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Abstract
Belowground materials from two miscanthus species were ground into fragments for preparing polyethylene composites. Both species show a lot of similarities in terms of polysaccharides, lignin and cell wall-linked *p*-coumaric and ferulic acids contents. The structures of polysaccharides and of lignins are markedly different in the miscanthus belowground and aboveground biomass. The non-cellulosic fraction of the samples comprises a high level of xylose, with the arabinose to xylose ratio about twice as high as that observed

for analogous stem samples, suggesting that belowground arabinoxylans are more substituted than stem ones. The mechanical properties of the belowground miscanthus-polyethylene composites correlate with several of their compositional traits, with similar trends as for plant stem-polyethylene composites with positive correlations for lignin and *p*-coumaric acid contents and negative correlations for most non-cellulosic sugars.

Keywords
Miscanthus; Belowground materials; Polysaccharides; Cell wall phenolics; Composites; Mechanical properties

1 Introduction

The use of plant biomass for manufacturing bio-based polymer composites has attracted a large interest due to its multiple advantages such as a decrease of weight compared to glass fiber-filled composites and a good environmental footprint (Gallos et al., 2017; Ramesh et al., 2017; Shah, 2013; Girijappa et al., 2019). Among the many plants which have been considered for reinforcing polymers is miscanthus, an interesting grass producing annually a large amount of biomass (Arnoult & Brancourt-Hulmel, 2015). One of the advantages of miscanthus is its low nitrogen needs (Zapater et al., 2017) due to the efficient recycling of nitrogen for the next growth period by its rhizomes (Strullu et al. 2011). This low-fertilizer demand promoted studies regarding the mechanisms of such nitrogen accumulation in the rhizomes (Li et al., 2016); Keymer & Kent, 2014; Dželetović & Glamočlija, 2015). Thus, the use of miscanthus combines a high biomass production with a low environmental impact (Zub et al. 2010). The preparation of polymer composites is done with a filler constituted by elongated miscanthus stem fragments. They act as reinforcing agents in polymer composites and it was shown that they can effectively produce polymer composites with good mechanical properties, using a wide range of matrices like polypropylene and polylactic acid (Bourmaud & Pimbert, 2008), poly(3-hydroxybutyrate-co-3-hydroxyvalerate)/poly(butylene adipate-co-terephthalate) (Nagarajan et al., 2013), poly(3-hydroxybutyrate-*co-* valerate)/polylactide (Nanda et al., 2013), polyesters (Rao et al., 2007), poly(vinyl alcohol) (Kirwan et al., 2007), poly(butylene succinate)/poly(butylene adipate-*co*-terephthalate) (Muthuraj et al., 2017a), poly(hydroxybutyrate-co-hydroxyvalerate) (Muthuraj et al., 2017b) or starch-based polymers (Johnson et al., 2005), sometimes removing different extractives by chemical treatments (Kirwan et al., 2007).

Nearly no domestication or breeding has been undertaken to identify new plant genotypes provided with traits favorable to the industrial production of polymer composites. To this end, there is a need to study the influence of compositional characteristics which may influence composite properties. In a recent work, we used different miscanthus genotypes to prepare polyethylene or polypropylene composites from stem fragments and by means of a robust protocol for preparing such composites (Girones et al., 2016). Recently, we evaluated miscanthus belowground fragments (rhizomes plus roots) for preparing polyethylene matrix composites and found that their low axial ratio and cellulose content led to composite mechanical properties inferior to those from stem-based composites (Chupin et al., 2017a). In this preliminary study, we employed only one miscanthus species (*M. x giganteus*) without considering the possible correlations between cell wall components and the composite mechanical properties. In the present work, we used the belowground biomass of two contrasted miscanthus species, *M. sinensis* and *M. x giganteus* and we made a more detailed study of their cell wall polysaccharides and phenolics in order to address the following questions:

- How do the belowground cell walls of these two species compare in terms of polysaccharides, lignin and cell wall-linked CA and FA?
- Do the mechanical properties of polyethylene composites made from these miscanthus belowground materials correlate with some of their compositional traits?

Due to the very different physiological roles of below and above matter in plants, we make the hypothesis that the mechanical properties of polymer composites prepared with belowground miscanthus fillers will have different correlations with the biochemical composition as compared with similar composites prepared with plant stem fillers.

2 Materials and Methods

2.1 Materials

The polymer matrix was a low-density polyethylene (PE), LDPE 1965T from Sabic© (melt flow index, MFI of 65 dg/min at 190 °C and 2.16 Kg, density of 919 kg/cm3). A high density maleic anhydride-grafted polyethylene, OREVAC 18507 provided by Arkema, with MFI of 5 g/10 min at 190 °C and 2.16 kg, was used as coupling agent.

Two miscanthus species were used: *Miscanthus x giganteus* from ADAS, United Kingdom (GIG) and *Miscanthus x sinensis* Goliath from Nursery Chombart, France (GOL), which is a large hybrid obtained from a cross between *Miscanthus x sinensis* and *Miscanthus sacchafloris*. Plants were grown in the INRA experimental unit in Estrées-Mons, in the Hauts-de-France region of Northern France (49°53N, 3°00E) on three different randomized blocks (B1, B2, B3) with three different nitrogen contents (N0 = 0 kg N/ha, N1 = 80 kg N/ha and N3 = 240 kg N/ha). They were harvested in February 2015, at the end of their 8th year of cultivation. The belowground materials (roots plus rhizome) were collected.

2.2 Preparation and characterization of the belowground fragments

The GIG belowground materials were ground by means of a coffee mill (Carrefour home). The GOL belowground materials being too hard to be directly ground in the coffee mill, their initial size was reduced in a Hellweg M50 granulator (Germany) equipped with a 2.5 mm grid. The fragments were ground later in the coffee mill. About 20 g of samples of both genotypes were sieved in a Retsch AS200 Digit shaker (Retsch, Germany) operating at 40 mm amplitude for 5 minutes. Six sieves with open pore size of 1000, 600, 400, 300, 200, and 100 µm were employed. Only the fragment fraction collected in the 300-200 µm sieve was used to prepare the composites. The procedure was repeated several times until the required amount for composite preparation was reached. Figure 1 shows the GIG and GOL belowground materials before grinding (a), after sieving (b), after compounding (c), and finally after injection molding (d).

Figure 1. Physical aspect of GIG (left) and GOL (right) belowground materials a) before grinding, b) after sieving (fraction collected with 200-300 µm openings), c) after mixing in the internal mixer, and d) after injection molding.

2.3 Composite preparation

An important issue regarding the study of detailed and tiny correlations between structural or chemical characteristics of a filler and properties of composites is to ensure that all parameters linked to the preparation of the filler and to the processing are kept under control and fixed. To reach this goal, a robust protocol for preparing fragments of plants and processing them with a polymer was established and validated (Girones et al., 2016). It allows to be sure that any difference seen when comparing different genotypes of a plant species is coming from the characteristics of the filler and not to the variation of parameters such as the size of the filler, their dispersion or distribution or the coupling between filler and matrix. Such protocol was successfully applied for studying plant fragment-based composites (Chupin et al., 2017a; Vo et al., 2020). The preparation used in this work follows this procedure. The composition of the

composite was kept constant at 35 wt% of belowground fragments, 1.75wt% of coupling agent (5 wt% on biomass basis) and 68.25wt% of polymer.
Prior to compounding, belowground fragments were overnight dried in an air-circulating oven operating at 70 °C. All composites were prepared in a Haake Rheomix 600 intensive kinetic mixer, equipped with counter-rotating ‘roller’ rotors. Rotors speed was fixed at 60 rpm and temperature of the mixing chamber was set at 150 °C. Belowground fragments, polymer pellets and coupling agent were introduced into the mixer in three steps (at t = 1 min, 3 min, and 5 min) during the first 5 minutes of the process, and then let be mixed for 4 minutes, for a total duration of 9 minutes. After compounding, miscanthus belowground-based composites were granulated in a blade mill provided with a 5 mm mesh. Test specimens were injection-molded in a Haake Minijet-II (Thermo Fisher Scientific, Germany) using steel molds complying either ISO-527-2-1BA (for tensile bar) or ISO-179 (for impact bar) specifications. Cylinder and mold temperatures of the injection molding machine were fixed at 150 °C and 40 °C, respectively. Injection and post-injection pressure were respectively set at 400 and 300 bars.

2.4 Mechanical characterization
Composite test bars were kept in a conditioned room at 23 °C and 50% humidity for at least 5 days before testing. Tensile tests were carried out in a Zwicki Z2.5 tensile testing machine (ZwickRoell) operating at 0.02 mm/s (1.2 mm/min). Tensile strength, Young’s modulus, elongation at maximum strength and elongation at break were measured. A pendulum Ceast 9050 (Instron Company, France) with a 1 J swing arm was employed to perform Charpy V-notch impact tests. 2 mm indents on impact bars (dimension = 80 x 10 x 4 mm3) were conducted by means of a single tooth Ceast NotchVIS manual notching machine (Instron, France). At least 5 specimens were tested per sample and per mechanical test.

2.5 Compositional analysis
The belowground samples ground to about 0.1 mm were subjected to exhaustive extraction with water then ethanol in an ASE350 accelerated solvent extractor (Dionex). The extract-free samples, yields given by weight%, mainly composed of cell walls, were then dried at 45°C for 48 h before the analyses of cell wall components, as follows. No correction was made for the few % of water which may remain in samples.
The analyses of non-cellulosic polysaccharides and cellulose were performed by two successive hydrolyses using trifluoroacetic acid and sulphuric acid as previously described (Chupin et al., 2017a). The neutral monosaccharide composition of non-cellulosic polysaccharides was measured using high-performance anion exchange chromatography with pulse amperometric detection, as previously described (Harholt et al., 2006; Vo et al., 2020). Lignin content was measured as Klason lignin (KL), using the Klason procedure as previously reported (Méchin et al., 2014). The determination of ester-linked CA and FA units was performed by mild alkaline hydrolysis, followed by solid phase extraction and HPLC chromatography, as recently described (Sibout et al., 2016).

2.6 Statistical analysis
Pearson correlation matrices were calculated and designed using the corrplot package (0.84 version) of the statistical software R (3.6.2 version).

3- <u>Results and discussion</u>

3.1 Cell wall polysaccharides and phenolics from the belowground materials of GOL and GIG miscanthus species

All these analyses were performed from the biological triplicates harvested in winter and from three different randomized blocks with three nitrogen fertilization levels (N=0, 80 and 240 kg/ha). The extract-free samples, mainly composed of cell walls, were recovered with an extraction yields ranging between 78 and 83 %, whatever the N level or the miscanthus species. These yields are about 10% lower than those obtained from winter-harvested stems. This result means that the belowground biomass contains more soluble components than the aboveground one in winter, a phenomenon in agreement with the storage role of rhizomes. The analyses of cell wall polysaccharides and phenolics from these extract-free belowground samples are shown in Table 1 while detailed block data are given in Table1S.

Table 1: Amounts of A) cell wall polysaccharides and B) cell wall phenolics in extract-free belowground samples from GIG and GOL miscanthus species. The data represent means (and SD) from biological triplicates. The main neutral sugars of non-cellulosic polysaccharides are xylose (Xyl), arabinose (Ara), Glucose (Glc) and Galactose (Gal). Lignin content is expressed as Klason Lignin (KL).

A) Cell wall polysaccharides in mg/g							
N level kg/ha	Miscanthus species	Cellulose	Non-cellulosic polysaccharides	Xyl	Ara	Glc	Gal
0	GIG	281 (20)	332 (24)	143 (5)	28 (2)	152 (27)	7 (1)
	GOL	233 (9)	357 (16)	146 (2)	33 (1)	168 (18)	10 (0)
80	GIG	298 (30)	309 (9)	142 (6)	24 (2)	136 (12)	5 (1)
	GOL	220 (18)	372 (15)	144 (6)	32 (5)	186 (14)	10 (2)
240	GIG	279 (14)	332 (23)	140 (3)	29 (2)	153 (26)	8 (0)
	GOL	226 (12)	376 (9)	145 (2)	34 (2)	186 (4)	11 (1)

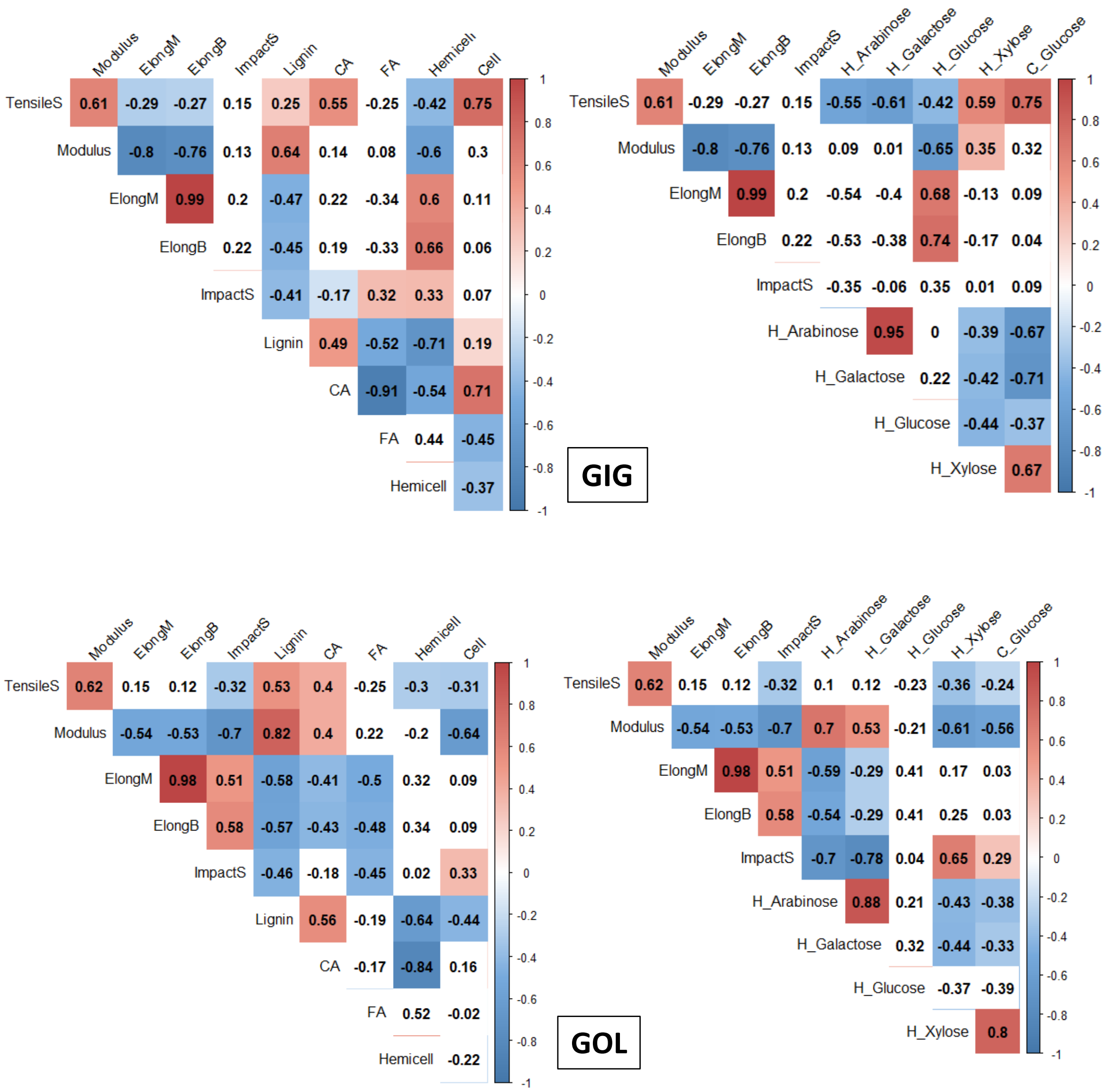
B) Cell wall phenolics in mg/g				
N level kg/ha	Miscanthus species	Lignin (KL)	Ester-linked CA	Ester-linked FA
0	GIG	228 (0)	17.4 (0.3)	3.3 (0.1)
	GOL	253 (1)	10.4 (0.3)	3.9 (0.2)
80	GIG	232 (0)	18.4 (0.8)	3.0 (0.1)
	GOL	246 (1)	9.8 (0.5)	2.6 (0.2)
240	GIG	226 (1)	16.3 (0.5)	3.6 (0.1)
	GOL	247 (1)	9.3 (0.2)	2.4 (0.1)

In agreement with Chupin et al. (2017a), the cellulose content of winter-harvested miscanthus belowground materials is nearly half that of stems while the level of non-cellulosic polysaccharides is higher. The neutral sugar composition of the non-cellulosic fraction reveals a high content of glucose which suggests that a substantial fraction of the starch reserves survived the water and alcohol extraction steps employed to prepare the extract-free belowground samples. The belowground starch abundance is higher in winter, up to values close to 100 mg per g of dry matter for GOL and GIG species (Purdy et al., 2015). It is therefore very likely that some non-cellulosic glucose originates from belowground starch. The non-cellulosic fraction of the samples comprises a high level of xylose, in agreement with the fact that arabinoxylans are major hemicelluloses of grass secondary cell walls (Vogel, 2008). The arabinose to xylose ratio (Ara/Xyl) reflects the arabinosylation degree of xylan chains. Whatever the nitrogen level, this Ara/Xyl ratio is higher in the GOL samples than in the GIG ones, which indicates that the two species differ in the structure of their belowground arabinoxylans. In addition, this ratio (ranging from 0.17 to 0.23) is also about twice as high as the one reported for analogous stem samples (0.11 ± 0.01 for GIG and 0.12 ± 0.01 for GOL). In other words, belowground arabinoxylans are markedly more substituted than stem ones. Together with the lower cellulose content, this higher substitution level might hinder molecular interactions between the chains of cell wall polysaccharides and therefore decrease the mechanical properties of the plant fibers. These Ara substituents of grass arabinoxylans are often acylated at *O*-5 by FA and a fraction of these FA esters can be ether-linked to lignins (Jacquet et al., 1995). In addition to the major Xyl, Glc and Ara sugars, the non-cellulosic fraction of the belowground samples contains a low amount of galactose (Gal) units. These Gal units might be linked to pectin components, even though pectins are minor constituents of grass secondary cell walls (Vogel, 2008).

In addition to polysaccharides, the belowground cell walls contain substantial lignin amounts that are slightly higher for the GOL species and at all nitrogen levels tested. These lignin amounts are close to the ones observed for winter-harvested whole stems of *M. x gig*. (226.5 ± 0.9 mg/g) or *M. sin*. (206.2 ± 2.4 mg/g). By their water expelling effect, these high levels of hydrophobic lignin polymers may participate to the hydrogen bond-mediated cohesion of cellulose microfibrils and thereby to their mechanical strength. In addition to feruloylated arabinoxylans, another unique feature of grass cell walls is the occurrence of *p*-coumaric acid units prominently ester-linked to lignin units (Ralph, 2010). Whatever the nitrogen level, the CA content was found to be higher in GIG than in GOL (Table 1), a phenomenon already observed for winter-harvested stem internodes from GIG (21.54 ± 1.4 mg/g) and GOL (16.40 ± 0.7 mg/g) species. In addition, this *p*-coumaroylation level (Table 1) was also found to be lower in belowground samples than in stem ones. It is noteworthy that, by contrast to the lignin amount which does not seem to be affected by the nitrogen level, the highest nitrogen level is associated with the lowest *p*-coumaric acid content in both samples. Taken together and not unexpectedly, these compositional analyses revealed that the structure of polysaccharides and of lignins are markedly different in the miscanthus belowground and aboveground biomass.

The correlations between all compositional and mechanical parameters are given in Table 2.

<u>Table 2</u>: Correlation coefficients between mechanical properties of composites and biochemical composition of belowground fragments used to prepare composites. TensileS: tensile strength; ElongM: elongation at maximum; ElongB: elongation at break; ImpactS: impact strength; CA: ester-linked *p*-coumaric acid; FA: ester-linked ferulic acid; hemicell: total amount of non-cellulosic polysaccharides, Cell: cellulose. In blue, negative correlations, in red, positive correlation. The magnitude of the correlation is expressed by the intensity of the color.



3.2 Correlations of biochemical composition with mechanical properties of belowground-based polyethylene composites

The complete results of tensile and impact tests performed on composites are reported in Table 2S. The mean values averaged over the three blocks are given in Table 3. On average, the tensile strength is about 10 MPa, the tensile modulus about 550 MPa, the elongation at break about 5% and the impact strength about 3.5 kJ/m2. Considering that for the neat matrix, the tensile strength is about 7.2 MPa and the tensile modulus about 130 MPa, the ratio of the tensile strength of the composite over the one of the neat matrix is about 1.4 while the equivalent ratio as for the modulus is 4.2. This is in the same range of ratio than for maize stem-based PE composites (Vo et al., 2020) and sorghum stem-based PE composites (Vo et al., 2017) with 30% concentration of biomass ratio, showing that miscanthus belowground materials have a moderate reinforcement effect, as for maize and sorghum stems. By contrast, the reinforcement capability of miscanthus stems is much higher, with tensile strength ratio of about 2.5 and modulus ratio of about 15 (Chupin et al., 2017a). A striking result of this work is the large difference of the values of tensile moduli between the first paper on belowground-based PE composites (Chupin et al., 2017a), i.e. about 257 MPa, and this work, i.e. about 550

MPa, although the matrix is the same PE. The difference of concentration of biomass filler, 35% in this paper and 30% in (Chupin et al., 2017a) cannot explain such a huge difference. Composites have a third component in addition of PE and biomass, a coupling agent whose role is to increase the bonding of biomass to the filler. In (Chupin et al., 2017a), this coupling agent is a terpolymer having 3% of maleic acid. In the present paper, the coupling agent is a maleic acid-PE with a high content of maleic acid (the value is kept confidential by the manufacturer) specifically designed for coupling lignocellulosic biomass and PE. It is the effect of this coupling agent which is giving such a doubling of the tensile modulus.

Table 3: Mechanical properties averaged over the three blocks for GIG and GOL belowground-based composites for the three nitrogen (N) levels. Tensile strength and modulus are in MPa, elongation at break in % and impact strength in kJ/m^2. The data represent means (and SD) from biological triplicates.

	N level kg/ha	Tensile strength	Tensile modulus	Elongation at break	Impact strength
GIG	0	10.3 (0.2)	593 (25)	5.0 (0.4)	3.5 (0.1)
GIG	80	10.4 (0.1)	605 (5)	5.0 (0.0)	3.7 (0.0)
GIG	240	10.3 (0.1)	604 (21)	4.8 (0.2)	3.7 (0.2)
GOL	0	9.8 (0.1)	531 (7)	5.1 (0.2)	3.3 (0.1)
GOL	80	9.7 (0.1)	525 (17)	5.4 (0.3)	3.3 (0.2)
GOL	240	9.6 (0.2)	533 (7)	5.1 (0.2)	3.3 (0.1)

As shown in Table 2, the correlation directions of the tensile modulus and the tensile strength with all the biochemical parameters are similar. This should be expected since a linear strength-stiffness relationship is often seen with short fiber filled composites (Sobczak et al., 2012; Vo et al., 2017). Usually, the higher the elongation at break, the higher the impact strength, as seen for GOL. The reason why there is no correlation for GIG is unknown. There is no apparent relationship between mechanical properties (tensile strength, tensile modulus, elongation at break, impact strength) and nitrogen inputs. These results agree with what was found by Chupin et al. (2017a) studying GIG.
The tensile strength and modulus are positively correlated with the cellulose content and negatively correlated with non-cellulosic polysaccharides in GIG, in agreement with previous results from composites with GIG belowground materials (Chupin et al, 2017a) or maize stem (Chupin et al., 2020) as well as short fiber composites (Charlet et al., 2007) or flax woven fabrics (Acera Fernández et al., 2016). It can be understood considering that cellulose chains are constituting the solid backbone of lignocellulosic tissues due to their excellent intrinsic mechanical properties (Saito et al., 2013). It is unclear why cellulose is negatively correlated with modulus and strength for GOL. Hemicelluloses are negatively correlated with strength and modulus, due to their weaker molecular interactions and low thermal resistance (Chupin et al., 2017b). The influence of cellulose on impact strength and elongation at break is not correlated for GIG composites. GOL composites show a weak correlation with impact strength. Lignin and *p*-coumaric acid contents are positively correlated with tensile strength and modulus for both genotypes and with tensile modulus for GOL. This correlation between these two biochemical parameters can be understood since the major fraction of *p*-coumaric acid is bound to lignins. Similar correlations of the mechanical properties with lignins and *p*-coumaric acid were found with maize (Chupin et al., 2020). The role of ferulic acid is not clear. There is a weak positive correlation to impact for GIG and negative correlations with elongation and impact strength. FA is not following the correlations of hemicellulose. The major non-cellulosic sugars of the belowground samples are xylose and glucose. Non-

cellulosic glucose shows no correlation with mechanical properties for GOL while there are negative correlations for tensile strength and modulus and positive ones for elongation in the case of GIG. Xylose shows a different picture, with positive correlations to modulus and strength for GIG and inverse negative correlations for GOL. From the above-mentioned correlations and data, it is possible to draw the following conclusions. GOL and GIG-based composites show similarities which are reminiscent of what has been found for plant stems (Kaack et al., 2003; Kong et al., 2013; Kaak and Schwarz, 2001) or other plant stem composites (Chupin et al., 2020), which is the favorable influence of cellulose and lignin and the unfavorable influence of hemicellulose on modulus and strength. However, GOL-based composites do not follow such a scheme for cellulose. An interesting result, in agreement with results found by Chupin et al. (2020), is that *p*-coumaric acid content is highly favorable to obtain composites with good modulus and strength. We may postulate that the composites prepared with GIG belowground materials have better mechanical performances than the ones prepared with GOL because GIG belowground materials have larger cellulose and *p*-coumaric acid contents and a lower hemicellulose content than GOL. GIG has a larger content of non-cellulosic glucose than GOL. Although fragments prepared by grinding below-ground miscanthus materials seem to behave like plant stems when preparing polymer composites, there are differences between the two genotypes which are intriguing, such as for example the unfavorable influence of cellulose content on tensile strength and modulus for GOL or the inverse correlations found for xylose between GOL and GIG. There is no easy explanation for such counter-intuitive results besides speculation that other factors linked to structural organization effects could play some roles.

4 <u>Conclusions</u>

The belowground cell walls of GOL and GIB species show a lot of similarities in terms of polysaccharides, lignin and cell wall-linked *p*-coumaric and ferulic acids contents. There is no clear influence of the level of nitrogen content. The content and structure of polysaccharides and of lignins are markedly different in the miscanthus belowground and aboveground biomass, which explains why fragments prepared from these two parts of the plant give very different reinforcing capacities when added to a polymer.

The cellulose content of the studied belowground miscanthus biomass is roughly half that of miscanthus stems while the non-cellulosic fraction has a high level of xylose. The cell walls contain substantial amounts of lignin that are slightly higher for the GOL species, in the same order of magnitude as the ones observed for winter-harvested whole stems. The lignin *p*-coumaroylation level, lower than in stem, was found to be higher in GIG belowground materials than in GOL ones, as observed for stem internodes. The arabinose to xylose ratio is also about twice higher than the one observed for analogous stem samples, suggesting that belowground arabinoxylans are more substituted than stem ones.

The mechanical properties of the belowground miscanthus-polyethylene composites correlate with several of belowground compositional traits, with similar trends as for miscanthus stems or plant stem-polyethylene composites. Considering that the fragments used to prepare polymer composites obtained after grinding have a low aspect ratio (Chupin et al., 2017a), the fact that the amount of cellulose, the polymer which is imparting good mechanical properties, is lower that when using stems, is one of the major explanations for the low mechanical properties of belowground miscanthus-polyethylene composites compared to stem-based ones. Lignin and *p*-coumaric acid contents are strongly positively correlated with tensile strength for both genotypes. These hydrophobic lignin polymers may participate to the hydrogen bond-mediated cohesion of cellulose microfibrils and thereby to their mechanical strength. Similar correlations of the mechanical properties with lignins and *p*-coumaric acid were found when using maize stems to prepare composites (Vo et al., 2020). There is a negative correlation between non-cellulosic molecules contents and the mechanical properties, as observed when using plant stems. GIG composites have better mechanical performances than the ones prepared with GOL due to its larger cellulose and *p*-coumaric acid contents and its lower hemicellulose content. The two main differences between GOL and GIG relate to the influence of cellulose and xylose contents on tensile strength and modulus. The hypothesis that the mechanical properties of polymer composites prepared with belowground miscanthus fillers will have different correlations with the biochemical composition as compared with the same composites prepared with plant stem fillers turned out to be incorrect. Both above and below ground matters have the same correlations with polysaccharides and phenolics. One interesting finding is the role of *p*-coumaric and ferulic acids which are respectively positively and negatively correlated to the mechanical properties of plant fragment-based polymer composites (this work for belowground miscanthus; Vo et al., 2020 for maize stems and an unpublished work from the same author group for miscanthus stems). However, a recent paper (Chupin et al., 2020) reports inverse correlations between these two hydroxycinnamic acids and the intrinsic mechanical properties of the miscanthus stem fragments. Considering that the mechanical properties of composites are directly positively correlated to the intrinsic properties of the filler, this discrepancy has no simple explanation. More work is needed to first confirm the fact that *p*-coumaric and ferulic acids are respectively negatively and positively correlated to the mechanical properties of plant stem fragments of other plants such as sorghum or maize. If confirmed, there will be a need to understand the role of these acids on the mechanical properties of plant fragments, considering also their role during the breaking of stems to produce the small pieces used for reinforcing polymers.

5- Author contributions

E. Di Giuseppe: Investigation, Writing- Original draft preparation; J. Girones: Investigation; L. Vo: Investigation; E. Gineau: Investigation; C. Lapierre: Methodology, Investigation, Writing; Maryse Brancourt-Hulmel: Methodology, Formal analysis; Stéphanie Arnoult-Carrier: Investigation; P. Navard: Conceptualization; Writing, Reviewing and Editing.

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Supplementary Materials

Polysaccharides and phenolics of miscanthus belowground cell walls and their influence on polyethylene composites

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Table 1S. Amounts of A) cell wall polysaccharides and B) cell wall phenolics in extract-free belowground samples from GIG and GOL miscanthus species. The data correspond to values obtained from three different field blocks B1/B2/B3. The main neutral sugars of non-cellulosic-polysaccharides are xylose (Xyl), arabinose (Ara), Glucose (Glc) and Galactose (Gal). Lignin content is expressed as Klason lignin (KL).

A) Cell wall polysaccharides in mg/g							
N level kg/ha	Miscanthus species	Cellulose	Non cellulosic polysaccharides	Xyl	Ara	Glc	Gal
0	GIG	274/303/265	351/339/305	138/148/143	28/26/30	175/157/122	7.3/6.6/7.8
	GOL	240/223/235	353/343/375	147/146/144	35/32/33	159/155/188	11.4/9.4/10.6
80	GIG	300/267/328	307/319/300	141/136/147	22/26/23	137/148/124	5.3/6.7/5.0
	GOL	201/236/223	388/366/360	139/151/141	37/26/32	201/180/177	11.6/8.8/10.7
240	GIG	290/263/283	306/350/341	142/142/137	31/28/28	123/169/166	8.5/8.3/7.8
	GOL	213/233/233	366/384/378	140/149/145	34/36/33	181/187/190	11.6/12.1/10.1

B) Cell wall phenolics in mg/g				
N level kg/ha	Miscanthus species	Lignin (KL)	Ester-linked CA	Ester-linked FA
0	GIG	226.4/228.5/229.2	17.3/17.7/17.1	3.2/3.4/3.4
	GOL	257.4/261.3/241.6	10.4/10.7/10.0	3.8/3.7/4.1
80	GIG	232.4/233.8/229.1	18.2/17.8/19.3	3.0/3.1/3.0
	GOL	251.1/239.9/248.2	9.4/6.6/10.3	3.9/3.4/3.8
240	GIG	235.1/221.1/221.6	16.8/16.0/16.2	3.5/3.7/3.6
	GOL	254.9/243.9/240.9	9.3/9.1/9.5	3.7/4.0/4.1

Table 2S. Mechanical properties of PE-based composites reinforced with GIG and GOL belowground fragments.

Rhizome	Bloc nr.	Nitrogen content kg/ha	Tensile strength (MPa)	Tensile modulus (MPa)	Elongation at break (%)	Impact strength (kJ/m^2)
Neat LDPE			7.2 (0.8)	130 (10)	na	44.8 (1.1)
GIG	**B1**	**0**	10.2 (0.1)	565 (8)	5.3 (0.2)	3.4 (0.2)
	B2		10.5 (0.09)	614 (4)	4.9 (0.1)	3.6 (0.1)
	B3		10.3 (0.09)	600 (17)	4.6 (0.3)	3.4 (0.1)
	mean and std. dev.		10.3 (0.2)	593 (25)	5.0 (0.4)	3.5 (0.1)
	B2	**80**	10.3 (0.08)	608 (12)	4.9 (0.3)	3.7 (0.1)
	B3		10.5 (0.07)	601 (6)	5.0 (0.2)	3.7 (0.2)
	mean and std. dev.		10.4 (0.09)	605 (5)	4.97 (0.03)	3.68 (0.01)
	B1	**240**	10.3 (0.1)	627 (7)	4.5 (0.2)	3.6 (0.1)
	B2		10.2 (0.1)	590 (5)	5.0 (0.2)	3.9 (0.2)
	B3		10.4 (0.1)	594 (6)	4.9 (0.1)	3.7 (0.1)
	mean and std. dev.		10.3 (0.1)	604 (21)	4.8 (0.2)	3.7 (0.2)
GOL	**B1**	**0**	9.9 (0.05)	537 (8)	5.0 (0.3)	3.1 (0.1)
	B2		9.7 (0.1)	534 (12)	4.9 (0.3)	3.4 (0.2)
	B3		9.8 (0.1)	523 (4)	5.3 (0.3)	3.3 (0.1)
	mean and std. dev.		9.8 (0.09)	531 (7)	5.1 (0.2)	3.3 (0.1)
	B1	**80**	9.8 (0.08)	540 (18)	5.3 (0.1)	3.3 (0.2)
	B2		9.7 (0.04)	507 (6)	5.7 (0.4)	3.6 (0.1)
	B3		9.6 (0.08)	527 (7)	5.1 (0.2)	3.2 (0.2)
	mean and std. dev.		9.7 (0.09)	525 (17)	5.4 (0.3)	3.3 (0.2)
	B1	**240**	9.8 (0.2)	534 (12)	4.9 (0.3)	3.1 (0.1)
	B2		9.5 (0.1)	521 (5)	5.2 (0.2)	3.3 (0.2)
	B3		9.5 (0.2)	515 (9)	4.9 (0.5)	3.4 (0.1)
	mean and std. dev.		9.6 (0.2)	522 (7)	5.1 (0.2)	3.3 (0.1)