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1   **Wind-evoked anemotropism affects the morphology and mechanical**

2   **properties of Arabidopsis**

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20 **Highlight**

21 *Arabidopsis thaliana* adapts to wind through alterations in morphology, mechanical
22 properties, tissue organisation and exhibits positive anemotropism. The observed response
23 differs from previously reported changes induced by mechanical perturbations.

24 **Abstract**

25 Plants are known to exhibit a thigmomorphogenetic response to mechanical stimuli by
26 altering their morphology and mechanical properties. Wind is widely perceived as
27 mechanical stress and in many experiments its influence is simulated by applying
28 mechanical perturbations. However, it is known that wind-induced effects on plants can
29 differ and at times occur even in the opposite direction compared to those induced by
30 mechanical perturbations. In the present study the long-term response of *Arabidopsis*
31 *thaliana* to a constant unidirectional wind was investigated. We found that exposure to
32 wind resulted in a positive anemotropic response and in significant alterations to
33 Arabidopsis morphology, mechanical properties, and anatomical tissue organisation that
34 were associated with the plant's acclimation strategy to a windy environment. Overall, the
35 observed response of Arabidopsis to wind differs significantly from previously reported
36 responses of Arabidopsis to mechanical perturbations. The presented results suggest that
37 the Arabidopsis' response is sensitive to the type of mechanical stimulus applied, and that it
38 is not always straightforward to simulate one type of perturbation by another.

Introduction

Current predictions on global warming show that the global average temperature will increase by 1.5⬜C compared to pre-industrial levels by 2030-2050 (Allen et al., 2018). Among other climatic changes, this will lead to alterations in wind speeds and patterns. Global climate simulation models show that the predicted increase in the global temperature will lead to a significant increase in surface wind speeds over the UK and Northern Europe (Hosking et al., 2018). Similar trends have been found for other parts of the world, e.g. analysis of the wind speed data for China demonstrated an increase in the wind speeds measured during spring and summer months over the last 40 years (Zhang et al., 2020).

Strong winds and storms have a negative impact on food security, since for crop plants (e.g. wheat, rice, maize) wind induced stem and root lodging significantly affects yields (Berry et al., 2004). An overview of the reported yield reductions in major crop plants due to lodging shows that it can reach up to 80% in wheat, 83.9% in rice, 65% in barley, 40% in oats, and 20% in maize (Shah et al., 2017). Wind also has an economic impact on wood production, since windthrow due to strong winds poses a serious threat to forests (Mitchell, 2013). The review by Schelhaas et al. (2003) showed that damage from windstorms to forests in Europe contributes over 50% to the total damage from all natural causes.

In their natural environment, plants are typically subjected to low and moderate wind conditions on a regular basis, since they cannot shelter themselves from wind. This interaction can have either beneficial or detrimental effects, and plants have developed a number of strategies to adapt and survive. Detailed reviews of these effects and strategies are given in De Langre (2008) and Gardiner et al. (2016).

Wind is a complex environmental factor (Ennos, 1997; Jones, 2013) that among other effects regulates the microclimate of plants, can alter photosynthesis (Smith and Ennos, 2003; Burgess et al., 2016), and change heat and mass transfer in plants (Jones, 2013). One of the most direct effects of wind on plants is the mechanical stress exerted on plants through the drag force. This, as in the case of mechanical stress induced by mechanical perturbations (e.g. brushing, touching), evokes physiological and morphological responses in plants known under the term thigmomorphogenesis first introduced by Jaffe (1973). Typically, thigmomorphogenesis leads to the inhibition of the stem length and

72   increase of its diameter, thus plants develop shorter but thicker stems (see e.g. Biddington,

73   1986; Jaffe and Forbes, 1993). In addition, mechanical perturbations reduce a plant's

74   aboveground biomass (Niklas, 1998; Kern et al., 2005). Similar effects were noticed in

75   plants, especially trees, that are exposed to wind and experience mechanical stress, mainly

76   in the form of bending (Lawton, 1982; Biddington, 1986). These observations inspired

77   numerous studies where mechanical perturbations, such as bending or touching, were

78   utilised to mimic the influence of wind on various plants (e.g. Gartner, 1994; Niklas, 1998).

79   However, the wind plant interaction involves fluid (air) to solid (plant) contact and

80   can be considered a fluid dynamic perturbation as opposed to a mechanical perturbation in

81   the form of brushing or touching where direct solid to solid contact is present. Different

82   types of perturbations may induce different effects on plants. Indeed, factorial experiments

83   where the response to wind and mechanical perturbations was studied separately on the

84   same plant species have shown that wind can have different and even opposite effects to

85   mechanical perturbations (Smith and Ennos, 2003; Anten et al., 2010). The different

86   response of the same plant to wind compared to mechanical perturbations suggests that

87   implicit extrapolations of results obtained from tests with mechanical perturbations (e.g.

88   brushing, flexing) to the effects of wind is not always correct (Anten et al., 2010).

89   *Arabidopsis thaliana*, a small annual herbaceous plant, is widely used in plant science

90   as a model organism to study different processes and mechanisms especially in the field of

91   plant genetics and molecular biology (Koornneef and Meinke, 2010). Arabidopsis Columbia

92   (Col-0) is commonly utilised as the reference genotype in plant science, but many other

93   natural accessions as well as mutants of this plant exist and have been subjected to a wide

94   range of investigations (Lamesch et al., 2012). Brulé et al. (2016) suggested that Arabidopsis

95   can be used, with certain limitations, as a model plant to investigate the influence of

96   different parameters on plant stiffness. The response of Arabidopsis to various mechanical

97   perturbations was explored in a number of studies and is well documented (Braam, 2005;

98   Chehab et al., 2009; Paul-Victor and Rowe, 2011). In addition, investigation of

99   thigmomorphogenesis in Arabidopsis at molecular level identified a set of touch-induced

100   genes whose expression is enhanced in response to various stimuli (Braam and Davis, 1990).

101   In general, mechanical perturbations of Arabidopsis result in a response that is

102   common to many other plants, i.e. reduction of the stem length. Mechanical

103   characterisation of the primary inflorescence stems showed that perturbed plants were less

104 rigid and had decreased elastic modulus compared to the control group, thus suggesting

105 that Arabidopsis follows "short and flexible strategy" for the stem development in order to

106 cope with mechanical stress (Paul-Victor and Rowe, 2011). From these experiments it was

107 conjectured that exposure to wind will have the same effects on Arabidopsis Col-0.

108 However, as mentioned previously, wind can have different and even opposite effects on

109 plants compared to those from mechanical stimuli. Indeed, a limited number of studies

110 where periodic wind treatment was applied to Arabidopsis show that even where the

111 response was in the same direction the magnitude of changes was not the same (e.g.

112 Bossdorf and Pigliucci, 2009). However, in these experiments the response to wind was

113 analysed as an average across a number of Arabidopsis ecotypes and the response specific

114 to Col-0 cannot be inferred. In addition, the mechanical characterisation of inflorescence

115 stems did not form part of this study. Consequently, the question remains open whether

116 the response of Arabidopsis Col-0 to mechanical stress induced by fluid dynamic

117 perturbations, i.e. wind, is the same as has been previously documented for mechanical

118 stress induced by mechanical perturbations, such as brushing or touching. In addition, the

119 effect of wind on mechanical properties of Arabidopsis, such as stem bending rigidity and

120 Young's modulus of elasticity, remains untested.

121   In the present study the influence of a constant unidirectional wind on Arabidopsis

122 ecotype Col-0 is investigated. The results show that wind treated plants exhibit a positive

123 anemotropic response and their morphology is significantly altered. In addition, exposure to

124 wind modified mechanical properties, anatomical tissues organisation and ion content of

125 the primary inflorescence stems. The observed changes were related to the acclimation

126 strategy of Arabidopsis to survive and develop under constant unidirectional wind. Overall,

127 it was found that wind-induced changes to Arabidopsis differ from those reported

128 previously as a result of mechanical perturbations in the form of brushing. These

129 observations suggest that Arabidopsis is sensitive to the type of stimulus applied and that

130 the substitution of one type of perturbation by another is not straightforward. This study

131 contributes to the systematic understanding of the thigmomorphogenetic response of

132 Arabidopsis and provides new insights into the response of plants to wind.

133 **Materials and methods**

134 **Plants**

5

135 In this study, the response of Arabidopsis to wind was characterised using a range of

136 techniques. Two experiments were carried out in order to obtain all measurements since

137 the number of plants that could be subjected to the wind treatment simultaneously was

138 limited to 21 in each experiment. Because of the different preparations, the quantitative

139 results differ between the two data sets although the qualitative results are the same. For

140 this reason, we report the results from the two data sets separately. In the first experiment,

141 seeds of Arabidopsis ecotype Columbia-0 were sown in a single pot and kept at 4°C for 48

142 hours. The pot was then placed in the growth chamber with a long-day cycle (16 hours of

143 light and 8 hours of darkness), temperature at 22°C, light intensity at 150 µmol m^{-2} s^{-1},

144 and humidity at 60 %. After approximately two weeks, the seedlings were transplanted into

145 individual pots and were kept in the same growth chamber. After 15 days, when the flower

146 bearing stem was about to start its development, plants were randomly separated into two

147 groups and moved to the glasshouse. In the second experiment, the procedure was the

148 same except that the plants were grown inside the glasshouse from its start. The first group

149 (21 plants in both experiments) was subjected to a constant unidirectional flow in a purpose

150 built wind tunnel (experimental group), while the second group (19 plants in the first and 21

151 plants in the second experiment) was cultivated in the same glasshouse but without wind

152 influence (control group). The conditions in the glasshouse for both groups were as follows,

153 long-day cycle, minimum light intensity at 150 µmol m^{-2} s^{-1}. The temperature and

154 humidity inside the glasshouse exhibit diurnal variation and only minimum and maximum

155 temperatures are controlled. However, when the wind tunnel was on, loggers placed inside

156 the wind tunnel test section (Lascar EL-GFX-2) and outside of the test section (Elitech RC-61)

157 showed that the conditions in terms of temperature and humidity experienced by the plants

158 are the same in both groups.

159 **Wind treatment**

160 To study the influence of wind on Arabidopsis, a specialised wind tunnel was designed and

161 built (Supplementary Fig. S1). The description of the wind tunnel together with the

162 characterisation of its test section using hot-wire anemometry is presented in

163 Supplementary data 1. The wind tunnel provides a well-controlled and characterised

164 constant wind environment. In both experiments, plants were subjected to a unidirectional

165 flow of a constant speed of 5 m/s with turbulence intensity of 2%. According to Bossdorf

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166 and Pigliucci (2009), who applied wind treatment of the same speed to Arabidopsis, this
167 value is higher than the typical wind speed at few decimetres above the surface, based on
168 the mean wind speeds data over Central Europe. In the United Kingdom, the mean wind
169 speed at 10 m height above the surface level can reach up to 7.5 m/s (MetOffice, 2020).
170 Assuming a logarithmic wind profile (Manwell et al., 2010), this value extrapolates to a
171 maximum average speed of 2.4-3 m/s at the height of 20-30 cm above ground in open
172 agricultural areas. Consequently, the wind speed of 5 m/s utilised in this study can be
173 considered as a high wind for small plants like Arabidopsis.

174   The wind was applied for 24 hours a day and the total duration of the treatment was
175 up to 17 days. Inside the test section, 21 pots with a single Arabidopsis plant were arranged
176 in 6 staggered rows (4 pots in the odd rows and 3 pots in the even). In order to minimise the
177 influence of the pot position and ensure even exposure to the wind, the pots were swapped
178 between rows and within each row every 4 to 5 days. The orientation of the plants with
179 respect to the flow direction was preserved. The top edges of the pots were in line with the
180 test section floor and the plants were regularly watered from the bottom.

181 **Phenotyping**

182 To investigate the morphological response of Arabidopsis to the wind treatment,
183 phenotyping of plants in both experimental and control groups was conducted at two times
184 during each experiment. The recorded parameters were the length of the primary
185 inflorescence stem, the number of stems (basal branches), and the number of branches. The
186 first phenotyping was conducted 34 to 38 days after sowing (DAS) when the average length
187 of the primary inflorescence stem in the control group plants was over 170 mm. All the
188 parameters were measured again 40 to 48 DAS in both experiments (6 to 10 days after the
189 first phenotyping). The second reported experiment was shorter in time compared to the
190 first one (12 and 17 days of the wind treatment respectively), however, the duration of the
191 experiment does not affect the trends in the wind induced changes to the Arabidopsis
192 phenotype. In addition, the average diameters of the top and bottom parts of the primary
193 inflorescence stems were measured from the photographs taken during the mechanical
194 tests in the first experiment. In the second experiment, after the end of the wind treatment,
195 the aboveground fresh biomass of 10 plants from both groups was assessed. After oven
196 drying at 70°C, the dry biomass was also determined.

Mechanical characterisation

In the first experiment, the changes in mechanical properties of Arabidopsis primary inflorescence stems as a result of wind treatment were characterised by assessing their bending rigidity and Young's modulus of elasticity. The former characterises the ability of the material to resist bending, while the latter its resistance to elastic deformations. Mechanical characterisation was conducted for all plants from the experimental (n=21) and control (n=19) groups on two segments taken from the same stem. The first segment was taken from the basal part of the stem, where the cells and tissues are the oldest and were subjected to the wind for the longest time. This part of the stem is referred to as "bottom part of the stem". The second segment was taken from the apex part of the stem, that was subjected to wind for a shorter period of time compared to the bottom part and is comprised of younger cells and tissues. This part of the stem is referred to as "top part of the stem". By investigating two different segments of the same stem it is possible to check the distribution of the mechanical properties along its length and to study whether the changes resulting from the wind treatment are consistent. Both segments were cut using a razor blade and, if necessary, cleared of branches, fruits, flowers, and young floral buds. The tip part of the stem, containing the growth zone, was removed from the top part of the stem prior to testing.

Mechanical characterisation of Arabidopsis primary inflorescence stems was conducted using the dynamic forced vibration method (Zhdanov et al., 2020). In this method, the mechanical properties of the tested stem segments are estimated through their multiple resonant frequencies (f_i) using Euler-Bernoulli beam theory (Blevins, 1979):

$$f_i = \frac{\lambda_i^2}{2\pi L^2} \sqrt{\frac{EI}{m}}, i = 1,2,3, \dots, n \tag{1}$$

where L is the length of the stem, I is the second moment of area, m is the mass per unit length, and λ_i is a dimensionless parameter that is obtained from the characteristic equation corresponding to the applied boundary conditions and vibration mode. In the present study, stem segments were tested with clamped-clamped boundary conditions. In all tests, the length of the tested stem segment was equal to 50 mm, corresponding to the distance between the clamping points. To evaluate I, a widely used approximation that a

226 segment of Arabidopsis stem has a circular cross-section of constant diameter along its
227 length was utilised (see e.g. Turner and Somerville, 1997; Bichet et al., 2001). The diameter
228 was determined from the photographs of the tested stem segment taken after each test
229 using the ImageJ software (Schneider et al., 2012) as the averaged diameter over several
230 locations. The mass of the segment was determined directly after each test using a precision
231 balance, m was then evaluated as the ratio between the mass and the length of the
232 segment. For the bottom part of the stem the determined mechanical proprieties were
233 averaged over the first three natural frequencies while for the top part the first four natural
234 frequencies were used. The multiple resonant frequency method was previously validated
235 against a standard three-point bending tests (Zhdanov et al., 2020). The tests were
236 performed immediately after the cutting of each segment to avoid changes in the
237 mechanical properties of the stem segments due to dehydration and decrease in turgor
238 pressure.

239 **Anatomical measurements**

240 In the second experiment, the anatomical tissue composition of the primary inflorescence
241 stem was investigated for ten plants from each group. Segments were taken from the basal
242 and apex ends, i.e. the same two parts of the stem were considered as for the mechanical
243 characterisation. The transverse segments were sectioned manually from the centre of
244 these stem parts using a razor blade and stained with 0.02% toluidine blue. As a result of
245 histochemical staining it was possible to differentiate three representative tissues, namely
246 the outer part that consists of epidermis and cortex, the middle part that mostly
247 accommodates lignified tissues (coloured in blue), and the innermost part - pith (coloured in
248 purple). The samples were observed on a Zeiss Stemi SV11 microscope and photos were
249 captured. The relative areas of the three aforementioned representative tissues were
250 measured from the images using ImageJ software.

251 **Ca^{2+} measurements**

252 In the second experiment, bottom stem segments from eight plants in each group were
253 collected and their fresh weight was determined. After drying for 48 hours and recording
254 the dry weight, the stems were homogenised, and dry material was extracted in 1M HCl.
255 The insoluble material was removed through centrifugation retaining supernatant. Aliquots
256 of the supernatant were used to determine Ca^{2+} content utilising flame photometry (Model

257 410 flame photometer, Sherwood). All measurements were quantified against calibration
258 standards.

259 **Statistical analysis**

260 All statistics of measured quantities are reported as mean ± standard deviation of n
261 observations. The post-hoc statistical analysis was carried out with a non-parametric
262 Wilcoxon rank-sum test. This test was chosen due to the small sample sizes of different
263 lengths and because some of the data did not follow the normal distribution. The tests were
264 performed in Matlab (R2015b, MathWorks, USA) using the in-built ranksum function.
265 Statistically significant difference was established at $p \leq 0.05$.

266 # Results

267 Due to the destructive nature of most of the conducted tests and limited room inside the
268 wind tunnel test section, two sets of experiments were conducted in this study. The
269 experiments were carried out at two different times of the year, hence the conditions inside
270 the glasshouse were slightly different in terms of temperature and maximum light intensity.
271 In addition, performing two sets of experiments gave possibility to increase the sample size
272 for statistics of the reported parameters. Where possible, the same tests were conducted in
273 both sets of experiments (phenotyping). Despite the quantitative differences between the
274 results obtained in the reported experiments, qualitatively they led to the same conclusions.

275 **Wind treatment induces changes in Arabidopsis phenotype**

276 The effect of wind treatment on the recorded morphological parameters was low compared
277 to the control group during the first phenotyping in both experiments (see Fig. 1). The
278 primary inflorescence stem of plants in the experimental group was on average shorter by
279 8.81% and 10.52% in the first and second experiments respectively (Fig. 1A). However, the
280 observed decrease is statistically significant ($p < 0.05$) only in the second experiment. The
281 average number of stems in the wind treated plants at this stage was 2.33 ± 0.73 and 2.24 ±
282 0.78 in the first and second experiments respectively (Fig. 1B). These values were lower
283 compared to the control group where the number of stems was 2.79 ± 1.13 and 3.95 ± 0.86
284 respectively. The difference in average number of stems between the wind treated plants
285 and the control group was statistically significant only in the second experiment. The same
286 effect was observed for the number of branches (Fig. 1C), namely plants from the

287 experimental group had fewer branches in both experiments 2.81 ± 0.6 and 4.95 ± 1.47
288 respectively compared to 3.26 ± 0.81 and 9.14 ± 2.43 in the control groups. As in the case of
289 the number of stems, this difference was statistically significant in the second experiment
290 only.

291   During the second phenotyping, which was conducted 10 and 6 days after the first
292 one in the first and second experiments respectively, the wind effects became more
293 substantial and statistically significant (see Fig. 1). The length of the primary inflorescence
294 stem in both experiments was reduced only slightly compared to the control group plants
295 (4.71% and 13.98%), but, in contrast to the first phenotyping, this difference became
296 statistically significant ($p < 0.05$) in all cases (Fig. 1A). In addition, both experiments showed
297 that plants subjected to the unidirectional constant wind have fewer stems and branches
298 compared to the untreated plants (Fig. 1B, C). The number of stems in the wind treated
299 plants recorded in both experiments was 5.1 ± 0.7 and 3.24 ± 0.62 respectively. These
300 values are significantly lower ($p < 0.05$) compared to 5.68 ± 0.75 and 4.81 ± 0.75 observed in
301 the plants from the corresponding control groups. The highest effect of the wind treatment
302 was measured for the number of branches. Plants from the experimental group had on
303 average 24.05 ± 4.96 and 9.38 ± 2.44 branches (in experiments 1 and 2 respectively), this is
304 lower by approximately 1/3 compared to the average number of branches in the plants
305 from the control group (34.47 ± 8.0 and 20.48 ± 4.91) and this difference is statistically
306 significant ($p < 0.0001$).

307   Wind treatment also resulted in a decrease of the diameter of the primary
308 inflorescence stem segments that was measured after the first experiment (see Fig. 2). The
309 bottom parts of the stem had significantly ($p < 0.05$) lower diameter compared to those of
310 the control group plants. The diameter of the top parts of the stem was also reduced, but
311 the difference was statistically insignificant ($p > 0.05$). The plants grown under a constant
312 unidirectional wind had a significantly ($p < 0.05$) lower aboveground fresh biomass (see
313 Table 1). Fresh weight of the Arabidopsis Col-0 exposed to wind was less than half of the
314 weight of the control plants. In addition, a significant ($p < 0.01$) reduction by almost 48% in
315 the dry aboveground biomass of the plants from the experimental group was also observed.
316 On the other hand, the biomass ratio that was calculated as the percentage of moisture
317 evaporated during oven drying was almost the same in both groups.

318 **Wind changes mechanical properties of Arabidopsis stems**

319 The primary inflorescence stems of Arabidopsis plants grown under constant wind were
320 inclined in the direction of the wind but did not show any signs of mechanical damage. The
321 mechanical properties, namely bending rigidity and Young's modulus of elasticity, of
322 segments taken from the tip and basal parts of the same stems were evaluated using the
323 dynamic forced vibration method (Fig. 3). Wind treatment resulted in a significant increase
324 ($p < 0.05$) in Young's modulus of elasticity of both tested segments (Fig. 3B). The average
325 value of the elastic modulus of the bottom part of the stem of plants exposed to the wind
326 was 1119 MPa, that is 17% higher compared to the untreated plants. The increase in the
327 modulus of elasticity of the segments taken from the top part of the stem was more than
328 12% in the same stems. While the intensive property (E) of the stem material increased, its
329 extensive property (EI) was only slightly ($p > 0.05$) lower for all tested stem parts (Fig. 3A).

330 **Wind changes anatomical structure of Arabidopsis stems**

331 Exposure to wind induced substantial changes to the anatomical structure of Arabidopsis
332 primary inflorescence stems in terms of their tissue organisation (see Table 2 and Fig. 4).
333 Plant stems from the experimental group have significantly ($p < 0.05$) more lignified
334 interfascicular tissue compared to the control group in their bottom part. In addition, wind
335 treatment resulted in a significant ($p < 0.05$) decrease of the pith tissue area in the same
336 part of Arabidopsis stems. On the other hand, the relative area of cortex together with
337 epidermis was not affected by the wind treatment and remained almost the same in the
338 bottom part of the stems in experimental and control groups. The changes in the tissue
339 organisation in the top part of the primary inflorescence stems demonstrate the same
340 trends to those observed in the bottom part as a result of exposure to wind (Table 2).

341 **Wind changes Ca^{2+} content of Arabidopsis stems**

342 Ion content measurements conducted after the second experiment showed that wind
343 treatment resulted in a significant increase ($p < 0.001$) in Ca^{2+} content in the Arabidopsis
344 primary inflorescence stems (Fig. 5). The weight of calcium per gram of the fresh weight on
345 average was almost twice higher in the stems of plants grown under constant unidirectional
346 wind compared to the stems of control group plants.

347 **Discussion**

348 **Morphological response of Arabidopsis**

349 As was shown in previous studies (Smith and Ennos, 2003; Anten et al., 2010), the effect of
350 wind can differ considerably from the effect of mechanical perturbations in the same plants.
351 Indeed, the changes to Arabidopsis Col-0 that were observed in this study differ from those
352 reported for the same ecotype in previous investigations where wind influence was
353 mimicked by mechanical perturbations. Regular brushing of Arabidopsis led to the reduction
354 of the stem length by approximately 50% (Paul-Victor and Rowe, 2011) and a similar
355 response was recorded as a result of physical touch (e.g. Braam and Davis, 1990). In
356 contrast, in the present experiments the highest observed decrease in the length of the
357 primary inflorescence stem subjected to a constant wind was only 14% compared to the
358 untreated plants. These observations are consistent with those reported by Bossdorf and
359 Pigliucci (2009) where the average reduction of the plant height of various natural
360 populations of Arabidopsis was approximately 13.2% when subjected to a periodic wind
361 treatment.

362 In addition to the inhibition of the stem length, a common thigmomorphogenetic
363 response in many plants includes an increase in the stem diameter. This was reported for
364 numerous plants as a result of mechanical bending (see e.g. Goodman and Ennos, 1996;
365 Telewski, 2006; Coutand et al., 2009). However, the present results show that the mean
366 diameter of the primary inflorescence stem measured for its bottom and top parts was
367 reduced in the wind exposed plants compared to the control group. Lower stem diameter
368 was also reported in trees as a result of wind influence. For example, exposure of *Cecropia*
369 *schreberiana* to the natural wind environment resulted in significantly lower stem diameters
370 in wind exposed plants compared to those in plants sheltered from the wind (Cordero,
371 1999). Slightly lower stem diameter was also observed in lodgepole pine (*Pinus contorta*
372 Douglas ex Louden) subjected to a constant wind in a controlled wind tunnel environment
373 (Rees and Grace, 1980). In addition, influence of wind, without mechanical flexure, led to a
374 decrease in the stem diameter of sunflowers (*Helianthus annuus* L.) in factorial experiments
375 conducted by Smith and Ennos (2003). When both wind and flexure were combined a slight
376 increase in the stem diameter was observed, however, as pointed by the authors the
377 amount of mechanical flexure received by plants in this group was considerably higher than
378 the wind could create. Interestingly, a reduction of the stem diameter in Arabidopsis Col-0

379 was also reported as a result of brushing (Paul-Victor and Rowe, 2011), showing, in this
380 respect, similarities of the response to the wind and brushing in these plants.

381 We found that plants grown under constant unidirectional wind had significantly
382 lower number of stems and branches compared to the untreated plants. As will be
383 discussed below, this can be a part of the acclimation strategy of Arabidopsis to deal with
384 wind loadings. The reduced branching in Arabidopsis was also reported as a result of a
385 periodic wind exposure of different flow speeds Bossdorf and Pigliucci (2009), but not for all
386 17 tested natural populations. The same behaviour was reported earlier by Pigliucci (2002)
387 for 11 natural accessions of Arabidopsis that were subjected to a periodic wind treatment of
388 different durations. While the number of branches in some accessions decreased, a similar
389 or even increased number was observed in others. On the other hand, increased branching
390 was reported for *Potentilla reptans* L as a response to the mechanical brushing that was
391 applied to mimic a wind environment (Liu et al., 2007).

392 Another effect of mechanical perturbations on plants is the decrease of
393 aboveground biomass (Niklas, 1998; Kern et al., 2005). This can be linked to the overall
394 reduction of the plant's size as a result of thigmomorphogenesis. In the present study, both
395 fresh and dry aboveground biomass was reduced for plants grown under a constant
396 unidirectional wind. This is attributed to the changes in the Arabidopsis morphology where
397 lower number of stems and branches together with slightly shorter stems of reduced
398 diameter were observed in plants subjected to wind. The decrease of Arabidopsis dry
399 biomass as a result of wind treatment is consistent with previous observations by Bossdorf
400 and Pigliucci (2009). Although changes in the biomass were not quantified for Arabidopsis
401 subjected to mechanical perturbations in the studies by Braam and Davis (1990) and Paul-
402 Victor and Rowe (2011), it can be reasonably presumed that it was decreased due to the
403 significant reduction in size of the plants. The very close values of the biomass ratio in both
404 experimental and control groups in the present study point to the same amount of water in
405 both groups at the time of harvest and throughout the experiments. Thus, the changes
406 observed as a result of wind treatment were not due to the water loss in plants from the
407 experimental group through increased evaporation. In addition, similar water content can
408 be associated with water saving strategies of plants under wind, but further investigation of
409 this aspect is required.

410   Comparing the changes in Arabidopsis morphology some similarities between
411   responses to wind and mechanical brushing can be noted. In both cases, a reduction of the
412   plant biomass and decrease in the stem diameter are observed, even though the latter is
413   not a common response to mechanical bending in plants. However, the inhibition of the
414   stem length is much lower in wind exposed plants compared to those subjected to brushing.
415   The observed differences in the responses to constant unidirectional wind as in the current
416   study and mechanical contact (e.g. Paul-Victor and Rowe, 2011) can be attributed to the
417   fact that in the first case Arabidopsis can adapt to the constant stress coming from a single
418   direction by adapting its shape and structure while it is not possible to achieve this when
419   stress is applied from multiple directions, as in the case of brushing.

420   **Anemotropic response of Arabidopsis to a constant unidirectional wind**

421   Environmental stimuli have an effect on the specific orientation of plant growth, which is
422   known as tropism. A tropic response can be either positive, i.e. towards the stimulus, or
423   negative, i.e. away from it. The response of plants to touch and other mechanical contacts is
424   known as thigmotropism. By analogy, the response of plants to wind can be termed as
425   anemotropic or anemotropism. In the present experiments, Arabidopsis ecotype Col-0
426   grown under the constant unidirectional wind exhibited anemotropic response (Fig. 6). The
427   young seedlings demonstrated positive anemotropic response to wind, by directing their
428   primary growth in the direction opposite to the direction of the flow (Fig. 6A, B). To the best
429   of the authors' knowledge, this is the first time an anemotropic response to a unidirectional
430   wind has been documented in any plant system (Telewski, 2012), although previous work on
431   conifers already suggested the existence of this type of tropic response (Rees and Grace,
432   1980; Berthier and Stokes, 2006). As Arabidopsis developed under the wind and its size
433   increased the shape of the plants became windswept, however the upwind bending of the
434   stems was preserved (Fig. 6C, D). The windswept growth form is widely found in trees for
435   which it is a crucial acclimation response to the wind environment (Telewski, 2012). It
436   should be noted that the Arabidopsis stem is known to exhibit gravitropism when inclined at
437   an angle from the vertical orientation. In case of the gravitropic response, the stem returns
438   back to the vertical orientation within few hours (Fukaki et al., 1996). Curvature of
439   Arabidopsis stems grown under constant unidirectional wind was preserved over time (see
440   Fig. 6), suggesting that the observed response is mainly due to anemotropism. However, an

441   interaction between anemotropic and gravitropic responses cannot be fully excluded since

442   gravity was inevitably present in the experiments. As was shown in the studies where

443   Arabidopsis was grown in a centrifuge (e.g. Dümmer et al., 2015; Chauvet et al., 2016), the

444   combination of gravity with an additional force results in a growth response in the direction

445   parallel to their resultant force. In the present study, the additional force was exerted by the

446   wind, which could explain the observed curvature of the stem and the fact that Arabidopsis

447   grown under constant wind did not develop parallel to the wind direction.

448   **Acclimation of Arabidopsis to a constant unidirectional wind**

449   To grow and survive in windy environments plants have developed a number of acclimation

450   strategies. These strategies include streamlining and reconfiguration, damping and wind

451   induced pruning (Gardiner et al., 2016). Reconfiguration, the term introduced by Vogel

452   (1984) to describe change of plant shape under wind, and streamlining are common for

453   most plants regardless of size and growing environment. In terms of this strategy, plants

454   reduce their frontal area and aerodynamically optimise their shape to experience less force

455   from the wind. The details of the physics and mechanics behind streamlining and

456   reconfiguration can be found in e.g. Gosselin (2019). Plants mainly experience force from

457   the wind in the form of fluid dynamic drag, which is defined as:

$$D = \frac{1}{2}\rho A C_D U_\infty^2, \tag{2}$$

459   where ρ is the density of the fluid, A the frontal area of the plant, C_D the drag coefficient

460   and U_∞ flow velocity. For plants it was shown that drag does not scale as U^2 but rather as

461    $U^{2+\nu}$, where ν is the Vogel exponent (Vogel, 1984, 1989). In order to reduce the drag force

462   experienced from wind, plants can modify only two parameters in the equation 2, namely

463   their drag coefficient and frontal area.

464   In the present study, Arabidopsis is expected to exhibit a long-term acclimation

465   strategy to a unidirectional wind environment. As mentioned above, Arabidopsis grown

466   inside the wind tunnel developed in the downstream direction and its shape became

467   windswept. In trees, a windswept form is a result of long term streamlining that occurs as a

468   response to regular wind exposure. Trees with windswept crowns are known to experience

469   substantially less drag (Telewski and Jaffe, 1986a), i.e. values of their C_D in equation 2 are

470   reduced. Taking into account similarity in the long-term response to continuous wind, it can

16

471 be presumed that Arabidopsis optimised its shape into a more streamlined one and thus

472 experienced less drag.

473   In addition, phenotyping showed a significantly lower total number of branches

474 (including basal branches) in the wind exposed plants. This in turn decreased their frontal

475 area compared to the untreated plants that had more branches and stems. From equation

476 2, a reduction in the frontal area directly leads to a decrease in the drag force the plant

477 experiences from the wind. Hence, the reduced number of stems and branches in the

478 experimental group compared to the control group can be considered as part of the long-

479 term acclimation strategy of Arabidopsis Col-0 to constant unidirectional wind.

480 **Changes in the mechanical properties of Arabidopsis stems**

481 The observed changes to the mechanical properties of Arabidopsis are opposite to those

482 reported as a result of brushing, where bending rigidity and Young's modulus of elasticity of

483 inflorescence stems were significantly reduced (Paul-Victor and Rowe, 2011). In the present

484 study, only a marginal decrease in the bending rigidity of Arabidopsis stems was recorded

485 for the experimental group (Fig. 3A). It should be noted that this property is dependent on

486 the stem geometry, namely the second moment of area. This parameter, in turn, is a

487 function of the stem diameter, which was lowered as a result of the wind treatment (Fig. 2).

488 As was discussed earlier, plants experience a drag force from the wind that is dependent on

489 the characteristic dimension of the plant (projected frontal area). Consequently, the

490 reduced stem diameter is also important for reducing the wind induced drag force. Paul-

491 Victor and Rowe (2011) suggest that changes in mechanical properties are related to

492 changes in the developmental rate and combined with alterations in plant morphology are

493 adaptive to the growth environment. Therefore, a possible explanation to the contrasting

494 results between the two types of treatment may be the fact that under unidirectional wind

495 Arabidopsis did not bend in opposite directions as in the case of brushing, and hence

496 increased flexibility was not required to adapt to the growth environment. On the other

497 hand, wind treatment increased the modulus of elasticity of the primary inflorescence

498 stems of Arabidopsis (Fig. 3B). Higher elastic modulus means that a larger stress needs to be

499 applied in order to produce the same strain (deformation). This may also form a part of the

500 acclimation strategy, making the plant material more rigid so it can withstand the loads

501 from the continuous wind exposure. In addition, increased modulus of elasticity

502 compensates the influence of lower stem diameter on the bending rigidity, so stems sway
503 less in the wind compared to the case where both E and D are reduced.

504   Other studies, where the influence of different types of perturbations (wind, flexing,
505 rubbing, etc) on the mechanical properties of plants was assessed, show that the changes
506 can be in any direction. Bending rigidity of stems was reported to decrease (e.g. Telewski
507 and Jaffe, 1986b; Cordero, 1999), increase (e.g. Hepworth and Vincent, 1999; Kern et al.,
508 2005; Niez et al., 2019) or remain unaffected (e.g. Jaffe et al., 1984). Young's modulus of
509 elasticity also can increase (e.g. Telewski and Jaffe, 1986a; Gladala-Kostarz et al., 2020) or
510 decrease (e.g. Jaffe et al., 1984; Cordero, 1999; Kern et al., 2005) as a result of various
511 mechanical treatments including wind. The present results support the hypothesis proposed
512 by Newcombe (1895) that there is no universal change in the mechanical properties of
513 plants as a response to mechanical stress (see Telewski (2016) for review). The changes are
514 likely to adjust to the exact type of perturbation and depend on many factors, e.g.
515 frequency of perturbations, amount of stress induced per perturbation, direction of
516 perturbation, etc. Thus, as discussed in Coutand et al. (2000), it is important to characterise
517 perturbations experienced by plants as well as their growth environment. In the present
518 study, the wind conditions inside the test section were characterised using hot-wire
519 anemometry and it was ensured that other environmental variables were the same for both
520 groups. Experiments conducted at different times of the year (see Methods) confirmed that
521 changes to the mechanical properties of Arabidopsis primary inflorescence stems resulting
522 from continuous exposure to wind at a constant flow speed of 5 m/s are repeatable. In
523 addition, as was shown for loblolly pines (Telewski and Jaffe, 1986b) and hybrid poplars
524 (Pruyn et al., 2000) the response to mechanical perturbations depends on the plant
525 genotype.

526   In future studies, various Arabidopsis ecotypes and mutants can be subjected to
527 different wind speeds and the corresponding changes to their mechanical as well as
528 morphological properties can be quantified. This will give the possibility to assess how
529 Arabidopsis adapts to different wind conditions and how these conditions affect the
530 aforementioned properties.

531 **Changes in the stem anatomy and ion content related to the changes in the mechanical**
532 **properties**

533   Lignin is known to strengthen cell walls and supportive fibres and thus provides rigidity to
534   plants (Smith et al., 2013; Brulé et al., 2016). As was shown with lignin deficit Arabidopsis
535   mutants, irx4, reduction of lignin leads to the decrease in the mechanical properties of
536   Arabidopsis stems (Jones et al., 2001). Consequently, the observed changes in the relative
537   areas of structural tissues as a result of wind influence in the present study suggest direct
538   correlation between an increase in the area of the lignified tissues and increase in the
539   modulus of elasticity of the tested stem segments.

540   The larger relative area of lignified tissues in Arabidopsis stems observed in wind
541   exposed plants is opposite to the effects of mechanical perturbations in the form of
542   brushing. Paul-Victor and Rowe (2011) reported a decrease in this parameter in the stems of
543   brushed plants. This was partially attributed to the recorded reduction in the mechanical
544   properties of perturbed plants compared to the control group.

545   Ko et al. (2004) showed that lignification in Arabidopsis inflorescence stems is
546   related to their weight and length. Furthermore, it was found that an artificial increase of
547   stem weight promoted further formation of lignified tissues. As in the experiments by Ko et
548   al. (2004), where addition of the weight to the stem tip increased the gravitational force
549   exerted on Arabidopsis stems, in the present study, stems were also made to experience an
550   additional force, namely the drag force that was induced by the constant unidirectional
551   wind. This suggests a possible explanation to the observed increase of the lignified tissues in
552   the wind treated plants compared to the control group.

553   In addition to an increase in the area of lignified tissues, stems of wind exposed
554   plants had higher Ca^{2+} content. An increase in calcium is known to occur in response to
555   different types of mechanical stress in plants (Telewski, 2006). Furthermore, Ca^{2+} ions
556   contribute to cell wall stiffening by taking part in the formation of pectate gels (Jiang et al.,
557   2005; Höfte et al., 2012). Based on this, it can be presumed that elevated level of Ca^{2+} may
558   also contribute to the increased modulus of elasticity of plant stems from the experimental
559   group.

560   **Wind and water stress in plants**

561   In general, it can be expected that wind leads to water stress in plants through increase in
562   transpiration from plant surfaces. In many cases, the increased evaporation from plants in
563   wind is a result of the decrease in cuticle resistance which occurs through abrasive wear and

564 damage due to flexing of leaves and collisions with other leaves (Jones, 2013). This was
565 experimentally shown for a number of plants, e.g. *Festuca arundinacea* (Grace, 1974), *Picea*
566 *sitchensis* and *Pinus sylvestris* (Van Gardingen et al., 1991). However, several theoretical and
567 experimental studies show that, in certain cases, wind has a small influence on the
568 evaporation rate in plants and even a decrease in transpiration has been observed
569 (Monteith, 1965; Drake et al., 1970; Rees and Grace, 1980; Dixon and Grace, 1984). It should
570 be noted that Rees and Grace (1980) and Dixon and Grace (1984) conducted experiments in
571 a wind tunnel and their findings thus are directly relevant to the current study.

572 The present study focusses on the changes in the Arabidopsis morphology and
573 mechanical properties as a result of continuous unidirectional wind treatment at a constant
574 speed. The growth conditions between the control and experimental groups were identical
575 except for the wind treatment. Moreover, the wind environment was created in a purpose-
576 built wind tunnel, that was precisely characterised, and through repeated experiments the
577 results were shown to be reproducible. Any changes in the plant microclimate came directly
578 from the wind and are expected to occur in the natural environment under the same
579 conditions resulting in similar changes to the plant structure and mechanical properties. The
580 top edges of the plant pots were in line with the wind tunnel floor, hence the leaf rosettes
581 were located very close to the wind tunnel floor inside the boundary layer, where flow
582 speed is significantly reduced. Consequently, the disturbance from wind to the leaf rosettes
583 of Arabidopsis was reduced to a minimum throughout the experiments and neither flexing
584 nor rubbing of leaves with each other were observed. The other aboveground parts of
585 Arabidopsis swayed in the wind but did not touch parts of neighbouring plants or other
586 parts of the same plant. Based on this, it can be presumed that damage to the cuticle was
587 also reduced or even eliminated thus avoiding increase in transpiration from the plants
588 through decrease in the cuticle resistance.

589 **Conclusions**

590 In this study, Arabidopsis ecotype Col-0 was subjected to a continuous wind treatment in a
591 purpose-built wind tunnel. Exposure to wind resulted in a positive anemotropic response,
592 recorded for the first time in any plant system, and in pronounced changes to the plant
593 structure. In addition, mechanical properties, anatomical tissue organisation and ion

594 content of the primary inflorescence stems were modified. Overall, the observed changes

595 can be interpreted as a part of Arabidopsis' acclimation strategy to wind.

596 **Supplementary data**

597 Supplementary data are available at JXB online.

598 Fig. S1. Wind tunnel design. (A) CAD model. (B) Wind tunnel inside the glasshouse

599 Fig. S2. Representative examples of the mean velocity contours at several locations along

600 the test section length for three settings of the variable transformer. The flow direction is

601 into the page. (A) Variac setting 70, $x/h = 0.875$; (B) Variac setting 70, $x/h = 2.775$; (C) Variac

602 setting 70, $x/h = 3.975$; (D) Variac setting 130, $x/h = 0.875$; (E) Variac setting 130, $x/h =$

603 2.775; (F) Variac setting 130, $x/h = 3.975$; (G) Variac setting 230, $x/h = 0.875$; (H) Variac

604 setting 230, $x/h = 2.775$; (I) Variac setting 230, $x/h = 3.975$. Velocity is normalised by the

605 mean flow velocity in the test section, and where h is the test section half height

606 **Data availability statement**

607 The data supporting the findings of this study are available from the corresponding author,

608 Oleksandr Zhdanov, upon request.

615 **Author contributions**

616 **OZ**: conceptualisation, investigation, formal analysis, visualisation, writing - original draft,

617 writing - review & editing. **MRB**: conceptualisation, supervision, resources. **HZB**:

618 conceptualisation, supervision. **AB**: conceptualisation, supervision, writing - review &

619 editing.

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Table 1: Overview of the changes to the Arabidopsis biomass (mean±s.d.) that were assessed after the end of the unidirectional wind treatment with a constant flow speed of 5m/s in the second experiment. p-values are obtained from the two-sided Wilcoxon rank sum tests comparing medians of the corresponding parameters in the control (n = 10) and experimental (n = 10) groups.

	Experiment 2		
	control plants	wind treated plants	p-value
fresh biomass, g	1.78±0.55	0.87±0.33	0.001
dry biomass, g	0.23±0.06	0.12±0.04	0.001
biomass ratio, %	86.7±0.6	85.6±0.3	0.0312

Table 2: Contribution of tissues to the total cross-sectional area of the Arabidopsis primary inflorescence stem segments (mean±s.d.) that was assessed after the end of the unidirectional wind treatment with a constant flow speed of 5 m/s in the second experiment. p-values are obtained from the two-sided Wilcoxon rank sum tests comparing medians of the corresponding parameters in the control (n=10) and experimental (n=10) groups.

	Tissue contribution, %		
	control plants	wind treated plants	p-value
Bottom part of the stem			
Pith	38.92±3.33	31.64±3.23	< 0.001
Lignified tissues	24.76±2.69	31.02±2.33	< 0.001
Cortex + epidermis	36.43±3.06	37.34±3.6	0.68
Top part of the stem			
Pith	35.6±1.88	31.47±2.4	< 0.01
Lignified tissues	22.99±1.83	26.21±1.08	< 0.001
Cortex + epidermis	41.41±2.3	42.32±1.96	0.32

Figure 1: Changes to the morphological parameters as a result of unidirectional wind treatment with a constant flow speed of 5m/s in both conducted experiments. (A) Length of the primary inflorescence stem. (B) Number of stems. (C) Number of branches. Error bars represent standard deviations. Statistically significant difference ($p \leq 0.05$) from the two-sided Wilcoxon rank sum tests comparing medians of the corresponding parameters in the control (n = 19 and n = 21 for the first and second experiments respectively) and experimental (n = 21 for both experiments) groups is marked with (*).

Figure 2: Changes to the Arabidopsis stem segments diameter as a result of the unidirectional wind treatment with a constant flow speed of 5 m/s measured in the first experiment. Error bars represent standard deviations. Statistically significant difference ($p \leq$ 0.05) from the two-sided Wilcoxon rank sum tests comparing medians of the corresponding parameters in the control (n = 19) and experimental (n = 21) groups is marked with (*).
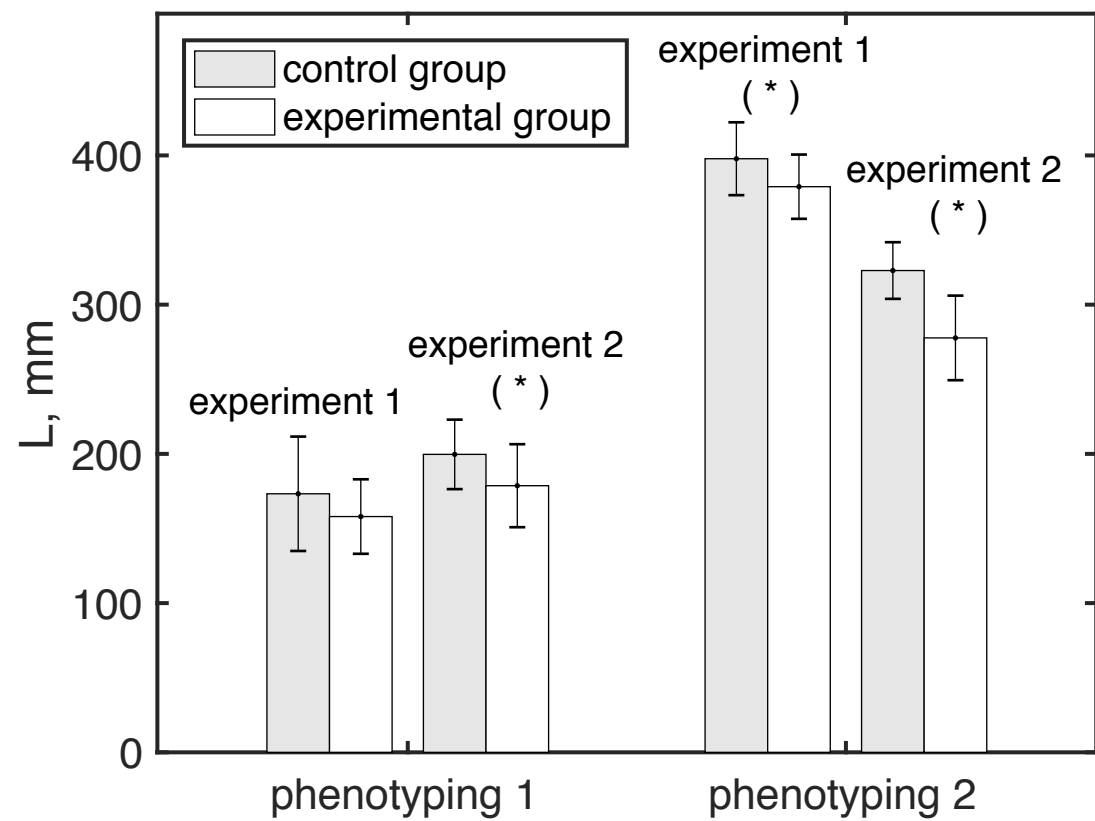
Figure 3: Changes to the mechanical properties of Arabidopsis stem segments as a result of the unidirectional wind treatment with a constant flow speed of 5 m/s in the first experiment. (A) Bending rigidity. (B) Young's modulus of elasticity. Error bars represent standard deviations. Statistically significant difference ($p \leq 0.05$) from the two-sided Wilcoxon rank sum tests comparing medians of the corresponding parameters in the control (n = 19) and experimental (n = 21) groups is marked with (*).

Figure 4: Representative examples of the primary inflorescence stem cross-section showing anatomical tissue organisation of its bottom part. (A) Plant form the control group. (B) Plant from the experimental group. co - cortex, ph - phloem, xy - xylem, if interfascicular tissue, pi - pith.
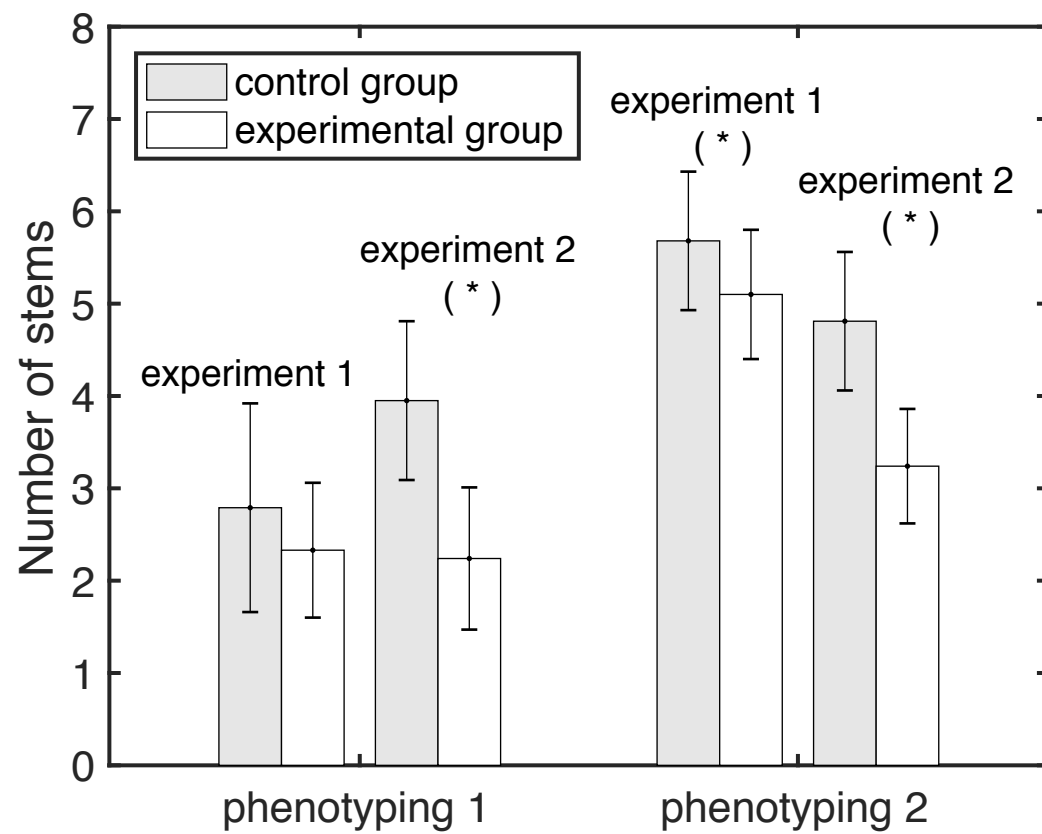
Figure 5: Changes to the Ca^{2+} content of Arabidopsis stems as a result of the unidirectional wind treatment with a constant flow speed of 5 m/s in the second experiment. Error bars represent standard deviations. Statistically significant difference ($p \leq 0.05$) from the two-sided Wilcoxon rank sum tests comparing medians of the corresponding parameters in the control (n = 8) and experimental (n = 8) groups is marked with (*).

Figure 6: Arabidopsis ecotype Col-0 grown under the constant unidirectional wind exhibits positive anemotropic response. (A) Young Arabidopsis seedlings (30 DAS) from experimental and control groups. (B) Young Arabidopsis seedlings (30 DAS) inside the wind tunnel test section. (C) Arabidopsis plants (34 DAS) from experimental and control groups. (D) Arabidopsis plants (34 DAS) inside the wind tunnel test section. Note that in (A) and (C) plants from the experimental group were removed from the wind tunnel and the curvature of their stems is increased compared to plants in (B) and (D) where Arabidopsis is subjected to a constant unidirectional wind of 5 m/s. For reference in all parts of the figure the diameter of the pot is 76 mm.
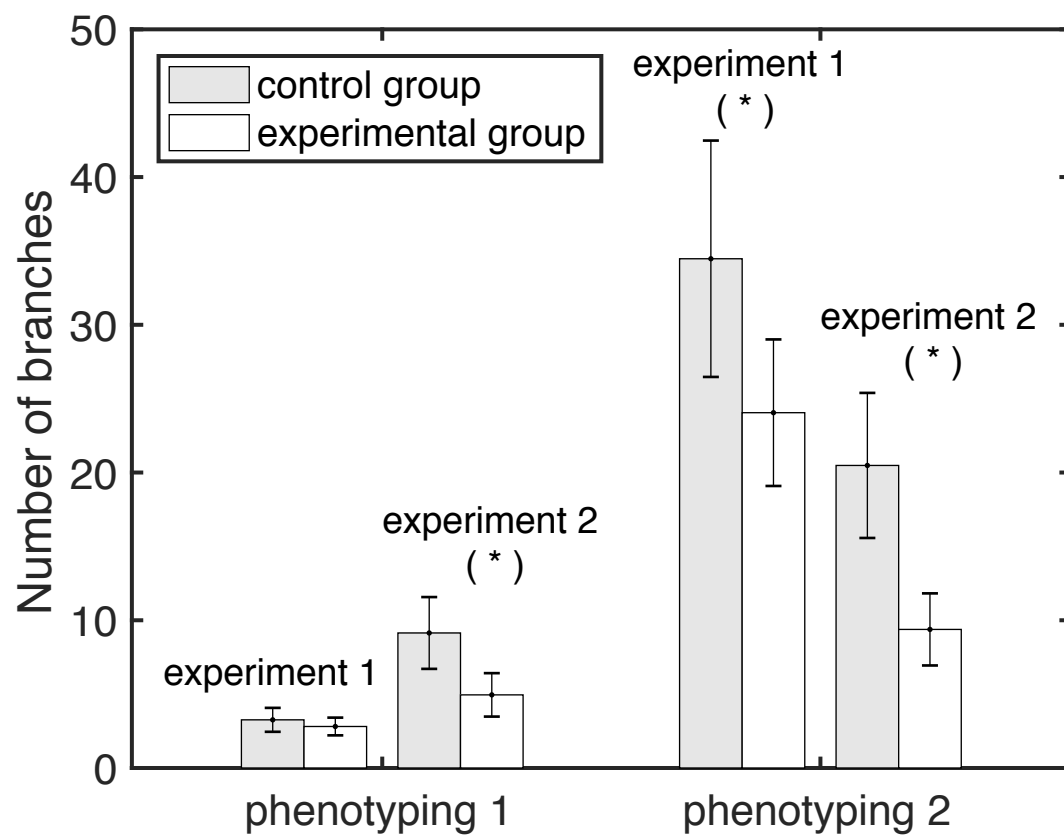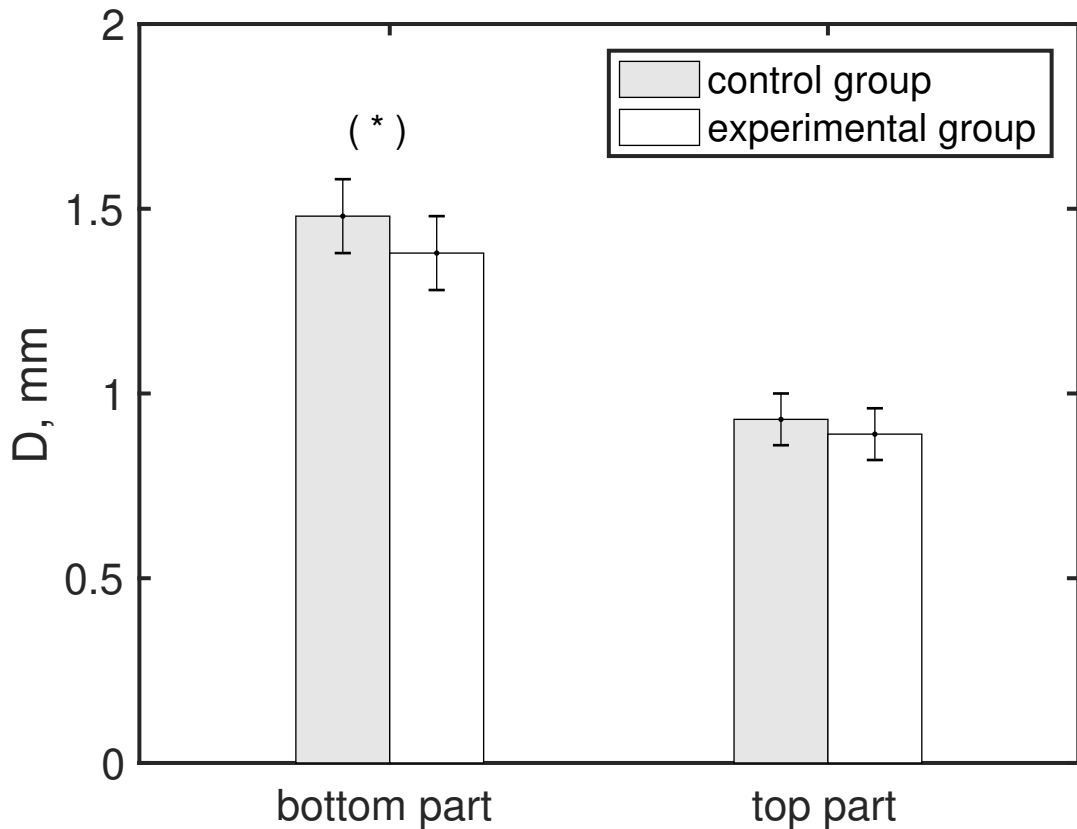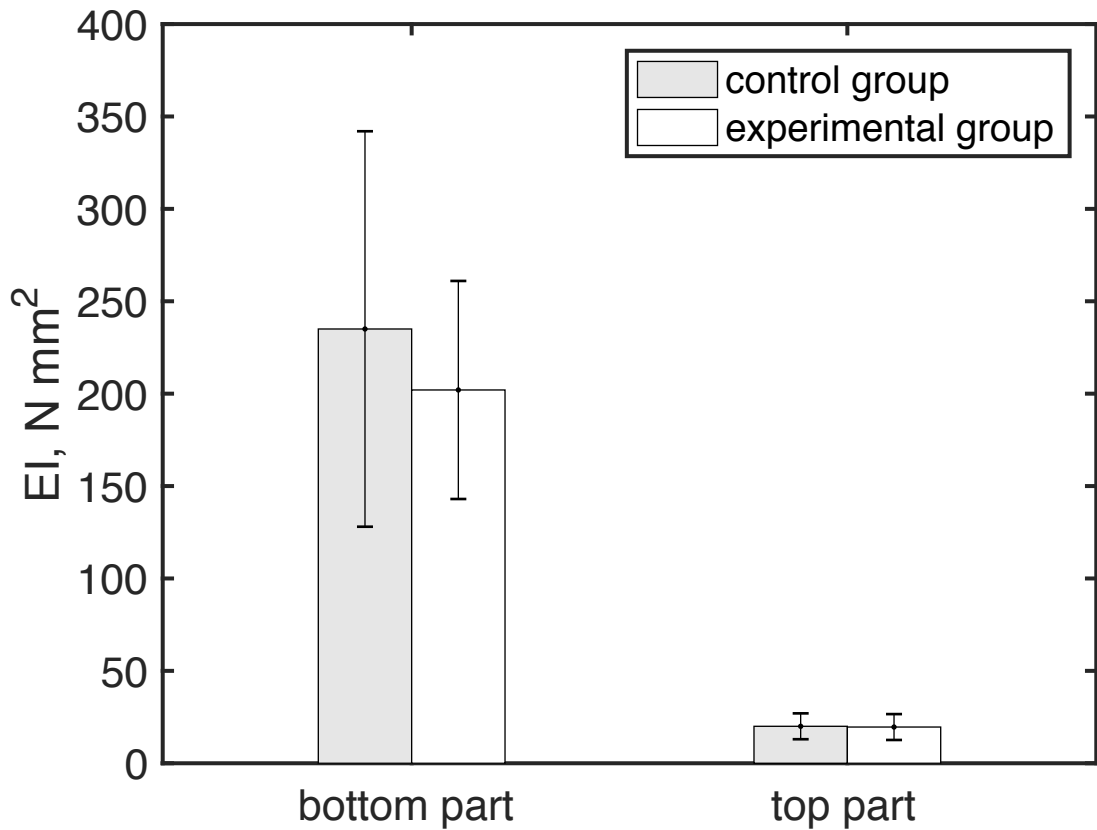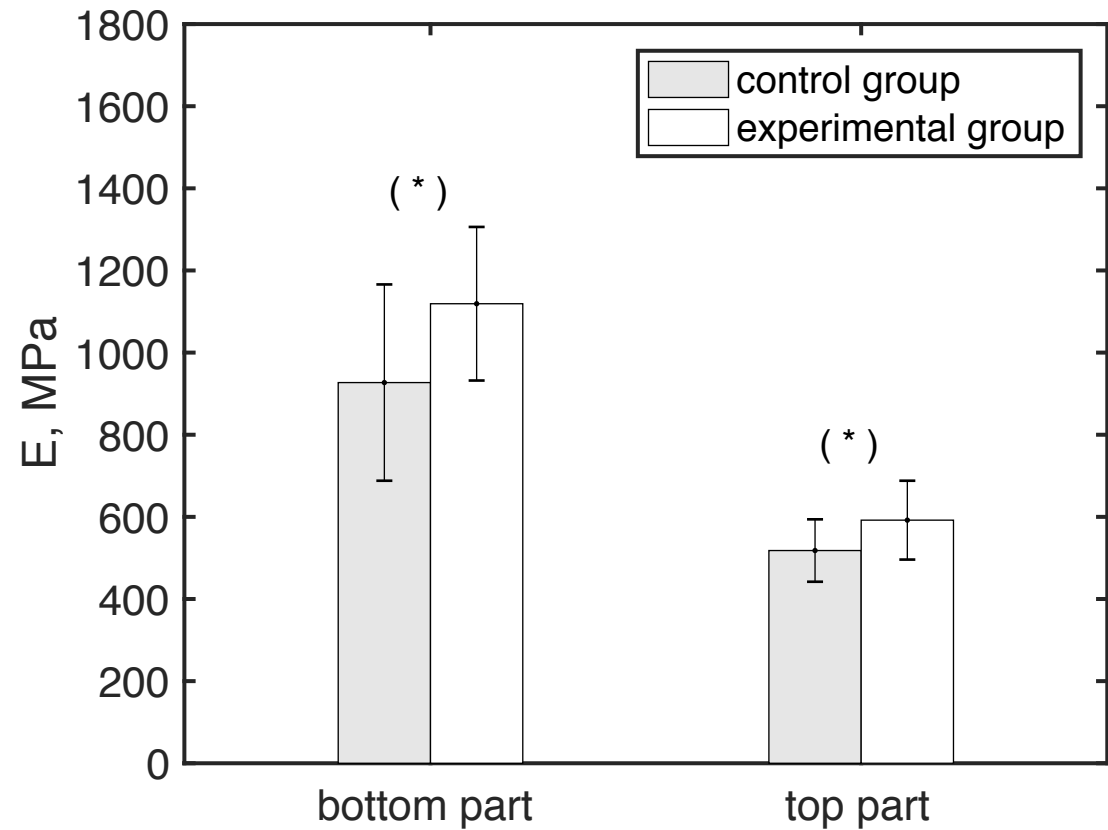
A

B

C

A

B

0.1mm

A

experimental group   control group

flow direction

B

flow direction
U=5m/s

C

experimental group   control group

flow direction

D

flow direction
U=5m/s