
235 (i.e.<LOD) was detected at 3 cm depth, (R2-D3). In the third ring, R3, and fourth ring, R4,
236 PAT was detected up to 2 cm and 1 cm in depth, respectively. On the other hand, no PAT
237 (<LOD) was detected at 5 cm distance from the center of the lesion (R5).

238 For apples stored at 20°C, for a 1 cm lesion, PAT was detected at low levels up to 2
239 cm in distance from the center of the lesion and also up to 2 cm in depth (Fig. 3b). For a 2 cm
240 lesion, PAT concentrations were again higher inside the lesions, R1-D1 (c.a. 50000 ng/g of

241 apple), than on the outer sections as PAT concentrations sharply decreased with distance.
242 PAT was not detected (<LOD) at 3 cm depth (R1-D3). For a 3 cm lesion, PAT concentrations
243 were not different in the 2 inner rings, R1 and R2, for 1 cm depth. The accumulation of PAT
244 in these inner rings was not observed for apples stored at 8°C. PAT was no longer detected at
245 5 cm from the center of the lesion (R5-D1). For 3 cm lesions, PAT concentrations in D3
246 samples were highly variable and greater at 20°C (c.a. 25000 ng/g and c.a. 15000 ng/g for R1-
247 D3 and R2-D3, respectively) than at 8°C (values close to LOQ for R1-D3 and <LOD for R2-
248 D3).

249 The increase in lesion size induced by *P. expansum* over time for apples stored at 8°C
250 and 20°C is shown in Fig. 4a, and Fig. 4b, respectively. Artificial inoculation efficiently
251 mimicked a spontaneous mold contamination. At 8°C, white mycelium appeared on the
252 surface of the apples by day 10, for a lesion diameter of about 1 cm. A greenish blue color due
253 to conidial production was clearly visible by day 14, for a lesion diameter of about 1.5 cm. At
254 20°C, the visual detection of mycelium and conidia was less obvious. But at day 8, for about a
255 4 cm diameter lesion, green mold was detected. By plotting the rot diameter as a function of
256 time, growth rates were determined from the linear correlations to be 1.58 mm/day and 7.44
257 mm/day at 8 and 20°C, respectively.

258

259 4. Discussion

260

261 The 3 selected *P. expansum* strains (3.72, 3.78, 3.80) were previously studied by Morales
262 et al. (2007, 2008). In our study, the 2 latter strains did not produce different PAT
263 concentrations on YES medium after 7d incubation at 25°C. Similarly, Morales et al. (2008)
264 showed that for a 1.10^6 conidia/ml concentration inoculated on apples stored at 20°C, no
265 significant differences between PAT productions were observed between the two strains.

266 However, PAT concentrations on YES medium (determined in our study), expressed in ng/g
267 dry weight, cannot be directly compared to PAT concentrations expressed as ng/g of apple.

268 At 20°C, the *P. expansum* 3.72 strain produced maximum PAT concentration of about
269 300 ng/g on Golden Delicious apples for a 2.5 cm lesion (Morales et al., 2008). However, in
270 the study, an entire cylinder containing the 2.5 cm lesion as well as a 1 cm diameter
271 surrounding the lesion was sampled regardless of depth. In accordance with Marín et al.
272 (2006), it was clear in our study that PAT concentration was much higher in surface samples
273 (1 cm in depth) than in the deeper samples. For example, at 20°C, for a 2 cm lesion, PAT
274 concentrations reached levels that were much higher than 300 ng/g in the one centimeter inner
275 ring of the lesion (50000 ng/g) and up to 2 cm in depth (15000 ng/g), whereas no PAT was
276 detected at a 3 cm depth. In another study, *P. expansum* strain PE39, produced up to 2000
277 ng/g PAT for a 2.5 cm diameter lesion by using the same sample method than Morales et al.
278 (2008), but the storage temperature was 25°C and the apple cultivar was Fuji (Welke et al.,
279 2011). However, for 2 cm lesions, PAT concentration was shown to reach 8000 ng/g apple in
280 approximately 20 g decayed portions as described by Bando et al. (2009).

281 For apples inoculated with the *P. expansum* 3.78 strain and stored at 20°C, the mean PAT
282 concentrations in the decayed portion (c.a. 2 cm) were 9670 ng/g and 3310 ng/g for Golden
283 and Fuji apples, respectively (Marín et al. 2006). These concentrations were lower than those
284 reported in our study (close to 50000 ng/g). But, in contrast to our study, a 3 cm cylinder was
285 sampled and the studied strains were different. Also, variations in PAT production are often
286 observed among strains as shown by our data. In accordance with Marín et al. (2006), for a 2
287 cm lesion, PAT was not detected at a depth greater than or equal to 2 cm. According to Baert
288 et al. (2007), the shape of the rot in the apple was a cone. In our study, the rot was
289 characterized by a constant ratio of the lesion's surface diameter to depth that was equal to 1.6
290 (Fig. 5). Therefore, the development of the mycelium was observed to be less in depth than on

291 the surface. This could be explained by less oxygen being available in the increasing depths of
292 the apple versus on the surface and possibly to the intrinsic apple factors (sugars, pH,...).

293 PAT accumulation was previously shown to be confined to the decayed area (Bandoh et
294 al., 2009). Marín et al. (2006) reported that about 2-6% of the total PAT concentration
295 migrates into the surroundings tissue, while Rychlik and Schieberle (2001) showed that PAT
296 decreased by 2 log at 1 cm distance from the rotted tissue. In accordance with these results, no
297 PAT was detected (<LOD) in our study at 1 cm away from the lesion. Therefore, up to a 3 cm
298 diameter sized lesion, it is recommended to the consumer to cut off at least 1 cm around and
299 below the mold spot. This recommendation is in accordance with Morales et al. (2008) that
300 did not find any PAT in the surroundings tissues, 1 cm around a 2.5 cm lesion. No experiment
301 was performed for 4 cm diameter sized lesions but, by extrapolation, a 6 cm cone of apple
302 which represents approximately half the apple, should be removed. In this case, it would be
303 preferable that the apple be discarded as unwanted mold associated off-flavors may also be
304 produced such as geosmin (Frisvad et al., 2004).

305 A decrease in PAT concentration in the rotted lesion of apples was observed with
306 decreasing temperature from 24-25°C to 4°C (Mc Callum et al. 2002; Northolt et al., 1978).
307 Later, it was shown that the effect of temperature on PAT production was also strain-
308 dependent (Baert et al., 2007). This effect was very important at temperatures lower than or
309 equal to 4°C. The effect of temperature on PAT production was not significant in the range of
310 8-10°C up to 20°C (Baert et al., 2007; Northolt et al, 1978). Except for D3 samples obtained
311 for 3 cm lesions, no differences in PAT concentrations were found between 8 and 20°C in our
312 study. This was partly due to high standard deviations between biological replicates as can be
313 expected as individual apples were used for each replicate.

314 The increase in lesion size was clearly slower at 8°C than at 20°C. At 20°C, the growth
315 rate of *P. expansum* on apples was 7.44 mm/day. This value was very close to the one (7.16

316 mm/day) reported by Baert et al. (2007). Unfortunately, these authors did not grow *P.*
317 *expansum* on apples at 8°C. In another study, the increase in rot diameter on “Golden
318 delicious” apples at 8 and 20°C were 2.17 and 6.14 mm/day, respectively (Northolt et al.,
319 1978). At 20°C, the calculated growth rate (6.14 mm/day) was below what we found in the
320 present study (7.44 mm/day), but at 8°C, the strain used by these authors showed a faster
321 growth rate (2.17 mm/day) than our isolate (1.58 mm/day). This effect may be explained by
322 the fact that some *P. expansum* isolates grow better at refrigerated temperatures (1°C) than at
323 20°C and *vice versa* (Garcia et al., 2011). At 8°C, *P. expansum* did not produce significant
324 **PAT** concentrations until a green mold color due to conidial production was visible, but this
325 simple correlation was not observed at 20°C. Nevertheless, in order to delay *P. expansum*
326 induced lesions on apples, it can be recommended to store apples at cool or cold temperatures.

327

328 **5. Conclusions**

329

330 Recommendations for consumers should be based on a “worst case scenario” postulate,
331 assuming that consumers cannot distinguish between a mycotoxigenic and non-mycotoxigenic
332 fungal strain. Among isolated molds, *P. expansum* is known to be responsible for blue rot of
333 pome fruits that causes between 70% and 80% of decay in stored fruit (Viñas et al., 1993). *P.*
334 *expansum* is also considered as the major PAT producer in fruits and related products
335 (Morales et al., 2007). Almost all *P. expansum* strains are PAT producers (Andersen et al.,
336 2004), although PAT production varies among strains. A very high PAT producer was
337 selected for this study. In order to increase food safety, the farm to fork approach also
338 includes the consumer. By artificially contaminating apples by a high PAT producing *P.*
339 *expansum* strain, we showed that mycotoxin concentration is related to the extent of the mold
340 induced lesion. The use of predictive mycology, based on considering the biological agent

341 responsible for mycotoxin production (Dantigny and Bensoussan, 2013) should be explored
342 as a useful approach to provide accurate recommendations on how moldy products should be
343 handled by the consumer.

344

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351

352

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421

422 Table 1. Patulin quantification parameters used for Q-TOF LC-MS analyses. RT: retention
423 time, MW: molecular weight, ESI: electrospray ionization, LOD: limit of detection, LOQ:
424 limit of quantification, R²: regression coefficient.

425

Metabolite	Chemical formula	RT (min)	MW (g/mol)	Quantifier ion (<i>m/z</i>)	ESI mode	PAT recovery	LOD (ng/g)	LOQ (ng/g)	R ²
Patulin	C ₇ H ₆ O ₄	0.8	154.027	153.019	-	90.6%	293	887	0.99

426

427

428

Figure captions

Figure 1. Sampling design to obtain 1 to 5cm width apple rings and 1 to 5cm depth apple slices for PAT quantification from *Penicillium expansum* contaminated apples.

Figure 2. Patulin production by 3 *Penicillium expansum* isolates (strains 3.72, 3.78 and 3.80) after 7 days growth on YES medium at 25°C. Results are expressed in ng of patulin per g of dry mycelial weight.

Figure 3. Patulin quantification determined for different sized lesions by *Penicillium expansum* 3.78 on Golden Delicious apples after incubation at 8°C (a) and 20°C (b). Lesion sizes are given in cm; R1 to R5: increasing circle sizes; D1 to D3: increasing depths. Results are expressed in ng of patulin per g of apple and correspond to average values of 3 biological replicates.

Figure 4. Evolution of *Penicillium expansum* lesions on 70 mm diameter Golden Delicious apples during incubation at 8°C from day 2 to 25 (A) and at 20°C from day 2 to 9 (B).

Figure 5. Relationship between surface diameter and depth of the blue rot on apples due to *Penicillium expansum* growth.

Figure 1.

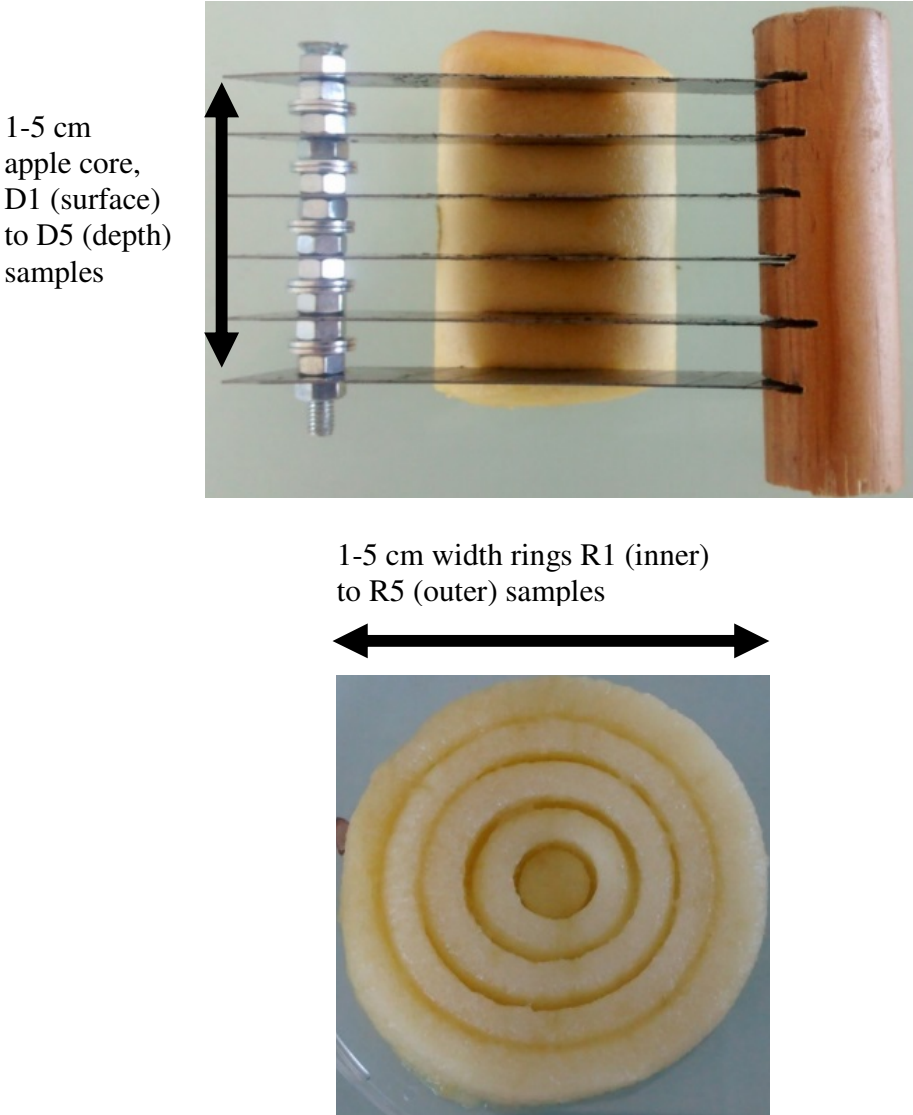


Figure 2.

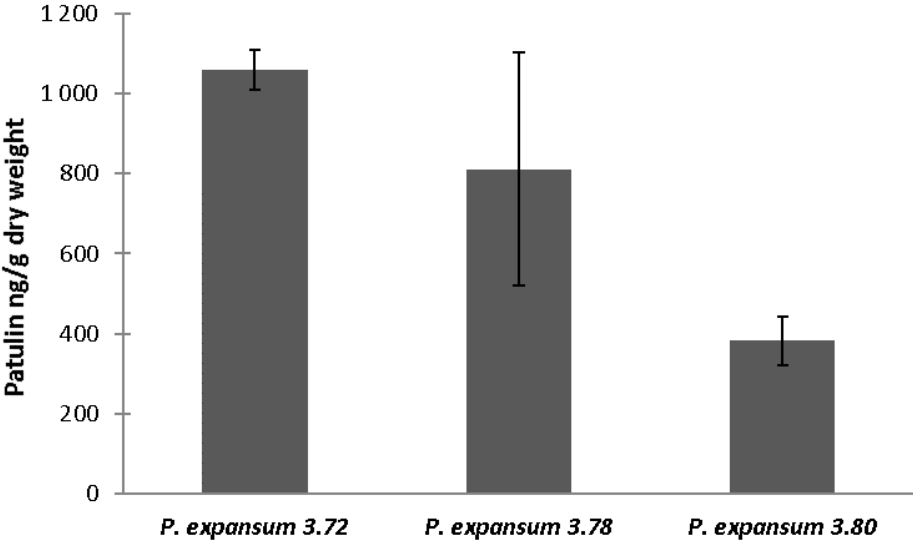
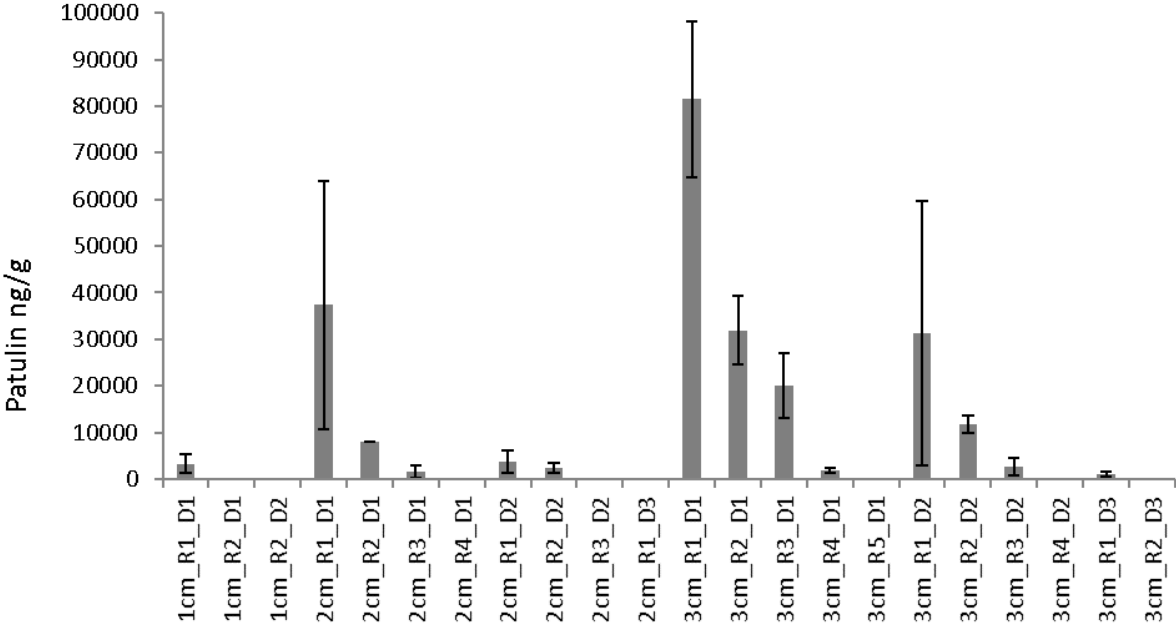


Figure 3.

A



B

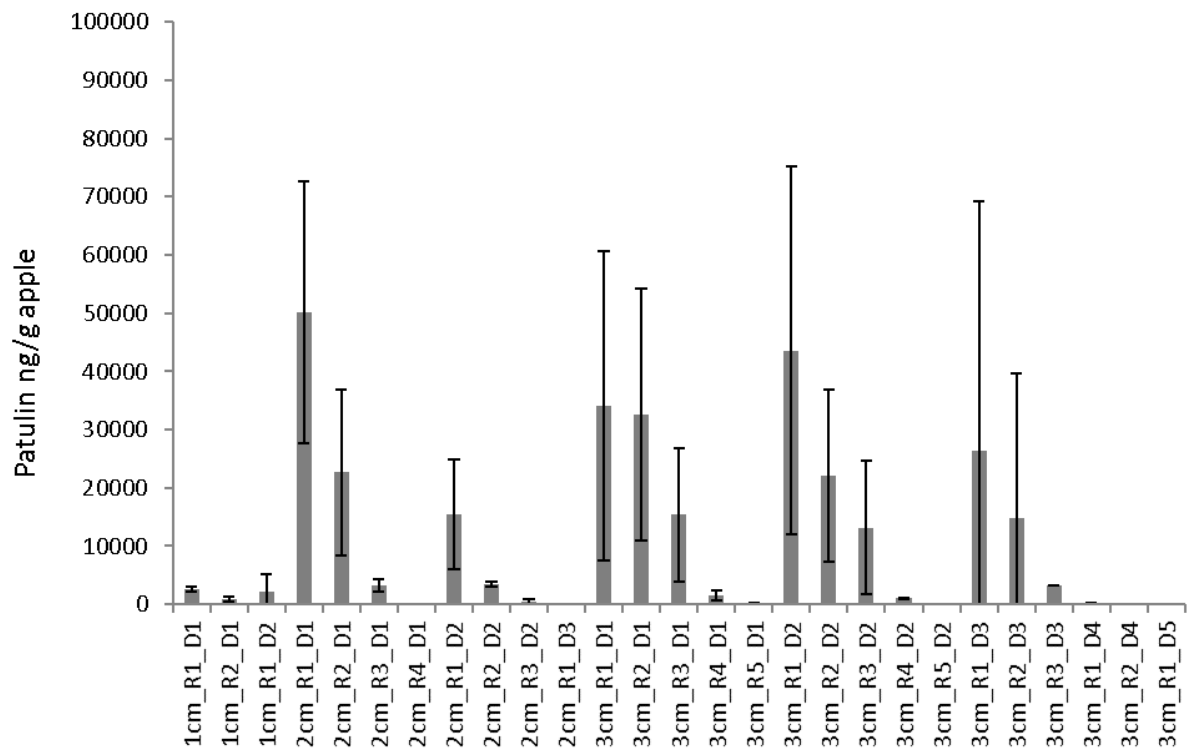
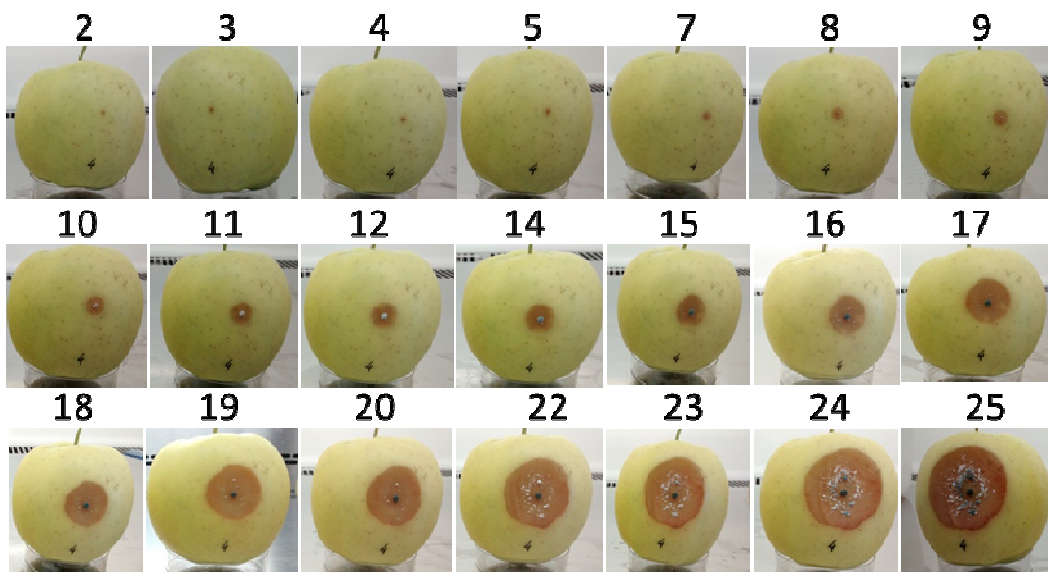
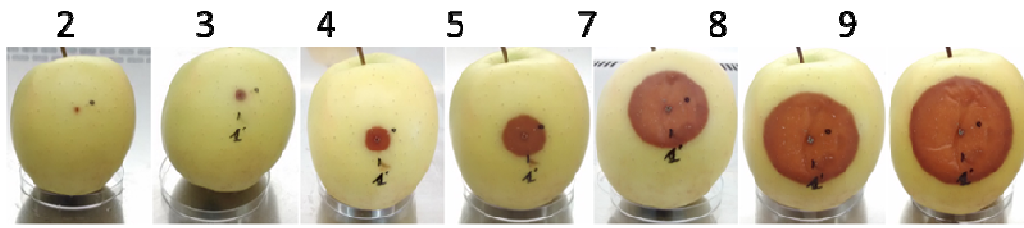


Figure 4.



A



B

Figure 5.

