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1 **Colonization dynamic of various crop residues by *Fusarium***
2 ***graminearum* monitored through real-time PCR measurements**

3

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5

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9

10 Abbreviated running headline: *Fusarium graminearum* survival in residues

11

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25 **Abstract**

26

27 **Aims:** To evaluate the effect of the type of crop residues on the colonization dynamic of *Fusarium*
28 *graminearum* in soil.

29 **Methods and Results:** The ability of *F. graminearum* to survive in the presence of various crop
30 residues was assessed on Petri dishes and in microcosms. These microcosms comprised soil that had
31 or had not been previously disinfested with or without amendment with various crop residues. The
32 colonization dynamic of *F. graminearum* was monitored through real-time PCR.

33 *Fusarium graminearum* development was higher in disinfested soil than in non-disinfested one. The
34 fungal growth was enhanced to various extents according to the type of crop residues, except for
35 mustard residues which inhibited it. The biochemical and physical properties of the residues were
36 likely to account for the differences in the survival of *F. graminearum*.

37 **Conclusions:** *F. graminearum* is a poor competitor in soil but it can use maize, wheat, and rape
38 residues to ensure its survival. Conversely alfalfa, which is assimilated by microorganisms very
39 easily, avoids long-lasting survival of the fungus. And finally mustard producing glucosinolates could
40 be used as an intermediate crop to reduce the inoculum amount.

41 **Significance and Impact of Study:** This study is contributing to the knowledge about *F.*
42 *graminearum* saprotrophic abilities and proposes interesting paths to limit its survival in soil.

43
44 **Keywords**

45
46 Saprotrophic development, primary inoculum, soil microbial interactions, plant pathogenic fungus,
47 *Fusarium* head blight.

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50

51 **Introduction**

52

53 Fusarium diseases affect crop production worldwide (Turkington et al. 2014). They mainly infects
54 wheat (*Triticum aestivum* L. subsp. *aestivum* and *Triticum turgidum* L. subsp. *durum* (Desf.) Husn.;
55 Fusarium Head Blight (FHB)), but also maize (*Zea mays* L.; Fusarium ear rot) and barley (*Hordeum*
56 *vulgare* L.; Parry et al. 1995). Their development affect grain quantity due to important yield losses,
57 and also grain quality due to mycotoxins produced by the fungi responsible for the infection. These
58 mycotoxins are of major concern for human and animal health as they are maintained during the
59 storage (Joint FAO/WHO Expert Committee on Food Additives 2001).

60 Several *Fusarium* species as well as *Microdochium nivale* (Fr.) Samuels & I.C. Hallet (var. *nivale*
61 and var. *majus*) are involved in the development of the disease. *Fusarium graminearum* Schwabe,
62 the anamorph phase of *Gibberella zeae* (Schwein.) Petch, is the most prevalent species in many parts
63 of the world (Goswami and Kistler 2004). Like other *Fusarium* species within the complex, *F.*
64 *graminearum* survives saprotrophically on infested crop residues (maize stalks, wheat straw and other
65 host plants; (Sutton 1982). These residues are believed to harbour the primary inoculum responsible
66 for the infection on wheat heads (Summerell et al. 1990).

67 Both the quantity and the type of residues affect pathogen survival. Field observations showed that
68 increases in inoculum amount and in disease severity are correlated with increasing quantities of
69 residues (Blandino et al. 2010). The residues quality is also a significant factor since *F. graminearum*
70 survival appears to be better ensured by wheat kernels than by other parts of the plant (Pereyra and
71 Dill-Macky 2005). This can be partly explained by the composition of residues, especially by their
72 C:N ratio (Khonga and Sutton 1988). This ratio depends on the plant species, on the plant organ, and
73 on the decomposition stage of residues (Nicolardot et al. 2001). The C:N ratio influences fungal
74 growth, as well as the production of asexual (macroconidia) and sexual structures (Pereyra and Dill-
75 Macky 2005), the latter being the main survival structures of the fungus on residues (Fernando et al.
76 2000).

77 Agricultural practices, and mainly crop residues management, play an important role in the control
78 of primary inoculums (Bailey and Lazarovits 2003). For instance, using FHB–susceptible plant
79 cultivars before wheat in the rotation scheme can dramatically favour the survival of the primary
80 inoculum, while non–susceptible host plants can hinder it (Schaafsma et al. 2005). Variations exist
81 among host plants: the disease is more severe after maize than after wheat or barley, as it depends on
82 the quantities of residues produced and on the differences in plants sensitivity (Champeil et al. 2004).
83 Unfortunately, the effects of other plants used as intermediate crops in rotations such as alfalfa
84 (*Medicago sativa* L.) and oilseed mustard (*Brassica juncea* (L.) Czern.) have not been studied
85 precisely enough within the framework of a possible control of *F. graminearum*, especially as
86 mustard incorporation have already been used successfully to reduce the survival of other plant
87 pathogens (Friberg et al. 2009). These plants have particular chemical compositions, which could
88 therefore increase or reduce *F. graminearum* survival. Alfalfa is rich in nitrogen and mustard is rich
89 in glucosinolates (Fahey et al. 2001). Soil tillage is also important for the development of the disease.
90 No tillage or minimum tillage practices increase the disease risk as compared to ploughing which
91 decreases it (Dill-Macky and Jones 2000). Residues left on the surface favour ascospores discharge
92 and macroconidia splash dispersal (Paul et al. 2004; Manstretta and Rossi 2015). Moreover, the
93 decomposition of residues is faster and more complete in soil than on the surface (Pereyra et al. 2004).
94 The decomposition stage of crop residues determines the nutritive quality of the substrate available
95 for the pathogen (Frankland 1998). The modification of this nutritive quality during the
96 decomposition process could be both a cause and a consequence of the successive colonizations of
97 the residues by fungal populations as previously shown in forest litters as well as in various arable
98 ecosystems (Hattenschwiler et al. 2005). The ability of a specific species to keep growing on the
99 residues depends on its ability to use available nutrients from a specific stage of decomposition as
100 well as to compete for them with other organisms (Naef et al. 2006). From this outlook, *F.*
101 *graminearum* quantities rapidly decrease as residue decomposition increase (Pereyra and Dill-Macky
102 2008).

103 Therefore, a promising alternative for preventing FHB would be to control the development of *F.*
104 *graminearum* primary inoculum in its natural habitat. There is still a need to improve knowledge
105 about this topic as it is difficult to understand the complex interactions between the parameters
106 affecting it. Until the 90's, the detection and the quantification of plant fungal pathogens were based
107 on plating techniques onto selective media (Singleton et al. 1992). PCR-based molecular techniques,
108 which are usually more sensitive, more specific and much faster than conventional techniques, have
109 been used for about twenty years to detect and quantify the causal agents of FHB , and in particular
110 *F. graminearum* (Nicholson et al. 1998). Such techniques have mostly been used to detect pathogens
111 on plant material (Brandfass and Karlovsky 2006) and on crop residues (Kohl et al. 2007). When *F.*
112 *graminearum* is studied in soil, plate count is still frequently used (Steinkellner and Langer 2004),
113 mostly because of the difficulty to work with DNA in soil environment (Sanzani et al. 2014). To date,
114 few studies have been including the notion of *F. graminearum* dynamic in soil and crop residues
115 using real-time PCR measurement (Palazzini et al. 2013). They rather focused on a situation at a
116 given time than on monitoring (Lukas et al. 2014).

117 The aim of this study was to bring better knowledge about the saprotrophic competence of *F.*
118 *graminearum* in soil and in crop residues by focusing on the notion of colonization dynamic. Besides,
119 the control of soil-borne microflora on colonization dynamic of *F. graminearum* was also tested. In
120 order to do so, the colonization dynamic of *F. graminearum* was monitored for several weeks through
121 real-time PCR in microcosms containing disinfested or natural soil amended or not with various crop
122 residues

123

124

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126

127 **Materials and methods**

128

129 *Fungal strain*

130 The strain of *Fusarium graminearum* (number MIAE00376) was provided by the collection
131 ‘Microorganisms of Interest for Agriculture and Environment’ (MIAE, INRA Dijon, France,
132 <http://www6.dijon.inra.fr/umragroecologie/Plateformes/ERB/Microorganismes-du-Sol>).

133 This strain was chosen because it has been successfully used as inoculum for field experiments
134 (unpublished). It was isolated from infested maize residues. Its morphological characterization was
135 done using phenotypic features assessed through macroscopic and microscopic observations and its
136 genetic identification was carried using molecular tools by sequencing the ITS (Genbank accession
137 number KU885384; White et al. 1990; Gardes and Bruns 1993) and the *trf-1 α* regions (KU885385;
138 Macia-Vicente et al. 2008). The 280-bp product specifically amplified by primers Fg16NF (ACA
139 GAT GAC AAG ATT CAG GCA CA) and Fg16NR (TTC TTT GAC ATC TGT TCA ACC CA)
140 was also sequenced (KU885386; Nicholson et al. 1998). The strain chemotype was identified as 15-
141 ADON type.

142 The strain was preserved on Potato Dextrose Agar medium (PDA) at room temperature and was also
143 stored at a temperature of –80 °C by cryopreservation in glycerol.

144 Fungal inoculum was produced in wheat bran liquid medium (distilled water complemented by 1 %
145 wheat bran, autoclaved at 120 °C, 20 min) adapted from Hassan and Bullerman (2009) and placed on
146 a rotary shaker (150 rpm) at 25 °C. After seven days of growing, the cultures were filtered through
147 sterile cheesecloth (approximately 50– μ m mesh size) to remove mycelium and remaining pieces of
148 bran. The conidial concentration was assessed within the aid of a 1–mm³ Malassez counting chamber
149 (Preciss, France). The concentration was then adjusted by dilution in sterile distilled water according
150 to the experimental requirements.

151

152

153 *In vitro radial growth of F. graminearum on medium amended with various crop residues*

154 Crop residues of wheat (*T. aestivum* subsp *aestivum*), grain maize (*Z. mays*), rape (*Brassica napus*
155 L.), alfalfa (*M. sativa*) and mustard (*B. juncea*) were picked at the INRA Experimental Unit of
156 Epoisses (Côte d'Or, France [5°05'E; 47°14'N]). Wheat and rape straws were collected from remains
157 left in the field after harvest in July 2009. Maize stalks were collected from remains left in the field
158 after harvest in November 2009. Entire plants of alfalfa and mustard were harvested during plant
159 growth in October 2010. The samples were air-dried, coarsely chopped and ground using a Prep'line
160 coffee grinder (SEB, France). These preparations were then sifted through 200- μ m mesh sieves and
161 then γ -irradiated [5K Gy; Ionisos, Dagneux, France] to sterilise them without affecting their physical
162 and chemical properties. The C:N ratios of the residues were assessed with Dumas method (Dumas
163 1831) by SAS laboratory (Ardon, France). Duplicated analyses were performed for each type of
164 residue.

165 To estimate the radial growth of *F. graminearum* on crop residues, water agar medium (2 %) was
166 autoclaved 20 min at 120 °C and then was supplemented with crop residues (1 l of water agar
167 containing 25 g of dry γ -irradiated crop residues). Ten microliters of titrated suspension of *F.*
168 *graminearum* spores (10^3 conidia.ml⁻¹) were placed in the centre of each Petri dish. Five replicates
169 were included for each residue type as well as for the water-agar residue-free control. Fungal radial
170 growth was measured daily on two orthogonal diameters of the fungal colony for each Petri dish for
171 six days.

172
173 *Effect of soil disinfection (biotic factor) and of wheat straw presence (trophic factor) on the*
174 *colonization dynamics of F. graminearum in microcosms*

175 The four treatments were: (i) residue-free disinfested soil; (ii) residue-free natural soil; (iii)
176 disinfested soil with wheat straw; and (iv) natural soil with wheat straw. The total incubation time of
177 the experiment lasted twelve weeks. The first sampling was made on the first day of the experiment,
178 and then the sampling was done twice a week during the first two weeks, once a week from week two
179 to week four, and finally every two weeks until the twelfth week (eleven samplings). For each

180 treatment and each sampling date, three microcosms were prepared. The soil was picked at the INRA
181 Experimental Unit of Epoisses. It had a silty–clayey texture (sand 6.1 %, silt 57.7 %, clay 36.2 %), a
182 pH of 7.1, 2.5 % of organic matter, a C:N ratio of 9.7 and a Cation Exchange Capacity of 20.1
183 cmol(+).kg⁻¹ soil. The soil (4 % moisture content) was sifted through 2–mm mesh sieves. Forty grams
184 (dry weight) of soil were distributed into tins boxes (5 cm diameter, 6 cm height). According to
185 the treatments, microcosms were autoclaved 1 hr at 105 °C for three consecutive times and left one
186 week to allow the evaporation of remaining toxic gas.

187 Wheat straw was prepared as previously described. According to the treatments, microcosms were
188 supplemented with 1 g of γ -irradiated wheat straw. The mixture was prepared by manual shaking for
189 30 sec. The moisture content in each microcosm was adjusted under sterile conditions to 80 % of
190 Water Holding Capacity of the soil (maximal quantity of water held by the soil) with a titrated
191 suspension of *F. graminearum* conidia. The final concentration was 10³ conidia.g⁻¹ of soil.
192 Microcosms were covered with tins lids to ensure sterile conditions and avoid drying, and then
193 were incubated at 20 °C in a thermostatic incubator.

194 At each sampling date three microcosms of each treatment were randomly and definitely taken away
195 from the group of available microcosms and were manually checked during 30 s to ensure there
196 homogeneity. For each sampled microcosm, 1 g of soil was stored at –20 °C for DNA extraction.

197
198 *Effect of the type of crop residue (trophic factor) on the colonization dynamics of F. graminearum in*
199 *microcosms*

200 The six treatments of natural soil were: (i) residue–free or amendment with residues of (ii) wheat,
201 (iii) maize, (iv) rape, (v) alfalfa, and (vi) mustard. The total incubation time of the experiment lasted
202 six weeks. The first sampling was made on the first day of the experiment, and then the sampling
203 frequency was once a week until week four, and a final sampling on the sixth week (six samplings).
204 The microcosms were prepared as previously described.

205

206 *DNA extraction*

207 Two grams of acid-washed glass beads with a diameter of 106 μm , eight sterile glass beads with a
208 diameter of 2 mm and 4 ml of lysis buffer containing 50 mmol.l^{-1} Tris-HCl (pH 8.0), 20 mmol.l^{-1}
209 EDTA (pH 8.0), 100 mmol.l^{-1} NaCl and 1 % (w/v) sodium dodecyl sulphate were added to each
210 sample. Samples were shaken for 30 sec at 4 m.s^{-1} in a FastPrep[®]-24 Instrument (MP Biomedicals,
211 LLC, USA) to physically disrupt the matrix. Then DNA was extracted as described previously by
212 Edel-Hermann et al. (2004). Briefly, after chemical DNA extraction at 70 °C, samples were purified
213 twice using polyvinylpyrrolidone spin columns to remove co-extracted humic acids, and then
214 once using GeneClean[®] Turbo kit (Q-BIOgene, Inc., USA). DNA samples were stored at 4 °C before
215 their use.

217 *Real-time PCR*

218 Primers

219 Primers Fg16NF (ACA GAT GAC AAG ATT CAG GCA CA) and Fg16NR (TTC TTT GAC ATC
220 TGT TCA ACC CA) specifically amplifying *F. graminearum* were used to obtain a 280-bp product
221 (Nicholson et al. 1998). Primers were purchased lyophilised with UltraPureGold[™] quality
222 (Eurogentec S.A., Belgium). They were dissolved to a 10- $\mu\text{mol.l}^{-1}$ concentration in DNase/RNase
223 free water.

225 Standard curve production

226 The specific fragment amplified by Fg16NF/Fg16NR was cloned through Kit pGEM[®]-T easy vector
227 systems II (Promega, USA) following the manufacturer's instructions. Then the plasmid was purified
228 with QIAfilter Plasmid Midi Kit (QIAGEN, Germany) and linearised using *Sal I* restriction enzyme
229 (Promega, USA). The concentration of linearised plasmid solution was adjusted to 10⁹ copies. μl^{-1} of
230 the specific fragment. Dilutions were performed to obtain solutions whose concentrations ranged
231 from 10⁹ down to 10² DNA copies. μl^{-1} .

232

233 PCR reaction conditions and cycling parameters

234 PCR reactions were performed in an ABI PRISM[®] 7900HT Sequence Detection System (Applied
235 Biosystems[™], USA) in a final volume of 25 μ l. Each sample contained 12.5 μ l of Absolute QPCR
236 SYBR Green ROX MIX 2X (Thermo Fisher scientific Inc., USA), 0.5 μ l of each primer, 1 μ l of either
237 sterile water (control) or DNA sample, and 10.5 μ l of sterile water. Two series of the standards (10^2
238 to 10^9 copies) were assessed during each experiment.

239 All PCR reactions were run using the following protocol: initial denaturation and polymerase
240 activation at 95 °C for 15 min, followed by 45 cycles of 95 °C for 15 s, 64 °C for 30 s, 72 °C for 30
241 s, and a final 5 min extension at 72 °C. During the process, fluorescence detection was carried out
242 during the annealing step. A melting curve analysis followed the cycling protocol: the samples were
243 heated at 95 °C for 1 min, cooled to 55 °C for 1 min and then temperature was increased from 70 to
244 90 °C at the rate of 1.75 °C.min⁻¹, with continuous measurement of the fluorescence to check the
245 amplified fragment purity. Three technical replicates were performed for each biological sample.

246 The detection limit of the PCR reaction was defined as the minimal number of DNA copies needed
247 to surely distinguish it from the background noise measured in the negative control sample. This limit
248 varies with each PCR reaction. It was set to ten times the quantity of copies detected in the negative
249 sample.

250 The presence of PCR inhibitors was checked by spiking 10^4 copies of *F. graminearum* specific
251 fragment in different samples of each modality. Spiked and non-spiked samples were compared to
252 check for inhibitory effects.

253

254

255 *Statistical analyses*

256 The data were logarithmically transformed to satisfy conditions of normality and of variance equality,
257 and then the three sets of experiments (i.e. in vitro radial growth of *F. graminearum* and the two sets

258 of experiment in microcosms) were analyzed by three models of analysis of variance (ANOVA;
259 Cohen 1988).

260

261 Model 1: In vitro radial growth of *F. graminearum* on medium amended with various crop residues.

262 The residue type and the incubation time were used as independent variables.

263

264 $Fungal\ radial\ growth_{ij} = \mu + Residue\ type_i + Incubation\ time_j + Residue\ type * Incubation\ time_{ij} + \epsilon_{ijk}$

265 (1)

266

267 where μ is a constant, *Residue type* is the effect of the type of residue ($i = 1, \dots, 5$), *Incubation time*
268 is the effect of the incubation duration ($j = 1, \dots, 5$), *Residue type*Incubation time* is the interaction
269 effect and ϵ is the residual error.

270

271 Model 2: Effect of soil disinfestation and of wheat straw presence on the colonization dynamics of *F.*

272 *graminearum* in microcosms. The soil disinfestation, the presence of wheat straw and the sampling

273 date were used as independent variables. The starting time of the experiment was not included in the

274 model.

275

276 $Number\ of\ fungal\ DNA\ copies_{ijk} = \mu + Soil\ disinfestation_i + Presence\ of\ straw\ residues_j + Sampling$

277 $date_k + Soil\ disinfestation * Presence\ of\ straw\ residues_{ij} + Soil\ disinfestation * Sampling\ date_{ik} +$

278 $Presence\ of\ straw\ residues * Sampling\ date_{jk} + Soil\ disinfestation * Presence\ of\ straw$

279 $residues * Sampling\ date_{ijk} + \epsilon_{ijkl}$ (2)

280

281 where μ is a constant, *Soil disinfestation* is the effect of the soil disinfestation ($i = 1, 2$), *Presence of*

282 *straw residues* is the effect of the presence of straw residues ($j = 1, 2$), *Sampling date* is the effect of

283 the incubation duration ($k = 1, \dots, 10$), *Soil disinfestation*Presence of straw residues*, *Soil*

284 *disinfestation*Sampling date, Presence of straw residues*Sampling date* and *Soil*
285 *disinfestation*Presence of straw residues*Sampling date* are the interactions effects and ε is the
286 residual error.

287

288 Model 3: Effect of the type of crop residue on the colonization dynamics of *F. graminearum* in
289 microcosms. The residue type and the sampling date were used as independent variables. The starting
290 time of the experiment was not included in the model.

291

$$292 \text{ Number of fungal DNA copies}_{ij} = \mu + \text{Residue type}_j + \text{Sampling date}_j + \text{Residue type} * \text{Sampling date}_{ij} \\ 293 + \varepsilon_{ijk} \quad (3)$$

294

295 where μ is a constant, *Residue type* is the effect of the type of residue ($i = 1, \dots, 6$), *Sampling date* is
296 the effect of the incubation duration ($j = 1, \dots, 5$), *Residue type*Sampling date* is the interaction effect
297 and ε is the residual error.

298

299 Significance of differences between means was assessed using the Newman–Keuls method (Zar
300 1999). The ANOVA models and the Newman–Keuls tests were carried out using XLSTAT
301 (Addinsoft™, France) with $\alpha = 5 \%$.

302

303

304

305

306

307 **Results**

308

309 *In vitro radial growth of F. graminearum on medium amended with various crop residues.*

310 The radial growth of *F. graminearum* varied according to the type of crop residue, and so did radial
311 extension speed (Table 1). Three types of residues, i.e. wheat, maize and rape, allowed a statistically
312 greater growth than the control (Figure 1), but no statistical difference was noticed among them. The
313 measurements were stopped after six days because the fungus had reached the edges of the Petri
314 dishes for these three treatments. No growth was observed on medium complemented with mustard
315 residues after six days, which provoked a significant interaction between *Residue type* and *Incubation*
316 *time*. A contamination in the medium supplemented with alfalfa residues made the results unusable
317 for this kind of residue.

318

319 *Effect of soil disinfestation (biotic factor) and of wheat straw presence (trophic factor) on the*
320 *colonization dynamics of F. graminearum in microcosms*

321 Although the curves displayed similar shapes in response to the different treatments, four clear and
322 significantly distinct patterns of DNA amount were observed (Figure 2). *Fusarium graminearum*
323 established at a higher density in the disinfested soil than in the natural soil, which meant that the
324 indigenous microflora could slow down the development of *F. graminearum*. In either disinfested or
325 natural soil, development and final densities increased when sterile wheat straw was added to the soil.
326 The three studied factors, i.e. the soil disinfestation, the presence of wheat straw and the sampling
327 date, as well as the level 2 interactions, were statistically significant (Table 2). Thus, the highest
328 number of *F. graminearum* DNA copies was found in the disinfested soil supplied with wheat straw,
329 while the lowest was found in the natural, residue-free soil. Between these two values, more DNA
330 copies were recovered in the disinfested, residue-free soil than in the natural soil supplied with wheat
331 straw. These results highlighted the role of natural soil-borne microflora in the regulation of *F.*
332 *graminearum* development, as well as the stock of resources supplied by the presence of wheat straw.
333 Finally, the final decrease in the number of DNA copies observed for each curve was statistically
334 significant.

335

336 *Effect of the type of crop residue (trophic factor) on the colonization dynamics of F. graminearum in*
337 *microcosms*

338 The curve shapes for this experiment were the same as for the previous one. The supply of different
339 types of crop residues in natural soils led to the establishment of *F. graminearum* at significant various
340 densities in the amended soils (Table 3; Figure 3). The sampling date showed no significant effect on
341 the amount of DNA copies, unlike the interaction between residue type and sampling date. The
342 number of recovered DNA copies was higher in soil amended with maize, wheat, rape and alfalfa
343 residues than in non-amended soil. The number of DNA copies recovered in soil amended with
344 mustard residues was lower than in non-amended soil, which proved that mustard residues did not
345 enhance the development of *F. graminearum*, and moreover seemed to reduce its survival. The
346 evolution of the DNA copies number was quite the same in soils amended with maize, wheat and
347 rape residues. It was higher in soils amended with maize and wheat residues than in soil amended
348 with rape residues during the first two weeks of the experiment, but not at the end. The soil amended
349 with alfalfa residues displayed a different effect towards *F. graminearum* colonization dynamic. The
350 quantity of *F. graminearum* DNA copies in soil amended with alfalfa residues was significantly the
351 highest after three weeks of experiment, but dropped dramatically to a lower value than the ones in
352 soils amended with maize, wheat and rape residues at the last sampling date.

353

354 *C:N ratios of the residues*

355 The C:N ratios of the residues varied from 14 for alfalfa to 116 for rape with respective ratios of 22,
356 33 and 76 for mustard, maize and wheat.

357

358

359 **Discussion**

360 *General shape of the colonization dynamic of F. graminearum*

361 The shapes of the curves were similar for all treatments in the first set of microcosms. First a priming
362 effect was observed. It could be due to the activation of fungal growth, during which *F. graminearum*
363 grows from inoculated spores to active mycelium through the use of resources directly available from
364 its environment (Harris 2005). At the beginning of the experiment, a bias was observed in soil
365 amended with straw residues since the quantity of fungal DNA copies was high compare to soil
366 without residue. Wheat straw was contaminated before the disinfestation process. Γ -rays kill
367 organisms without completely destroying DNA, but it is assumed that such residual DNA disappeared
368 rapidly during the experiments (Romanowski et al. 1992). However, the results of the first sampling
369 time had not been included in ANOVA models (2) and (3), which were therefore more relevant. After
370 the priming effect, the subsequent kind of stagnation in DNA copy numbers could match to the period
371 of adaptation to the habitat, during which fungal enzymatic machinery targeting the complex trophic
372 resources was activated (Debeire et al. 2014). Eventually, the final decrease of the curves could be
373 due to the exhaustion of available resources, during which *F. graminearum* evolves from mycelium
374 to new conidia and ascospores (Bateman et al. 2007).

376 *Effect of soil disinfestation on the colonization dynamic of F. graminearum*

377 The soil disinfestation was the main factor influencing the colonization dynamics of *F. graminearum*:
378 the quantities of fungal DNA copies in the disinfested soil, whether supplied with wheat residues or
379 not, were one-hundred-fold higher than in the natural soil. We can therefore conclude that *F.*
380 *graminearum* development was regulated by soil-borne communities. This result was expected
381 because *F. graminearum* is known to be not highly persistent in soil, and particularly in comparison
382 with other *Fusarium* species such as *F. oxysporum* (Pereyra and Dill-Macky 2008). The fungal
383 abundance in the soil used in this study had been count through plating method around 10^5 colony
384 forming unit.g⁻¹ of soil (Friberg et al. 2009), among which *F. oxysporum* strains were abundant while
385 *F. graminearum* strains were not (Edel-Hermann et al. 2015). The development of pathogen

386 populations on residues could be reduced by managing the indigenous microbial communities of the
387 soil in order to increase soil suppressiveness (Weller et al. 2002).
388 Moreover, all residues tested in this study had previously been disinfested, but the initial residue–
389 borne communities would probably have increased the regulatory effect. Trying to promote the
390 growth of indigenous or introduced microorganisms which are able to outcompete plant pathogenic
391 fungi within the crop residues was recently suggested (Lakhesar et al. 2010). Particular species were
392 found to suppress the sporulation of *F. graminearum* macroconidia and ascospores as well as
393 perithecia production on wheat straw and maize stalks under controlled conditions (Bujold et al. 2001;
394 Luongo et al. 2005). However, their efficiency under field conditions remained limited.
395 In this present experiment, the effect of macrofaunal (earthworms...) and microfaunal (nematodes...)
396 communities on the colonization dynamic of *F. graminearum* had not been tested, but it would also
397 have an effect under natural conditions. *Lumbricus terrestris* L. *Aphelenchoides saprophilus* Franklin
398 and *Folsomia candida* Willem impacted significantly the decomposition rate of *Fusarium*–infested
399 wheat straw into the soil and also the degradation rate of *Fusarium*–produced mycotoxins (Oldenburg
400 et al. 2008; Wolfarth et al. 2013). Therefore, multitrophic interactions within the soil could regulate
401 and control such soil–borne and residue–borne pathogenic inoculums.

402

403 *Effect of the presence of wheat straw on the colonization dynamic of F. graminearum*

404 In both disinfested soil natural soil, the presence of wheat residues provided a favourable habitat for
405 fungal development. The residues could constitute either trophic resources by providing nutrients, or
406 structural resources by altering soil environments. *Fusarium graminearum* produces a wide range of
407 enzymes in the presence of plant material, and several of them degrade cellulose, hemicelluloses and
408 pectin (Debeire et al. 2014). Therefore *Fusarium graminearum* owns the enzymatic machinery to
409 degrade compounds of plants primary cell walls and to use residues as nutrients, as revealed by the
410 fungal radial growth on media supplied with crop residues. The final significant decrease of DNA

411 copies number observed in each treatment confirmed the poor ability of *F. graminearum* to remain at
412 high amount in soil over time.

413

414 *Effect of the C:N ratio of the residues on the colonization dynamic of F. graminearum*

415 On Petri dishes as well as in the second set of microcosms, the fungal growth was higher in presence
416 of crop residues than in the controls without residues, except for mustard residues. No difference was
417 observed on Petri dishes between the effects of maize, wheat and rape residues. Conversely, maize,
418 wheat and rape residues were top down ranked for the number of recovered DNA copies after the
419 starting time of the experiment in microcosms, but the differences were not significant at each
420 sampling day. Maize and wheat are hosts of the disease. As a result, having these crops as preceding
421 crops increases disease risks (Champeil et al. 2004). Surprisingly, rape, which is not a host plant, also
422 favoured fungal growth. The survival of *F. graminearum* is improved by increased residues amounts
423 (Blandino et al. 2010). However, this last parameter is not an explanation of the differences observed
424 in this study since all residues were added in the same proportion to the soil (1 g dry
425 matter.microcosm⁻¹). Champeil et al. (2004) noticed that wheat and durum wheat produce similar
426 amounts of residues, but the level of infection of wheat after these crops is different. Inoculum
427 production varies with the plant species. It is not the same on a given plant over time, and it depends
428 on the plant organ (Khonga and Sutton 1988; Pereyra and Dill-Macky 2008). This can be partly
429 explained by the C:N ratio of the residues. This ratio varies depending on the decomposition stage of
430 the residues. It influences fungal growth as well as the production of macroconidia and sexual
431 structures. Rich residues (low C:N ratio) provide a favourable habitat, allowing longer saprotrophic
432 development before perithecia production as compared to poor residues (high C:N ratio; Khonga and
433 Sutton 1988). In this study, maize, wheat and rape were ranked depending on their C:N ratio value.
434 The low C:N ratio of maize (33) best promoted fungal growth, while the high C:N ratio of rape (116)
435 least promoted it. The ratio of maize was particularly low in the experiment mainly because maize

436 leaves were used for technical reasons. The results could have been different if more maize stalks
437 (C:N ratio around 130) had been used (Nicolardot et al. 2001).

438

439 *Effect of the mustard residues on the colonization dynamic of F. graminearum*

440 Maize, wheat and rape were first chosen for this study since they are major rotational crops in the
441 region (Burgundy, France; Agreste 2015). Rape was also tested because it is a member of
442 Brassicaceae family. This family is known for its deleterious action on microbial communities
443 through the release of allelopathic molecules in its rhizosphere (Morra and Kirkegaard 2002).
444 However, growth in water agar medium supplied with rape residues showed no clear action of
445 antifungal products since no difference was noticed between rape, maize and wheat. Conversely,
446 when water medium agar was supplied with mustard residues, no growth of *F. graminearum* was
447 observed. The presence of such deleterious secondary metabolites was therefore highlighted in
448 mustard. *Fusarium graminearum* growth was also significantly lower in soil amended with mustard
449 residues than in non-amended soil although its C:N ratio was favourable (22), confirming the
450 inhibition effect the mustard had got on fungal growth. This result was consistent with the effect of
451 incorporated mustard on other plant pathogens (Friberg et al. 2009). This inhibition could only
452 originate from mustard since the residues were disinfested. Sinigrin is the main glucosinolate found
453 in mustard (Bellostas et al. 2007). Some studies showed that among several cereal root pathogens, *F.*
454 *graminearum* was moderately sensitive to isothiocyanates resulting from the hydrolysis of
455 glucosinolates, and particularly to sinigrin (Kirkegaard et al. 1996). The type and the amount of
456 glucosinolates produced depend on Brassicaceae species, on the variety within these species, on plant
457 maturity, on the part of the plant considered and on environmental plant growth conditions (Bellostas
458 et al. 2007). This could explain why the colonization dynamics of *F. graminearum* differed in the soil
459 with the mustard residues, which contain high levels of sinigrin, as compared to the soil with the rape
460 residues, which contain low levels of glucosinolates.

461

462 *Effect of the alfalfa residues on the colonization dynamic of F. graminearum*

463 Finally, the effect of C:N ratio was confirmed by the experiment in alfalfa-supplied soil. *Fusarium*
464 *graminearum* growth in soil amended with alfalfa residues was significantly the highest of the set at
465 the beginning of the experiment. Alfalfa had the lowest C:N ratio (14) of all types of tested residues.
466 However, the number of DNA copies decreased twenty-one days after inoculation, and reached a
467 lower value than those observed with the other promoting residues at the end of the experiment. These
468 observations were consistent with the contention that the substrate had been exhausted or
469 decomposed. Alfalfa was expected to mineralise faster than mature wheat or rape straw which had a
470 higher C:N ratio (Pascault et al. 2010). Therefore, the rapid exhaustion of the trophic resource could
471 lead to a drastic decrease in *F. graminearum* density. Further studies should focus on the role of
472 legumes and their subsequent crop remains on *F. graminearum* survival.

473

474 In conclusion, due to the saprotrophic phase of *F. graminearum* life cycle, there is a real need to
475 understand better how it survives in soil and in residues. *Fusarium graminearum* appears to be a poor
476 competitor over time, particularly compared with other *Fusarium* species, e.g. *F. oxysporum*. Yet, it
477 seems to overcome this difficulty since it can still cause FHB. This study showed that *F. graminearum*
478 development was under control of soil-borne community, but was buffered by the presence of
479 residues. Crop residues promoted the fungal development to different extents depending on their type.
480 Consequently, several techniques should be combined to control primary inoculum production by the
481 phytopathogenic fungus: i.e. the crop residues management through rotations and soil tillage, the
482 stimulation of the antagonistic microflora, and the use of preventive preceding crops. For this last
483 parameter, mustard could be used as it produces antifungal compounds as well as legumes for which
484 the fast mineralization destroys *F. graminearum* habitat.

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486

487

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489

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494

495 **Conflict of Interest**

496

497 The authors certify that this work was carried out in a public research organization and in no way
498 constitutes a potential source of conflict of interest with other public or private research organizations.

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652 Table 1: In vitro radial growth of *F. graminearum* on medium amended with various crop residues -
 653 ANOVA table for the model 1.

<i>Fungal radial growth</i>			
Source of variation	<i>df</i>	<i>MS</i>	<i>F</i>
<i>Residue type</i>	4	1.46	7171.64***
<i>Incubation time</i>	4	0.76	3707.94***
<i>Residue type*Incubation time</i>	16	0.05	240.50***

654 *df*: degrees of freedom, *MS*: Mean Square, *F*: Fisher's F

655 * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$

656

657

658 Table 2: Effect of soil disinfestation and of wheat straw presence on the colonization dynamics of *F.*
 659 *graminearum* in microcosms - ANOVA table for the model 2.

<i>Number of F. graminearum DNA copies</i>			
Source of variation	<i>df</i>	<i>MS</i>	<i>F</i>
<i>Soil disinfestation</i>	1	87.91	1735.02***
<i>Presence of wheat straw residues</i>	1	21.41	422.51***
<i>Sampling date</i>	9	0.26	5.09***
<i>Soil disinfestation*Presence of wheat straw</i>	1	0.37	7.37**
<i>Soil disinfestation*Sampling date</i>	9	0.16	3.18**
<i>Presence of wheat straw*Sampling date</i>	9	0.06	2.48*
<i>Soil disinfestation*Presence of wheat straw*Sampling date</i>	9	0.06	1.26

660 *df*: degrees of freedom, *MS*: Mean Square, *F*: Fisher's F

661 * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$

662

663 Table 3: Effect of the type of crop residue on the colonization dynamics of *F. graminearum* in
664 microcosms - ANOVA table for the model 3.

Source of variation	Number of <i>F. graminearum</i> DNA		
	<i>df</i>	<i>MS</i>	<i>F</i>
<i>Residue type</i>	5	30.83	430.71***
<i>Sampling date</i>	4	0.10	1.35
<i>Residue type*Sampling date</i>	20	0.32	4.42***

665 *df*: degrees of freedom, *MS*: Mean Square, *F*: Fisher's F

666 * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$

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667 Figures captions
668

669 Figure 1: In vitro radial growth of *F. graminearum* on media amended with various crop residues.
670 The control was provided by the radial growth of *F. graminearum* on water agar medium without
671 any type of residue. Each measure was obtained by the average of five independent experimental
672 runs. For each one, the value was obtained by the average of two measurements of the fungal
673 colony diameter on two orthogonal axes.

674 Type of residue: (□) rape, (■) mustard, (●) wheat, (×) maize and (○) control without residue
675 Statistical groups based on Newman–Keuls test with $\alpha=0.05$ for the Residue type: maize, wheat
676 and rape: a; control: b, mustard: c. Treatments are significantly different when followed by
677 different letters.

678
679 Figure 2: Effect of soil disinfestation and of wheat straw presence on the colonization dynamics
680 of *F. graminearum* in microcosms.

681 Each measure was obtained by the average of three independent experimental runs. The value of
682 each one was obtained by the average of three successive measurements by real–time PCR.

683 Soil disinfestation/presence of wheat residues: (■) disinfested soil/wheat residues, (□)
684 disinfested soil/without residue, (●) natural soil/wheat residues and (○) natural soil/without
685 residue.

686 Statistical groups based on Newman–Keuls test with $\alpha=0.05$ for the Soil disinfestation*Presence
687 of wheat straw interaction: disinfested soil / wheat residues: a; disinfested soil / without residue:
688 b; natural soil / wheat residue: c; .natural soil / without residue: d. Treatments are significantly
689 different when followed by different letters.

690

691 Figure 3: Effect of the type of crop residue (trophic factor) on the colonization dynamics of *F.*
692 *graminearum* in microcosms.

693 Each measure was obtained by the average of three independent experimental runs. The value of
694 each one was obtained by the average of three successive measurements by real-time PCR.

695 Type of residue: (□) rape, (■) mustard, (●) wheat, (×) maize, (▲) alfalfa and (○) control
696 without residue

697 Statistical groups based on Newman-Keuls test with $\alpha=0.05$ for the Residue type*Sampling date
698 interaction at the end of the experiment: maize: ab; wheat: ab; rape: bc; alfalfa: c; no amendment:
699 d and mustard: e. Treatments are significantly different when followed by different letters.

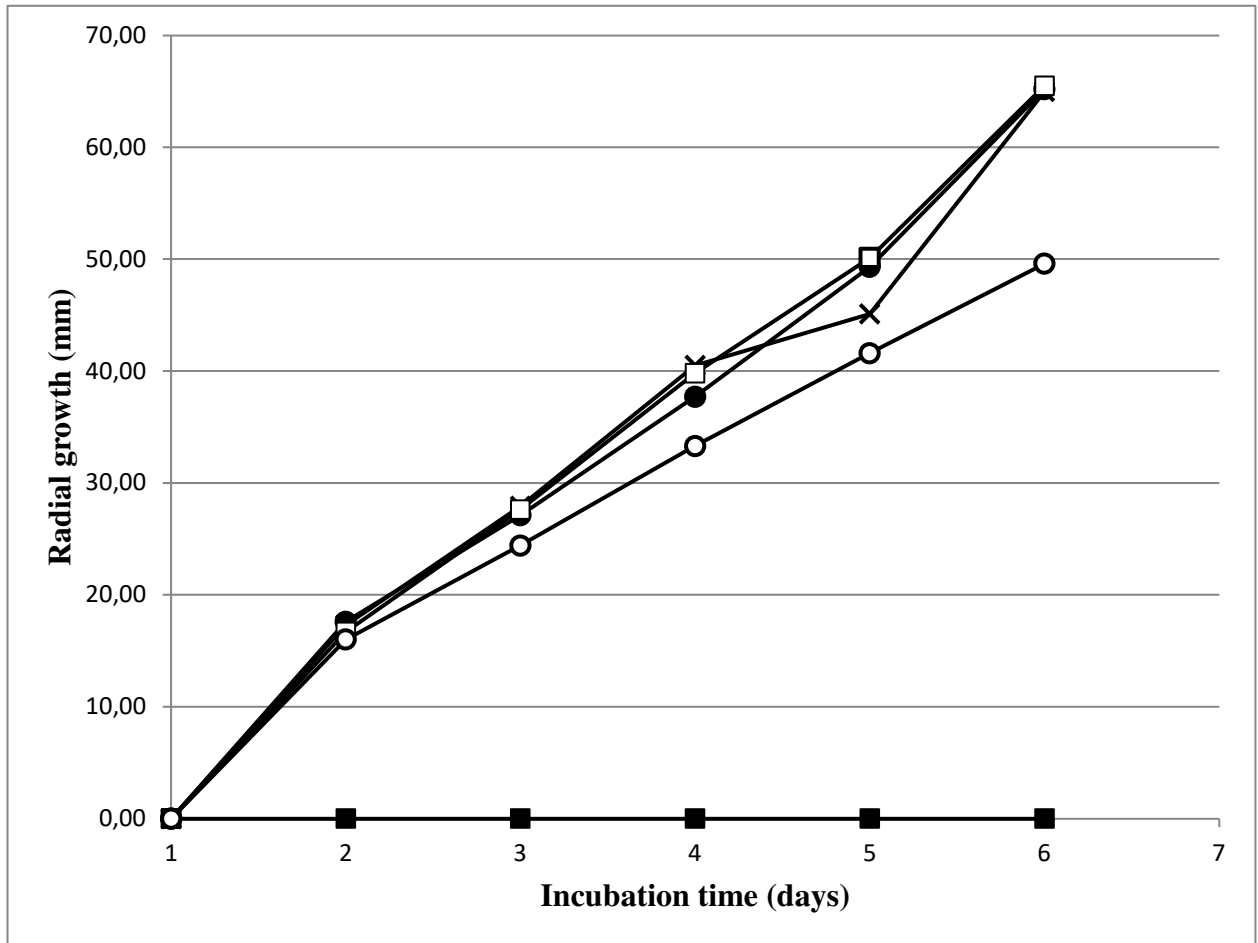
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701 Figure 1

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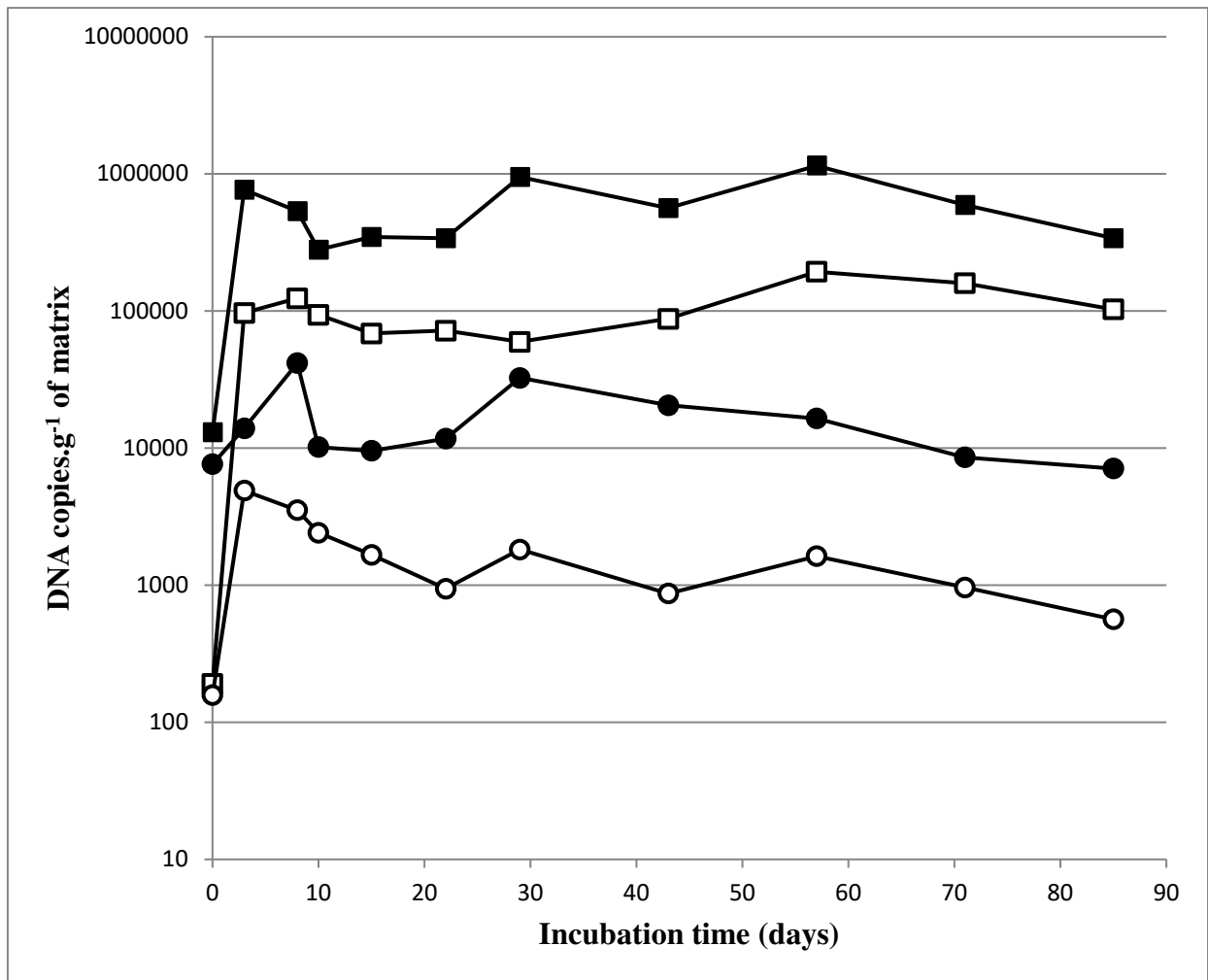
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704 Figure 2

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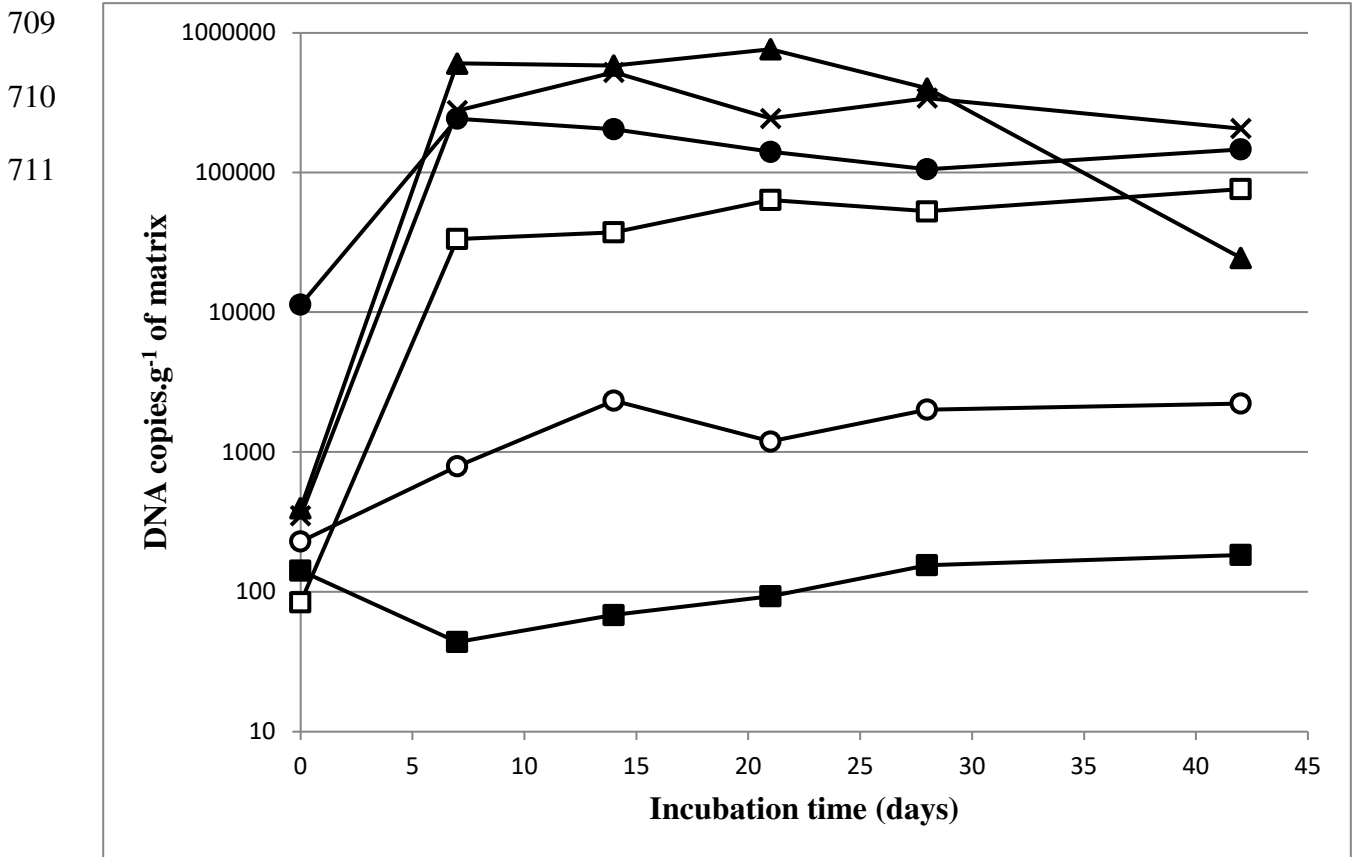
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708 Figure 3



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