Manipulating resource allocation in plants

Article

Accepted Version

1   **Manipulating resource allocation in plants**

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13   Running title: Manipulating resource allocation

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15   Keywords: Resource allocation, senescence, abscission, leaf, pod, Arabidopsis,

16   Brassica

17

18   Wordcount: 6291

19   Five figures in total. Three in colour in print and online.

20 ***ABSTRACT***

21 The distribution of nutrients and assimilates in different organs and tissues is in a

22 constant state of flux throughout the growth and development of a plant. At key

23 stages during the life cycle profound changes occur and perhaps one of the most

24 critical of these is during seed filling. By restricting the competition for reserves in

25 Arabidopsis plants the ability to manipulate seed size, seed weight, or seed content

26 has been explored. Removal of secondary inflorescences and lateral branches

27 resulted in a stimulation of elongation of the primary inflorescence and an increase

28 in the distance between siliques. The pruning treatment also led to the

29 development of longer and larger siliques that contained fewer, bigger seeds. This

30 seems to be a consequence of a reduction in the number of ovules that develop

31 and an increase in the fatty acid content of the seeds that mature. The data show

32 that shoot architecture could have a substantial impact on the partitioning of

33 reserves between vegetative and reproductive tissues and could be an important

34 trait for selection in rapid phenotyping screens to optimise crop performance.

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INTRODUCTION

Plants accumulate and redistribute macro and micronutrients throughout their life cycle. Ultimately the plant is seeking to optimise the way it responds to environmental cues and to integrate these with the genetically determined elements of development in order to maximise its reproductive potential and ensure the production and survival of the next generation. Plants, like other living organisms, can be divided into those that have an r- or a K- reproductive strategy (MacArthur and Wilson, 1967; Pianka, 1970). Most weed species adopt an r-strategy and produce many thousands of small seeds with minimum investment of resource per seed; in contrast, many trees invest in relatively few reproductive units per individual but each unit is associated with a substantial quantity of resource to help protect and nurture the subsequent generation (Pianka, 1970). For example, the reproductive propagule of an *Arabis* weed species is a seed weighing less than 1mg and sunflower seeds are about 100mg each, whereas coconuts typically weigh over 1kg.

In order to optimise reproductive potential plants need to (re)mobilise nutrients from sources to sinks in a highly orchestrated way. This process is spatially and temporally dependent on the stage of the plant's life cycle and the reproductive strategy of the plant under consideration. For instance, an annual plant will ultimately die after seed filling is complete; in this case the seeds are the means by which the genetic information of an individual over-winters and survives until growing conditions become favourable once again. In some species seed dormancy can last months or even years and provides a highly effective means of spreading

63 the timing of germination and potentially avoiding adverse environmental

64 challenges (Barton, 1961). Work at the Millennium Seed Bank at the Royal Botanic

65 Gardens, Kew revealed that 200 year old seeds, collected by the Dutch explorer Jan

66 Teerlink during a voyage to the Cape of Good Hope in 1803, were still viable. The

67 species included a *Leucospermum* from the family Proteaceae, and two species

68 from the family Fabaceae including an Acacia and a legume called *Liparia villosa*,

69 indicating a great range of species for which long-term dormancy is a viable option.

70 For annual plants such as Arabidopsis, under optimal growth conditions, the

71 process of resource allocation ensures that ultimately nearly all the nitrogen

72 resources produced during the photosynthetic period will be remobilised from the

73 vegetative tissues into the developing seeds. In contrast, perennial plants commit a

74 more limited proportion of resources to the reproductive phase of their life cycle as

75 it is advantageous to retain some photosynthetic capacity for as long as the

76 environmental conditions allow. For this reason, it has been speculated that the

77 anthocyanins produced in autumnal leaves provide photoprotection to enable

78 maximal re-absorption during times when the leaves are vulnerable to the effects

79 of high light accompanied by low temperatures (Archetti et al., 2009). For a

80 perennial plant it is less essential to ensure reproductive success every year due to

81 their longer life spans, and tolerance to environmental stress is often achieved by

82 slower growth in addition to niche adaptations such as an evergreen habit. Bulbous

83 plants provide an example where temperature is key to regulating source-sink

84 balance; lower temperatures result in delayed leaf senescence and larger bulbs due

85 to an improved equilibrium between carbon fixation capacity and sink strength

86 (Gandin et al., 2011). Having lived for over 4800 years the bistlecone pine,

87   Methuselah, is a prime example of a plant's ability to endure abiotic stresses. Grime

88   (1977) proposed that resource-allocation strategies in plants could be divided into

89   three categories: Competitive, Stress-tolerant and Ruderal Strategies (denoted C-,

90   S- and R-strategies respectively). Grime summarised the consequences of C-, S- and

91   R-strategies on resource allocation for plant growing in three broad habitat

92   categories (Table 1) and it is interesting to review these in the light of considering

93   crop plants and the habitats under which they are grown in a modern agricultural

94   environment.

95   ***RESOURCE ALLOCATION STRATEGIES OF EXISTING CROP PLANTS***

96   The major cereal crops have undergone many centuries of domestication and, in

97   the developed world, are typically grown in environments where water and

98   nutrients are plentiful. Selection by 'man' has promoted C-strategy plants that

99   compete well in a monoculture and make large changes in root/shoot biomass as a

100 consequence of stress, meaning that when these crops are grown in environments

101 experiencing prolonged stress they survive less well (see Table 1). This may be one

102 explanation why the major grain crops of the world do not yield well in countries

103 which habitually experience high levels of abiotic (or biotic) stresses , unlike plants

104 native toarid environments which can respond quickly to resource pulses. For

105 instance, high temperatures and low rainfall across southern Australia in 1982,

106 1994 and 2004 restricted the total wheat yield to less than 10 million tonnes,

107 whereas the favourable environment in the 1983/4 season that followed the major

108 drought in 1982 produced in excess of 22 million tonnes (Sutton, 2009). In contrast,

109 more recently domesticated crop plants, such as *Brassica* species, retain the weedy

110   characteristics of their wild progenitors and continue to adopt an R-strategy. In

111   consequence, these plants produce seed very rapidly, especially when experiencing

112   abiotic stress (Sinniah et al., 1998), and ensure that the genepool is carried

113   forwards to the next generation. In these situations it would appear that some seed

114   can be produced with such speed that very little resource reallocation is required;

115   this is perhaps not surprising since the seed in a Brassicaceous plant is itself

116   photosynthetic in the initial stages of its development, but it is surrounded by a

117   photosynthetic pod that can supply photosynthates at a highly localised site (Hua at

118   al., 2011). However, if *Brassica* crops are grown under conditions of minimal abiotic

119   stress they undergo a normal pattern of leaf and pod senescence that suggests

120   resource reallocation does occur to enhance seed number and quality.

121   Observations of *Brassica rapa* and *Brassica napus* inflorescences indicates that

122   seed development is frequently terminated following development of the first-

123   formed pods and resumes at the end of flowering, leading to regions of the

124   inflorescence without mature siliques (Figure 1). McGregor (1981) also reported

125   high levels of pod abortion in *B. campestris* and *B. napus* which was attributed to

126   over production of flowers and pod formation sites, so that a plant retains spare

127   pods which can fully develop should any become damaged, indicating that pod

128   abortion and the development of excess pod formation sites is normal and not just

129   a response to injury or biotic/abiotic stress. The ability to selectively abort pods

130   during periods of high abiotic stress, and resume once the stress has diminished,

131   would enable plants to become S-strategists. Bosac et al. (1994) and Stewart et al.

132   (1996) both found that exposing the racemes of *B. napus* or *B. campestris* to ozone

133   caused pod abortion, pod abscission or fewer seeds per pod. Black et al. (2000)

134 made the same observation and raised the possibility that for a wild plant this was

135 potentially disadvantageous; if seed size is too low then plants may lose their

136 competitive advantage against those growing nearby.

137 Crop breeding programmes still largely focus on increasing yield, particularly in the

138 light of the goal to feed an ever-increasing global population. Most crops originate

139 from R-strategy plants, yet breeding programmes are frequently orientated

140 towards C-strategy plants. In those countries where land use for crops is marginal

141 and abiotic stress levels are high it might be more desirable to select for S-strategy

142 plants. Although S-strategy plants can be overgrown by competitors in a wild

143 habitat, in farmed land this is manageable through weed control or less dense seed

144 planting rates. A crop ideotype would be an S-strategy plant with the ability of an R-

145 strategy plant to reproduce rapidly when the environmental conditions become

146 harsh, thus ensuring at least a minimum yield each year.

147 ***MANIPULATING SOURCE:SINK RATIOS***

148 Using the R-strategy plant Arabidopsis as a model to determine how the

149 manipulation of resource allocation can impact on seed quality and yield it has been

150 possible to explore how a crop ideotype might be optimised and developed in a

151 breeding programme. Plants were grown under near optimal conditions in order to

152 maximise seed number. The objective was to ascertain how much plasticity in

153 resource allocation was retained by a plant and whether there was potential to

154 increase seed size, yield and quality to make an R-strategy plant invest additional

155 resources into seeds. If the mechanism of resource allocation can be better

156 understood then it can be manipulated in crop plants that still harbour weedy traits

157 from their ancestors in order to produce improved varieties. Such varieties might

158 be 'tailored' to suit differing environmental conditions. Selective 'pruning' of parts

159 of the inflorescence was carried out to alter source-sink relationships across the

160 plant. Control plants were compared to those that had either secondary

161 inflorescences removed, or all secondary inflorescences and lateral branches from

162 the main inflorescence removed, thus representing a decrease in sink strength as

163 the pruning treatments became more severe (see Fig. 2A).

164 Analyses of the growth morphology of the plants revealed that the main

165 inflorescence became significantly elongated as pruning became more severe, but

166 that the number of pods on the main stem remained the same as a consequence of

167 larger spaces between the pod formation sites (Fig. 2B, C). The rationale for

168 stimulating inflorescence elongation is unclear, as any competition for light would

169 have been reduced not enhanced by the pruning treatment. Whether this change

170 resulted from an alteration in cell number or size has yet to be determined,

171 however Dale (1959) also observed stem elongation in disbudded cotton plants.

172 Apical dominance was mechanically reinforced by the selective removal of lateral

173 branches and secondary stems and this seems to have resulted in the main

174 inflorescence becoming even more dominant, but the advantage of this is not clear.

175 An alternative explanation is that without the drain of lateral branches on

176 photosynthate reserves the pruned plants were able to implement a more extreme

177 shade avoidance response and elongate more than their highly branched

178 competitors in order to capture the available light more effectively. Rosette leaf

179 number and biomass increased significantly with the pruning of lateral and

180   secondary branches (sinks), but the rate of senescence of individual leaves was the

181   same as the control plants. This finding is in agreement with that of Nooden and

182   Penney (2001) who found that stem removal increased the longevity of the plant

183   but not of individual rosette leaves, enabling the rosette to remain a source of

184   photosynthates and other metabolites for much longer (Bennett et al., 2011; Fig

185   2D). In common with findings in soybean (Seddigh and Jolliff, 1986), it would appear

186   that the strength of the sink is the dominant factor in determining resource re-

187   allocation from rosette leaves into the pods. Without the pull from multiple

188   branches containing developing seeds, the rosette continues to develop without

189   overall senescence in the absence of other environmental cues such as day length

190   or temperature change. Other experiments in soybean have shown that in the

191   absence of sufficient sink strength the stomata close, leading to a reduction in

192   photosynthesis, although the consequence of this on the distribution of existing

193   photoassimilates and the timing of senescence was not established (Setter and

194   Brun, 1980). However, some authors contest the theory that reproductive

195   development is intrinsically linked to leaf senescence in Arabidopsis. Hensel et al.

196   (1993) reported that the reproductive organs had no influence on leaf senescence

197   and rosette leaf age was the only driver of senescence; Nooden and Penny (2001)

198   took the idea a step further and argue that loss of meristem activity represents the

199   primary signal for whole plant senescence. Early research on source-sink

200   relationships in plants developed the 'self-destruct hypothesis' (Sinclair and De Wit,

201   1976) which assumed that increased nutrient remobilisation out of the leaves was

202   the driver for early senescence. Later researchers in the field found that the rate of

203   photosynthesis in leaves was reduced when sink strength was reduced

204 (Wittenbach, 1983; Crafts-Brander, 1984) and Seddigh and Jolliff (1986) proposed

205 that increasing sink strength would drive increased production of carbon and

206 nitrogen by the leaves, thus switching the emphasis back in line with our current

207 thinking that sink strength 'pulls' resources from the rosette (Bennett et al., 2011).

208 Wild type Arabidopsis plants have been shown to have sub-maximal levels of

209 nitrogen remobilisation from the leaves into developing seeds, but if over-

210 expression of cytosolic pyruvate, orthophosphate dikinase (PPDK) is engineered

211 then nitrogen is exported more rapidly from senescing leaves and seeds on the

212 transgenic lines were larger than wild type (Taylor et al., 2010). This work

213 demonstrates that rosette nitrogen is present in excess of that demanded by the

214 sink (pods) but it is only metabolised in proportion to the pull from the developing

215 pods. The PPDK over-expressing transgenics provide evidence that it is possible to

216 alter the dynamics of remobilisation and source-sink relationships and the capacity

217 for additional resource accumulation is present in the seeds, even in a species such

218 as Arabidopsis where lipid is the primary form of storage metabolite.

219 ***RESOURCE ALLOCATION TO THE PODS CAN BE ALTERED***

220 Reduction in sink strength from pruning lateral and secondary branches altered pod

221 morphology compared to the control. Resultant pods were longer and with a bigger

222 area (Fig. 2E), however, the total number of seeds within each pod was significantly

223 reduced, yet the total weight of seeds in the most extreme manipulation was

224 significantly heavier (Fig. 2F). These observations indicate that when the number of

225 reproductive sites was reduced the number of seeds that even start to develop

226 within a pod is less than the total that could be theoretically sustained within a pod,

227   as opposed to lots of seeds being formed and then some aborted. These data

228   suggest that the sink strength is determined by the number of pods rather than the

229   number of seeds. For a weedy species such as Arabidopsis there is a logic to the

230   argument that the minimum unit that a plant 'measures' is a pod rather than a

231   seed. Since pod shatter will release all the seeds within that pod simultaneously it

232   makes ecological sense that the rate of development of all seeds within a single pod

233   is highly coordinated. If the number of pods is too small to pull sufficient resources

234   from the rosette then the co-ordination sequence could be reprogrammed to

235   enable a few large, viable seeds to develop rather than lots of small seeds with

236   impaired viability. The mechanism by which a pod is able to 'measure' the amount

237   of resource remains unexplained but our observations indicate that if sink strength

238   is low then not all the potential sites of attachment in a pod are used to develop

239   seeds. As a consequence low numbers of seeds develop per pod to guarantee that

240   all the seeds formed will meet a minimum threshold of resources required for

241   viability. One hypothesis is that R-Strategy annual plants operate a 'minimum

242   viability threshold for reproductive success' and ensure that some seeds will be

243   produced with the best chance of survival. If conditions remain favourable and

244   further resources are available then the plant will invest in more units of

245   reproduction (pods), thus increasing sink strength and mobilising resources more

246   fully from the rosette. This is evident when pods from the main inflorescence of un-

247   pruned plants are compared to those from the lateral branches. The pods on the

248   lower section of the main inflorescence are the first formed and contain the

249   heaviest seeds, whereas the later formed pods on lateral branches contain lighter

250   and significantly fewer seeds (Fig. 3). Seeds from pods on lateral branches have high

251 viability, but further detailed experiments are necessary to establish if there is a

252 fitness cost if these smaller seeds are selected over multiple generations. Other

253 researchers have raised the possibility that an R-strategy plant such as Arabidopsis

254 makes a late, or even continuous, decision about the volume of seed set based on

255 the available nutrients during the reproductive stage, rather than it being

256 predetermined by growth and development during the vegetative phase (Schulze et

257 al., 1994).

258 The observation that seed size within a pod can be manipulated by source/sink

259 manipulation raises the question whether this is the consequence of altered

260 partitioning of resource allocation into each seed or pod. Seeds accumulate a

261 mixture of lipids, proteins and carbohydrates as they develop. In Arabidopsis the

262 protein and carbohydrate are mostly in the embryo, whereas the endosperm

263 contains the majority of the lipid component. The experiments reported here have

264 revealed that enhancing the source strength through selective stem removal causes

265 a trend towards an increase in the fatty acid content of the seeds, although protein

266 content did not alter (Fig. 4A, B). This suggests that the developing embryo is of a

267 fixed size, but the endosperm component can increase beyond a minimum

268 threshold level. Partitioning of individual fatty acids did not change, indicating that

269 the ratio is genetically determined and therefore fixed. In Arabidopsis the dominant

270 fatty acids are linoleic acid, eicosenoic acid and linolenic acid which are omega 6,

271 omega 9 and omega 3 unsaturated fatty acids respectively. These were found in

272 similar proportions to that established by other workers (Katavic et al., 1995;

273 Penfield et al., 2004), although less oleic acid (omega 9) was detected than both of

274 these groups who found comparable levels in both the embryo and endosperm of

275 Arabidopsis seeds.

276 ***IMPLICATIONS FOR CROP PRODUCTION***

277 An analysis of all the different parameters we have measured in relation to

278 resource allocation in Arabidopsis has revealed that seed and pod number per plant

279 is more closely related to protein and lipid content than seed mass or pod area (Fig.

280 5). This analysis suggests that if the target is to increase the number of viable seeds

281 per pod the consequence should be further mobilisation of lipid and protein

282 content into each individual pod which will result in a redistribution of these

283 resources amongst a greater number of seeds within those pods. However, the

284 overall mass of seeds per pod is unlikely to be increased so the usefulness of

285 increasing seed number is limited to production scenarios where the aim is to

286 increase the number of seeds that can give rise to viable plants e.g. seed production

287 for the leafy vegetable market. If the target is to increase seed size and nutrient

288 content *per se* then it is necessary to alter the source-sink relationship within the

289 plant and to start to shift the architecture away from that of an obligate R-strategy

290 plant and towards that of an S- or C-strategy plant.

291 There are many reasons why the typical architecture observed in an R-strategy

292 plant that has been adapted for crop production is less than optimal. Most ruderal

293 plants either have a highly branched habit, if they are dicotyledonous, with minimal

294 coordination of pod development and shattering between branches or, in the case

295 of monocotyledonous plants, the natural architecture is to produce numerous

296 tillers with a consequential separation of heading dates and maturation rates on

297   different tillers. In a few examples (e.g. wheat, barley, rice) long-term selection of

298   domesticated crops has enabled much closer development of seed heads present

299   on different tillers, but in years when the environment is less favourable, for

300   example when late season rain affects a wheat crop, new tillers are produced that

301   mature several weeks after the main inflorescence. In crops that have received far

302   less attention from plant breeders, such as the numerous small grain crops grown

303   in Africa and Asia, the coordination between flowering heads is extremely poor,

304   leading to a considerable reduction in the proportion of potential yield from each

305   plant that is actually harvested. For these reasons it would be desirable to replicate

306   the architecture enforced by our selective stem removal experiments, but it is

307   important to appreciate the potential consequences of the change for seed yield

308   and quality.

309   It is well known that adjusting the seed rate (planting density) for numerous crops

310   can have a large influence on plant morphology. Plants grown closely together will

311   reduce the number of branches they produce and adopt a shade avoidance strategy

312   of elongating the main stem to capture the maximum amount of available light

313   (Robson et al., 1996). Whilst excessive stem height can lead to problems with

314   lodging there are ways of preventing this through the introgression of genes that

315   lead to dwarfing, such as *Rht* in wheat, and the resultant plants are then able to

316   concentrate their resource allocation into the seed head or pods on the primary

317   inflorescence. Since non-branched plants are grown in a much more densely

318   planted stand the actual yield per m^2 may not alter significantly, but the

319   coordination will be greatly improved by achieving a monoculture of primary

320   inflorescences from any crop, meaning more of the seeds formed will actually be at

321   the optimal harvest stage simultaneously.

322   What has become clear from the above findings and from existing literature is that

323   increasing the nutrient supply to the plants during the vegetative stage does not

324   guarantee an increase in yield and/or seed quality. Masclaux-Daubresse and

325   Chardon (2011) showed that nitrogen limitation negatively affected plant biomass,

326   yield, and harvest index but did not change the dry weight of individual seeds.

327   There is evidence that the leaves produce photosynthates to an excess of those

328   required by the developing pods and seeds and that there is a positive feedback

329   loop through which the pods (in the case of a Brassicaceous plant) pull the

330   resources they need from the rosette. Whilst supplying adequate nutrition to the

331   leaves and roots will avoid morphological constraints imposed by a starvation

332   stress, more nutrition is not necessarily a guarantee of greater yield (Allen &

333   Morgan, 1972) and a restricted nutrient supply does not necessarily mean that

334   senescence will occur more rapidly; Abdallah et al. (2011) demonstrated that short

335   or long-term sulphur limitation delays senescence and allows the plant to

336   remobilise much more sulphur and nitrogen out of its leaves. Agronomic

337   consideration should be given to treatments that would make plants increase the

338   rate of resource remobilisation from the source to the sink, for example increasing

339   the expression of PPDK (Taylor et al., 2010) or *Arabidopsis thaliana NITRATE*

340   *TRANSPORTER2.1* (*ATNRT2.1*; Chopin et al., 2007), or ways to adjust the feedback

341   mechanism between source and sink and how it changes through different stages

342   of development. Since these are rather subtle changes it is likely to fall to plant

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343 breeders to target suitable genes that will change developmental patterns of

344 resource allocation and tip the delicate balance of source sink relationships in the

345 appropriate direction at different stages of crop growth.

346 **PLANT BREEDING TARGETS FOR ALTERED ARCHITECTURE AND RESOURCE**

347 **ALLOCATION**

348 The control of branch formation in plants has become much better characterised

349 over recent years. Shoot and root branching is in part mediated by the *MAX* family

350 of genes that function through regulating the strigolactone pathway. Recessive

351 mutants of *MAX2* exhibit excessive branching (Stirnberg et al., 2002) and *MAX2*

352 encodes an F-Box leucine rich repeat protein that modulates strigolactone

353 signalling, reportedly by targeting the protein for ubiquitination and degradation by

354 the 26S proteasome. Strigolactones appear to act downstream of auxin, and the

355 two plant growth regulators interact so that auxin is able to mediate apical

356 dominance by inducing the expression of strigolactone biosynthesis genes (Brewer

357 et al., 2009). *MAX2* was originally isolated as *ORE9*, a gene involved in promoting

358 leaf senescence and thereby potentially increasing the source of metabolites

359 available for seed production (Woo et al., 2001). It is interesting to note that

360 *max2/ore9* mutants exhibit increased branching but show delayed plant

361 senescence. In contrast, silencing of the *BRANCHED1b* gene resulted in increased

362 branching in tomato, but no notable change in fruit maturation or seed

363 development (Martin-Trillo et al., 2001). *BRC1b*, along with *FINE CULM1* are

364 believed to be downstream of strigolactone synthesis and presumably downstream

365 of resource allocation during senescence, since fruit development and ripening are

366   not altered in the plants. Clearly the consequences of this genetic manipulation

367   differs from that brought about by pruning treatments and it would be interesting

368   to determine if the mutations above resulted in the production of more or heavier

369   seeds. Work by Abreu and Munné-Bosch (2009) with the *NahG* transgenics and *sid2*

370   mutations in Arabidopsis, both of which reduce salicylic acid synthesis, showed that

371   seed production was increased by 4.4-fold and 3.5-fold respectively, compared to

372   the wild type. Salicylic acid deficiency also resulted in higher seed nitrogen content

373   and increased pro-vitamin A and vitamin E than in wild type plants. *NahG* and *sid2*

374   both exhibited increased branching per plant, along with reduced seed weight per

375   100 seeds, increased seed number per fruit and per plant. These results support

376   those we found with manipulation of architecture by pruning; in both cases the

377   least branched phenotype has the highest seed weight and the fewest seeds per

378   pod.

379   Other mutants that have already been characterised have the potential to be

380   utilised in the development of crops with altered resource allocation, many of

381   which are also involved in plant growth regulator signalling (Quirino et al., 2000).

382   Improved resource allocation, and consequentially yield enhancement, was

383   achieved in rice by reducing the expression of the ethylene receptor *ETR2*, which

384   caused a 4% increase in thousand grain weight by adjusting starch acclimation and

385   increasing sugar translocation into the grains (Wuriyanghan et al., 2009). It is well

386   known that several mutants in ethylene signalling pathways result in delayed

387   senescence of the leaves in dicotyledonous plants (Grbic and Bleecker, 1995) and

388   this may have the potential to alter seed filling at a later stage in development. Ma

389 and Wang (2003) identified the *ETR1* homologue in wheat and initial experiments

390 suggest that it is involved in integrating signals from other hormones, such as

391 jasmonic acid, to regulate leaf senescence. Experiments that modulated cytokinin

392 signalling via a SAG12 feedback loop have shown that leaf senescence can be

393 significantly delayed if cytokinin content in the leaves is kept high (Gan and

394 Amasino, 1995) and that this can enhance yield, although the impact of this

395 manipulation has been shown to be dependent on nutrient availability and under

396 limited N supply the transgenics had significantly lower fruit dry weight (Wingler et

397 al., 2005). Using this strategy to specifically increase cytokinin content in the roots

398 of tomato plants has been reported to increase fruit yield by 30% (Ghanem et al.,

399 2011). Cytokinins also appear important in modulating stress responses; when IPT

400 was placed under the regulation of a stress-responsive SARK promoter in rice

401 source/sink modifications led to improved drought tolerance and increased grain

402 yield under water-stress (Peleg et al., 2011). As a staple food crop, rice architecture

403 has been extensively researched and a recently identified point mutation in

404 *OsSPL14* was reported to result in the "ideal" plant architecture by producing a

405 taller plant with fewer tillers, denser panicles and enhanced grain yield per panicle

406 (Jiao et al., 2010). The identification of such alleles, which not only affect rice

407 architecture but also yield, may provide targets for future breeding programs.

408 ***IMPLICATIONS FOR CROP DEVELOPMENT***

409 The example given in the section above is a prime illustration of how below-ground

410 events influence the development of above-ground development and architecture.

411 Soil compaction can have serious consequences for the development of above-

412   ground biomass. Dry mass in barley was reduced by over 50% when soil bulk

413   density was increased from 1.1 to 1.7 g.cm^{-3} (Mulholland et al., 1996) with a

414   concomitant increase in xylem abscisic acid content in the compacted samples.

415   Some attempts have been made to relate soil compaction in different soils types to

416   yield in cereals (Hamza and Anderson, 2005), but without the ability to directly

417   observe below ground root architecture it will be difficult to assess how resource

418   allocation is related to soil structure across the whole plant.

419   The need for rapid phenotyping methods for plants growing *in vivo* has led to the

420   advent of techniques such as microscale X-ray Computed Tomography (microCT)

421   which enables root systems to be investigated in sufficient detail to provide

422   information about architecture as well as biomass (Lucas et al., 2011). Other

423   phenomics centres are being established to investigate above-ground architecture

424   (Berger et al., 2010). Together these techniques will provide a better understanding

425   of how overall plant architecture is regulated, the relationship of architecture to

426   crop yield, an understanding of the variability that exists even between plants

427   grown in a monoculture, and therefore how best precision agriculture can be

428   exploited to optimise plant growth and seed/fruit development.

429   ***ACKNOWLEDGEMENTS***

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Table 1. Morphogenetic responses to desiccation, shading, or mineral nutrient stress of competitive, stress tolerant, and ruderal plants and their ecological consequences of three types of habitat (Reproduced from Grime, 1977).

Strategy	Response to stress	Habitat 1 [a]	Habitat 2 [b]	Habitat 3 [c]
Competitive	Large and rapid changes in root:shoot ratio, leaf area and root surface area	Tendency to sustain high rates of uptake of water and mineral nutrients to maintain dry matter production under stress and to succeed in competition	Tendency to exhaust reserves of water and/or mineral nutrients both in rhizoshere and within the plant; etiolation in response to shade increases susceptibility to fungal attack	Failure rapidly to produce seeds reduces chance of rehabilitation after disturbance
Stress tolerant	Changes in morphology slow and often small in magnitude	Overgrown by competitors	Conservative utilisation of water, mineral nutrients and photosynthate allows survival over long periods in which little dry matter production is possible	
Ruderal plants	Rapid curtailment of vegetative growth and diversion of resources into seed production		Chronically low seed production fails to compensate for high rate of mortality	Rapid production of seeds ensures rehabilitation after disturbance

[a] In the early stages of productive, undisturbed habitats (stresses mainly plant induced) and coinciding with competition.

[b] In other continuously unproductive habitats (stresses more or less constant and due to unfavourable climate and/or soil) or in the late stages of succession to productive habitats.

[c] In severely disturbed, potentially productive habitats (stresses either a prelude to disturbance e.g. moisture stress preceding plant fatalities or plant induced), between period of disturbance.

Figure 1. *Brassica napus* floral inflorescence showing regions of pod abortion.

Figure 2. Morphological influences on resource allocation in Arabidopsis. (A) The three selective stem removal treatments; from left to right: control, secondary branches removed, secondary and lateral branches removed (B) Height of the main inflorescence from each treatment (C) Distance between the pods on the primary inflorescence (D) Images of the rosettes at the end of flowering (E) Pod area and length for each treatment (F) Seed number and weight per pod. N=10 unless otherwise stated on graph. * indicates significant differences between the control and treatments (P <0.05; determined by ANOVA followed by Tukey's post-hoc analysis). Plants were grown under controlled conditions with a 16h light, 8h dark cycle. Temperature was maintained throughout at 20°C and 55% RH.

Figure 3. Comparison of thousand grain weight and the number of seeds per pod between the main inflorescence and lateral branches in un-manipulated plants. N=10. * indicates significant differences between the control and treatments (P <0.05; determined by ANOVA followed by Tukey's post-hoc analysis)

Figure 4. Resources allocated to the seeds. (A) Seed protein concentration from each treatment. Total protein quantification was performed using the Thermo Scientific Pierce BCA protein assay kit according to the manufacturer's protocol. The microtitre plate was read at an absorbance of 540nm. (B) Seed fatty acid profile to show amounts and proportional quantification of seeds from each treatment. N= 10 replicates per treatment. No significant differences were detected but the trend was for an increase in fatty acid content as the manipulations become more severe.

Seeds were freeze dried and the fatty acids measured via direct transmethylation (Smooker et al., 2011). Pentadecanoic acid and methyl heptadecanoate were used as internal standards, the former being present during the transmethylation process whilst the later was added just before the samples were run on the GC. 1μl aliquots of the heptane phase containing the fatty acid methyl esters (FAMEs) were analysed by gas chromatography with flame ionization detection (Agilent G1530a) using a CP-Sil 88 column (50 m length x 0.25 mm id., 0.25 μm film thickness; Chrompack). The GC conditions were: split mode injector (50:1), flame ionizer detector temperature 260°C, oven temperature 130°C for 3 min and increasing at 10°C/min for 6 min: total analysis time 20 min. FAMEs were identified by comparison to the Supelco FAME mix (Sigma-Aldrich).

Figure 5. Dendrogram of resource allocation variables generated by cluster analysis illustrating the relationship between plant architecture and the partitioning of resources.
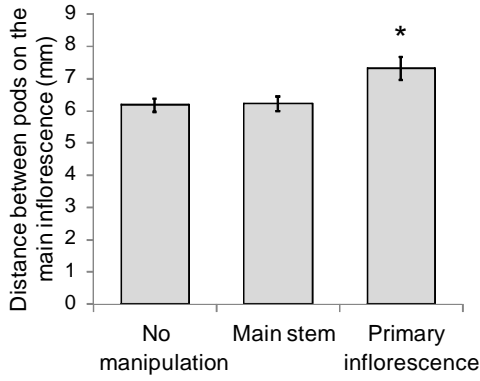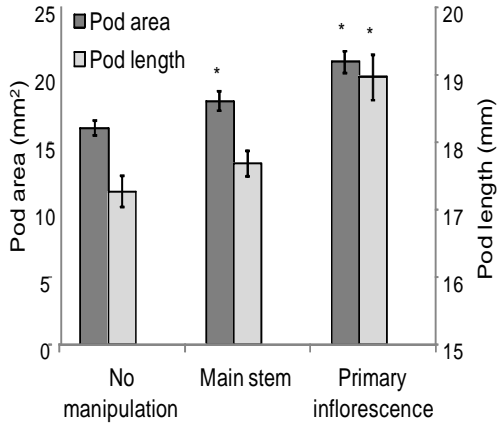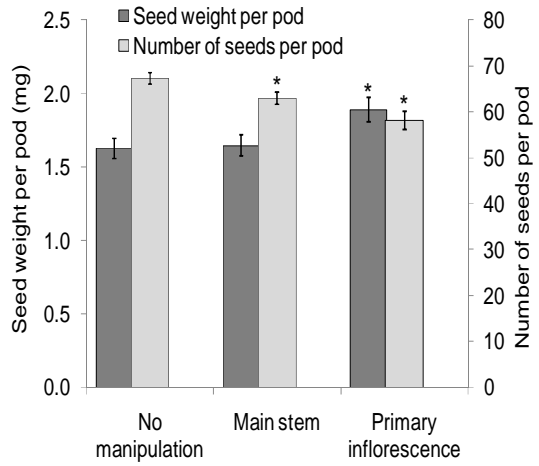
Figure 1.

Figure 2.

A

No manipulation | Main stem | Primary inflorescence

B

C

D

E

F

Figure 3.

Figure 4.

A

B

Figure 5.