1   **Reducing shade avoidance can improve Arabidopsis canopy performance against**

2   **competitors**

3

4   Chrysoula K. Pantazopoulou[1], Franca J. Bongers[1,2] and Ronald Pierik[1]

5   [1] *Plant Ecophysiology, Dept. of Biology, Utrecht University, The Netherlands*

6   [2] *State Key Laboratory of Vegetation and Environmental Change, Institute of Botany, Chinese*

7   *Academy of Sciences, Beijing, China*

8   **Correspondence: c.pantazopoulou@uu.nl** and **r.pierik@uu.nl**

9

10 **Abstract**

11

12 The loss of crop yield due to weeds is an urgent agricultural problem. Although

13 herbicides are an effective way to control weeds, more sustainable solutions for weed

14 management are desirable. It has been proposed that crop plants can communally

15 suppress weeds by shading them out. Shade avoidance responses, such as upward leaf

16 movement (hyponasty) and stem or petiole elongation, enhance light capture of

17 individual plants, increasing their individual fitness. The shading capacity of the entire

18 crop community might, however, be more effective if aspects of shade avoidance are

19 suppressed. Testing this hypothesis in crops is hampered by the lack of well-

20 characterized mutants. We therefore investigated if *Arabidopsis* competitive

21 performance at the community level against invading competitors is affected by the

22 ability to display shade avoidance. We tested two mutants: *pif4pif5* that has mildly

23 reduced petiole elongation and hyponasty and *pif7* with normal elongation but absent

24 hyponasty in response to shade. Although *pif4pif5* performed similar to wildtype, we

25 found that *pif7* showed significantly increased canopy biomass and suppression of

26 invading competitors as compared to its wildtype. Our data thus show that modifying

27 specific shade avoidance aspects has potential for plant community performance. This

28 may help to suppress weeds in crop stands.

29

30

31

32

33 **Highlight**

34 Hyponastic response in canopies facilitates light penetration and weed growth.

35 Inhibition of this response to neighbors increased canopy biomass, canopy closure and

36 suppression of competitors.

37

38 **Key words**

39 Shade avoidance, canopy architecture, planting pattern, competition, *Arabidopsis*

40 *thaliana*, hyponasty

41

42

43 **Introduction**

44 Competition from weeds accounts for substantial yield losses in global crop

45 production systems (Bridges, 1994; Liebman *et al.*, 2001; Oerke 2006). Weed control

46 is usually accomplished by the extensive use of herbicides, and although these can be

47 effective, they are costly and have negative side effects on people's health and the

48 environment (Buhler, 2002; Chauhan and Johnson, 2010). There is, therefore, an

49 urgent need for novel methods to suppress weeds in a cost-effective and sustainable

50 manner.

51 Light is the core energy source for all plants, and when grown at high planting

52 densities, individual plants consolidate light capture by growing away from the shade

53 cast by neighboring plants, a process called shade avoidance (de Wit *et al.*, 2016*a*).

54 Proximate plants are detected through the red (R) : far-red (FR) light ratio (R:FR) in the

55 light reflected between plants, a ratio that decreases because of selective absorption

56 of R light for photosynthesis and reflection of FR light. When weeds emerge in a crop

57 vegetation, FR reflection becomes more intense due to the presence of more plant

58 biomass, leading to strong reduction of R:FR (Pierik and Testerink, 2014). Several

59 studies have shown that under low R:FR conditions, crops develop phenotypes such

60 as inhibition of lateral branching (tillering) in wheat (Ugarte *et al.*, 2010), formation of

61 smaller tubers and longer stem in potato (Boccalandro *et al.*, 2003) and increased stem

62 elongation in maize (Dubois *et al.*, 2010). All these morphological changes have a

63 negative impact on crop productivity (Robson *et al.*, 1996; Boccalandro *et al.*, 2003).

64 One approach to improve crop yield and suppress weeds, is by optimizing the shading
65 capacity of the entire crop community, for example by optimizing planting patterns
66 and plant phenotypic responses to density. In Evolutionary Agroecology (a.k.a.
67 Darwinian Agriculture) it is proposed that shade avoidance responses that enhance
68 individual plant performance, actually reduce performance of the entire community
69 of crop plants (Weiner *et al.*, 2010).

70 Studies on cereal crops have shown that optimization of cropping pattern and density
71 based on the community, rather than the individual performance, led to a more
72 effective weed suppression and increased crop productivity (Weiner *et al.*, 2001;
73 Olsen *et al.*, 2005, 2006; Kristensen *et al.*, 2008). This has for example been shown to
74 increase wheat yield by up to 30% (Weiner *et al.*, 2001). This major improvement may
75 be explained by the fact that crop plants growing in uniform patterns can collectively
76 create a stronger shade over the weeds than those sown in for example row planting
77 patterns. In other words, crop plants can collectively suppress weeds much better
78 than individual plants would be capable of. Since shade avoidance responses have a
79 major impact on the 3D architecture of the plant, they strongly impact the shading
80 capacity of a uniform plant community. Here, we will investigate to what extent
81 modulation of shade avoidance responses at high planting density can improve
82 shading capacity, competitor (weed) suppression and canopy plant performance.

83 Shade avoidance responses are typically elicited upon detection of a reduced R:FR
84 ratio, and are further promoted by depletion of blue light when the canopy closes (
85 (Ballaré, 1999; de Wit *et al.*, 2016*b*). Shade avoidance responses include upward leaf
86 movement (hyponasty), elongation of stems and petioles and inhibition of branching
87 (Franklin, 2008; Pierik and de Wit, 2013; de Wit *et al.*, 2016*b*). These responses help
88 plants reposition their leaves away from the shade and into the light. Shade avoidance
89 responses have been observed in most crop species and also in a variety of wild
90 species, including the genetic model plant *Arabidopsis thaliana* (Ballaré, 1999;
91 Franklin, 2008; Martínez-García *et al.*, 2010; Casal, 2012; Gommers *et al.*, 2013).

92 In responses to low R:FR, phytochrome photoreceptors are inactivated (Ballaré, 1999;
93 Franklin *et al.*, 2003; Kozuka *et al.*, 2010) and this relieves their repression of
94 Phytochrome Interacting Factors (PIFs) (Li *et al.*, 2012; Jeong and Choi, 2013; Leivar
95 and Monte, 2014), a class of transcription factors that promote the expression of

96   growth promoting genes (Oh *et al.*, 2012; Zhang *et al.*, 2013). PIF4, PIF5 and PIF7 are

97   the dominant PIF proteins involved in shade avoidance in Arabidopsis (Lorrain *et al.*,

98   2008; Koini *et al.*, 2009; Hornitschek *et al.*, 2012; Li *et al.*, 2012; Pantazopoulou *et al.*,

99   2017).

100   Shade avoidance responses in crops are often associated with reduced yield, because

101   resource investments are rerouted from harvestable organs towards stem elongation

102   (Robson *et al.*, 1996; Boccalandro *et al.*, 2003; Carriedo *et al.*, 2016). These responses

103   furthermore create a more open canopy architecture, which allows more light

104   penetration that in turn facilitates weed growth.

105   Here, we will investigate if canopy planting patterns and modifications of shade

106   avoidance responses can indeed optimize canopy architecture to suppress

107   competitors by improved shading capacity. We will compare *Arabidopsis thaliana*

108   wildtype with well-described mutants for aspects of the shade avoidance syndrome;

109   an opportunity that does not (yet) exist in other plant species. We show that the *pif7*

110   mutant that lacks a hyponastic response, but has preserved petiole elongation

111   responses to neighbors, performs significantly better at high density, uniform planting

112   patterns, than does its corresponding wildtype. Modest inhibition of both elongation

113   and hyponasty in the *pif4pif5* double mutant on the other hand did not affect plant

114   performance. Consistent with our hypothesis, a canopy of *pif7* plants was better able

115   to suppress competing invaders than was a shade avoiding wildtype canopy.

116   Our data indicate that modifying plant canopy architecture through altered shade

117   avoidance characteristics provides great opportunity to control weed proliferation in

118   cropping systems in a sustainable way.

119

120

121   **Materials and methods**

122

123   **Canopy conditions and measurements**

124   Genotypes used in this study, as canopy plants were wild-type Col-0, *pif4-101 pif5-1*

125   (Lorrain *et al.*, 2008) and *pif7* (Leivar *et al.*, 2008) while *pif4-101 pif5-1 pif7-1* (de Wit

126   *et al.*, 2015) was used as the invading competitor. Canopy seeds were sown in a pot

127   with a surface area of 10.5x10.5 cm filled with a substrate of soil:perlite (2:1), with

4

128   additional nutrients [6 g of slow release fertilizer (Osmocote 'plus mini' Ammonium

129   Nitrate Based Fertilizer; UN2071; Scotts Europe BV, Heerlen, The Netherlands) and 6

130   g MgOCaO (17%; Vitasol BV, Stolwijk, The Netherlands]. The *pif4pif5pif7* plants were

131   sown in a different pot three days after canopy plants for germination. Sowing was

132   followed by stratification for four days (dark, 4°C). After stratification plants were

133   moved to a short-day growth chamber (9 h/15 h of light/dark period respectively; R:FR

134   was 2.3 and PAR = 150 µmol m^{-2} s^{-1}). When canopy plots were 15 days old (seeds of

135   the canopy were sown directly in plots), competitor *pif4pif5pif7* seedlings (12 days

136   old) were transplanted into the plot (Fig. S1). The canopy plots were grown for another

137   29 days and subsequently harvested. Measurements were performed on four plants

138   for each plot. Petiole and lamina length of the three longest leaves from each plant

139   were measured with a digital caliper. Individual plant leaf area was scanned and

140   determined with image-J software. Shoot dry weight was recorded with a digital scale,

141   after drying the tissue at 70°C oven for three days. Plot biomass and LAI were

142   calculated from the four individuals by extrapolating to the full plot and density. The

143   heights of the canopies were measured with a ruler while the canopy cover was

144   determined from top photographs using the Plant CV software (Gehan *et al.*, 2017). In

145   the canopy cover measurements by the PlantCV, we exclude the outer plants of the

146   canopy to avoid edge effects. The height and the canopy cover measurements were

147   taken every five days, starting from the day 20 of canopy growth. Seed output was

148   recorded in separate experiments with the same growth conditions, three months

149   after sowing. Every 10 days (starting from the sowing day) plants were watered with

150   nutrients, on all other days they were watered with tap water. When the first silique

151   from each pot turned brown, watering was stopped. The number of siliques was

152   measured, after two weeks of ripening. Petiole angles (hyponasty) (Fig.3 and Fig.4)

153   and lamina length (Fig. S5) of the fifth-youngest leaf were measured digitally with

154   image J. Pictures were taken every day for 13 days, starting at day 28 (t=0).

155

156   **R:FR measurements**

157   The R:FR measurements started at day 20 (before the competition starts, (de Wit *et*

158   *al.*, 2012)] by using the Spectrosense2-Skye light sensor with a glass fiber extension

159   with 0.6 cm light collection area. The sensor was placed inside of the canopy plot (Fig.

160   S1A) and measured the R:FR from four different directions and on four different

161   positions, resulting in 16 measurements per time/per pot. When canopy closure

162   occurred, the sensor was placed under the canopy, without causing any damage to

163   the plants or interfering with the canopy shade. The measurements were always taken

164   from the same position in all densities and patterns.

165

166   **Experimental design of the densities and patterns with or with the competitor**

167   ***pif4pif5pif7***

168   For the Col-0 canopy plants three different densities were used (16 plants per pot

169   (1111 plants m^{-2}), 25 plants per pot (2500 plants m^{-2}), 64 plants per pot (8264 plants

170   m^{-2}); hereafter low, medium and high density respectively) and two spatial patterns

171   [uniform (equal distance between the plants) and row (bigger distance between the

172   rows of the plants but smaller distance between the plants within the rows), See Fig.

173   S1B)]. In uniform pattern, the distance between the plants was 3 cm, 2 cm and 1 cm

174   in low, medium and high density respectively. In row pattern, the distance between

175   the rows was always 5 cm while within the rows the distance between the plants were

176   0.6 cm, 1.25 cm and 2 cm in high medium and low density respectively. For the

177   canopies consisting of *pif4pif5 and pif7* plants only high density-uniform pattern was

178   used. The number of competitor *pif4pif5pif7* plants and their positions which were

179   transplanted into the high density-uniform pattern plots were the same in all the

180   canopies [16 plants per pot (1111 plants m^{-2})] (Fig. S1C).

181

182   **Light experiments**

183   Individual plant responses to R:FR were studied for the different genotypes used here.

184   To reduce the R:FR light ratios in the control white (W) light conditions from Philips

185   HPI lamps (R:FR = 2.3, 160 μmol m^{-2} s^{-1} PAR), supplemental far-red LEDs (Philips Green

186   Power FR 730 nm) were used. FR supplementation resulted in R:FR = 0.2 (160 μmol m^{-2}

187   s^{-1} PAR). To mimic the true canopy shade, green filter (Lee filters Fern Green) was

188   used (resulting in R:FR = 0.35 and 35 μmol m^{-2} s^{-1} PAR). The light spectra of the

189   treatments were measured with an Ocean optics JAZ spectroradiometer (Fig. S2).

190

191   **FSP model**

192   A functional-structural plant (FSP) model (Vos *et al.*, 2009) of Arabidopsis rosettes,

193   previously used and described in (Bongers *et al.*, 2017, 2019; Pantazopoulou *et al.*,

194   2017), was used to simulate Arabidopsis plant types, using the simulation platform

195   GroIMP and its radiation model (https://**sourceforge**.net/projects/**groimp**/).

196   Arabidopsis rosettes were represented by a collection of leaves (represented by a

197   petiole and lamina) whose appearance rate and shape were based on empirical data

198   (Bongers *et al.*, 2017). The leaves individually grew in time in 3D based on light

199   interception, photosynthesis and plant-wide carbon allocation principles (for detailed

200   explanations of the principles see Evers, 2016; Bongers *et al.*, 2017). In addition, leaves

201   showed petiole elongation and hyponastic responses based on the virtual touching of

202   leaves and the perception of R:FR (Bongers *et al.*, 2017). Therefore, individual growth

203   and shade avoidance responses depended on the capture of light (represented by PAR

204   intensity) and the perception of R:FR within the simulated canopy.

205   The light source emitted PAR representing 220 µmol m^{-2} s^{-1} and a R:FR ratio of

206   2.3, which corresponded to the growth chamber experiments. 100 plants were placed

207   in a uniform grid of 10 × 10 with an inter-plant distance of 1 cm, of which only the

208   middle 16 plants were used for analyses. Plants grew for 44 days based on the PAR

209   captured, photosynthesis rates and carbon allocation patterns (Evers, 2016; Bongers

210   *et al.*, 2017). Each model time step, which represented 24 hours, hyponastic responses

211   could occur if leaves touched or if R:FR perception at the lamina tip was below 0.5

212   (Bongers *et al.*, 2017; Pantazopoulou *et al.*, 2017). The strength of the hyponastic

213   responses depended per model scenario; plants could increase their leaf angle with 0,

214   0.2, 1, 5, 10, 15 or 20 degrees per day. The angle of the leaves over time was therefore

215   a function of the number of times in which touch and/or low R:FR perception occurred

216   per individual leaf, with a maximum leaf angle of 80 degrees. The intensity of PAR that

217   reached the soil was captured by 64 virtual soil-tiles (each 0.25 cm^2) underneath the

218   16 middle plants.

219

220   **Statistics**

221   Data were analyzed by one or two-way ANOVA followed by LSD test. All the analyses

222   were performed with GraphPad.

223

7

224 **Results**

225 **The effect of planting density and pattern on Col-0 performance**

226 To investigate the effect of sowing pattern and density on *Arabidopsis thaliana*

227 (hereafter Col-0) performance, we grew canopy plots in three different densities (low,

228 medium and high) and two different patterns (uniform and row) (Fig. 1A). The R:FR

229 showed a reduction in all densities and patterns through time, reflecting the growing

230 canopy (Fig. 1B). However, the strongest and most rapid decline of R:FR was observed

231 in high density/uniform pattern, where the R:FR was decreased from approximately

232 2.0 to 1.1 after eight days of measurements hinting at a rapidly closing canopy (Fig.

233 1A). This was not the case for the row pattern in high density, where the R:FR was still

234 high, presumably because the inter row distance was higher than in the uniform

235 pattern. Low and medium density showed reduction of R:FR (less than 1.5) at day 36

236 (Fig. 1B), indicating that the canopy remained more open for a longer period of time.

237 The leaf area index (LAI) expresses the amount of leaf area per unit soil area and

238 reflects the closure status of the canopy. LAI increased more strongly in the uniform

239 than in the row pattern and mostly in the medium and high densities (Fig. 1C).

240 Interestingly, leaf lamina length decreased with increasing plant density, irrespective

241 of the planting pattern (Fig. S3A). The opposite was observed for petiole length, where

242 the high density induced the strongest elongation (Fig. S3B). Enhanced petiole

243 elongation, combined with reduced lamina size, are classic aspects of shade

244 avoidance.

245 Furthermore, there was a strong and significant effect of the density and planting

246 pattern on Col-0 biomass. The row pattern produced Col-0 plants with the smallest

247 dry weight, indicating that the intraspecific competition was higher in rows compared

248 to the uniform pattern. In terms of planting density, the total biomass of the plot in

249 high density and uniform pattern was higher than the other densities (medium, low)

250 than the row pattern (Fig. 1D).

251 The number of siliques per square meter for the different density and planting

252 patterns was consistent with biomass (Fig. 1E), which suggests that the uniform-

253 planting pattern at the high density would result in the highest yield per unit area.

254

255 **Steering canopy light penetration through variations in hyponasty**

256   Using a previously published 3D computational Arabidopsis model (Bongers *et al.*,

257   2017), we determined the percentage of light penetration in the canopy through time

258   and under control of different degrees of hyponasty. Seven different hyponastic

259   scenarios were simulated; from 0 degrees up to 20 degrees hyponastic growth (Fig. 2).

260   The simulations show that canopies, consisting of plants with minimal hyponastic

261   response to neighbors (e.g. 0,0.2, 1 and 5 degrees) create strong reduction of light

262   penetration inside the canopy; after 28 days, less than 5 % of the light reaches the

263   soil. On the other hand, scenarios with faster hyponasty allowed for less light

264   extinction and thus higher penetration of light inside the canopy. These simulations

265   support the notion that upward leaf movement responses to neighbors may facilitate

266   light penetration through the canopy, which can be beneficial for weed growth.

267   To test experimentally if altered hyponastic growth can regulate canopy

268   closure and light penetration, we selected previously published mutants with altered

269   shade avoidance characteristics. The *pif7*, *pif4pif5* double and *pif4pif5pif7* triple

270   knockouts have reduced hyponastic responses to shade cues in short-term

271   experiments (Pantazopoulou *et al.*, 2017) and we verified their responses to

272   prolonged shade cue conditions. Reduction in R:FR resulted in the elevation of Col-0

273   petiole angle (hyponasty) during the first two days (day 29 and 30), while petiole

274   elongation was promoted from day 28 until 34 (Fig. 3A-D). *pif7* had a similar petiole

275   elongation response as did Col-0 in all the treatments but its hyponastic response to

276   low R:FR was entirely absent, whereas its response to green shade (reproducing

277   canopy shade) was severely reduced (Fig. 3A & 3B). *pif4pif5* showed a phenotype

278   initially similar to wild-type both in terms of petiole angle and elongation, but the

279   petiole elongated slightly less through time in low R:FR. On the other hand,

280   *pif4pif5pif7* was unresponsive to low R:FR for both traits (Figure 3C & 3D). Green filter

281   triggered a continuous shade avoidance phenotype in Col-0 (hyponasty and petiole

282   elongation) from day 28 up to 36 (8 days) (Figure 3B & 3D). Hyponastic responses were

283   reduced in *pif4pif5* and not observed at all in *pif7 and pif4pif5pif7* under these severe

284   shade conditions (Fig. 3A & 3C). In general, Col-0 shade avoidance responses

285   (hyponasty & petiole elongation) were stronger in green shade than in low R:FR alone.

286   Overall, *pif4pif5* was less responsive than Col-0, whereas *pif4pif5pif7* was fully

287   insensitive to the different light conditions. Interestingly, *pif7* showed similar petiole

288   growth as Col-0 and a similarly absent hyponastic response as in *pif4pif5pif7*.

289   The impact of different magnitudes of hyponastic responses in canopy closure,

290   was tested by growing canopies of Col-0, *pif7* and *pif4pif5*. We decided not to use the

291   *pif4pif5pif7* triple mutant as a canopy plant, but as an invading competitor in

292   establishing canopies. High density, uniform planting patterns were used, since these

293   closed their canopies most effectively (Fig. 1). Here, we monitored the canopy closure

294   state through time by using the imaging analysis PlantCV (Fig. 4). Data showed that

295   *pif7* canopies developed a better soil cover than Col-0 and *pif4pif5* early in the canopy

296   development (day 20 until 25). The *pif4pif5* canopies remained more open than *pif7*

297   canopies for another five days but percentage of the covered soil area was not

298   significantly different from Col-0 canopies at day 30. At later stages all canopies had

299   developed nearly full closure without. The *pif4pif5* canopies display reduced petiole

300   elongation compared to *pif7* and Col-0 (Fig. 3D), resulting in a relatively low canopy

301   height for this double mutant (Fig. S4B). The height of *pif7* canopies was also reduced

302   as compared to Col-0 (Fig. S4B), presumably because of the reduced upward leaf

303   movement in this mutant (Fig. 3A)

304

305   **Performance of canopy and competitor plants during competition**

306   To test the impact of separate shade avoidance traits on competitor suppression and

307   canopy performance, we used as canopy plants the strong shade avoider Col-0, the

308   mild reduction of shade avoidance genotype *pif4pif5*, and *pif7* which does not show

309   hyponasty but does induce petiole elongation upon low R:FR (Fig. 3). As an invading

310   competitor we used *pif4pif5pif7,* which was planted between the canopy plants. To

311   estimate shade avoidance responses of the different genotypes in true canopies,

312   rather than independent light treatments, we measured petiole and lamina length at

313   the end of the canopy development. *pif7* canopy plants displayed the largest lamina

314   compared to Col-0 and *pif4pif5* canopy plants during competition. Petiole length was

315   enhanced upon competition in Col-0 and *pif7* but not in *pif4pif5* canopy plants (Fig.

316   S6). The strong lamina and petiole elongation but not hyponasty (fig. 3 and fig S4B)

317   responses of *pif7* during competition could have resulted in the higher biomass and

318   LAI compared to the other two genotypes (Fig. 5). This also had a strong effect on

10

319   *pif4pif5pif7* competitor performance. The faster closed canopy and plant growth of

320   *pif7* during competition was associated with a reduction in growth of *pif4pif5pif7*

321   competitors (Fig. 6A & 6B). On the other hand, the improved light exposure of

322   *pif4pif5pif7* competitor plants under the rapidly closed canopy of *pif4pif5* was

323   associated with enhanced biomass and leaf area (L.A.) of the competitor triple mutant

324   compared to the other genotypes (Fig. 5, 6A & 6B). Indeed, the *pif4pif5pif7* competitor

325   hardly survives under the *pif7* canopy while the percentage of survival between Col-0

326   and *pif4pif5* was similar (Fig. 6C)*.*

327

328

329   **Discussion**

330   The adaptability of to changing environments and their competitive potential makes

331   them a major threat for agricultural yields when they interact with crop plants (Oerke,

332   2006). Studies in the past decades found that crop sowing uniformity in high density

333   can positively affect yield and suppress weeds (Weiner *et al.*, 2001; Olsen *et al.*, 2006;

334   Kristensen *et al.*, 2008). A second way to improve crop yield and suppress weeds,

335   according to the principles of Evolutionary Agroecology would be by controlling the

336   shade avoidance properties of the crops in a way that would minimize light

337   penetration through the canopy down to the soil where weeds sprout (Weiner *et al.*,

338   2010). Since only very few well-defined shade avoidance mutants exist in crops

339   (Carriedo *et al.*, 2016; Kebrom and Mullet, 2016; Weiner *et al.*, 2017), we tested the

340   impact of shade avoidance modulation on canopy performance and weed suppression

341   in the model species *Arabidopsis thaliana*.

342   Although petiole elongation, combined with upward leaf movement (hyponasty), will

343   increase access to light at the individual plant level (Ballaré and Pierik, 2017;

344   Pantazopoulou *et al.*, 2017), the reduced leaf lamina growth that typically occurs in

345   shade avoiding *Arabidopsis* (de Wit *et al.*, 2015) may counterbalance the predicted

346   gain in photosynthesis of individual plants (Fritz *et al.*, 2018). Part of the shade

347   avoidance responses will have been triggered through the drop in R:FR inside the

348   canopies (Fig. 1B). However, shade avoidance responses, and especially hyponasty,

349   can on their turn also affect the R:FR inside the canopy by affecting the extent to which

350   a vertical canopy structure is formed in this otherwise horizontally growing rosette

11

351   species (de Wit *et al.*, 2012). Modulating shade avoidance traits in different canopy

352   structures may thus affect light distribution inside these canopies. Indeed, using a 3D

353   Arabidopsis plant model (Bongers *et al.*, 2017), we found that slow-down of

354   hyponastic growth upon shade detection in all canopy plants can clearly reduce light

355   penetration through the canopy down to soil level (Fig. 2). To test the consequences

356   of this scenario experimentally and also monitor the effect that these canopies can

357   have on competitor performance we used *Arabidopsis* mutants that had similar

358   hyponastic responses variations as used in the 3D plant model. As a competitor, we

359   used the *pif4 pif5 pif7* triple mutant*,* which remained unresponsive in terms of

360   hyponasty and petiole elongation under the long-term shade conditions. The

361   *pif4pif5pif7* would not be able to outgrow the canopy plants, allowing us to 1) mimic

362   the crop-weed competition where crops (like cereals) typically have a size advantage

363   and 2) record the effect of different canopy architectures (i.e. *pif7* and *pif4pif5*) on

364   competitor performance. The canopy architecture of *pif7* had a strong negative impact

365   on the performance of competitor *pif4pif5pif7* and a positive impact on its own canopy

366   biomass (fig. 5A). Under these conditions, the *pif4pif5pif7* competitor biomass and

367   survival rate were significantly lower than under Col-0 and *pif4pif5* canopy

368   architectures (Fig. 6A & 6C). We propose that the much faster closing of the *pif7*

369   canopy together with the larger LAI as compared to the Col-0 and *pif4pif5* canopies

370   (Fig. 4, S4 & fig. 5B), resulted in less light availability for the competitor, leading to

371   reduced performance of the competitor.

372   Interestingly, despite the fact that the *pif4pif5* canopy architecture showed mild

373   reduction of shade avoidance responses, the competitor *pif4pif5pif7* performed

374   similar in Col-0 and *pif4pif5* canopy. We speculate that the advantage of modestly

375   reduced shade avoidance in *pif4pif5* for communal competitor suppression might be

376   outweighed by its reduced overall growth rate, which still leads to a relatively open

377   canopy.

378   As mentioned above, *pif4pif5pif7* lacks any shade avoidance response to plant density,

379   signaled by low R:FR and green filter (fig. 3). This allowed us to study if the resident

380   canopy architecture can be optimized such that growth in the understory can be

381   inhibited by shading the invading competitors. Future studies could be designed to

382   include competitors that can show shade avoidance responses and thus have the

12

383 capacity to compete stronger against the dominant canopy. It would be possible then

384 that the invading competitors could even escape from the shade-casting canopy

385 altogether and enhance their individual fitness at the expense of the collective fitness

386 of the dominant canopy. If good mutants come available for upright-growing, stem-

387 forming plants, these could be used to test scenario's of a more vertically layered

388 canopy, representing many of the staple crops world-wide, for weed-suppression.

389 Our data show that losing one of the shade avoidance responses in Arabidopsis

390 canopy, hyponasty, has potential to suppress competitors. Translating this to crop-

391 weed competition scenario's depends on the architecture of the crop plant but could

392 potentially improve weed suppression and reduce yield losses due to weeds.

393

394

395 Author contributions: C.K.P. and R.P. designed research; C.K.P. and F.J.B. performed

396 research; C.K.P. and F.J.B analyzed data; and C.K.P. and R.P. wrote the paper.

397 The authors declare no conflict of interest.

398

399 **Acknowledgments**

404

405

406

407

408

409

410

411

412

413

414

415

References

Ballaré CL. 1999. Keeping up with the neighbours: phytochrome sensing and other signalling mechanisms. Trends in plant science **4**, 97–102.

Ballaré CL, Pierik R. 2017. The shade-avoidance syndrome: multiple signals and ecological consequences. Plant, cell & environment.

Ballaré CL, Scopel AL, Sánchez RA. 1990. Far-red radiation reflected from Adjacent Leaves: An Early Signal of Competition in Plant Canopies. Science **247**, 329–332.

Boccalandro HE, Ploschuk EL, Yanovsky MJ, Sánchez RA, Gatz C, Casal JJ. 2003. Increased phytochrome B alleviates density effects on tuber yield of field potato crops. Plant Physiology **133**, 1539–1546.

Bongers FJ, Douma JC, Iwasa Y, Pierik R, Evers JB, Anten NPR. 2019. Variation in plastic responses to light results from selection in different competitive environments—A game theoretical approach using virtual plants. PLoS computational biology **15**, e1007253.

Bongers FJ, Pierik R, Anten NPR, Evers JB. 2017. Subtle variation in shade avoidance responses may have profound consequences for plant competitiveness. Annals of botany, in press.

Bridges DC. 1994. Impact of weeds on human endeavors. Weed Technology **8**, 392–395.

Buhler DD. 2002. 50th Anniversary—Invited Article: Challenges and opportunities for integrated weed management. Weed Science **50**, 273–280.

Bullock D, Khan S, Rayburn A. 1998. Soybean yield response to narrow rows is largely due to enhanced early growth. Crop Science **38**, 1011–1016.

Carriedo LG, Maloof JN, Brady SM. 2016. Molecular control of crop shade avoidance. Current opinion in plant biology **30**, 151–158.

Casal JJ. 2012. Shade avoidance. The Arabidopsis book / American Society of Plant Biologists **10**, e0157.

Chauhan BS, Johnson DE. 2010. Implications of narrow crop row spacing and delayed Echinochloa colona and Echinochloa crus-galli emergence for weed growth and crop yield loss in aerobic rice. Field Crops Research **117**, 177–182.

Dubois PG, Olsefski GT, Flint-Garcia S, Setter TL, Hoekenga OA, Brutnell TP. 2010. Physiological and genetic characterization of end-of-day far-red light response in maize seedlings. Plant physiology **154**, 173–186.

Evers JB. 2016. Simulating Crop Growth and Development Using Functional-Structural Plant Modeling. Canopy Photosynthesis:From Basics to Applications.219–236.

Franklin KA. 2008. Shade avoidance. New Phytologist **179**, 930–944.

Franklin KA, Davis SJ, Stoddart WM, Vierstra RD, Whitelam GC. 2003. Mutant analyses define multiple roles for phytochrome C in Arabidopsis photomorphogenesis. The Plant Cell **15**, 1981–1989.

Fritz MA, Rosa S, Sicard A. 2018. Mechanisms Underlying the Environmentally Induced Plasticity of Leaf Morphology. Frontiers in genetics **9**.

Gehan MA, Fahlgren N, Abbasi A, Berry JC, Callen ST, Chavez L, Doust AN, Feldman MJ, Gilbert KB, Hodge JG. 2017. PlantCV v2: Image analysis software for high-throughput plant phenotyping. PeerJ **5**, e4088.

Gommers CMM, Visser EJW, St Onge KR, Voesenek LACJ, Pierik R. 2013. Shade tolerance: when growing tall is not an option. Trends in Plant Science **18**, 65–71.

14

463   **Hornitschek P, Kohnen M V., Lorrain S,** *et al.* 2012. Phytochrome interacting factors
464   4 and 5 control seedling growth in changing light conditions by directly controlling
465   auxin signaling. Plant Journal **71**, 699–711.
466   **Jeong J, Choi G**. 2013. Phytochrome-interacting factors have both shared and distinct
467   biological roles. Molecules and cells **35**, 371–380.
468   **Kebrom TH, Mullet JE**. 2016. Transcriptome profiling of tiller buds provides new
469   insights into PhyB regulation of tillering and indeterminate growth in sorghum. Plant
470   physiology **170**, 2232–2250.
471   **Koini MA, Alvey L, Allen T, Tilley CA, Harberd NP, Whitelam GC, Franklin KA**. 2009.
472   High temperature-mediated adaptations in plant architecture require the bHLH
473   transcription factor PIF4. Current Biology **19**, 408–413.
474   **Kozuka T, Kobayashi J, Horiguchi G, Demura T, Sakakibara H, Tsukaya H, Nagatani
475   A**. 2010. Involvement of auxin and brassinosteroid in the regulation of petiole
476   elongation under the shade. Plant Physiology **153**, 1608–1618.
477   **Kristensen L, Olsen J, Weiner J**. 2008. Crop density, sowing pattern, and nitrogen
478   fertilization effects on weed suppression and yield in spring wheat. Weed Science **56**,
479   97–102.
480   **Leivar P, Monte E**. 2014. PIFs: systems integrators in plant development. The Plant
481   Cell **26**, 56–78.
482   **Leivar P, Monte E, Al-Sady B, Carle C, Storer A, Alonso JM, Ecker JR, Quail PH**. 2008.
483   The Arabidopsis phytochrome-interacting factor PIF7, together with PIF3 and PIF4,
484   regulates responses to prolonged red light by modulating phyB levels. Plant Cell **20**,
485   337–352.
486   **Leivar P, Quail PH**. 2011. PIFs: pivotal components in a cellular signaling hub. Trends
487   in plant science **16**, 19–28.
488   **Li L, Ljung K, Breton G, Schmitz RJ, Pruneda-Paz J, Cowing-Zitron C, Cole BJ, Ivans LJ,
489   Pedmale U V, Jung H-S**. 2012. Linking photoreceptor excitation to changes in plant
490   architecture. Genes & development **26**, 785–790.
491   **Liebman M, Mohler CL, Staver CP**. 2001. *Ecological management of agricultural
492   weeds*. Cambridge University Press.
493   **Lorrain S, Allen T, Duek PD, Whitelam GC, Fankhauser C**. 2008. Phytochrome-
494   mediated inhibition of shade avoidance involves degradation of growth-promoting
495   bHLH transcription factors. Plant Journal **53**, 312–323.
496   **Martínez-García JF, Galstyan A, Salla-Martret M, Cifuentes-Esquivel N, Gallemí M,
497   Bou-Torrent J**. 2010. Regulatory Components of Shade Avoidance Syndrome.
498   Advances in Botanical Research.65–116.
499   **Norris RF, Elmore CL, Rejmánek M, Akey WC**. 2001. Spatial arrangement, density,
500   and competition between barnyardgrass and tomato: I. Crop growth and yield. Weed
501   Science **49**, 61–68.
502   **Oerke E-C**. 2006. Crop losses to pests. The Journal of Agricultural Science **144**, 31.
503   **Oh E, Zhu J-Y, Wang Z-Y**. 2012. Interaction between BZR1 and PIF4 integrates
504   brassinosteroid and environmental responses. Nature Cell Biology **14**, 802.
505   **Olsen J, Kristensen L, Weiner J**. 2006. Influence of sowing density and spatial pattern
506   of spring wheat (Triticum aestivum) on the suppression of different weed species.
507   Weed Biology and Management **6**, 165–173.
508   **Olsen J, Kristensen L, Weiner J, Griepentrog H-W**. 2005. Increased density and
509   spatial uniformity increase weed suppression by spring wheat. Weed Research **45**,

510   316–321.
511   **Pantazopoulou CK, Bongers FJ, Küpers JJ, Reinen E, Das D**. 2017. Neighbor detection
512   at the leaf tip adaptively regulates upward leaf movement through spatial auxin
513   dynamics. **114**.
514   **Pierik R, Testerink C**. 2014. The art of being flexible: how to escape from shade, salt,
515   and drought. Plant Physiology **166**, 5–22.
516   **Pierik R, de Wit M**. 2013. Shade avoidance: phytochrome signalling and other
517   aboveground neighbour detection cues. Journal of Experimental Botany **65**, 2815–
518   2824.
519   **Robson PRH, McCormac AC, Irvine AS, Smith H**. 1996. Genetic engineering of
520   harvest index in tobacco through overexpression of a phytochrome gene. Nature
521   biotechnology **14**, 995–998.
522   **Ross MA, Lembi CA**. 1999. Herbicide incorporation techniques and equipment.
523   Applied Weed Science. 2nd ed. Upper Saddle River, NJ: Prentice-Hall, 371–375.
524   **Schmitt J, Dudley SA, Pigliucci M**. 1999. Manipulative approaches to testing adaptive
525   plasticity: phytochrome-mediated shade-avoidance responses in plants. the
526   american naturalist **154**, S43–S54.
527   **Thornley JHM, Johnson IR**. 1990. A mathematical approach to plant and crop
528   physiology. A mathematical approach to plant and crop physiology.
529   **Ugarte CC, Trupkin SA, Ghiglione H, Slafer G, Casal JJ**. 2010. Low red/far-red ratios
530   delay spike and stem growth in wheat. Journal of experimental botany **61**, 3151–
531   3162.
532   **Vos J, Evers JB, Buck-Sorlin GH, Andrieu B, Chelle M, De Visser PHB**. 2009.
533   Functional–structural plant modelling: a new versatile tool in crop science. Journal of
534   experimental Botany **61**, 2101–2115.
535   **Weiner J, Andersen SB, Wille WK, Griepentrog HW, Olsen JM**. 2010. Evolutionary
536   Agroecology: the potential for cooperative, high density, weed-suppressing cereals.
537   Evolutionary Applications **3**, 473–479.
538   **Weiner J, Du Y, Zhang C, Qin X, Li F**. 2017. Evolutionary agroecology: individual
539   fitness and population yield in wheat (Triticum aestivum). Ecology **98**, 2261–2266.
540   **Weiner J, Griepentrog H, Kristensen L**. 2001. Suppression of weeds by spring wheat
541   Triticumaestivum increases with crop density and spatial uniformity. Journal of
542   Applied Ecology **38**, 784–790.
543   **de Wit M, Galvão VC, Fankhauser C**. 2016*a*. Light-mediated hormonal regulation of
544   plant growth and development. Annual review of plant biology **67**, 513–537.
545   **de Wit M, Kegge W, Evers JB, Vergeer-van Eijk MH, Gankema P, Voesenek LACJ,**
546   **Pierik R**. 2012. Plant neighbor detection through touching leaf tips precedes
547   phytochrome signals. Proceedings of the National Academy of Sciences **109**, 14705–
548   14710.
549   **de Wit M, Keuskamp DH, Bongers FJ, Hornitschek P, Gommers CMM, Reinen E,**
550   **Martínez-Cerón C, Fankhauser C, Pierik R**. 2016*b*. Integration of phytochrome and
551   cryptochrome signals determines plant growth during competition for light. Current
552   Biology **26**, 3320–3326.
553   **de Wit M, Ljung K, Fankhauser C**. 2015. Contrasting growth responses in lamina and
554   petiole during neighbor detection depend on differential auxin responsiveness rather
555   than different auxin levels. New Phytologist **208**, 198–209.
556   **Zhang Y, Mayba O, Pfeiffer A, Shi H, Tepperman JM, Speed TP, Quail PH**. 2013. A

557 quartet of PIF bHLH factors provides a transcriptionally centered signaling hub that
558 regulates seedling morphogenesis through differential expression-patterning of
559 shared target genes in Arabidopsis. PLoS genetics **9**, e1003244.
560
561

562
563
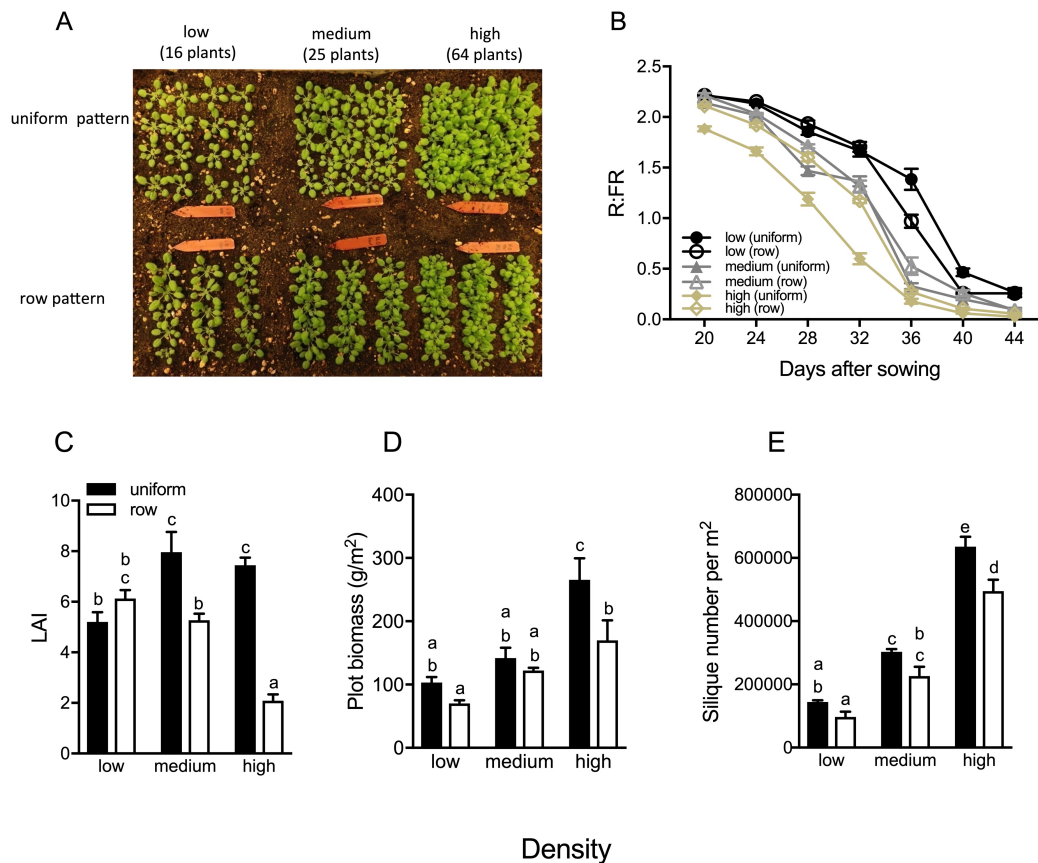
564
565 Fig. 1: Arabidopsis Col-0 in high density, uniform pattern produces more biomass and

566 canopy cover than at lower densities and row patterns. (A) In the upper row Col-0

567 plants grow in a uniform pattern (uniform), while in the lower row plants grow in row

568 pattern (row) at three different densities (low, medium, high). (B) The R:FR light ratio

569 measured inside Col-0 canopies, during the days of growth, in low (black lines),

570 medium (grey lines) and high (yellow lines) densities and two patterns (uniform and

571 row). (C-E) Leaf area index (LAI; C), plot biomass per m^2 (D) and seed output (silique

572 number per m^2 pot; E) at three different densities (low, medium, high) and two

573 different planting patterns (uniform, row). Data represent mean ± SE (n=5). Different

574 letters indicate statistically significant differences (two-way ANOVA with LSD test, $P < 0.05$).

575
576
577
578

18
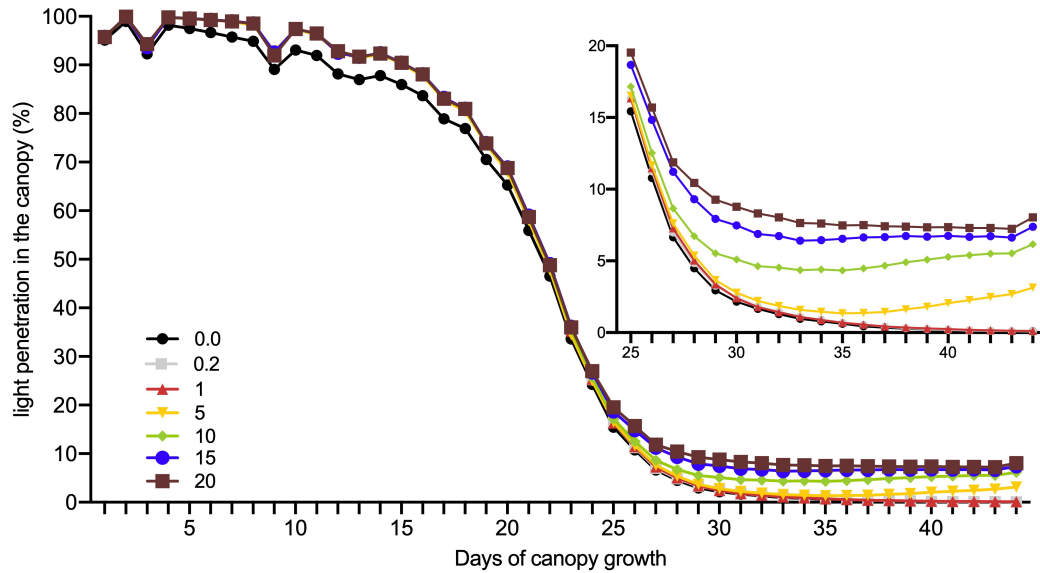
Fig. 2: Reduced hyponastic responses result in lower percentage of light penetrating the canopy and reaching the soil. Percentage of light reaching the soil is simulated with a 3D Arabi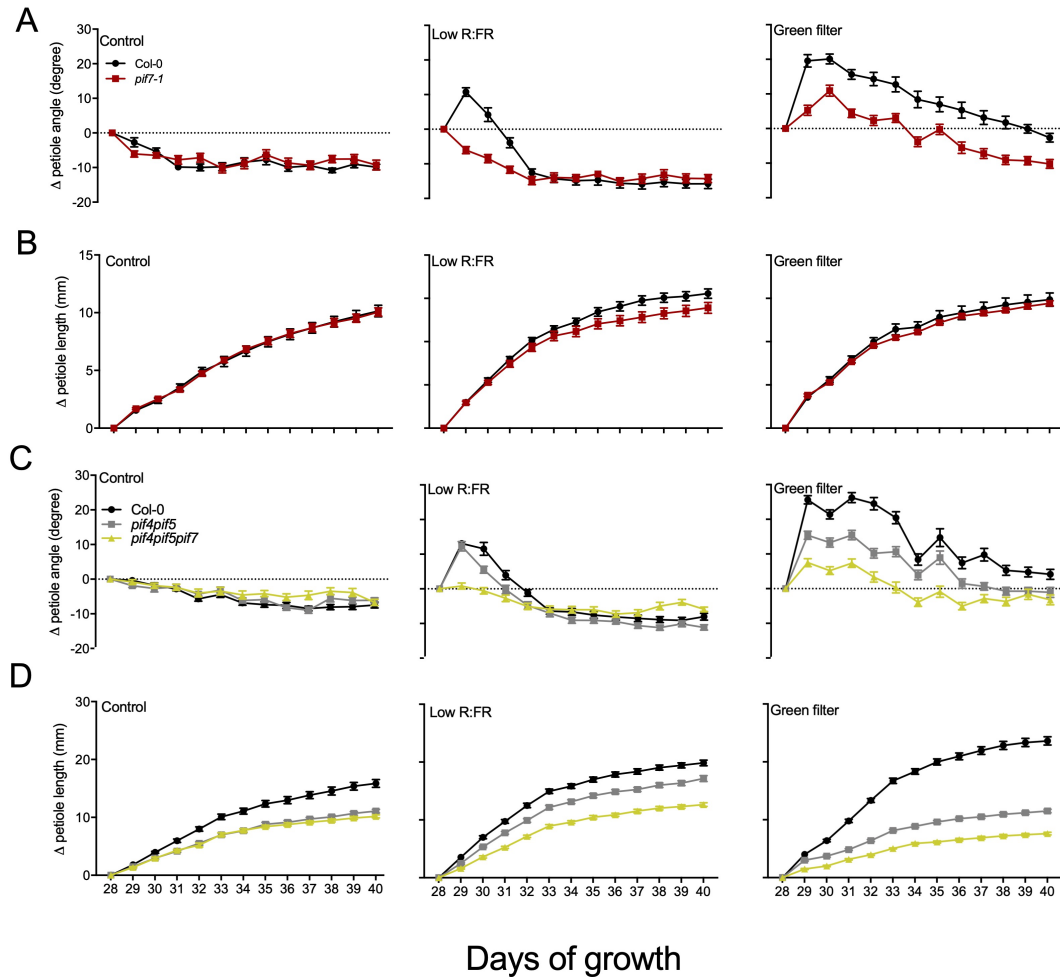dopsis computational model. Various canopy growth simulation scenarios consist of plants with different degrees of hyponastic responses to proximate neighbor plants, ranging from 0 to 20 degrees per day (see legend). Data represent mean ± SD (n=10).
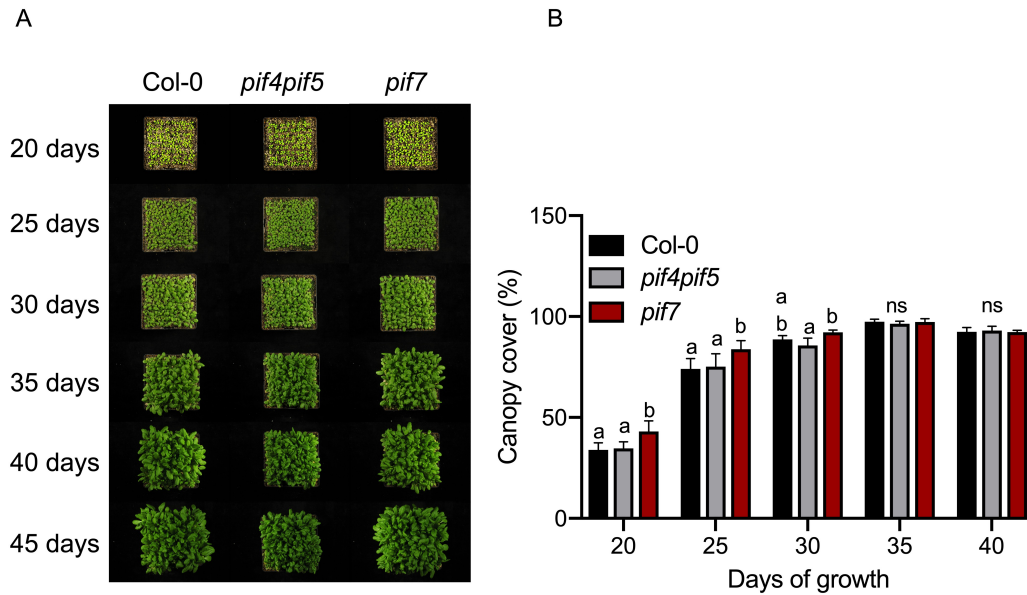
Fig. 3: Shade avoidance responses (change in petiole length (A, C) and change in petiole angle (B, D)) of Col-0, *pif7*, *pif4pif5* and *pif4pif5pif7* upon white light (control), low R:FR and green filter exposure. Light treatments lasted 13 days and started when plants were 28 days old. Data represent mean ± SE (n=15).

20

596
597 Fig. 4: The *pif7* mutant creates a faster closed canopy than Col-0 and *pif4pif5*. (A)

598 Pictures illustrate how the canopies of Col-0 (left), *pif4pif5* (middle) and *pif7* (right)

599 plants develop and close soil exposure to light. (B) The percentage of soil covered by

600 the same canopies: Col-0 (black bars), *pif4pif5* (grey bars) and *pif7* (red bars) plants,

601 through time. The Col-0, *pif4pif5* and *pif7* canopies plants grew at high density,

602 uniform pattern. Data represent mean ± SE (n=5). Different letters indicate statistically

603 significant differences (two-way ANOVA with LSD test, $P < 0.05$. ns=not significant).

604
605
606
607
608
609
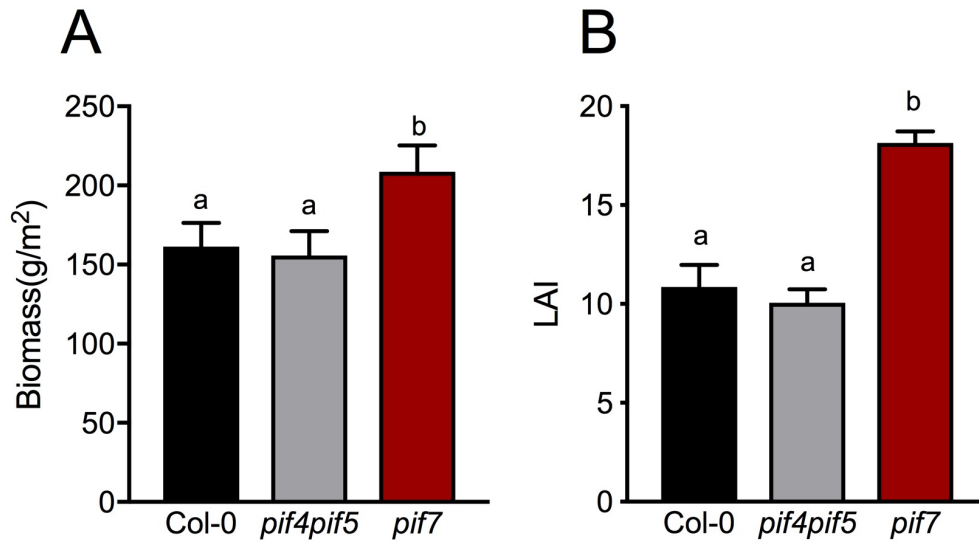
Fig. 5: The *pif7* canopies grew larger than Col-0 and *pif4pif5* under high-density competing conditions. (A) Biomass and (B) LAI of canopies consisting of Col-0 (black bar), *pif4pif5* (grey bars) or *pif7* (red bars), growing at high density, uniform pattern, measured after 44 days of growth. Data represent mean ± SE (n=5). Different letters indicate statistically significant differences (two-way ANOVA with LSD test, $P < 0.05$).
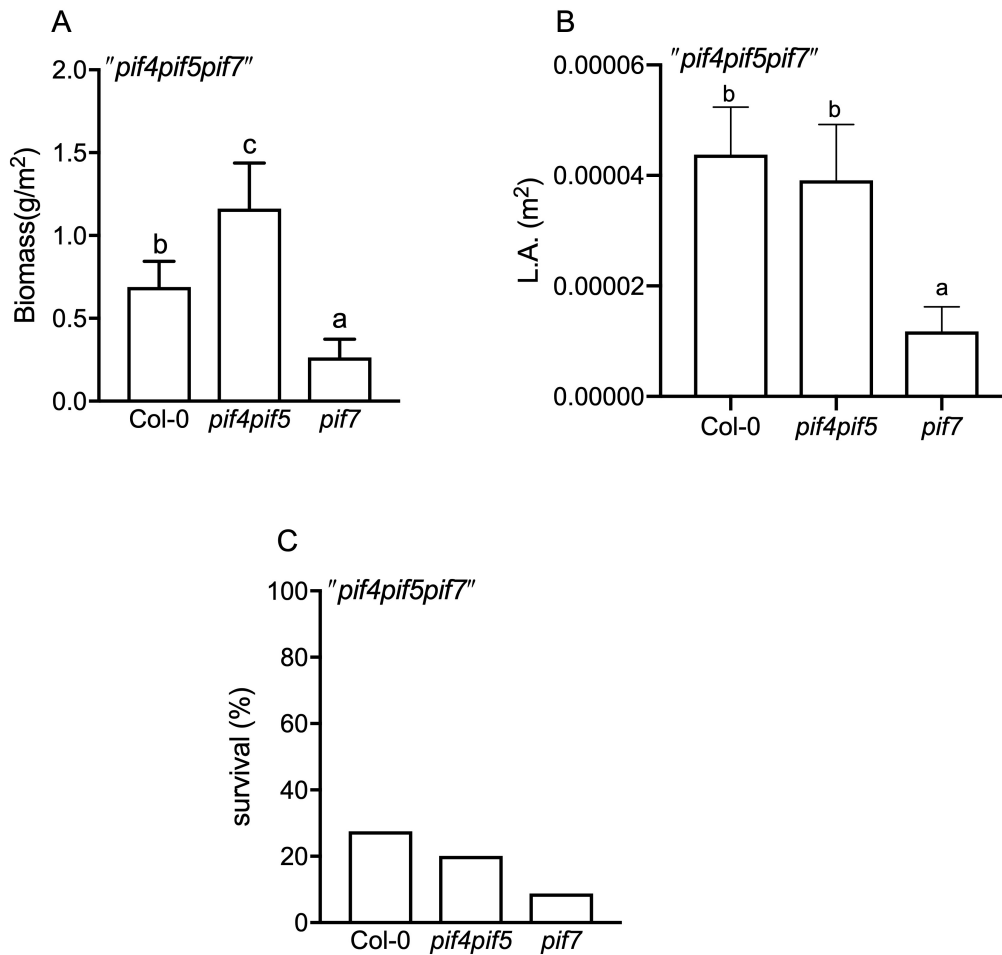
617
618   Fig. 6: The *pif7* canopies suppressed the competitor*pif4pif5pif7* more effectively than

619   did Col-0 and *pif4pif5* canopies. The competitor's (A) biomass, (B) leaf area and (C)

620   percentage of survival, under the canopies of Col-0, *pif4pif5* and *pif7* for 44days. The

621   plants grew at high density, uniform pattern. Data represent mean ± SE (n=5). Different

622   letters indicate statistically significant differences (two-way ANOVA with LSD test, $P < 0.05$).

623