

1 Short title: Electrical responses in poplar
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11 **Stem bending generates electrical response in poplar**

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15 One sentence summary: Poplar stem bending induces an electrical response with high speed
16 and strong decrement.

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34

35 **Abstract**

36 Under natural conditions, plants experience external mechanical stresses such as wind
37 and touch that impact their growth. A remarkable feature of this mechanically induced growth
38 response is that it may occur at distance from the stimulation site, suggesting the existence of
39 a signal propagating through the plant. In this study, we investigated the electrical response of
40 poplar trees to a transient controlled bending stimulation of the stem that mimics the
41 mechanical effect of wind. Stem bending was found to cause an electrical response that we
42 called ‘gradual’ potential, similar in shape to an action potential. However, this signal
43 distinguished from the well-known plant action potential by its propagation up to 20 cm along
44 the stem and its strong dumping in velocity and amplitude. Two hypotheses regarding the
45 mode of propagation of the ‘gradual’ potential are discussed.

46 **Key words:**

47 Trees, bending, electrical signaling, ‘gradual’ potential, decrement

48 **Introduction**

49 Plants perceive mechanical disturbances from their environment and adjust their
50 growth according to a process called thigmomorphogenesis (Jaffe, 1973). In natural
51 conditions, trees experience wind every day, applying recurrent force loadings that lead to
52 mechanical strain of stem and branches. As a physiological response, trees in windy
53 environments show a low primary growth and an increase of their diameter (Telewski, 2006).
54 The responses to mechanical disturbances can be local or remote. As shown by Coutand et al.
55 (2000), bending of a tomato stem leads to a rapid cessation of primary growth away from the
56 stimulated area. Although, this rapid response has not been confirmed in poplar after a single
57 basal flexion of the stem (Tixier et al., 2014), a slowdown in primary growth was observed
58 following daily repeated flexions for weeks (Niez et al., 2019). These observations suggest
59 the ability of the plants to transport mechanically-induced information over a long distance.

60 Several long-distance signaling ways can be considered from the literature essentially
61 studied after wounding stimuli: chemical signaling (Choi et al., 2016), hydraulic pulse
62 (Malone, 1993; Lopez et al., 2014) and electrical signaling (Mousavi et al., 2013; Hedrich et
63 al., 2016). Although these pathways are generally studied separately, they are likely to interact
64 (Farmer et al., 2014; Van Bel et al., 2014). The electrical signaling hypothesis has not yet
65 been studied in case of non-wounding mechanical stimulations such as organ bending
66 triggered by wind.

67 In plants, two types of electrical signals were described: the action potential (AP) and
68 the slow wave (SW) or variation potential. The SW is characteristic of a response to a
69 solicitation producing wounds. It is consisted of a rapid depolarization phase followed by a
70 short plateau step where the transmembrane potential remains stable, and finally a
71 repolarization step lasting variably from 1 to 45 minutes. Its propagation speed is of the order
72 of $\text{mm}\cdot\text{s}^{-1}$ (Sambeek and Pickard, 1976; Roblin and Bonnemain, 1985; Julien et al., 1991).
73 The SW is positively correlated with stimulus intensity and its amplitude decreases with
74 increasing distance from the stimulation site (Stahlberg et al., 2005). Several studies reported
75 the SW involvement in the activation of defense mechanisms such as the proteinase inhibitor
76 induction (Wildon et al., 1992) and jasmonates signaling pathway (Mousavi et al., 2013). The
77 AP is a rapid and transient depolarization (a few seconds to 2 min) of the plasma membrane
78 with all-or-nothing characteristics, propagating with a tissue-specific rate and without
79 decrement (Zawadzki et al., 1991; Fromm and Spanswick, 1993; Stankovic et al., 1998). The
80 signal propagates preferentially through the phloem, which shares common properties with
81 neurons in animals, namely the existence of membrane continuity by which electrical
82 excitations could be transmitted from one cell to another via plasmodesmata, pores connecting
83 the cytoplasm of one cell to that of its neighbour (Fromm and Lautner, 2007; Van Bel et al.,
84 2014). AP has mainly been studied in the context of rapid motor activity of plants, such as
85 carnivorous or *Mimosa pudica* plants, in which a propagation rate of the order of $5 \text{ cm}\cdot\text{s}^{-1}$ has
86 been measured (Sibaoka, 1969). APs have also been triggered in plants without rapid motor
87 activity such as *Lupinus* or willow (Paszewski and Zawadzki, 1976; Fromm and Spanswick,
88 1993).

89 The rapid propagation of AP could explain the rapid onset of remote responses such as
90 longitudinal growth inhibition observed in plants after stem bending. As highlighted by recent
91 studies, trees are particularly impacted by mechanical stimulations related to wind (Alonso-
92 Serra et al., 2020). Therefore, the aim of this study is to investigate the electrical signals
93 triggered after a transient bending of poplar stems by electrophysiological measurements.

94 **Results**

95

96 To test the effect of stem bending on the production of an electrical signal that
97 propagates, the first challenge was to develop an original device (Fig.1) in which the
98 measuring electrodes were kept immobile during the stem bending. In our case, we attached
99 the stem to two fixed points. This mechanical configuration avoided the possible rotation of

100 the cross sections of the stem and thus, the movement of its non-solicited part. This ensured
101 that the electrodes were kept totally immobile when the stem was bent.

102 A single, transient and rapid bending (2s) of the apical part of poplar stem induced a
103 depolarization wave (Fig. 2A) propagating basipetally up to 20 cm with a significant
104 decrement. The mean amplitude near the flexion point was around 34 mV (e1), and dropped
105 to 24 mV after 5 cm (e3), to reach a mean close to zero at 20 cm (e6 = 2.6 mV) (Fig. 2B). The
106 passage of the depolarization wave was followed by a slight hyperpolarization of the four
107 measuring electrodes closest to the stimulation. Then, a slow (up to 30 minutes) and
108 oscillation-free return to the initial potential was observed. The half-life time of the
109 depolarization wave also decreased with the position of the electrode; from 44.2 s for e1 (the
110 closest electrode from the bent zone) to 34.3 s for e3 and 6.8 s for e6, the farthest electrode
111 (Table 1A). The average propagation speed of the depolarization wave was evaluated between
112 each electrode (Table 1B). Close to the bent zone, the speed ranged from 12.9 to 19.4 cm.s⁻¹
113 between e1 and e3, while it dropped significantly to a range of 5.8 to 3.3 cm.s⁻¹ between the
114 farthest electrodes e3 and e6. Triggering and propagation of the electrical signal in response to
115 stem apical bending was found in 100 % of poplars (N= 10).

116 A transient and rapid bending of the basal stem segment also generated a
117 depolarization wave (Fig. 2C) propagating acropetally up to 20 cm. The mean amplitude near
118 the bent zone was around 32.4 mV (e1), and dropped to 21.2 mV after 5 cm (e3), to finally
119 reach a mean close to zero (e6 = 3 mV) after 20 cm (Fig. 2D). Following the depolarization
120 phase a slight hyperpolarization was observed on the four measuring electrodes closest to the
121 stimulation. Then, a slow and oscillation-free return to the initial potential occurred. The half-
122 life time of the depolarization wave also decreased with the position of the electrode; from 51
123 s for e1 to 42.3 s for e3 and 9.7 s for e6 (Table 1A). The average propagation speed of the
124 depolarization wave was evaluated between each electrode. Close to the bent zone, the speed
125 ranged from 15.1 to 16.9 cm.s⁻¹ between e1 and e3, while it dropped significantly to a range
126 of 8.4 to 1.2 cm.s⁻¹ between the farthest electrodes e3 and e6 (Table 1B). As with the bending
127 of the apical segment, triggering and propagation of the electrical signal in response to stem
128 basal bending was found in 100 % of poplars (N=10).

129 In order to compare the nature of the electrical signals propagated following different
130 types of stimulation, we therefore tested the response of poplars in the case of a leaf burn. As
131 shown in Fig. 3, a rapid depolarization was observed for each electrode following a leaf burn;
132 starting with those placed closest to the stimulation site. This depolarization wave propagated

133 both acropetally and basipetally in the poplar stem. The return to the initial potential was slow
134 (5 to 45 minutes) and irregular. The amplitude tended to be greater near the stimulation,
135 ranging from 5 to 70 mV. The propagation speed was about 0.02 cm.s^{-1} .

136 As complementary experiments, we tried to generate an AP by applying cold water to
137 the stem on eight different poplars individuals (Fig. 4). Whereas three plants did not react, a
138 local depolarization (no propagation) was measured on four of them, with a mean amplitude
139 of $21.3 \text{ mV} \pm 5.5$. Only one plant showed a clear depolarization event propagating over a
140 distance of at least 1 cm but less than 3 cm with a velocity of around 0.5 cm.s^{-1} and an
141 amplitude of 30 mV.

142 **Discussion**

143 We studied this signaling following three types of stimulations, each triggering
144 electrical signals with different characteristics. In response to a leaf burn, a depolarization
145 typical of a SW was generated in the poplar trees (Fig. 3). The characteristics of this SW are
146 identical than those described in the literature for herbaceous species: a fast depolarization
147 that tends to decrease with distance followed by a slow and irregular return to the initial
148 potential and a propagation velocity of the order of 0.01 cm.s^{-1} (Sambeek and Pickard, 1976;
149 Roblin and Bonnemain, 1985; Julien et al., 1991). On the one hand, the recording of the SW
150 in response to wounding allows validating our experimental setup used for measuring the
151 propagation of electrical signals in poplar. On the other hand, the comparison of the two
152 electrical responses clearly suggests that the one caused by the bending of the stem is not a
153 slow wave.

154 The shape of the electrical response induced by stem bending, a rapid depolarization
155 followed by a repolarization at the initial potential, shows similarities with AP as described by
156 Pickard, 1973. The maximum amplitude reached near the bent zone was around 34 mV; a low
157 value compared to AP measured in various plant species. The position of the six electrodes
158 along the stem allowed following the electrical signal over a distance of 20 cm. These
159 measurements enlightened a significant attenuation of the amplitude and velocity with the
160 increasing distance from the stimulation point (Figs. 2B; 2D and Table 1B). On the contrary,
161 the literature reports that the plant AP propagates at constant rate and without decrement
162 (Sukhov et al., 2018). Although very few studies have focused on studying it over a distance
163 greater than 10 cm, this was observed by Zawadzki et al. (1991) where an AP electrically
164 generated on a stem of *Helianthus annuus* propagates over at least 15 cm of stem at constant

165 rate and without decrement, later confirmed by Stankovic et al. (1998). This decrement is a
166 first major difference that distinguishes our signal from a classical plant AP.

167 Despite its action potential-like shape, the electrical response recorded following stem
168 bending does not match with the definition of AP: it differs by the slow decreasing of the
169 amplitude during the propagation of the signal. Moreover, it differs from the cold induced
170 putative action potential described in *Populus trichocarpa* by a speed 30 times higher
171 (Lautner et al., 2005). Furthermore, the amplitude of the AP observed by Lautner et al.,
172 (2005) did not seem to show a decrement, although it is not specified if the distance between
173 the two electrodes was higher or less than 10 cm. To take into account this typical
174 characteristic of bending-induced electrical response, we propose to descriptively call this
175 electrical signal as a 'gradual' potential.

176 We propose two hypotheses to explain the decrement of the 'gradual' potential. The
177 first hypothesis assumes an electrotonic propagation of the 'attenuated' potential. In this case,
178 the decrement can be explained by the poor performance of the excitable and connected cells
179 (especially the phloem) as a propagation medium for an electrical signal. Indeed, the electrical
180 resistance caused by the shrinkage of the membrane at the plasmodesmata, and the
181 capacitance of the non-myelinated membrane (companion cells) cause the dissipation of the
182 electrical signal (Hedrich et al., 2016). The possible re-amplification of the signal along the
183 phloem by voltage-dependent channels would then not be sufficient to compensate for this
184 dissipation. Indeed, no equivalent to Ranvier's nodes has been identified in plants to date. The
185 transmission of the excited state to neighboring cells would tend to decrease, which could
186 account for the drop in propagation speed. The second hypothesis suggests that the 'gradual'
187 potential observed following a transient bending of the stem could result from the opening of
188 mechanosensitive channels following the passage of a hydraulic pressure wave in the vascular
189 tissues, itself induced by the bending (Lopez et al., 2014, Louf et al., 2017). This hydraulic
190 pulse is itself attenuated over the distance because of lateral leaks and the mechanical
191 flexibility of the pipes, which absorb some of the initial energy.

192 **Conclusion**

193 Transient bending of poplar stems generates a rapid depolarization wave that to
194 propagates rapidly up to 20 cm from the stimulation zone with decrement. The characteristics
195 of this depolarization wave do not fit with the definitions neither of a slow wave nor of the
196 well-known action potential. Also, we propose to call this signal 'gradual' potential. Its

197 propagation mechanism remains unknown. That is why current research is now focusing on
198 the hypothesis of hydro-electric coupling.

199 **Materials and methods**

200 **Plant material and culture conditions**

201 Young poplars (*Populus tremula*×*alba*, clone INRA 717-1B4) were obtained by *in*
202 *vitro* micropropagation (Leple et al., 1992). Once they reached a height of about 4 cm, they
203 were gradually acclimatized on a hydroponic solution (Martin et al., 2009) through decreasing
204 relative humidity. Trees were then placed in a growth chamber (16 h/8 h light/dark cycle at 40
205 $\mu\text{mol.m}^{-2}.\text{s}^{-1}$ and 22 °C/18 °C with air relative humidity of 60%). Four months after
206 micropropagation, the poplars were used for experiments: at this stage, stems were about 77.8
207 cm (± 1.5 SE) tall with an average diameter of 5.8 mm (± 0.3 SE).

208 Prior to electrophysiology experiments plants were moved into a Faraday cage in
209 ambient laboratory (16 h/8h light/dark cycle at 20 $\mu\text{mol.m}^{-2}.\text{s}^{-1}$ and 22 °C/20 °C). Plants were
210 set vertically and fixed in two points of the stem with clamping rings (Fig. 1). Foam was
211 rolled around each part of the stem before tightening the clamping rings to avoid stem wounds
212 and to allow possible stem diameter variations. The root system was plunged in a 20 L vat
213 filled with hydroponic solution.

214 **Bending treatments**

215 The leaves of the bent segment were removed with a razor blade to avoid uncontrolled
216 mechanical stimuli. The speed and the magnitude of the bending stimulation were controlled
217 by a motorized arm (hybrid stepping motors 17PM-H311-P1, Minebea.Co) that pushed the
218 stem against a plastic template; which had a constant radius of curvature. The speed of the
219 motor was fixed in order to manage a flexion time of 2 s (round trip) using interface software
220 MegunoLink. The strain magnitude on the stem periphery was controlled by the radius of
221 curvature of the plastic template. This method allowed applying the same strain level along 12
222 cm of the bent segment. The setup was adjusted in order to apply a maximal peripheral
223 longitudinal strain of the bark of around 2% (Moulia et al., 2015). This value is high enough
224 to generate significant thigmomorphogenetic responses (Niez et al., 2019).

225 Two types of bending treatments were applied. For transient apical bending, the
226 template was placed 35 cm below the apex (Fig. 1A). For transient basal bending, the
227 template was placed 25 cm above the roots (Fig. 1B).

228 **Burning treatment**

229 The burn was carried out at a leaflet along the midrib with a match. The flame was
230 held 1 cm below the leaflet for 4 to 6 seconds.

231 **Cold treatment**

232 The cold treatment was applied by placing a drop of cold water (5°C) with a pipet tip
233 near the upper clamping ring.

234 **Monitoring of extracellular electrical signals**

235 In order to minimize artefactual interferences, one single poplar was placed in a Faraday
236 cage. All electronic materials were located outside the cage. Six measuring electrodes (tinned
237 copper wire (359-835), 0.25 mm diameter, RS Components) were inserted into the stem,
238 passing through all the tissue to the pith, and measured electrical potential simultaneously
239 near the bending area and up to 20 cm from it. After the installation was completed, the plant
240 was left undisturbed for stabilization during 24 to 48 hours.

241 The reference electrode (RC3 model, World Precision Instruments) was made of an
242 Ag/AgCl wire immersed in the nutrient solution of the plants. The electrical potential
243 recorded is the difference between a measuring electrode and the reference electrode with
244 respect to ground. The first measuring electrode e1 was inserted 2 cm below (apical bending)
245 or above (basal bending) the stem-to-template contact (Fig. 1). The respective distances of
246 each electrode from e1 were 2 cm (e2), 5 cm (e3), 10 cm (e4), 15 cm (e5), and 20 cm (e6).
247 The six measuring electrodes and the reference electrode were connected to a cDAQ-9171
248 (National Instrument) electronic card. The electronic card was used as an impedance amplifier
249 (10 G Ω) and A/D converter. DAQExpress 1.0.1 software (National Instrument) monitored the
250 potential difference with a sampling rate of 200 Hz. The graphs and analyzes were built using
251 MatLab® software.

252 The analysis of the recordings provided several parameters of the signal. The amplitude
253 was defined as the maximum difference value compared to the baseline before bending. The
254 half-life time of the signal was defined as the duration at half amplitude (Fig. 2A). The
255 average propagation speed of the signal was calculated between each electrode as the ratio of
256 the distance between two consecutive electrodes to the delay between the detection of a
257 change in potential difference.

258 **Statistical analysis**

259 All measured and computed data were statistically analysed using R software. Kruskal
260 and Wallis's tests and Dunn's tests were performed to compare results in terms of amplitude,
261 duration and speed of the signal ($p < 0.05$).

262

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267 Tables

268 **Table 1.** Evolution of the half-life time (A) and the mean of the propagation speed (B) of a
269 depolarization wave induced by an apical or basal bending of the stem.

270 A

Electrode number	Half-life time (s)	
	Apical bending	Basal bending
e1	44.2 ± 6.7	51 ± 8.3
e2	32.9 ± 4.5	43.3 ± 7.1
e3	34.3 ± 3.5	42.3 ± 4.4
e4	21.6 ± 5	35 ± 3.6
e5	15.1 ± 4.9	32.3 ± 3.9
e6	6.8 ± 4.4	9.7 ± 9.7

271

272 B

Interval from the bent-zone (cm)	Average speed (cm.s ⁻¹)	
	Apical bending	Basal bending
[0;2]	12.98 ± 2.4	15.12 ± 5.4
[2;5]	19.41 ± 3.8	16.87 ± 2.7
[5;10]	5.85 ± 1.7	12.27 ± 2.7
[10;15]	3.31 ± 1.7	8.43 ± 1.1
[15;20]	3.36 ± 2.7	1.19 ± 1.2

273

274 **Figure legends**

275 **Figure 1: Experimental designs for measuring electrical signals in stem after an apical**
276 **(A) or a basal (B) transient bending.** The poplar tree is placed in a Faraday cage; fixed in two
277 points of the stem with clamping rings (r). The roots incubate in a nutrient solution. The stem
278 is bent at 2.5 cm.s⁻¹ with a motorized arm (m) that pushes the stem along a plastic constantcurvature template (t) .
279 The electrodes are placed in the immobile portion of the stem. Electrode
280 e1 is inserted 2 cm below the stem-template contact. Then the distances are: e1-e2 = 2 cm, e2-
281 e3 = 3 cm, e3-e4 = 5 cm, e4-e5 = 5 cm and e5-e6 = 5 cm. The potential difference is monitored
282 along 20 cm of straight stem, below (A) or above (B) the bent region.

283 **Figure 2: Monitoring of the electrical signal generated after a transient stem bending**
284 **and recorded at different positions in the stem in *Poplar tremula x alba*.** Recording of the
285 electrical signals measured by the six electrodes after a transient apical (A) or basal (C) stem
286 bending applied on the stem (arrow). The half-life time of the signal was defined as the time
287 during which the signal is higher than half the maximal amplitude. Evolution of the amplitude of
288 depolarization wave after apical (B) or basale (D) stem bending depending on the distance from
289 the bent-zone. Values represent the mean (\pm SE), (n=10). Letters indicate the values that are
290 statistically different (Kruskal and Wallis's tests and Dunn's tests p < 0.05).

291 **Figure 3 : Monitoring of the electrical signal generated after a burnt leaf (bl) in *Poplar***
292 ***tremula x alba*.** For each electrode, slow wave consists in a fast depolarization followed by a
293 slow and irregular return to the initial potential. Here the amplitude ranges between 20 to 70 mV
294 and the velocity is around 0.02 cm.s⁻¹(A). The time of the stimulation is indicated by the black
295 arrow. The poplar tree is placed in a Faraday cage; fixed in two points of the stem with clamping
296 rings (r). Stimulation consists of burning the leaf with a match for about 4 s (B). The electrodes
297 are placed along the stem as follows: e1-e2 = 2 cm, e2-e3 = 3 cm, e3-e4 = 5 cm, e4-e5 = 5 cm
298 and e5-e6 = 5 cm.

299 **Figure 4 : Monitoring of the electrical signal generated after the application of droplet of**
300 **water at 5°C to the stem (w) in *Poplar tremula x alba*.** The time of the stimulation is indicated
301 by the black arrow (A). The poplar tree is placed in a Faraday cage; fixed in two points of the
302 stem with clamping rings (r). The electrodes are placed along the stem as follows: e1-e2 = 2 cm,
303 e2-e3 = 3 cm and e3-e4 = 5 cm (B).

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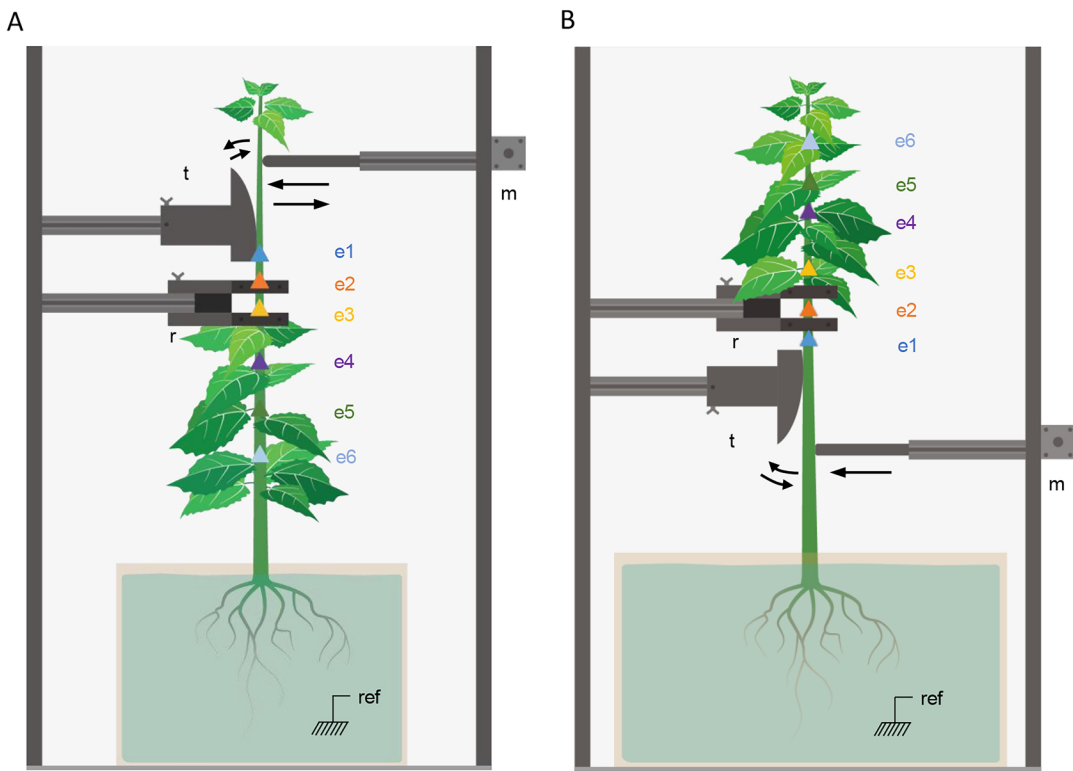


Figure 1: Experimental designs for measuring electrical signals in stem after an apical (a and b) or a basal (c) transient bending. The poplar tree is placed in a Faraday cage; fixed in two points of the stem with clamping rings (r). The roots incubate in a nutrient solution. The stem is bent at 2,5 cm.s⁻¹ with a motorized arm (m) that pushes the stem along a plastic constant-curvature template (t). The electrodes are placed in the immobile portion of the stem. Electrode e1 is inserted 2 cm below the stem-template contact. Then the distances are : e1-e2 = 2 cm, e2-e3 = 3 cm, e3-e4 = 5 cm, e4-e5 = 5 cm and e5-e6 = 5 cm. The potential difference is monitored along 20 cm of straight stem, below (b) or above (c) the bent region.

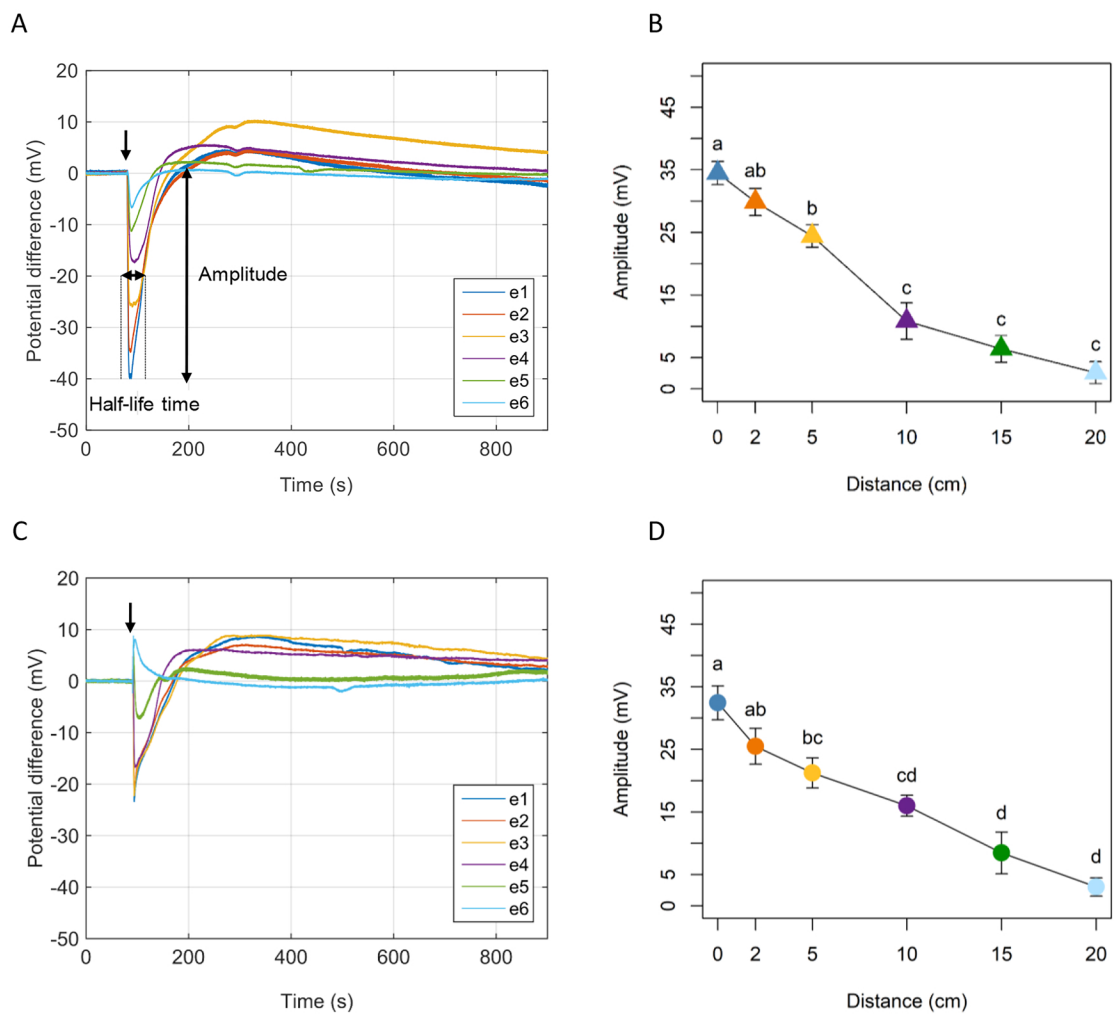


Figure 2: Monitoring of the electrical signal generated after a transient stem bending and recorded at different positions in the stem in *Poplar tremula x alba*. Recording of the electrical signals measured by the 6 electrodes after a transient apical (A) or basal (C) stem bending applied on the stem (arrow). The half-life time of the signal was defined as the time during which the signal is higher than half the maximal amplitude. Evolution of the amplitude of depolarization wave after apical (B) or basale (D) stem bending depending on the distance from the bent-zone. Values represent the mean (\pm SE), (n=10). Letters indicate the values that are statistically different (Kruskal and Wallis's tests and Dunn's tests $p < 0.05$).

Table 1: Evolution of the half-life time (A) and the mean of the propagation speed (B) of a depolarization wave induced by an apical or basal bending of the stem.

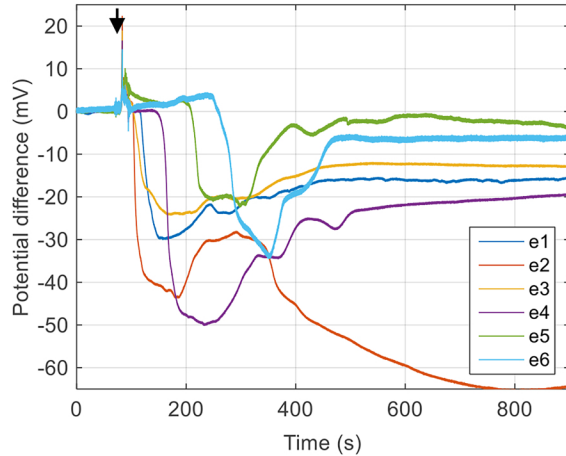
A

Electrode number	Half-life time (s)	
	Apical bending	Basal bending
e1	44,2 ± 6,7	51 ± 8,3
e2	32,9 ± 4,5	43,3 ± 7,1
e3	34,3 ± 3,5	42,3 ± 4,4
e4	21,6 ± 5	35 ± 3,6
e5	15,1 ± 4,9	32,3 ± 3,9
e6	6,8 ± 4,4	9,7 ± 9,7

B

Interval from the bent-zone (cm)	Average speed (cm.s ⁻¹)	
	Apical bending	Basal bending
[0;2]	12,98 ± 2,4	15,12 ± 5,4
[2;5]	19,41 ± 3,8	16,87 ± 2,7
[5;10]	5,85 ± 1,7	12,27 ± 2,7
[10;15]	3,31 ± 1,7	8,43 ± 1,1
[15;20]	3,36 ± 2,7	1,19 ± 1,2

A



B

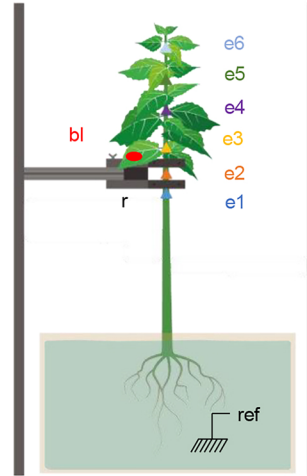
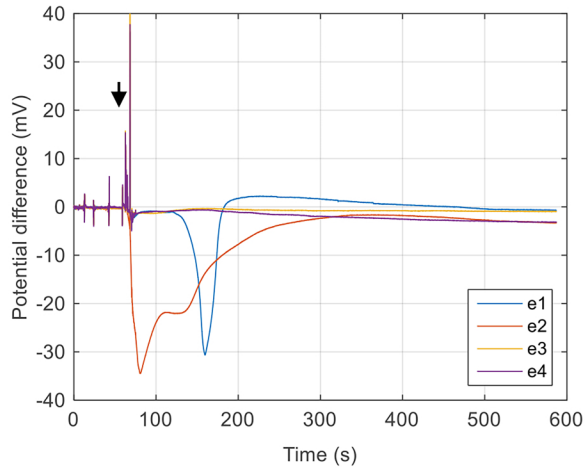


Figure 3 : Recording of a slow wave propagating from a burnt leaf (bl). The poplar tree is placed in a Faraday cage; fixed in two points of the stem with clamping rings (r). Stimulation consists of burning the leaf with a match for about 4 s. The time of the stimulation is indicated by the black arrow. The electrodes are placed along the stem as follow : e1-e2 = 2 cm, e2-e3 = 3 cm, e3-e4 = 5 cm, e4-e5 = 5 cm and e5-e6 = 5 cm. For each electrode, slow wave consists in a fast depolarization followed by a slow and irregular return to the initial potential. Here the amplitude ranges between 20 to 70 mV and the velocity is around $0.02 \text{ cm}\cdot\text{s}^{-1}$.

A



B

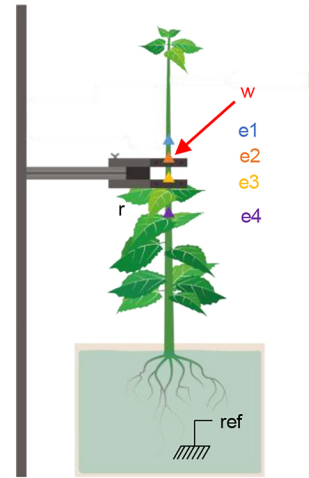


Figure 4 : Recording of an action potential in response to the application of droplet of water at 5°C to the stem (w). The poplar tree is placed in a Faraday cage; fixed in two points of the stem with clamping rings (r). The time of the stimulation is indicated by the black arrow. The electrodes are placed along the stem as follow : e1-e2 = 2 cm, e2-e3 = 3 cm, e3-e4 = 5 cm, e4-e5 = 5 cm and e5-e6 = 5 cm.

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