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1 **Exploring the impact of verticillium wilt disease on the mechanical properties of elementary**  
2 **flax (*Linum usitatissimum* L.) fibres**

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16

17 **Abstract**

18 Verticillium wilt is a disease caused by the fungus *Verticillium dahliae* (*V. dahliae*) which is  
19 widespread in flax (*Linum usitatissimum* L.) cultures. *V. dahliae* negatively impacts the fibre  
20 yield with up to 60 % loss at the scutching step of fibre transformation. Yet, little is known

21 about the consequences of *V. dahliae* on the mechanical properties of flax fibres. In this study,  
22 we investigated the tensile characterisation of elementary flax fibres impacted by *V. dahlia*.  
23 Using elementary tensile tests, we observed an important decrease in the mechanical  
24 properties of the studied flax fibres, with a 32 % decrease of the tensile strength at break and a  
25 15 % decrease of the strain at break. Further investigation of the flax fibres cell wall using  
26 atomic force microscopy (AFM) in peak-force non-quantitative mechanical mapping mode,  
27 showed a 23 % decrease in the longitudinal indentation modulus of flax fibre cell walls,  
28 measured directly in their cross section. This is arguably due to the important enzymatic  
29 arsenal of *V. dahliae* revealed by a bioinformatic analysis of secreted carbohydrate-active  
30 enzymes targeting plant cell wall components. The coupling of this analysis with electron  
31 microscopy observations and sugar analysis highlighted the degradation mechanisms of this  
32 fungus which significantly affects the mechanical performance of flax fibres.

33

34 **Keywords:** AFM PF-QNM; carbohydrate-active enzymes; elementary fibre; *Linum*  
35 *usitatissimum L.*; tensile properties; *Verticillium dahliae*

36

## 37 **1. Introduction**

38 Environmental concerns encourage the use of flax fibres (*Linum usitatissimum L.*) for both  
39 technical and textile applications as a result of their outstanding tensile mechanical properties  
40 and limited environmental impact (Baley & Bourmaud, 2014; Le Duigou et al., 2011). Flax fibres  
41 are made up of multi-layered cell walls, but weather conditions directly affect the growth of  
42 flax, in particular during the filling of the elementary fibres (Goudenhoft et al., 2018). Hydric  
43 stress delays the formation of flax fibres, specifically during the elongation phase

44 (Chemikosova et al., 2006). This results in shortened fibres and altered cell wall formation due  
45 to a change in biochemical composition (Chemikosova et al., 2006), and more globally results  
46 in shorter flax stems (Kariuki et al., 2016) that negatively impact the fibre production during  
47 the scutching process.

48 *Verticillium dahliae* (*V. dahliae*) is a pathogen that causes the Verticillium wilt disease to flax  
49 stems. *V. dahliae* is found at the stage of dormancy in the soil as microsclerotia, that consists  
50 of a compact cluster of mycelium, which can confer more than 14 years survival to the fungus  
51 (Bressan et al., 2016). *V. dahliae* is extremely polyphagous with more than 200 potential host  
52 species including potato and salad (Bressan et al., 2016; Mol, 1995). Fungal growth is  
53 stimulated by an increase in temperature and humidity in the soil, in particular by root exudate  
54 (Blum et al., 2018; Valade et al., 2019), but climate change may also encourage the  
55 germination of *V. dahliae*.

56 This vascular fungus penetrates by the roots of flax at an early stage of growth (vegetative  
57 stage) when the stems are approximately 10 cm high (Goudenhoofst et al., 2019). *V. dahliae*  
58 then progresses acropetally inside the conducting vessels of the xylem with raw sap. This  
59 environment is relatively poor in polysaccharides or mineral resources (Klosterman et al.,  
60 2011). Yet, the evolution of *V. dahliae* in this environment prevents it from having to compete  
61 with other microorganisms present in the different tissues of the plant (Yadeta & Thomma,  
62 2013). Blum et al. (2018) have carried out inoculation test with *V. dahliae* during hydroponic  
63 cultivation of flax by dipping the stems in a solution containing conidia (spores produced by *V.*  
64 *dahliae*). After one week post inoculation (wpi), the cell differentiation zone was completely  
65 colonized by *V. dahliae* and most of hyphae were oriented parallel to the longitudinal axis of  
66 the roots. Located at the intercellular junction, the hyphae developed several swollen  
67 structures similar to appressoria that allow the fungus to penetrate into a host cell. The entry

68 of *V. dahliae* into the xylem causes a series of defence reactions from the plant, such as by the  
69 formation of tyloses, pectin gels or gums, phenolic compounds or inorganic sulfur (Blum et al.,  
70 2018). Leaves wilting appeared approximately three to four wpi and at eight wpi the fibre  
71 bundles were not yet reached by *V. dahliae*. The presence of mycelium in the primary  
72 superficial tissue cells of flax roots was also noted by Marchal (Marchal, 1940). These filaments  
73 formed chalydospores, which are disposed in small chains or gathered into bunches to  
74 constitute microsclerotia.

75 The systematic development of *V. dahliae* causes the wilting of the plant due to a disturbance  
76 of the flow of raw sap in the xylem. Nevertheless, the first symptoms, which also include a  
77 wilting of the leaves starting at the base of the stem, and a chlorose (discoloration) of the stem  
78 are not sufficient to establish a reliable diagnostic at this stage because these symptoms are  
79 very similar to that caused by hydric stress of other fungal pathogens such as *Fusarium* spp.  
80 (Blum et al., 2018). The symptoms are more distinctive at the flower stage of flax and  
81 especially during retting, with a metallic blue coloration of the stems and easily separable fibre  
82 bundles from the woody core of the stem. *V. dahliae* can cause up to a 60 % loss in long fibre  
83 yield at the scutching step of fibre transformation, and consequently an important production  
84 of flax tows (Arvalis, 2019). Thus, the decrease in long fibre yield is due to the fibre breakage  
85 induced by the disease but the number of single fibres in the plant does not really change, the  
86 main difference is the change in fibre morphology, inducing a large number of short fibres, i.e  
87 flax tows.

88 The control of the development of *V. dahliae* is particularly difficult because of its important  
89 persistence in the soil (Bressan et al., 2016), and also because of its polyphagia (Mol, 1995).  
90 There are currently only very few resistant plant species and none of them concern fibre flax.  
91 Rotational cultures (every 6 or 7 years regarding flax) are therefore necessary with the

92 introduction of non-host or resistant species between the cultivation of at-risk species in order  
93 to limit the development of pathogens such as verticillium wilt. There are currently no  
94 fungicides nor biocontrol of seeds, vegetation or soil. Other advices include the management  
95 of cultural residues, in particular by exporting the straws from the fields (which is already the  
96 case for fibre flax) and the cleaning of the harvest material to avoid the contamination of  
97 neighbouring parcels (Valade et al., 2019). New detection methods based on DNA analysis  
98 allowed to quantify and to establish maps of the infected field zone. Knowledge of the density  
99 of *V. dahliae* in a parcel before sowing can also be an interesting way to protect future cultures  
100 (Bressan et al., 2016).

101 The consequences of *V. dahlia* on flax fibre yields are important, but its impact at the scale of  
102 elementary fibres is still unknown. Its consequences for a use in technical applications, for  
103 example as biocomposite reinforcement material and textile, is, to the best of our knowledge,  
104 unexplored. This study aims at the mechanical characterization of elementary fibres of a flax  
105 sample infected by *V. dahliae* on fibre bundles to understand its evolution. This investigation  
106 was further completed, at the cell wall scale, with measures by atomic force microscopy (AFM)  
107 in peak-force quantitative nanomechanical mapping mode. Scanning electron microscopy  
108 observations, bioinformatic genomic assessment and biochemical assessment were also used  
109 to better explain the impacts of *V. dahliae* on the mechanical properties of flax at the scale of  
110 cell walls and of elementary fibres.

111

112 **2. Materials and Methods**

113 **2.1. Flax fibres and bundles**

114 A sample of reference long scutched flax (*Linum usitatissimum L.*) fibres from the Avian variety  
115 was provided by Van Robaeys Frères (France). It was cultivated in 2017 in France in the Picardy  
116 region and corresponds to a good quality sample regarding a use in the textile field. A sample  
117 of fibres of the same variety cultivated in 2020 also in the Picardy region, and impacted by *V.*  
118 *dahlia* was studied in comparison. For these two cultivation years, the cumulative rain fall and  
119 mean temperatures as well as mean maximum and minimum temperatures were analogous.

120 The two batches were dew-retted for approximatively 6 weeks. The level of infection was not  
121 quantified but corresponds to the infection present on the stems following retting. The  
122 elementary fibres and fibre bundles were extracted by hand from the median part of the  
123 stems. Only the fibres from the middle of the stem were studied.

124

125 **2.2. Molecular authentication (DNA extraction, PCR, and sequencing).**

126 Approximately 100 mg of mycelium present at the surface of infected flax samples (Fig.1.a)  
127 was scraped and genomic DNA was extracted using the NucleoSpin PlantII Kit (Macherey–  
128 Nagel, France). The ribosomal DNA internal transcribed spacer region (rDNA-ITS) was then  
129 amplified by PCR using the ITS5/ITS4 primers as described in (Navarro et al., 2021) DNA  
130 amplification was performed in a Mastercycler Nexus GSX1 (Eppendorf, Montesson, France)  
131 and the PCR product was sequenced by Genewiz (Leipzig, Germany). The sequence was  
132 compared with sequences in the GenBank database using blastn.

133

134

### **2.3. Scanning electron microscopy (SEM) observations**

135

The flax fibres and bundles were observed using a Jeol JSM 6460LV (France) scanning electron

136

microscope (SEM). Each sample was first sputter coated with a thin layer of gold (Edwards

137

Sputter Coater, France) in high vacuum to avoid them from charging during analysis.

138

139

### **2.4. Tensile testing of elementary fibres**

140

The flax fibres were extracted by hand and glued to paper frames with a gauge length of 10

141

mm. Their diameter was then measured by optical microscopy (Olympus AX70) at six positions

142

along each fibre and the cross-sectional area was calculated from the mean measured

143

diameter. The tensile tests were carried out using an MTS machine (France) with a 2 N force

144

sensor and a tensile speed of 1 mm/min. The compliance of the set-up was measured and used

145

for the calculation of the strain and tangent modulus **according to the NF T25-501-3 standard.**

146

The latter was measured from the last linear zone of the stress strain curve of each fibre, in

147

agreement with the NF T 501-2 standard.

148

149

### **2.5. Atomic force microscopy (AFM)**

150

The longitudinal indentation modulus of the flax cell walls (measured on fibre cross section)

151

was investigated by atomic force microscopy (AFM, Bruker, USA) in peak force quantitative

152

nanomechanical mapping mode (PF-QNM). Flax fibres bundles were first cut in 10 mm

153

segments and glued upon 5 mm long paper frames. The embedding protocol is precisely

154

described in (Goudenhoofft et al., 2018). Transversal sections of the samples were obtained

155

with an ultramicrotome (Leica Ultracut R) equipped with diamond knives (Diamond Histo and



156 Ultra AFM). The indentation modulus of the cell walls was measured with a specific tip with a  
157 stiffness constant of 139 N/m, a tip radius between 15 and 35 nm, a scan frequency of 8  $\mu\text{m/s}$   
158 and a maximum force of 200 N were used for the measures. The tip and probe cantilever  
159 calibration protocol is described by Arnould et al. (Arnould et al., 2017). In order to control the  
160 AFM measurements, the indentation modulus of several positions across the thickness of the  
161 G cell wall layer but also of the embedding resin were measured by nanoindentation prior to  
162 AFM measurements. Image treatment was carried out using the Gwyddion software (Nečas &  
163 Klapetek, 2012).

164

## 165 **2.6. Biochemical composition analysis**

166 Wet chemical analysis was used to evaluate the monosaccharide content of both the reference  
167 and the verticillium infected fibres. First, sample homogenization was carried out by  
168 cryogrinding approximately 1g of each flax fibre bundles samples (SPEX 6700 freezer Mill). The  
169 latter were then hydrolysed in 12 M H<sub>2</sub>SO<sub>4</sub> (Sigma Aldrich) for 2 h maintained at 25 °C by a  
170 heating plate, then an additional hydrolysis was carried out during 2 h at 100 °C with 1.5 M  
171 H<sub>2</sub>SO<sub>4</sub> in which inositol was used as internal standard. Individual neutral monosaccharides  
172 (arabinose, rhamnose, fucose, glucose, xylose, galactose and mannose) were analysed as their  
173 alditol acetate derivatives (Blakeney et al., 1983) by gas-liquid chromatography (Perkin Elmer,  
174 Clarus 580, Shelton, CT, USA) equipped with an DB 225 capillary column (J&W Scientific,  
175 Folsom, CA, USA) at 205°C, with H<sub>2</sub> as the carrier gas. Furthermore, Galacturonic Acid (GalA)  
176 and Glucuronic Acid (GlcA) were determined by an automated m-hydroxybiphenyl method  
177 (Thibault, 1979) and merged as Uronic acid (UrAc). Standards of carbohydrate solutions with  
178 three known concentrations were used for calibration. Analyses were performed in three

179 independent assays. The total monosaccharide content is the sum of each monosaccharide  
180 amount, and is expressed as the percentage of the dry matter mass.

181 The lignin content was quantified by colorimetric analysis for each sample in triplicates  
182 subsequently to the acetyl bromide method (Hatfield & Fukushima, 2005). Sample mass was  
183 approximately 20 mg per assay, the chemicals used were laboratory grade from Sigma Aldrich,  
184 and the lignin content is reported as the percentage of the dry matter mass.

185

## 186 **2.7. Analysis of *V. dahlia* genome for secreted carbohydrate-active enzymes**

187 All putative proteins of *Verticillium dahlia* genome (Klosterman et al., 2011) were compared to  
188 the entries in the CAZy database (Drula et al., 2022). A homemade pipeline which combines  
189 BlastP (<https://blast.ncbi.nlm.nih.gov/Blast.cgi>) and HMMER3 (<http://hmmer.janelia.org/>)  
190 tools was used to compare protein models with the sequences of the CAZy modules. The  
191 proteins with E-values smaller than 0.1 were further screened by a combination of BlastP  
192 searches against libraries created from the sequences of the catalytic and non-catalytic  
193 modules. HMMER3 was used to query against a collection of custom-made hidden Markov  
194 model (HMM) profiles constructed for each CAZy family. This was followed by a manual  
195 inspection by expert curators to resolve borderline cases. Peptide signals were predicted using  
196 Phobins (Käll et al., 2004).

197

198

199 **3. Results and Discussion**

200 **3.1. SEM observations**

201 The presence of the fungal pathogen was confirmed on infected flax samples using the  
202 barcoding ITS gene. After DNA extraction and sequencing, the best match was *V. dahliae* with  
203 high confidence (more than 99% sequence identity). We also evaluated the presence of *V.*  
204 *dahliae* with SEM observations of the infected flax sample as compared to reference flax fibres  
205 (**Figure 1**). It is possible to notice that the reference flax fibre bundles are comparatively clean  
206 with little organic residues at their surface. The fibres are individualised and reasonably  
207 accessible, as shown in **Figure 1b** and d. However, the fibres of flax infected by *V. dahliae*  
208 fibres **display** many microsclerotia at the surface of the bundles (**Figure 1c, e**), and fungal  
209 filaments which correspond to *V. dahlia* mycelium (Marchal, 1940). These microsclerotia are  
210 clinging to the cell walls and colonize the flax bundles continuously along their length.

211

212

**Figure 1**

213

214 *V. dahliae* develops in the flax xylem during its growth and progresses acropetally with the raw  
215 sap towards the plant's apex. Studies of incubation have shown that this progression is  
216 performed during several weeks and that after 8 wpi, *V. dahliae* had not yet reached the fibres  
217 situated at the periphery of the stem (Blum et al., 2018), the authors suggested that fibre  
218 degradation could take place when the stem is unable to defend itself anymore. The growing  
219 of flax takes place during 12 to 16 weeks, and it is possible that the fibre infection takes place  
220 before their maturity, but also following stem pull-out and during the retting phase. From

221 these images, it is not possible to know at which growth stage the fibres were colonized by *V.*  
222 *dahliae*, but the microsclerotia are extremely present following sample retting and storing.

223

224

### Figure 2.

225

226 **Figure 2** shows the visual longitudinal aspect of an elementary fibre infected by *V. dahlia*  
227 observed by SEM. Examination reveals microcracks that are visible lengthwise. Such defects  
228 are not present on the reference elementary flax fibres. Furthermore, the infected fibres show  
229 an important surface roughness, resulting from concomitant mechanical and enzymatic  
230 degradation mechanisms caused by *V. dahliae*.

231

232

### 3.2. Tensile mechanical properties of the elementary flax fibres

233 The results of the tensile tests are given in **Table 1** for both the reference flax fibres and the *V.*  
234 *dahliae* sample. A clear decrease is noticeable in the mechanical properties for the latter  
235 contaminated sample. The tangent modulus of the colonized fibres shows a -20 % reduction  
236 compared to the reference sample, for which the modulus is measured at  $43.6 \pm 17.0$  GPa.  
237 Despite a value that is located in the lower standard deviation of the mean tangent modulus  
238 measured at  $52.5 \pm 8.5$  GPa from testing 50 different flax fibre batches (Baley & Bourmaud,  
239 2014), the measured modulus is in agreement with the literature. Furthermore, a Mann-  
240 Whitney statistical nonparametric test was carried out on the two populations and confirms  
241 that the two populations are significantly different with a significant level of 0.05.

242

243

### Table 1

244

245 The infection by *V. dahliae* also causes an important decline in the tensile strength at break of  
246 the elementary fibres, measured at  $773 \pm 374$  MPa for the reference sample, against  $522 \pm 322$   
247 MPa for the *V. dahliae* sample, which represents a -32 % relative difference. Finally, the strain  
248 at break of the elementary fibres is also affected, with a -15 % decrease measured for the *V.*  
249 *dahliae* sample compared to the reference sample, at  $1.78 \pm 0.69$  % and  $2.09 \pm 0.59$  %  
250 respectively. A Mann-Whitney statistical test also confirms for both the strength and the strain  
251 at break that the reference and Verticillium wilt fibre samples are significantly different.

252 Lefeuvre et al. (Lefeuvre, Bourmaud, et al., 2015) have shown the importance of the fibre  
253 sampling zone on their cross section area and tensile mechanical properties. Furthermore, a  
254 correlation has been observed between the tangent modulus of flax fibres and their measured  
255 diameter before tensile testing (Charlet et al., 2009). Yet, the diameter of the flax fibres from  
256 the two tensile-tested samples is similar at  $15.23 \pm 3.13$   $\mu\text{m}$  and  $16.21 \pm 3.39$   $\mu\text{m}$  for the  
257 reference and the *V. dahliae* sample, respectively, suggesting the representative results.

258 **Figure 3** allows a comparison of the cumulative probability of the strength at break of both the  
259 reference and the *V. dahliae* sample. The figure brings in light that 80 % of the fibres affected  
260 by *V. dahliae* have a strength at break at 650 MPa, whereas this corresponds to that of 50 % of  
261 the reference sample. The first and third quartile of the *V. dahliae* sample are at approximately  
262 200 and 600 MPa, against 366 and 968 MPa for the reference flax fibres.

263

264

### Figure 3

265

266 The damages visible on the outer surface and cross-section of the fibres following *V. dahliae*  
267 (**Figure 2**), partly explain the loss of strength at break for this sample. The irregular section  
268 causes localised zones of strain concentration during the tensile tests. The strength at break is  
269 highly dependent on the presence of microscopic defects according to the Griffith theory  
270 (Griffith, 1921). These damages lead also to an overestimation of the real cross section of  
271 fibres at their rupture location, and therefore an underestimation of the strength at break and  
272 the tangent modulus. But because of the visual aspect of the transversal cross-section (**Figure**  
273 **4.b**) and of the negligible difference between the diameters of the fibres from both samples,  
274 the comparison of the range of their mechanical properties is possible. Nevertheless, the  
275 defects caused by *V. dahliae*, such as the ones shown in **Figure 2**, induce localized strain  
276 concentrations during tensile testing, which may create local damages at the origin of the  
277 rupture initiation of the fibre (**Table 1**). It is also possible that defect zones like kink-bands are  
278 possible sensitive zones to the action of enzymes (Thygesen et al., 2011), slightly decreasing  
279 the strength at break of the fibres during tensile tests.

280 The mechanical properties given in **Table 1** highlight the decrease in the tangent modulus. A  
281 modification in the cellulose and pectin content in the cell walls can explain this phenomenon,  
282 as well as the difference in the measured strength at break between both samples of the study  
283 (**Figure 3**) (Lefevre, Le Duigou, et al., 2015). Crystalline cellulose is indeed the major  
284 polysaccharide responsible for the mechanical properties of plant cell walls. It is present in the  
285 form of microfibrils well-ordered with a small helical angle with regards to the fibre axis in the  
286 S2-G layer of flax fibres. The cellulases and pectinases secreted by *V. dahliae* can modify the  
287 microstructure of the cell walls, and hypothetically impact the behaviour of the microfibrils  
288 during a tensile test. A lower amount of pectin combined with a modification of the internal

289 organisation of the cell walls in particular with the appearance of important defects can also  
290 decrease the load transfer between the cellulose microfibrils. In the literature, it is reported  
291 that a reduction of approximately 10 % in weight of non-structural polysaccharides content  
292 leads to a reduction by a factor 2 of the shear strength necessary to initiate the reorientation  
293 of the cellulose microfibrils in the cell walls of elementary flax fibres (Lefeuvre, Le Duigou, et  
294 al., 2015). Despite the important loss of tangent modulus and strength at break observed for  
295 the verticillium wilt sample with regard to the reference sample (**Table 1**), these elementary  
296 fibres have sufficient mechanical properties to be used as composite materials reinforcements.

297

### 298 **3.3. Longitudinal modulus of the cell walls measured by nanoindentation**

299 To quantify the evolution of the stiffness of cell walls of flax fibres, AFM analyses were carried  
300 out by means of a longitudinal solicitation in the transverse section of fibre bundles. **Figure 4a**  
301 and b show a cartography of the indentation modulus of the cell walls of respectively the  
302 reference and Verticillium wilt flax fibres obtained by the peak-force quantitative  
303 nanomechanical mapping imaging mode. **Figure 4c** presents the distribution of the indentation  
304 modulus of both sample but from the different observation zones. The mean indentation  
305 modulus of the reference sample is  $20.39 \pm 3.54$  GPa against  $15.72 \pm 2.61$  GPa for the *V.*  
306 *dahliae* sample, which represents a relative decrease of -23 %.

307

308

**Figure 4**

309

310 The modulus of indentation obtained by AFM for the reference fibres is coherent with the  
311 values of the literature, between 18 and 24 GPa (Goudenhoft et al., 2018)(Marchal,  
312 1940)(Nečas & Klapetek, 2012)(Blakeney et al., 1983). Despite the dependence of sample  
313 preparation and in particular to the stiffness of the embedding resin and the geometry of the  
314 tip used for the AFM measurements, the values obtained here are coherent for mature and  
315 healthy flax fibres.

316 In **Figure 4b**, the local indentation modulus of the fibres from the *V. dahliae* sample is shown.  
317 Unlike the reference sample, the fibres have an irregular cross-section and degraded cell walls.  
318 These irregularities are located at the outer periphery of the cells and sometimes reach the  
319 lumen, as visible in the detailed view of **Figure 5a**. The indentation modulus distribution profile  
320 along the white arrow of **Figure 5a**, as shown in **Figure 5b**, highlights a relatively constant  
321 stiffness at approximately 16 GPa throughout the cell wall surface, with a slight decrease of the  
322 stiffness at the external periphery of the fibres. The lumen is visible at a distance of 3.8  $\mu\text{m}$   
323 along the plotted profile, and another internal porosity seems to be present at a distance of 2  
324  $\mu\text{m}$ . These additional internal porosities have in fact been measured in elementary fibres in the  
325 literature by (Richely et al., 2021).

326

327

## Figure 5

328

329 *V. dahliae* can release numerous enzymes during the vascular infection of a plant, in particular  
330 cellulases and a multitude of pectin degrading enzymes. These enzymes are particularly well  
331 adapted to the environment in which *V. dahliae* evolves, specifically in the xylem fluids where  
332 the concentration in carbohydrates, amino acids and inorganic ions is weak (Klosterman et al.,



333 2011). The *V. dahliae* genome contains an important number of polysaccharide lyases and  
334 rhamnogalacturonan lyases (Klosterman et al., 2011). This diversity of enzymes illustrated the  
335 capacity of *V. dahliae* to degrade cell walls and allows *V. dahliae* to progress inside the vessels  
336 of the xylem. The degraded surface aspect of the elementary fibres is a consequence of the  
337 colonization of *V. dahliae*. Particularly, the pectinases and cellulases produced by *V. dahliae*  
338 can be at the origin of the important decrease in local stiffness of the cell walls measured by  
339 AFM due to a possible reduction of cellulose crystallinity. Local stiffness is indeed dependent  
340 on the cellulose content and on the chemical composition of the cell walls (Gindl et al., 2002;  
341 Gindl et al., 2004). The influence of pectin on the mechanical behaviour of elementary fibres  
342 has been investigated by Lefeuvre et al. (Lefeuvre, Le Duigou, et al., 2015). The extraction of  
343 polysaccharides presents in the amorphous matrix of the cell walls, notably in the pectin  
344 matrix, leads to a -30 % drop in the tensile strength at break of the fibres. A -29 % drop in the  
345 modulus of fibres has also been shown following the extraction of structural polysaccharides.

346

347

### Figure 6

348

349 The cell wall degradation of the *V. dahliae* sample fibres is also confirmed by SEM  
350 observations, shown in **Figure 6**. The fibres are mainly degraded at their periphery but also  
351 locally towards the centre of the lumen. The reference flax fibre bundles show in contrast the  
352 expected mature polygonal cell walls with a very small lumen here.

353

354 **3.4. Biochemical composition**

355 **Table 2** shows the results of the biochemical composition analysis carried out on fibres  
356 bundles of the reference and verticillium wilt flax sample. The hemicelluloses composition is  
357 overall very similar. A 22 % and a 38 % relative increase in mannose and xylose respectively for  
358 the *V. dahliae* fibre sample compared to the reference fibres can be noted. There is also a 28 %  
359 relative difference between the lignin content of the reference and the Verticillium wilt  
360 sample, but no significant difference in the amount of glucose between the two samples.

361

362 **Table 2**

363

364 Studies have shown that *V. dahliae* possesses an important enzymatic arsenal containing an  
365 important and complex amount of polysaccharide lyases, including in particular pectate lyases  
366 and rhamnogalacturonan lyases (Klosterman et al., 2011). Klosterman et. al suggested that this  
367 allowed the pathogen to use the pectin in the plant cell walls or from the gels released in the  
368 xylem by plant infection reaction mechanisms. The pectin degrading enzymes may directly  
369 contribute the progression of the *V. dahliae* within the xylem vessels, and eventually allow it to  
370 reach the flax fibre bundles (Blum et al., 2018).

371 The slight differences in lignin and other polysaccharide contents can be attributed to inter-  
372 variety scatter or to soil and climate differences between the cultivation areas and times of the  
373 samples. This modest difference could possibly be due to a defence reaction from the plant  
374 (Paul-Victor et al., 2017) but the time of infection is not precisely known in the present study.

375

376

### 3.5. Genomic analysis of *V. dahliae* carbohydrate-active enzymes

377

To evaluate the degradation potential of *V. dahliae*, we performed annotation of its genome

378

with a focus on enzymes targeting plant cell wall polysaccharides, *i.e.*, CAZymes predicted to

379

degrade cellulose, hemicelluloses or pectin. Out of the 489 CAZymes predicted to be involved

380

in polysaccharides catabolism, 311 CAZymes are predicted to be secreted, of which 172 are

381

predicted to be involved in cellulose, hemicelluloses and pectin degradation. When focusing

382

only on the plant cell wall degrading CAZymes secreted, we noticed that *V. dahliae* displays an

383

impressive array of enzymes belonging to the different CAZy classes (GH, glycoside hydrolases;

384

AA, auxiliary activity enzymes; CE, carbohydrate esterases; PL, polysaccharide lyases)

385

targeting the different component of plant cell wall (cellulose, hemicelluloses and pectin)

386

(**Figure 7**).

387

388

#### Figure 7

389

390

As previously observed, *V. dahliae* is predicted to secrete 72 pectin-degrading enzymes

391

including 38 polysaccharide lyases. Another interesting observation is the large array of lytic

392

polysaccharide monooxygenases (LPMOs) with 26 enzymes from the families AA9 and AA16,

393

predicted to be involved in the oxidative degradation of cellulose (Bennati-Granier et al., 2015;

394

Filiatrault-Chastel et al., 2019) together with their redox partners (e.g., AA3-AA8 cellobiose

395

dehydrogenase) required to trigger LPMO activity (**Table 3**). Interestingly, fungal LPMOs create

396

nicking points that lead to the disruption of the cellulose fibre structure with rupture of chains

397

(Villares et al., 2017). This bioinformatic analysis is in line with SEM and biochemical

398 assessment, supporting the high degradation potential of *V. dahliae*, being in capacity to  
399 simultaneously degrade major components of flax cell walls.

400

401

#### 402 **4. Conclusion**

403 Flax fibres are sensitive to numerous diseases during its cultivation, in particular to the fungus  
404 *V. dahliae* that develops inside the vessels of the xylem of flax during growth of the plant. Even  
405 if *V. dahliae* is a vascular parasite, *V. dahliae* can reach the fibres bundles, especially following  
406 the stem pull out, and can cause important damages to the fibre yield during scutching. Its  
407 consequences on the mechanical properties of elementary fibres are also substantial, with a -  
408 20 % decrease in the fibre stiffness and a -32 % decrease in fibre strength for the studied  
409 sample. This could be explained by the local cell wall damages caused by the CAZymes  
410 secreted by *V. dahliae*, which may be at the origin of strain concentrations and therefore  
411 premature fibre failure.

412 They also affect the volume of the cell walls, causing a -25 % decrease in the indentation  
413 modulus measured by AFM for elementary flax fibres compared to reference fibres. We  
414 suggest that the pectinases and cellulases (especially the LPMOs) predicted to be secreted by  
415 *V. dahliae* could be at the origin of such a modification in the cell walls affected by *V. dahliae*.  
416 However, the development stage of *V. dahliae*, the origin of the flax sample (location of the  
417 fibres along the stem, growth conditions, etc.) are all factors that must be taken into account  
418 when considering the mechanical property evolution of the fibres. Future work perspectives  
419 concern a possible progression of the Verticillium wilt disease from the xylem of the flax stem  
420 to the peripheral fibre bundles, but also a hypothetical evolution of the pathogen on the fibres

421 during their storage and use. In this objective, a hydroponic/root dipping method for plant  
422 cultivation and inoculation, and green fluorescent protein modified strains could be carried out  
423 to follow the *in-planta* development of *V. dahliae* at lab scale.

424 Without a preventive solution other than crop rotation in fields, the consequences of this  
425 disease, from which the symptoms are difficult to detect with certainty during the growth of  
426 flax stems, are significant. This is alarming regarding the yields at the different processing steps  
427 of flax fibres as *V. dahliae* can already cause up to a -60 % loss in fibre yields at the scutching  
428 step, and has important consequences on the mechanical properties of flax fibres as  
429 reinforcement of biocomposites or textiles.

430

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438

439

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554

555 **Figure captions**

556 **Figure 1.** Longitudinal SEM observations of a) raw infected flax stems b, d) the reference flax  
557 fibre sample, and of c, e) the *V. dahliae* flax fibre sample at different scales.

558 **Figure 2.** Longitudinal SEM observations of *V. dahliae* flax fibres showing a) microcracks in the  
559 cell wall and b) a rugous surface.

560 **Figure 3.** Cumulative probability of the strength at break of the reference and *V. dahliae*  
561 elementary flax fibre samples.

562 **Figure 4.** AFM measurements of a) the reference flax sample and b) the Verticillium wilt  
563 sample. The colour scale gives the related indentation modulus in GPa; c) Distribution of the  
564 modulus for the reference and the Verticillium wilt flax samples.

565 **Figure 5.** a) Detailed view of fibre cross section AFM measurements from **Figure 4b**. The scale  
566 on the left provides the related modulus in GPa. The orange zones show an example of the  
567 area used to calculate the mean fibre modulus at 15.85 GPa; c) Evolution of the modulus  
568 obtained across the profile shown by the white arrow in the image b).

569 **Figure 6.** Transversal SEM observations of elementary flax fibres of a) the reference sample  
570 and b) the *V. dahliae* sample.

571 **Figure 7.** Number of *V. dahliae* CAZymes (cellulases, hemicellulases and pectinases) predicted  
572 to be secreted. The CAZymes secreted were sorted by class (GH, AA, CE, PL) according to the  
573 CAZy classification. GH, glycoside hydrolases; AA, auxiliary activity enzymes; CE, carbohydrate  
574 esterases; PL, polysaccharide lyases. The list of CAZymes (sub)families is provided in Table 3.

575

576 **Table captions**

577 **Table 1.** Tensile mechanical properties of the reference and Verticillium wilt elementary flax  
578 fibre samples.

579 **Table 2.** Biochemical composition of the reference and Verticillium wilt flax fibre samples.

580 **Table 3.** List of *V. dahliae* CAZymes encoded by the genome and predicted to be secreted.

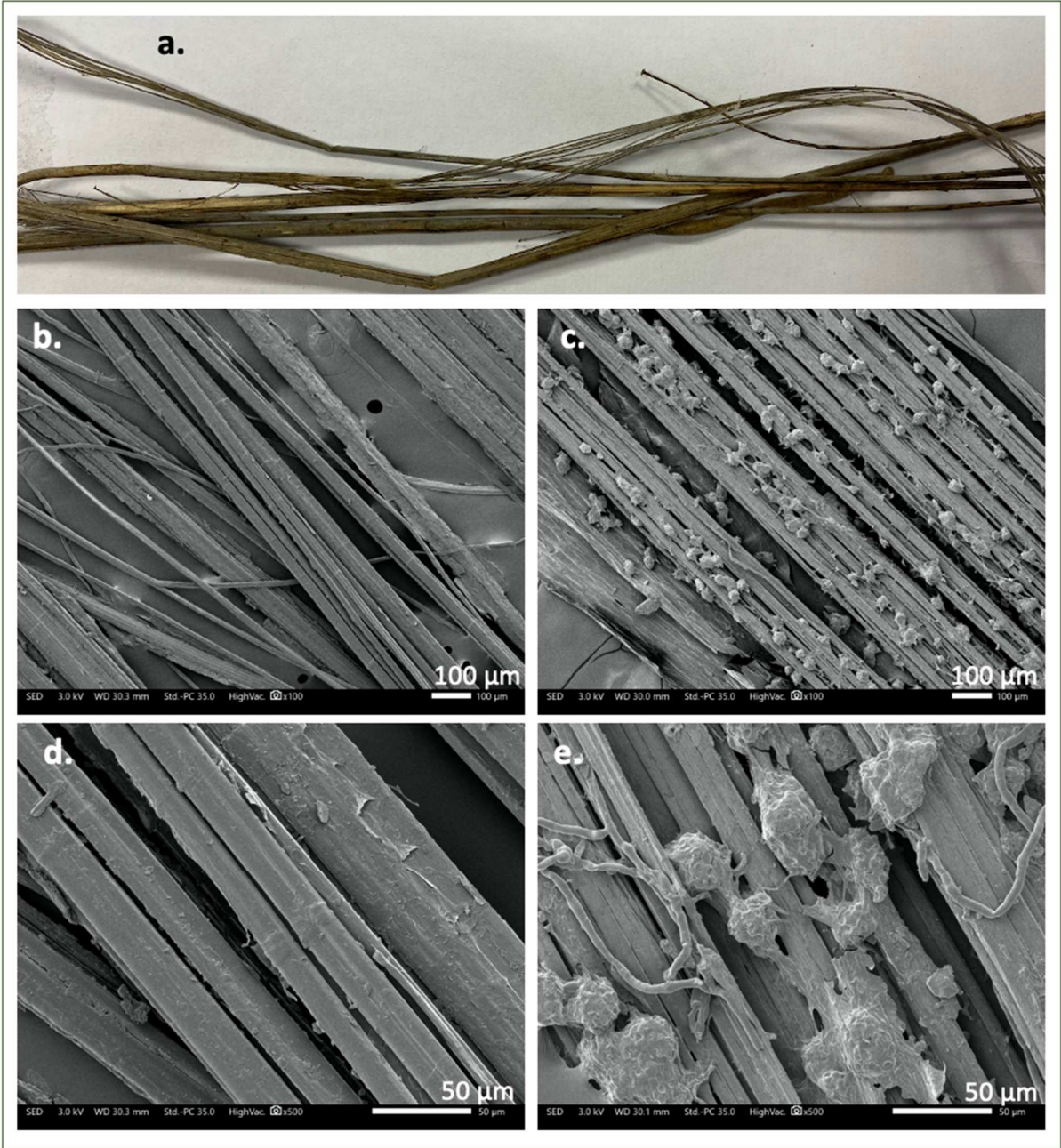
581 CAZymes are listed with an indication of their family (and subfamily when available), their

582 number, their putative activity and the polysaccharide predicted to be targeted.

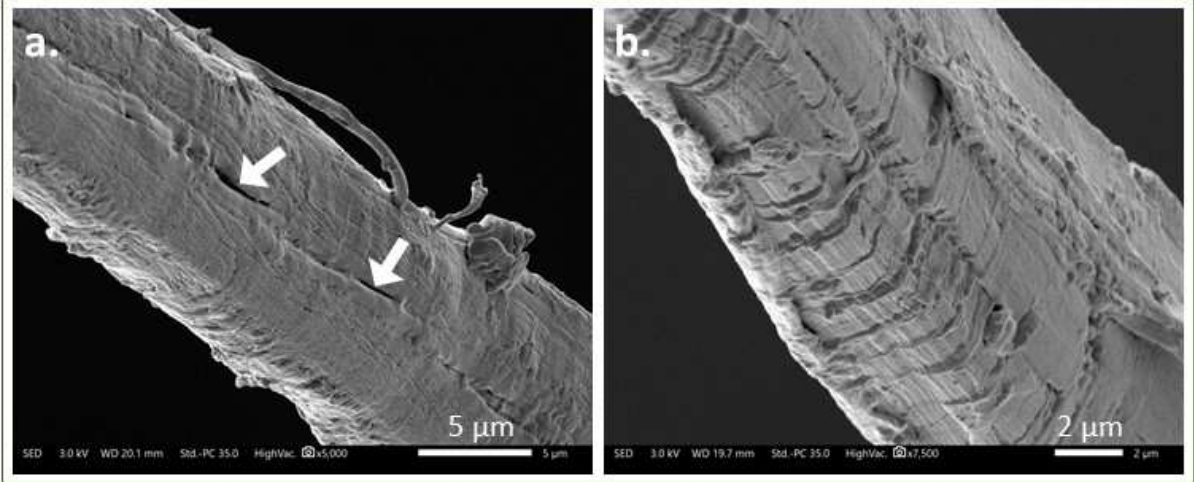
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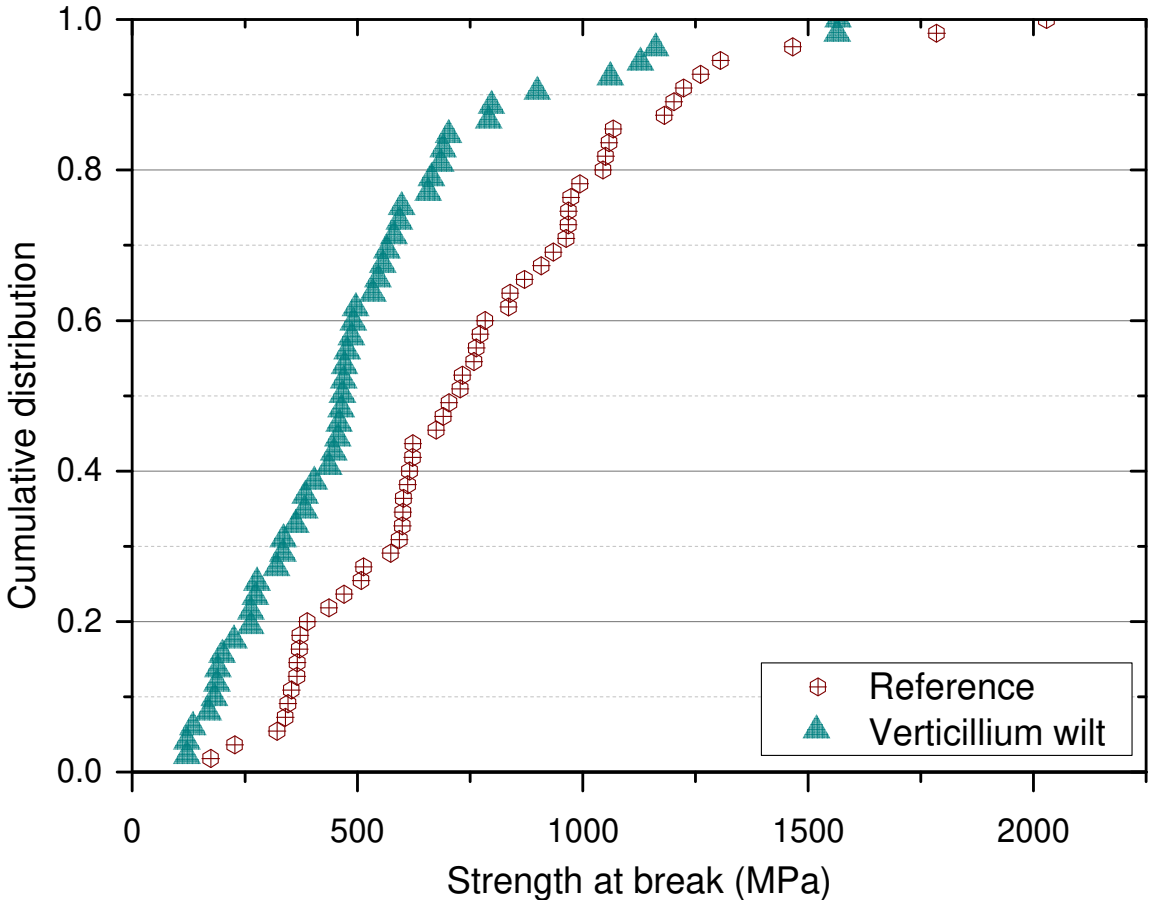
**Figure 1.** Longitudinal SEM observations of a) raw infected flax stems b, d) the reference flax fibre sample, and of c, e) the *V. dahliae* flax fibre sample at different scales.



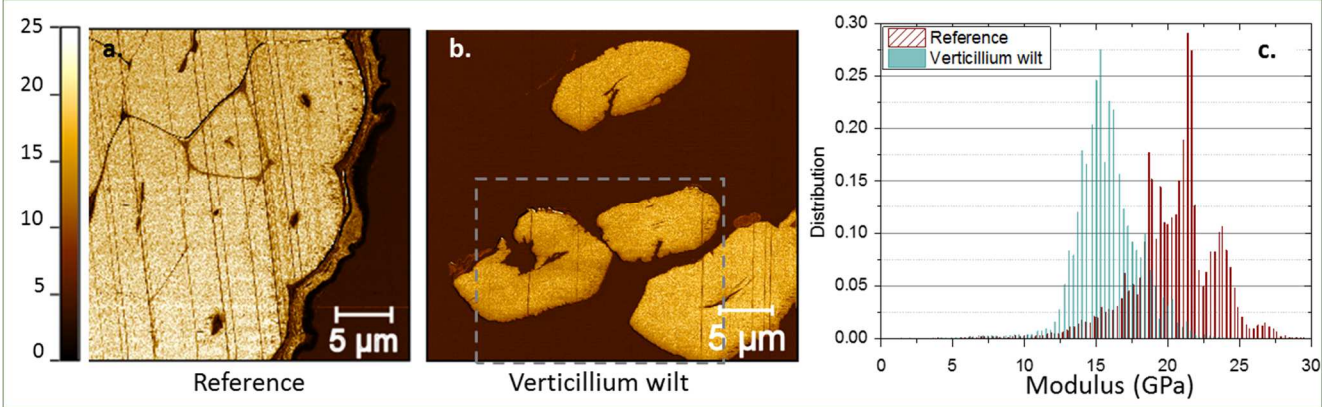
**Figure 2.** Longitudinal SEM observations of *V. dahliae* flax fibres showing a) microcracks in the cell wall and b) a rugous surface.



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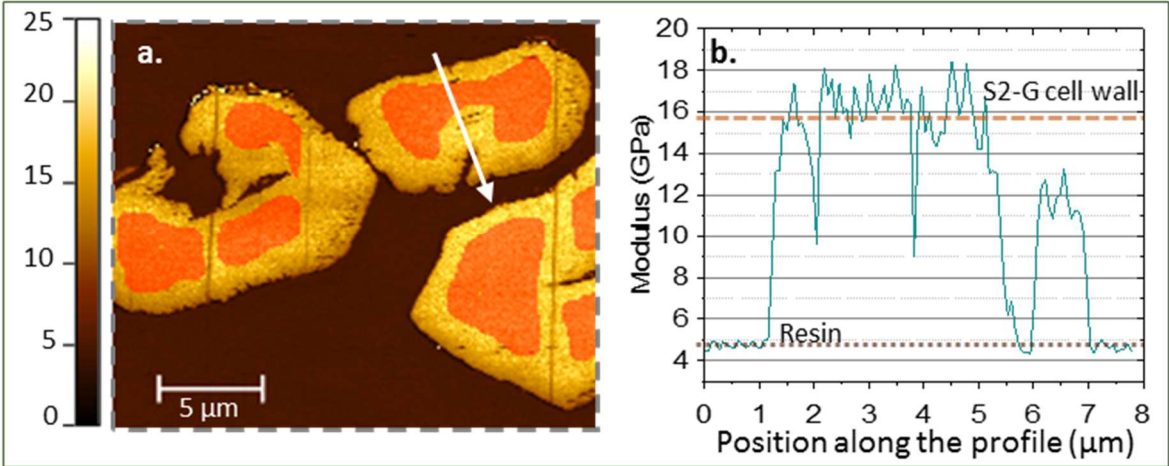


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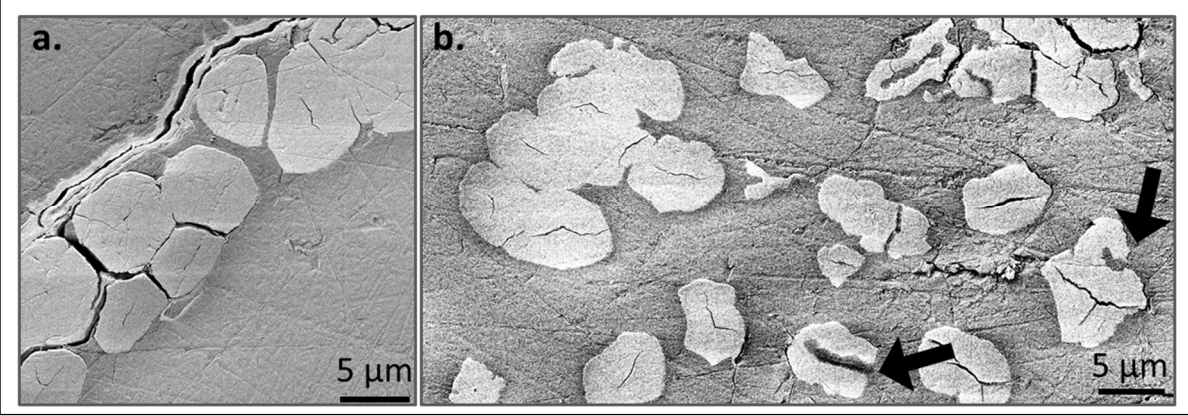




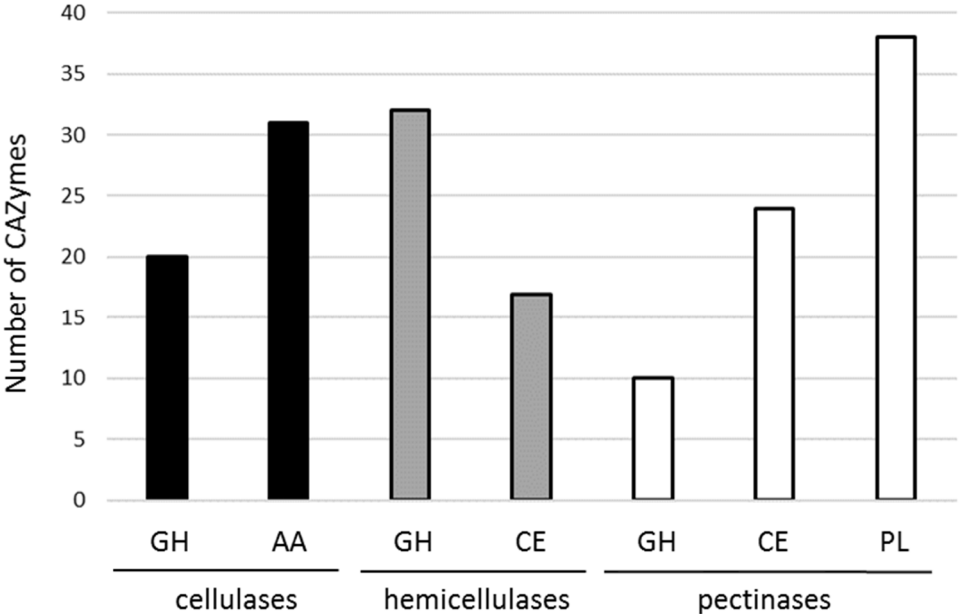
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**Table 1.** Tensile mechanical properties of the reference and Verticillium wilt elementary flax fibre samples.

Sample	Number of samples	Diameter ( $\mu\text{m}$ )	Tangent modulus (GPa)	Strength at break (MPa)	Strain at break (%)
Reference	55	15.2	43.6	773	2.09
<i>SD</i>		3.13	17.0	374	0.59
Verticillium wilt	52	16.2	34.9	522	1.78
<i>SD</i>		3.39	16.0	322	0.69

**Table 2.** Biochemical composition of the reference and Verticillium wilt flax fibre samples.

Biochemical composition (% of dry mass)	Reference		Verticillium wilt	
	Mean	<i>SD</i>	Mean	<i>SD</i>
Fucose	0.04	0.03	0.06	0.01
Arabinose	1.13	0.28	0.98	0.11
Rhamnose	0.74	0.06	0.72	0.02
Galactose	4.37	0.33	4.52	0.03
Xylose	0.85	0.18	1.17	0.14
Mannose	4.27	0.28	5.22	0.01
Galacturonic Acid	0.50	0.01	0.49	0.01
Glucuronic Acid	0.16	0.26	0.18	0.04
Glucose	69.79	1.01	65.14	4.19
Lignin	2.96	0.08	3.80	0.02

**Table 3.** List of *V. dahliae* CAZymes encoded by the genome and predicted to be secreted. CAZymes are listed with an indication of their family (and subfamily when available), their number, their putative activity and the polysaccharide predicted to be targeted.

CAZy (sub)Family and module associated	Number of enzymes predicted to be secreted per family	Putative activity	Polysaccharide targeted
AA9	20	Lytic polysaccharide monooxygenase	cellulose
AA9-CBM1	4	Lytic polysaccharide monooxygenase	cellulose
AA16	2	Lytic polysaccharide monooxygenase	cellulose

AA7	2	Glucosylglycosyl hydrolase	cellulose
AA8-AA12	1	Pyrroloquinoline quinone-dependent oxidoreductase	cellulose
AA8-AA3_1	1	Cellobiose dehydrogenase	cellulose
AA8-AA3_1-CBM1	1	Cellobiose dehydrogenase	cellulose
CBM1-GH5_4	1	Endo-glucanase	cellulose
CBM1-GH5_5	1	Endo-glucanase	cellulose
CBM1-GH6	1	Endo-glucanase	cellulose
GH6	3	Endo-glucanase	cellulose
GH7	3	Cellobiohydrolase	cellulose
GH7-CBM1	3	Cellobiohydrolase	cellulose
GH12	3	Endo-glucanase	cellulose
GH12-CBM1	1	Endo-glucanase	cellulose
GH45	1	Endo-glucanase	cellulose
GH45-CBM1	1	Endo-glucanase	cellulose
GH131	1	Endo-glucanase	cellulose
GH131-CBM1	1	Endo-glucanase	cellulose
GH5_7	3	Endo-mannanase	hemicellulose
GH5_7-CBM1	1	Endo-mannanase	hemicellulose
CBM1-GH5_7	1	Endo-mannanase	hemicellulose
GH10	2	Endo-xylanase	hemicellulose
GH10-CBM1	1	Endo-xylanase	hemicellulose
GH11	5	Endo-xylanase	hemicellulose
GH11-CBM1	1	Endo-xylanase	hemicellulose
GH27	2	Galactosidase	hemicellulose
GH27-CBM35	1	Galactosidase	hemicellulose
GH43_13	2	Arabinofuranosidase	hemicellulose
GH43_21	1	Arabinofuranosidase	hemicellulose
GH43_22-GH43_34	1	Arabinofuranosidase	hemicellulose
GH43_26	1	Arabinofuranosidase	hemicellulose
GH43_26-CBM42	1	Arabinofuranosidase	hemicellulose
GH43_29	1	Arabinofuranosidase	hemicellulose
GH43_30	1	Galactofuranosidase	hemicellulose
GH43_36	1	Arabinofuranosidase	hemicellulose
GH43-CBM1	1	Arabinofuranosidase	hemicellulose
CBM35-GH26	1	Endo-mannanase	hemicellulose
GH51	1	Arabinofuranosidase	hemicellulose
GH54-CBM42	1	Arabinofuranosidase	hemicellulose
GH67	1	Glucuronidase	hemicellulose
GH115	1	Glucuronidase	hemicellulose
CE1	4	Acetyl xylan / Feruloyl esterase	hemicellulose
CE1-CBM1	1	Acetyl xylan / Feruloyl esterase	hemicellulose
CE3	4	Acetyl xylan esterase	hemicellulose
CE4	4	Acetyl xylan esterase	hemicellulose
CBM1-CE15	1	Methyl glucuronoyl esterase	hemicellulose
CE16	3	Acetyl esterase	hemicellulose
CE4	1	Acetyl xylan esterase	pectin

CE5	13	Acetyl xylan esterase / cutinase	pectin
CE5-CBM1	1	Acetyl xylan esterase / cutinase	pectin
CE8	4	Pectin methylesterase	pectin
(CE8) <sub>5</sub>	1	Pectin methylesterase	pectin
CE12	4	Pectin acetyesterase	pectin
GH43_24-CBM35	1	Galactanase	pectin
GH53	1	Endo-galactanase	pectin
GH78	3	Rhamnosidase	pectin
GH93	1	Exo-arabinanase	pectin
GH105	3	Rhamnogalacturonyl hydrolase	pectin
GH146	1	Arabinofuranosidase	pectin
PL1	3	Pectate lyase	pectin
PL1_2	2	Pectate lyase	pectin
PL1_4	5	Pectate lyase	pectin
PL1_4-CBM1	1	Pectate lyase	pectin
PL1_7	4	Pectate lyase	pectin
PL1_9	1	Pectate lyase	pectin
PL1_10	1	Pectate lyase	pectin
PL3_2	10	Pectate lyase	pectin
CBM1-PL3_2	1	Pectate lyase	pectin
PL4	2	Rhamnogalacturonan endolyase	pectin
PL4_1	1	Rhamnogalacturonan endolyase	pectin
PL4_3	3	Rhamnogalacturonan endolyase	pectin
PL9_3	2	Pectate lyase	pectin
PL26	1	Rhamnogalacturonan exolyase	pectin
PL42	1	Rhamnohydrolase	pectin

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