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Auxin export from proximal fruits drives arrest in temporally-competent inflorescences

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1 ABSTRACT

2 A well-defined set of regulatory pathways control entry into the reproductive phase in flowering
3 plants. Conversely, little is known about the mechanisms that control the end of the reproductive
4 phase ('floral arrest'), despite this being a critical process for optimising fruit and seed production.
5 Complete fruit removal, or lack of fertile fruit-set in male sterile mutants, prevents timely floral
6 arrest in the model plant *Arabidopsis*, leading to a previous proposal that floral arrest results from a
7 cumulative fruit/seed-derived signal that causes simultaneous 'global proliferative arrest' (GPA).
8 Recent studies have suggested that floral arrest involves gene expression changes in the
9 inflorescence meristem that are at least in part controlled by the *FRUITFULL-APETALA2* pathway,
10 however there is limited understanding of how this process is controlled and the communication
11 needed at the whole plant level. Here, we provide a framework for the communication previously
12 inferred in the GPA model. We show that floral arrest in *Arabidopsis* is not 'global' and does not
13 occur synchronously between branches, but rather that the arrest of each inflorescence is a local
14 process, driven by auxin export from fruit proximal to the inflorescence apex. Furthermore, we
15 show that inflorescences are only competent for floral arrest once they reach a certain
16 developmental age. Understanding the regulation of floral arrest is of major importance for the
17 future manipulation of flowering to extend and maximise crop yields.

18

19 INTRODUCTION

20 A complex series of regulatory pathways that integrate both internal and environmental signals
21 regulate entry into the reproductive phase (the 'floral transition') in flowering plants [1]. These
22 initiation pathways have received much attention, but relatively little is known about the
23 mechanisms that control the end of the reproductive phase ('floral arrest'). This is somewhat
24 surprising, since the correct timing of floral arrest is a critical process for optimising fruit and seed
25 production, and hence reproductive success. In a seminal study from 1994, Hensel *et al.* examined
26 floral arrest in the model species *Arabidopsis thaliana*, and showed that floral arrest normally
27 occurs through a regulated process in which each inflorescence ceases to open flowers and enters
28 an arrested state [2]. This process was proposed to be triggered by fruits, since complete fruit
29 removal, or lack of fertilisation in *ms1* male sterile mutants, prevented timely inflorescence arrest
30 anywhere on the plant. Inflorescences eventually ceased flower production, but only through
31 terminal differentiation of the inflorescence meristem [2]. Analysis of reduced fertility and embryo-
32 lethal mutants suggested only fruit containing >30% fertile seed are able to trigger arrest, and that
33 seed are an essential part of the process [2]. Finally, it was observed that post-arrest fruit removal
34 leads to the re-activation of arrested inflorescences, and the production of new fruit, suggesting
35 floral arrest is a reversible state [2]. These observations led to a model in which floral arrest was
36 proposed to result from accumulation of a fruit/seed-derived signal that, at a threshold level, would
37 trigger simultaneous 'global proliferative arrest' (GPA) in all inflorescences [2].

38
39 After a long gap, two recent studies have provided new insights into floral arrest in *Arabidopsis*.
40 Wuest *et al.* showed that, transcriptionally, the arrested inflorescence meristem state strongly
41 resembles dormancy in axillary inflorescence buds, suggesting that the process of inflorescence
42 arrest could represent a direct reversal of bud activation [3]. In a second study, Balanza *et al.*
43 showed that *fruitfull* mutants undergo delayed floral arrest, and suggested that inflorescence arrest
44 requires a FRUITFULL-APETALA2 regulatory module, which may be under the control of the
45 miR156/miR172 ageing pathway [4]. However, much remains unclear about the mechanistic basis
46 for both inflorescence arrest itself, and the wider coordination of floral arrest across the plant. We
47 are especially interested in understanding the mechanism by which fruits bring about inflorescence
48 arrest, and therefore set out to understand this process in more detail.

49

50 RESULTS

51

52 **Floral arrest is not synchronous in Arabidopsis**

53 Our initial observations suggested that in the Col-0 ecotype, floral arrest may not be synchronous
54 and that inflorescences may arrest at different times. Since synchronous arrest is a key tenet of the
55 GPA model, we performed a more detailed re-assessment to confirm these observations. By
56 tracking the duration of flower production ('inflorescence duration') in each inflorescence in a
57 cohort of Col-0 plants, we found that inflorescence arrest across plants is not synchronous, with on
58 average ~5 days between arrest of the first and last inflorescences (**Fig. 1A, Fig. S1, Table S1**).
59 We measured the duration of three orders of inflorescence: primary (PI; the main bolting stem),
60 secondary (those arising from primary leaves, whether cauline or rosette) and tertiary (those
61 arising from leaves on the secondary inflorescences) (**Fig. S2**). The timing of arrest followed a
62 general basipetal pattern, with the PI and the secondary cauline (C) inflorescences arresting first at
63 similar times, followed by a wave of arrest across the secondary rosette (R) inflorescences (**Fig.**
64 **1A, Fig. S1**). Tertiary inflorescences arrest at approximately the same time as their parent
65 inflorescence (**Fig. S1**). This pattern corresponds to the general pattern of inflorescence activation
66 observed earlier in the experiment, in which secondary cauline inflorescences activate together,
67 followed by a basipetal wave of activation across the secondary rosette inflorescences (**Fig. 1A,**
68 **Fig. S1**). Thus, we propose that floral arrest occurs when active inflorescences reach the end of
69 their lifetime, and its timing is largely a reflection of the timing of inflorescence activation. In
70 instances where inflorescence activation is synchronous (probably including those in Hensel *et al.*),
71 floral arrest may also be near-synchronous, but this is not a key element of floral arrest.

72

73 We also observed an additional phenomenon of 're-flowering' in a number of experiments,
74 whereby after the arrest of most or all inflorescences, previously dormant axillary buds would
75 activate, giving rise to new inflorescences (**Fig. 1E**); although this is observed relatively frequently,
76 to our knowledge it has not been previously characterised in the literature. The re-initiation of
77 flowering was not observed in all plants, nor indeed in all experiments, and the number of
78 additional fruits produced through re-flowering varied between experiments, but was generally
79 greatest in those experiments with a higher initial fruit production (**Fig. 1D**). The existence of the
80 re-flowering phenomenon, and the ability of buds to activate in *de novo* manner following systemic
81 floral arrest further highlights the non-global, asynchronous nature of floral arrest. This also implies
82 that there may be multiple signals that are active at different stages which are driving floral
83 activation/arrest.

84

85

86

87 **Floral arrest is a temporally-regulated process**

88 In these analyses, we also observed that each order of inflorescence (primary, secondary, tertiary)
89 had a distinctive duration between activation and arrest. Although the activation and arrest of
90 individual inflorescences was not synchronous, the duration for inflorescences of the same order
91 was generally very similar. This was true both when comparing inflorescences within individual
92 plants, and when comparing IMs between different plants in the same experiment (**Fig. 1B**).
93 Furthermore, we observed that, across a wide range of different experiments run under similar
94 conditions (**Table S1**), the primary inflorescences in Col-0 had very similar durations, being active
95 for 22 ± 3 days post bolting (dpb) (**Fig. 1C**). We observed that the total 'floral duration' before floral
96 arrest was also consistent between experiments, occurring at around 27 ± 3 dpb (**Fig. 1C**). These
97 data suggest that inflorescence arrest may be a predominantly time-dependent process, requiring
98 inflorescences to become responsive to floral arrest signals, rather than one purely driven by
99 cumulative feedback inhibition from fruit-derived signals.

100

101 **Timely arrest in response to fruit presence is a local process in each** 102 **inflorescence**

103 The absence of synchronous arrest across inflorescences suggested that floral arrest is not
104 determined by a systemic signal. We confirmed that, as shown by Hensel et al [2], timely
105 inflorescence arrest requires fertile fruit, since removal of fruit everywhere on the plant was
106 sufficient to prevent inflorescence arrest anywhere on the plant (**Fig. 2A,C**). However, when we
107 performed localised continuous flower removal on secondary cauline inflorescences, we observed
108 that treated inflorescences did not undergo arrest despite plants having ~90% of their normal fruit-
109 set, whilst timely arrest was observed elsewhere on the plant (**Fig. 2D,E**). Together with the lack of
110 synchronicity, these data suggest that floral arrest is not a systemically-regulated process, but
111 rather consists of the independent, locally-regulated arrest of individual inflorescences.

112

113 **Delayed floral arrest in response to fruit absence occurs systemically**

114 Contrary to this model, the results of Hensel *et al.* clearly demonstrated an extension of PI duration
115 upon removal of secondary inflorescences, suggesting that systemic feedback from fruits can
116 modulate the duration of individual inflorescences. We repeated this debranching treatment and
117 confirmed that in the *Ler* and Col-0 backgrounds, it does indeed extend inflorescence duration and
118 fruit production of the PI, relative to untreated plants (**Fig. 3A,B**). Interestingly, we observed that
119 the duration of the PI in untreated *Ler* plants was longer than that in Col-0 by approximately 7-9
120 days (cf. **Fig. 3A** and **Fig. 2E**), suggesting there is variation in Arabidopsis ecotypes for
121 inflorescence duration. Similarly, when we removed tertiary inflorescences from secondary
122 inflorescences in Col-0, we observed a small extension to the duration of secondary
123 inflorescences, and a corresponding increase in the number of fruit they produce (**Fig. 3C**). Thus,
124 even though the general *presence* of fruit across the plant is not sufficient to trigger arrest of

125 individual fruitless inflorescences, the general *absence* of fruit is sufficient to extend the duration of
126 individual fully-fruited inflorescences. Collectively, our data suggest that fruit play two distinct roles
127 in floral arrest, systemically modulating inflorescence duration, and locally driving inflorescences to
128 undergo arrest. This likely indicates the existence of multiple fruit-derived signals that are involved
129 in floral arrest.

130

131 **Small numbers of fruit are sufficient to trigger inflorescence arrest**

132 Each of the treatments used by Hensel et al to support the GPA model caused a dramatic global
133 reduction in fertile fruit, and resulted in systemic delay of floral arrest. However, the intensity of
134 these treatments precluded more nuanced understanding of the role of fruit in inflorescence arrest,
135 and we therefore investigated the effect of more subtle treatments. We observed that if we
136 removed flowers continuously from inflorescences beyond their normal lifetime, and then allowed
137 plants to recover, each inflorescence arrested within a few days, despite having produced only a
138 small number of fertile fruits (approximately 6-10 per inflorescence) (**Fig. 2B**). This suggests that
139 relatively small numbers of fruit may be sufficient to trigger inflorescence arrest. Similarly, if we
140 used a dexamethasone-inducible *MS1:MS1-GR* construct to restore fertile fruit formation to the
141 *ms1-1* mutant (*Ler* background), from 12 days post anthesis of the first flower (dpa), we observed
142 regulated inflorescence arrest, unlike in untreated controls (**Fig. 4A**). However, the number of
143 fertile fruit per inflorescence was only around 45% of that in wild-type plants (**Fig. 4B**). To more
144 clearly delineate the number of fruit needed to trigger arrest, we performed differential flower-
145 removal treatments on secondary cauline inflorescences of the same plant, which if untreated
146 typically undergo arrest at the same time (**Fig 1A and Fig. S1**). On each plant, every other flower
147 was removed from one inflorescence (1/2), three of every four flowers were removed from another
148 inflorescence (3/4), and four of every five flowers were removed from a third inflorescence (4/5); a
149 fourth was left untreated (**Fig 5F**). Despite the resulting dramatic differences in fruit set, the treated
150 inflorescences on the same plant all underwent normal regulated arrest; although the more severe
151 treatments delayed inflorescence arrest by 2-3 days (**Fig. 5A**). The most severely-treated
152 inflorescences arrested despite only having produced 20% of the fruit produced by untreated
153 controls (**Fig. 5B**); the average of 7 fruit needed for arrest in this treatment is highly consistent with
154 the number produced in the plants shown in **Fig. 2B**. These data thus do not support a model in
155 which cumulative fruit-set upon each inflorescence is required for arrest.

156

157 **Proximal fruit are needed for temporally-competent inflorescences to arrest**

158 These data also present a paradox: approximately 7 fertile fruit are sufficient in certain
159 circumstances to trigger arrest, but most inflorescences produce far more than 7 fruit before
160 arresting. Given our earlier observations of inflorescence duration (**Fig. 1A,C**) and that
161 inflorescences on the same plant tend to arrest at approximately the same time despite individually
162 producing different fruit numbers (**Fig. 5A,B**), these data reinforce the idea that temporally-

163 acquired responsiveness to a fruit-derived signal is critical, rather than a threshold level of signal
164 being reached. We therefore tested how the timing of fruit production affects inflorescence arrest.
165 In a first experiment, we performed two treatments; 'early' plants had all flowers removed, until
166 around 30 flowers had been produced by the PI (12-13dpb), and were then allowed to continue
167 flowering normally. Despite producing far fewer fruit than control plants (**Fig. 5D**), the PI of 'early'
168 plants underwent arrest at the same time as untreated plants (approximately 21dpb) (**Fig. 5C**).
169 This mirrored the effect seen in the dexamethasone-inducible *MS:MS1-GR* line (**Fig. 4A**).
170 Conversely, 'late' plants were allowed to flower as normal until around 30 flowers had opened on
171 the PI (12-13dpb); subsequently all open flowers were removed from the plant for 20 days. Despite
172 producing the same number of fruit as 'early' plants during the first 21dpb (**Fig. 5D**), 'late' plants did
173 not undergo timely arrest (**Fig. 5C**). However, when flower removal treatment was ended in 'late'
174 plants at approximately 30dpb, the inflorescence was active for a further 7 days, producing around
175 7 fertile fruits before arresting (again consistent with the minimum fruit numbers established in **Fig**
176 **2B, 4C**). These data demonstrate that fruit are only able to trigger arrest when inflorescences have
177 become temporally competent to arrest, at the end of their normal lifetime.

178
179 To further examine the relationship between timing of fruit production and arrest, we performed an
180 experiment in which all fruit were removed from three secondary cauline inflorescences on the
181 same plant at 17dpb. One inflorescence per plant was subsequently allowed to produce fruit
182 normally until it arrested (X); this approximated the '50% early' treatment (**Fig 5F**). Another
183 inflorescence was allowed to produce 10 fruit from 17-20dpb, but then had all subsequent flowers
184 removed (Y) (**Fig 5F**). The final inflorescence had additional flowers removed until 20dpb, and was
185 then allowed to produce 10 fruit from 20-22dpb; all subsequent flowers were also removed (Z) (**Fig**
186 **5F**). The timing of arrest was then compared to the PI on the same plants. Treatment X
187 inflorescences produced ~24 fertile fruit, and arrested shortly after the PI (26dpb)(**Fig 5E**). Neither
188 treatment Y nor Z inflorescences underwent timely arrest, despite having produced sufficient fertile
189 fruit (**Fig. 5E**) However, most of the Y and Z inflorescences did eventually undergo a regulated
190 arrest (with bud cluster)(8/12 inflorescences for Y and 12/13 inflorescences for Z); the Z
191 inflorescences arresting somewhat earlier (31dpb) than the Y inflorescences (33dpb) (**Fig. 5E**).
192 Together with the experiment shown in **Fig 5A/B**, these data show that a small number of fruit
193 proximal to the inflorescence apex are sufficient to trigger arrest, once the inflorescence is arrest-
194 competent (**Fig 5F**). The further away fruit are from the meristem at the point the inflorescence
195 becomes arrest-competent, the lower the ability of those fruit to trigger arrest (**Fig 5F**); very distal
196 fruit are completely unable to trigger arrest.

197
198 Collectively, our data suggest that inflorescence arrest is a time-dependent process, in which
199 inflorescences become competent to arrest at a certain developmental age post-floral transition,
200 and then undergo almost immediate arrest, as long as they receive an inhibitory signal from fruit

201 they have recently produced. This developmental age does not directly reflect the absolute age of
202 the inflorescence, with the relationship between developmental age and absolute age likely varying
203 due to environmental influences or differences in growth history, and is reflected in the range of
204 fruit numbers produced between plants.

205

206 **Auxin export from fertile fruit triggers inflorescence arrest**

207 We next questioned how fertile fruit trigger floral arrest. Previous authors tentatively proposed that
208 fruit communicate with inflorescence apices by a phytohormonal signal, although provided no clear
209 evidence supporting this [2,3]. A number of phytohormones could be involved in delivering the
210 floral arrest signal and multiple signals may also be involved at the various developmental stages.
211 Gibberellin is an important regulatory signal produced during fruit development, and could act as
212 an arrest-inducing signal. To test this, we examined the quintuple *rga-t2 gai-t6 rgl1-1 rgl2-1 rgl3-1*
213 (*della*) mutant that lacks all DELLA proteins [12], and which as a result has effectively
214 constitutive gibberellin responses. We saw a dramatic increase in fruit number per inflorescence
215 in the *della* mutant, consistent with the known role of gibberellin in controlling meristem size and
216 activity [13] (**Fig. S3A**). However, the *della* mutant had an identical PI duration to the *Ler* wild-
217 type, suggesting that gibberellin is not a major factor regulating timely floral arrest (**Fig. S3B**).
218 However, given the differences in the rate of flower production ('florochron') between *della* and
219 *Ler*, we cannot rule out that gibberellin might play a smaller, quantitative role in floral arrest. The
220 much higher fruit production in the *della* mutant does not induce premature floral arrest, which
221 further indicates that arrest does not occur upon reaching a cumulative fruit-signal threshold.

222

223 Transcriptionally, the switch between activity and arrest in inflorescence meristems mirrors the
224 switch between activity and dormancy in axillary meristems (AMs) [2]. Since this switch in AMs is
225 controlled in part by auxin export from the AM into the stem [6,7], we hypothesised that auxin may
226 also be a key signal in floral arrest, especially given the high levels of auxin known to be produced
227 in fruits and seeds in many species [8,9,10,11]. Previous work in *Arabidopsis* has identified a curve
228 of hormone production in developing fruit, with a peak in auxin content at 6dpa [11]. To confirm
229 whether fertilisation increases the auxin content of *Arabidopsis* fruit, sterile (*ms1-1*) and fertile (*Ler*)
230 fruit were sampled at 6dpa, and auxin levels were quantified using UHPLC-MS/MS. This analysis
231 showed that auxin levels are much higher in fertile fruit (392pg/mg tissue) than sterile fruit
232 (16pg/mg tissue) (**Fig. 6D**), a difference further amplified by their 10-fold greater mass (**Fig. S4A**).
233 We next ascertained whether fertile fruit indeed transport auxin into the stem, by collecting auxin
234 exported from the pedicels of 6dpa fertile fruit from the PI. We found that individual fertile fruit
235 export ~75pg of auxin in 21 hours, which is 7.5 fold higher than equivalent sterile fruit (**Fig. 6E**).
236 Given that the equivalent pool of mobile auxin collected from the associated inflorescence stem is
237 ~100-200pg [7], it is clear that a small number of fertile fruit make a very significant contribution to
238 auxin levels in the inflorescence stem.

239

240 To directly test this model, we assessed whether exogenous application of auxin to sterile fruits
241 could restore timely arrest of the PI. We treated sterile fruit in the *ams* mutant, which like *ms1* fails
242 to undergo normal floral arrest [14], with the auxin analog NAA from 6dpa. This resulted in earlier
243 inflorescence arrest with the PI producing ~50 fruit, compared to ~80 in mock-treated plants (**Fig.**
244 **6A**). In auxin-treated *ams* plants, arrested inflorescences have the normal 'bud cluster' morphology
245 associated with the arrest of wild-type inflorescences (**Fig. 6C**). As expected, although auxin
246 treatment occurred throughout flowering, it only induced arrest at the time that inflorescences
247 normally become competent to arrest, at around 20dpa (**Fig. 6A**). When we applied NAA to the
248 uppermost 10 sterile fruit of *ams* individuals at 20dpa (and to any fruit subsequently formed in the
249 following 3 days), this rapidly induced a normal floral arrest (**Fig. 6B**) through the treatment of
250 relatively few (~18) sterile fruit (**Fig. 6B**), consistent with the role of proximal fruit triggering
251 inflorescence arrest only when the inflorescence is competent to do so. To rule out the possibility
252 that auxin application to sterile fruit activates synthesis of a 'second messenger' that actually acts
253 as an arrest signal, we performed NAA application at 23dpa to de-fruited pedicels in *ams* mutants.
254 This treatment was completely effective at inducing timely inflorescence arrest, unlike the mock
255 treatment, similar to the fruit application experiments (**Fig S4B**). This shows production of a second
256 messenger in fruit is not required for arrest, although it is possible a second messenger could still
257 be produced in the stem.

258

259 If auxin exported from fertile fruits triggers floral arrest, treatments affecting the auxin transport
260 system might be expected to inhibit the ability of fruit to export auxin, and drive arrest. To test this
261 idea, we analysed arrest in three mutants with reduced auxin transport, namely *pin3 pin4 pin7*
262 (*pin347*) which lacks three members of the PIN auxin efflux carrier family [15], *aux1 lax1 lax2 lax3*
263 (*aux1 lax123*) which lacks all members of the AUX/LAX family of auxin influx carriers [16] and
264 *smx16 smx17 smx18 (smx1678)* which has a 60% reduction in PIN1 abundance and auxin transport
265 in the stem [17]. These mutants have some pleiotropic phenotypes, but are broadly wild-type in
266 terms of their branching architecture [15,16,17]. Consistent with our hypothesis, two of these lines
267 had delayed inflorescence arrest; with a clear and lengthy delay in *aux1 lax123* and *smx1678* (**Fig.**
268 **S4C**). While *aux1 lax123* does reduce fruit fertility, *smx1678* mutants are normally fertile and set
269 seed well [17], showing the effect on inflorescence arrest in this line at least is not due to reduced
270 fertility. We do not believe that the arrest defect in *smx1678* mutants is connected to their primary
271 defect in strigolactone signalling, because mutants completely deficient in strigolactone signalling
272 and synthesis arrest at essentially the same time as wild-type (**Fig. S4E**). Taken together, our data
273 demonstrate that auxin is likely a key signal that triggers floral arrest in temporally-competent
274 inflorescences.

275

276 DISCUSSION

277 Our research provides clearer understanding of the process of floral arrest in Arabidopsis, and the
278 regulatory mechanisms that govern it. We show that floral arrest arises from the uncoordinated
279 local arrest of inflorescences, rather than a globally coordinated arrest, and that quasi-
280 synchronicity of floral arrest is a natural consequence of the quasi-synchronous inflorescence
281 activation. We show that inflorescences will only arrest when they become temporally-competent to
282 do so, which is likely a reflection of the developmental age of the inflorescence meristem. Our work
283 thus complements the recent work of Balanzà et al [4] who showed that age-related up- and down-
284 regulation of the FRUITFULL and APETALA2 transcription factors in inflorescence meristems was
285 associated with delayed floral arrest. FRUITFULL and APETALA2 are thus likely to be key factors
286 determining the competence of inflorescence meristems to arrest, and may integrate external
287 signals from the fruit [4].

288
289 We have shown that auxin exported from fruits triggers arrest in competent inflorescences. Auxin
290 exported from dominant shoot apices is a potent but indirect inhibitor of AM activation [5],
291 suggesting that auxin exported from fruits might act analogously to indirectly inhibit inflorescence
292 activity. This is corroborated by data from Wuest et al [3], who showed that arrested inflorescences
293 meristems have a similar transcriptome to pre-activation AMs in Arabidopsis, supporting the idea
294 that arrest might represent an inverse of AM/IM activation. Two major, non-mutually exclusive
295 mechanisms have been proposed for the inhibitory effect of apical auxin on AM activation. In the
296 'second messenger' model, cytokinin and strigolactones are synthesised in the stem, and are
297 transported into buds where they promote and repress AM activation, respectively. In this model,
298 apical auxin acts by repressing cytokinin and promoting strigolactone synthesis in the stem.
299 Conversely, in the 'canalization' model of shoot branching, it is proposed that AMs need to create a
300 'canalized' auxin transport link to the stem, in order to export auxin, and thus become active [7,18].
301 In this model, the presence of apical auxin reduces the auxin sink strength of the stem, limiting the
302 number of AMs that can create a canalized link, and therefore grow [7,18]. Building on this model,
303 we propose that arrest-competent inflorescence apices become inhibited and de-activated
304 because they are out-competed for auxin sink strength in the stem by the considerable quantity of
305 auxin exported from proximal fruit. This model in turn suggests that the arrest-competent state may
306 be associated with a rapid loss of auxin source strength in the inflorescence apex (**Fig. 6F**). The
307 result of losing the competition for auxin sink strength is that auxin transport connection between
308 the apex and the stem is 'de-canalized', preventing further apical activity. It is important to note that
309 in the canalization model it is not auxin accumulation in shoot apices that causes their growth
310 inhibition, it is the loss (or lack) of a canalized auxin transport link in itself. In support of this model,
311 we found that sub-apical application of the auxin transport inhibitor NPA, which completely blocks
312 export of auxin from the PI, was sufficient to trigger regulated arrest in sterile *ams* inflorescences
313 following the 20dpa timepoint (**Fig S4D**).

314

315 Our work thus potentially expands the canalization framework to a new developmental process, but
316 more work will be needed to test and model these ideas. We have also clearly shown that
317 gibberellin signalling does not have a role in controlling inflorescence duration despite the fact that
318 it can affect fruit production. Nonetheless, this does not exclude a role for other phytohormones, as
319 is seen in AM activation. The potential presence of additional signals is also reflected in the re-
320 initiation of flowering that is observed in previously “dormant” inflorescences (**Fig 1E**). This occurs
321 late in the plant life-cycle once the seeds are maturing. At this stage the seeds/pods will have lower
322 auxin levels, suggesting that additional signals may also be involved in this process. Overall, our
323 model refines Hensel et al’s GPA model [2], and provides a mechanistic framework which would
324 potentially allow for the duration of flowering to be extended or reduced to match local climatic
325 conditions, whilst also containing a key checkpoint so that flowering only ceases if fertile fruit have
326 recently been made. This paves the way to provide understanding of the end-of-flowering
327 syndromes in other species, which in turn has potential impact for extending and maximising future
328 crop yields.

329

330

331 MATERIALS & METHODS

332

333 **Plant growth conditions**

334 Plants for phenotypic and microsurgical experiments were grown on John Innes compost, under a
335 standard 16h/8h light/dark cycle (20°C) in controlled environment rooms with light provided by
336 white fluorescent tubes at a light intensity of $\sim 120\mu\text{mol}/\text{m}^2\text{s}^{-1}$. Plants for hormone profiling,
337 dexamethasone application and hormone application experiments were grown on John Innes No.3
338 compost under the same light/dark cycle but at 22°C/18°C, with light provided by fluorescent tubes
339 at an intensity of $\sim 150\mu\text{mol}/\text{m}^2\text{s}^{-1}$.

340

341 **Plant materials**

342 *Arabidopsis* wild-types Col-0 and Ler were used as indicated. The following lines have previously
343 been described before; *ms1-1* (Ler background) [19]; *AMS:AMS-GR ams* (used as *ams* mutants;
344 Col-0 background, *ams* is SALK_152147) [12]; *MS1:MS1-GR ms1-1* (Ler background) [20]; *rga-*
345 *t2 gai-t6 rgl1-1 rgl2-1 rgl3-1 (della;* Ler background) [12], *pin3-3 pin4-3, pin7-1* (Col-0
346 background) [15], *aux1 lax1 lax2 lax3* (Col-0 background) [16], *smxl6-4 smxl7-3 smxl8-1* (Col-0
347 background) [17].

348

349 **Phenotypic assessments**

350 We used the following nomenclature (**Fig. S4**). The primary embryonic shoot apex gives rise to
351 primary leaves and eventually forms the primary inflorescence. Flowering branches that form from
352 axillary buds in the axils of primary leaves are secondary inflorescences. Secondary inflorescences
353 formed from primary cauline leaves are cauline inflorescences (denoted C1 etc.), those from
354 primary rosette leaves are rosette inflorescences (denoted R1 etc.). Secondary inflorescences are
355 numbered in the order in which they activate, from the shoot apex downwards through the cauline
356 nodes, and then into the rosette nodes. Thus, C1 is the apical-most cauline inflorescence, C2 is
357 the second apical-most inflorescence, and so on. We have separated the numbering of the cauline
358 and rosette nodes, such that R1 is the apical-most rosette inflorescence. Branches that form from
359 secondary inflorescences are tertiary inflorescences, etc, and are named after the parental
360 branching system in rootward fashion (e.g. C2.1 = uppermost tertiary branch on the second cauline
361 inflorescence).

362

363 For the timing data in Figures 1A, 1B, 1C, 2D, 3A, 3C, 3D, 4C, 5B, 5C, S1, S2B and S3C plants
364 were assessed daily until visible flower buds were present at the shoot meristem. This date of floral
365 transition was recorded, and plants were assessed daily as appropriate for IM activation (scored
366 when buds were longer than 10mm) and IM arrest (scored when there were no more open flowers
367 on the IM). For fruit counts in Fig. 1D, 3B, 3E, 4C, 5B and S2A the number of inflorescences was

368 counted, and the number of fruits on each inflorescence recorded (or the number of fruits
369 removed). Fruit counts were made at final arrest unless otherwise stated.

370

371 For the DEX-induction experiment, *MS1:MS1-GR ms1-1* plants were treated with either a solution
372 consisting of 10ml distilled water, 25 μ M Dexamethasone (from a 25mM stock in ethanol), and 2 μ l
373 Silwet-77, or a mock containing the same but with only ethanol. Treatments were carried out at 11
374 and 12dpa and fruit number was subsequently counted at the time points indicated on the graph.
375 Following the arrest of the DEX-treated plants, the percent of fertility in all plants was evaluated
376 counting the number of fruit which had extended.

377

378 **Micro-surgical experiments**

379 Flower removal in Fig 2A-D, 4B-C 5A-B and 5C was performed every 1 to 2 days by removing all
380 open flowers on the plant between the stated time points. Branch removal in Figure 3AB, 3C, 3DE
381 was performed by cutting off branches at their base at the stated time point.

382

383 **IAA metabolite quantification**

384 For quantification of IAA and IAA metabolites, 6dpa fruits were sampled from mature flowering (ca.
385 15-18dpa) *ms1-1* and *Ler* plants. Fruit age had been tracked by marking their corresponding
386 flowers with thread at 6 days previously, at anthesis. For the export assay the same strategy was
387 used, but following excision fruits were placed pedicel-down in closed PCR tubes containing 50 μ l
388 2.5mM sodium diethyldithiocarbamate buffer and incubated for 21h in a growth room. The samples
389 were snap frozen in liquid nitrogen and stored at -80°C until analysis, either by GC-MS/MS as
390 described in Prusinkiewicz et al 2009 (eluates) or by UHPLC-MS/MS as described in [21], where
391 prior the UHPLC-MS/MS analysis the fruit tissues were extracted and purified according to [22].

392

393 **Hormone applications**

394 For the 5mg/g NAA lanolin treatments, 50 μ l of either 100mg/ml stock solution in DMSO or just
395 DMSO for the mock with 1 μ l of dye was added to 1g of molten lanolin (heated to 60°C) and
396 subsequently shaken until completely incorporated. Enough of the paste to create a thin layer was
397 then applied using a micropipette tip to the fruit. For the early/continual NAA application
398 experiments, the application regimen began at 6dpa of the first flower. For the late NAA application
399 experiment, treatment was initiated at 20dpa and only the top (i.e. proximal to the IM) 10 fruits, and
400 any produced above these in the subsequent 3 days were treated. For NAA removal and
401 replacement treatments, plants were de-fruited of the top 10 fruit at 23dpa and the resulting cut
402 pedicel was treated with NAA in lanolin as in the late treatments. For NPA treatments, an
403 approximately 1cm region directly below the apex of the PI was either treated with NPA (0.1mg/g,
404 from a 100mg/ml DMSO stock) in lanolin or a mock (1 μ l DMSO in lanolin) at 12dpa. Treatments

405 were conducted at the same time as fruit number counts, indicated by the time points on the
406 graphs.

407

408 **Experimental design and statistics**

409 Samples size for each experiment are described in the figure legends. For plant growth
410 experiments, each sample was a distinct plant. For auxin measurements, each sample was set of
411 tissue pooled from multiple plants; each sample was distinct. For data analysis, we tested data for
412 normality to determine the most appropriate statistical test, except when mixed-effects models
413 were used, where instead sphericity was not assumed and the Greenhouse-Geisser correction
414 was applied. For Sidak's multiple comparisons, individual variances were calculated for each
415 comparison.

416

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423

424 **AUTHOR CONTRIBUTIONS**

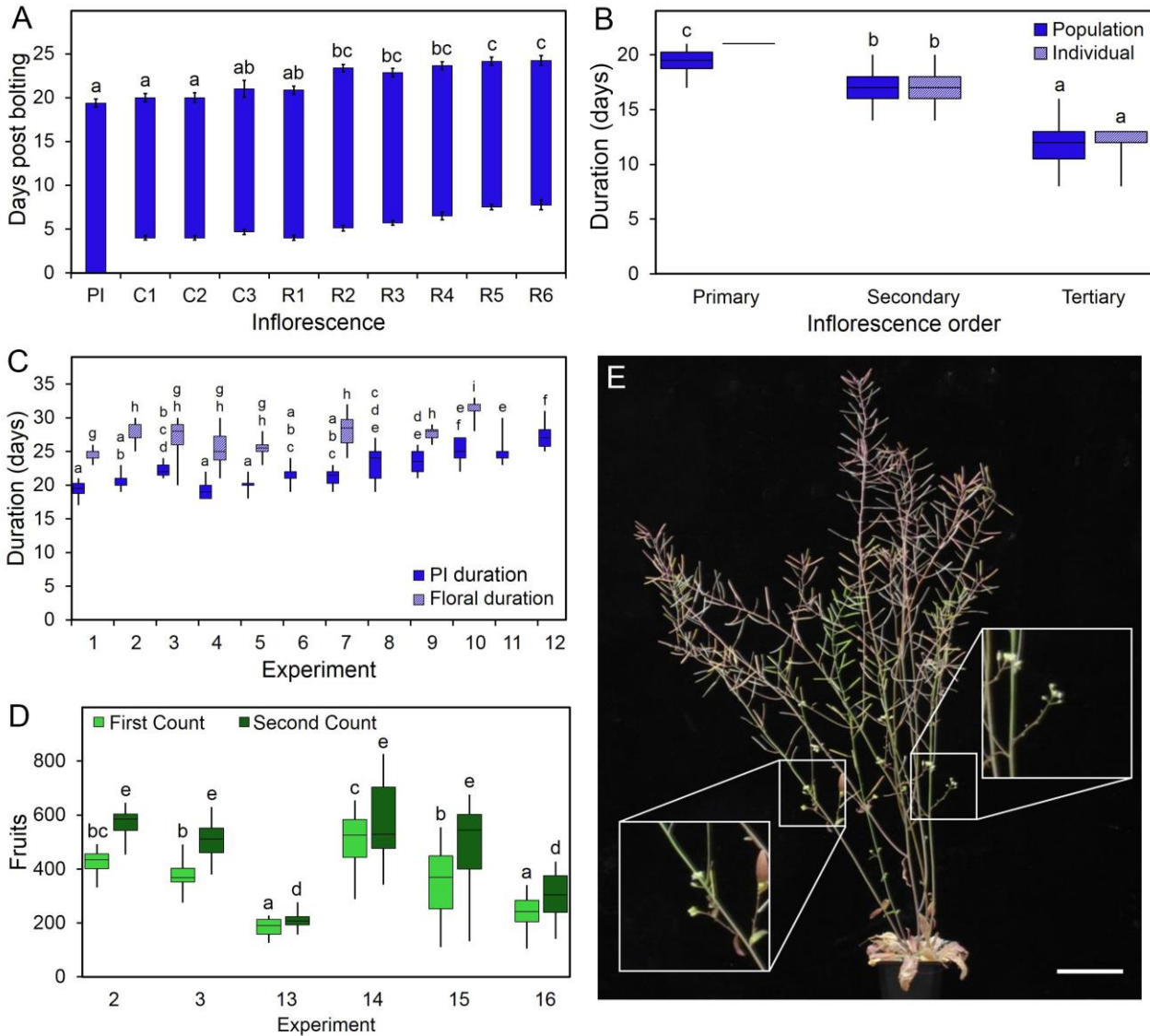
425 CW, AW, JS, KL performed experiments and analysed the data. TB, AB & ZW designed the study.
426 All authors contributed to writing the manuscript.

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- 486
- 487

FIGURES



488 **Figure 1. Inflorescence meristem arrest is a temporally-regulated process**

489 (A) Timing of inflorescence activation and arrest across different branches. PI = primary
 490 inflorescence, C1 = secondary cauline inflorescence 1 (the uppermost on the plant) etc., R1 =
 491 secondary rosette inflorescence 1 (the uppermost rosette inflorescence). The mean time after floral
 492 transition (bolting), until the activation of each inflorescence was measured, along with the
 493 subsequent time until its arrest, for a population of Col-0 plants. Each bar is the mean of 3-8 plants,
 494 since not all plants had each type of inflorescence. Any inflorescence type occurring on two or
 495 fewer plants was excluded from analysis. Error bars indicate s.e.m. Bars with the same letter are
 496 not statistically different from each other (ANOVA, Tukey HSD test).

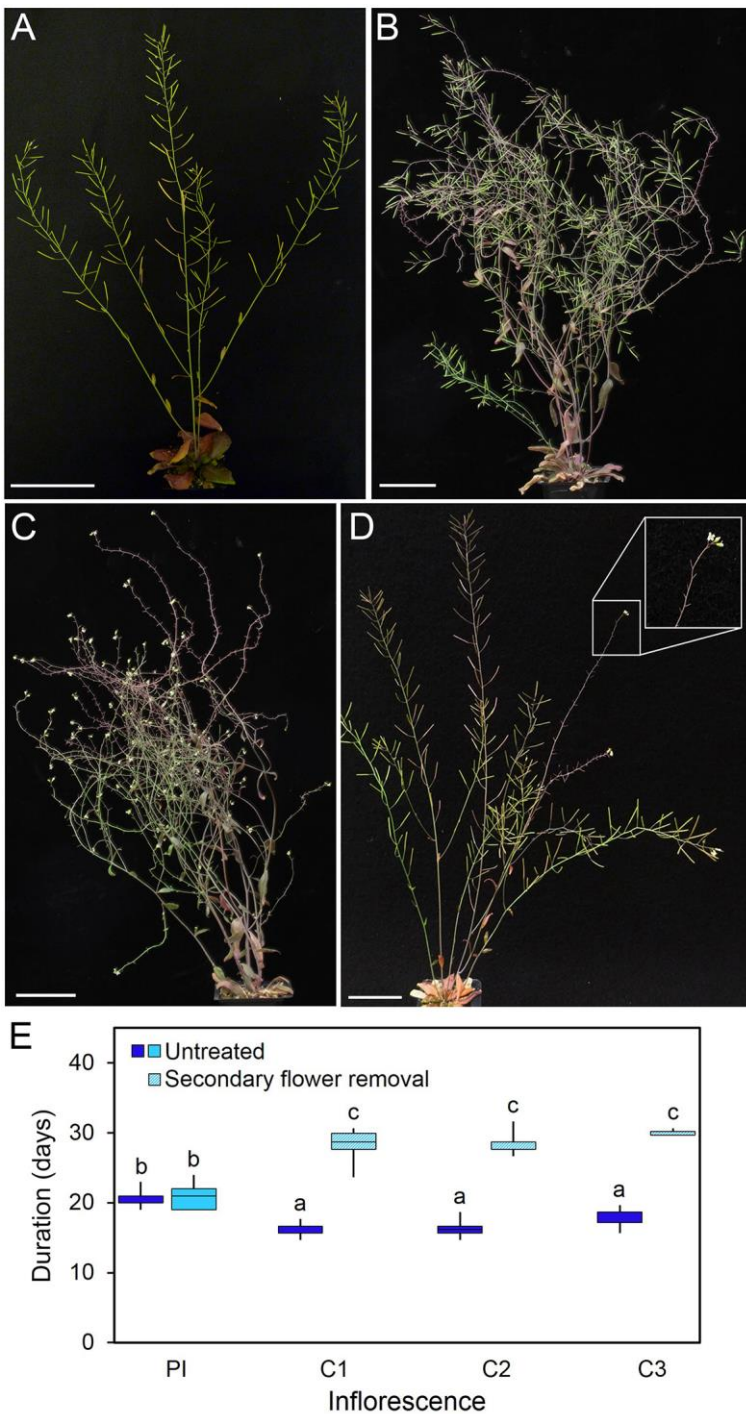
497 (B) Mean duration, from activation to arrest, of different classes of inflorescences, in a single Col-0
 498 plant, and (B) across a population of Col-0 plants. Bars indicate standard deviation. For the
 499 population, n=8 plants. Asterisks indicate statistically significantly different time of arrest from the
 500 primary inflorescence (ANOVA, Dunnett's test, n=3-8, * = p<0.05, ** = p<0.01, *** = p<0.001).

501 (C) Duration of the PI as an individual inflorescence, and total time from floral transition to *initial*
502 floral arrest of the whole plant (floral duration), in Col-0 plants grown in long days (16h light/8h
503 dark) in 12 independent experiments. $n=8-24$, bars indicate s.e.m. Bars with the same letter are
504 not significantly different from each other (ANOVA, Tukey HSD test).

505 (D) Mean total fruit production in long day-grown Col-0 plants across 6 separate experiments when
506 before re-flowering (light green bars) and after re-flowering ('second count', dark green bars),
507 $n=11-18$ depending on experiment, bars indicate s.e.m. Bars with the same letter are not
508 significantly different from each other (ANOVA, Tukey HSD test).

509 (E) Photograph showing re-flowering in Col-0, with new branches produced after initial floral arrest
510 highlighted in white boxes. Scale bar = 5cm.

511



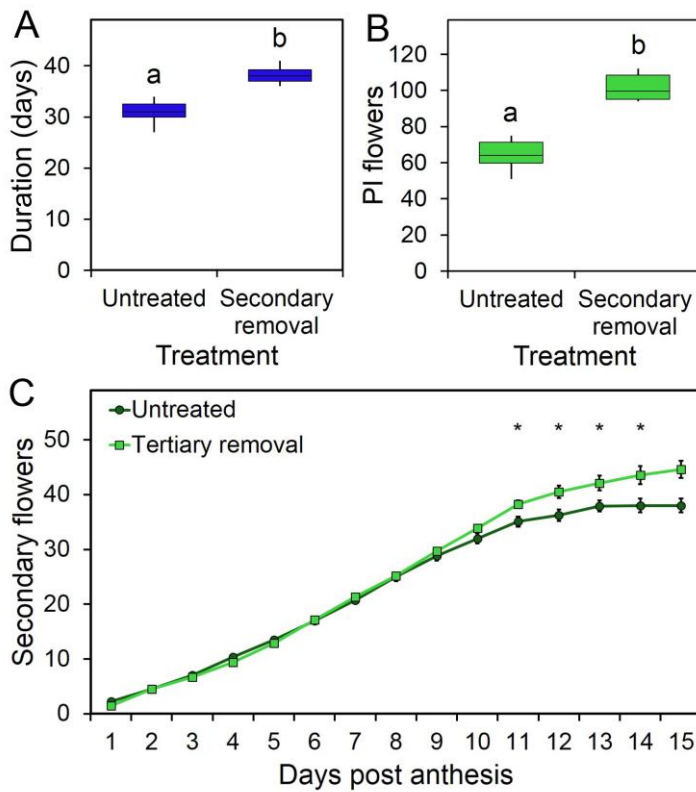
512 **Figure 2. Floral arrest is locally regulated by fruit presence**

513 (A-D) Floral arrest is delayed by continuous flower removal. Continuous daily removal of flowers
 514 across all inflorescences delays floral arrest in wild-type Arabidopsis (A, C), but when treatment is
 515 ended fruits develop, and arrest occurs within a few days (B). Local flower removal prevents arrest
 516 of individual inflorescences, but has no systemic effect (D).

517 (E) Inflorescence duration in response to local flower removal. Open flowers were removed from
 518 secondary cauline inflorescences (C1, C2, C3) every 1-2 days until 17 days post bolting (dpb),
 519 whereupon open flowers were removed daily. Inflorescence duration in secondary cauline
 520 inflorescences was significantly extended where flowers were removed (hatched light blue bars),

521 relative to secondary cauline inflorescences in untreated plants (dark blue bars). However, the
522 duration of primary inflorescences (which were not treated) was not different between treated (light
523 blue) and untreated (dark blue). $n=11-12$, bars indicate s.e.m. Bars with the same letter are not
524 statistically different from each other (ANOVA, Tukey HSD test).

525

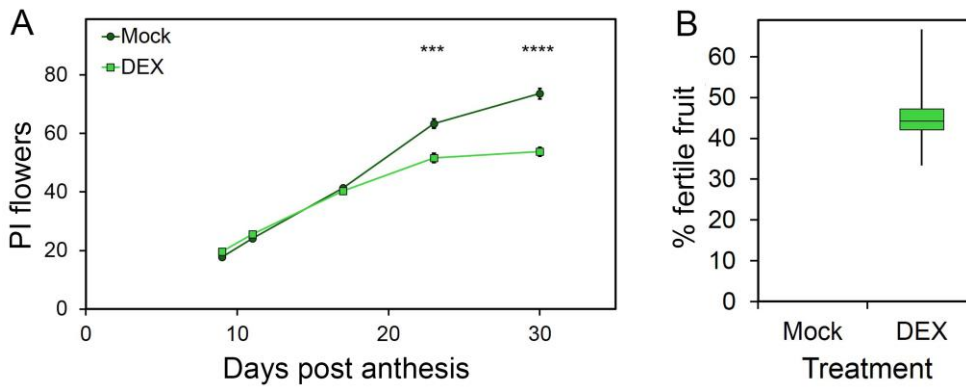


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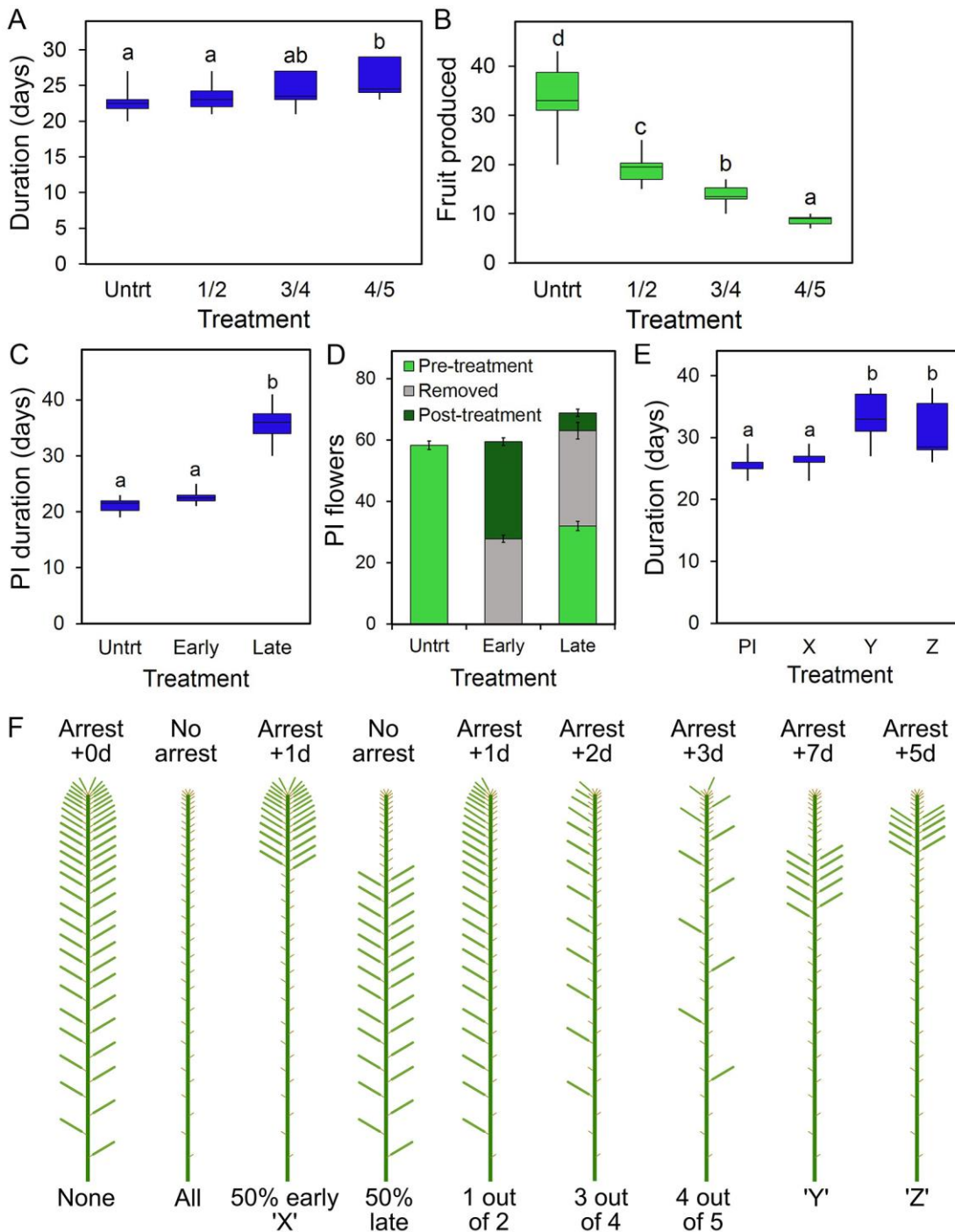
527

528 **Figure 3. Inflorescence duration is extended by global fruit absence**

529 (A, B) Effect of secondary inflorescence removal on the duration of primary inflorescences (PI) in
 530 the Ler ecotype of Arabidopsis. In treated plants, all secondary inflorescences were removed at 7
 531 days post bolting (dpb), and the timing of PI arrest was measured (A), as well as the number of
 532 flowers produced by the PI (B). $n=12$, bars indicate s.e.m. Bars with the same letter are not
 533 statistically different from each other (T-test, $p>0.05$). C) Effect of tertiary inflorescence removal on
 534 the duration of secondary inflorescences in the Col-0 ecotype of Arabidopsis. In treated plants, all
 535 tertiary inflorescences were removed at 6 days post anthesis, and the daily rate of flower opening
 536 after anthesis of the first flower on the secondary inflorescence was measured until inflorescence
 537 arrest. $n=11-12$, bars indicate s.e.m. Asterisks indicate statistically significant difference between
 538 the treatments (T-test with Bonferroni correction, $p<0.05$).



539 **Figure 4. Small numbers of fruit are sufficient for local inflorescence arrest**
 540 (A,B) Floral arrest is delayed by male sterility. Mock treated *MS1:MS1-GR ms1-1* plants are fully
 541 sterile and do not undergo timely primary inflorescence arrest, behaving the same as *ms1-1* sterile
 542 plants. However if fertility is restored by 25 μ m DEX treatment at 11 and 12 days post anthesis
 543 (dpa) of the first flower on the primary inflorescence, timely inflorescence arrest occurs. n=9-12,
 544 bars indicate s.e.m. Stars indicate significance as determined by Sidak's multiple comparisons
 545 following fitting of a mixed-effects model (**** = p <0.0001). (B) Application of DEX resulted in
 546 subsequent restoration of fertility, while mock-treated plants exhibited complete sterility. n=9-12,
 547 bars indicate s.e.m.



548 **Figure 5. Proximal fruit drive arrest in competent inflorescence meristems.**
 549 (A,B) Effect of fruit removal on inflorescence arrest. Secondary cauline inflorescences on the same
 550 plant were subjected to four different fruit removal treatments, removing either no fruit (untreated),
 551 one out of every two fruit (1/2), two out of every three fruit (3/4) or four out of every five fruit (4/5).
 552 The timing of secondary inflorescence arrest was measured (A), as well as the number of fruit
 553 produced by each inflorescence (B). $n=12$, bars indicate s.e.m. Bars with the same letter are not
 554 statistically different from each other (ANOVA, Tukey HSD test).
 555 (C, D) Effect of partial and differential fruit removal on inflorescence meristem arrest. In 'Early'
 556 plants, open flowers were removed from the whole plant every 1-2 days until approximately 30

557 flowers had been produced on the primary inflorescence, following which they were allowed to
558 flower normally. 'Late' plants were allowed to flower as normal until around 30 flowers had opened
559 on the primary inflorescence, then all subsequently-produced flowers were removed daily until
560 30dpb, when the inflorescence was allowed to produce fruit again. **(C)** Shows the inflorescence
561 duration of the PI for these different treatments. **(D)** Shows the number of flowers produced by the
562 PI in these treatments, coloured according to whether the flower was produced before (light green)
563 or after (dark green) treatment, or whether it was removed (grey). $n=11-12$, bars indicate s.e.m.
564 Bars with the same letter are not statistically different from each other (ANOVA, Tukey HSD test).
565 **(E)** Effect of timing of fruit production on inflorescence arrest. Secondary cauline inflorescences on
566 the same plant were subjected to three different treatments (X,Y,Z)(see **F**). In all treatments, fruit
567 produced up to 17 days post bolting (dpb) were removed. Treatment X inflorescences were then
568 allowed to make fruit until arrest. Treatment Y inflorescences were allowed to set 10 fruit from
569 17dpb, and then were subjected to continuous flower removal until arrest. Treatment Z
570 inflorescences were subjected to continuous flower removal until 20dpb, at which point they were
571 allowed to set 10 fruit, before flower removal was restarted until arrest. The primary inflorescences
572 on the same plant acted as untreated controls. The graph shows the mean time of arrest (days
573 post bolting) for inflorescences in each of these treatments. $n=13-14$, bars indicate s.e.m. Bars with
574 the same letter are not statistically different from each other (ANOVA, Tukey HSD test).
575 **(F)** Diagram summarising the effects of fruit removal quantity and timing on floral arrest, based on
576 experiments in Figure 5.

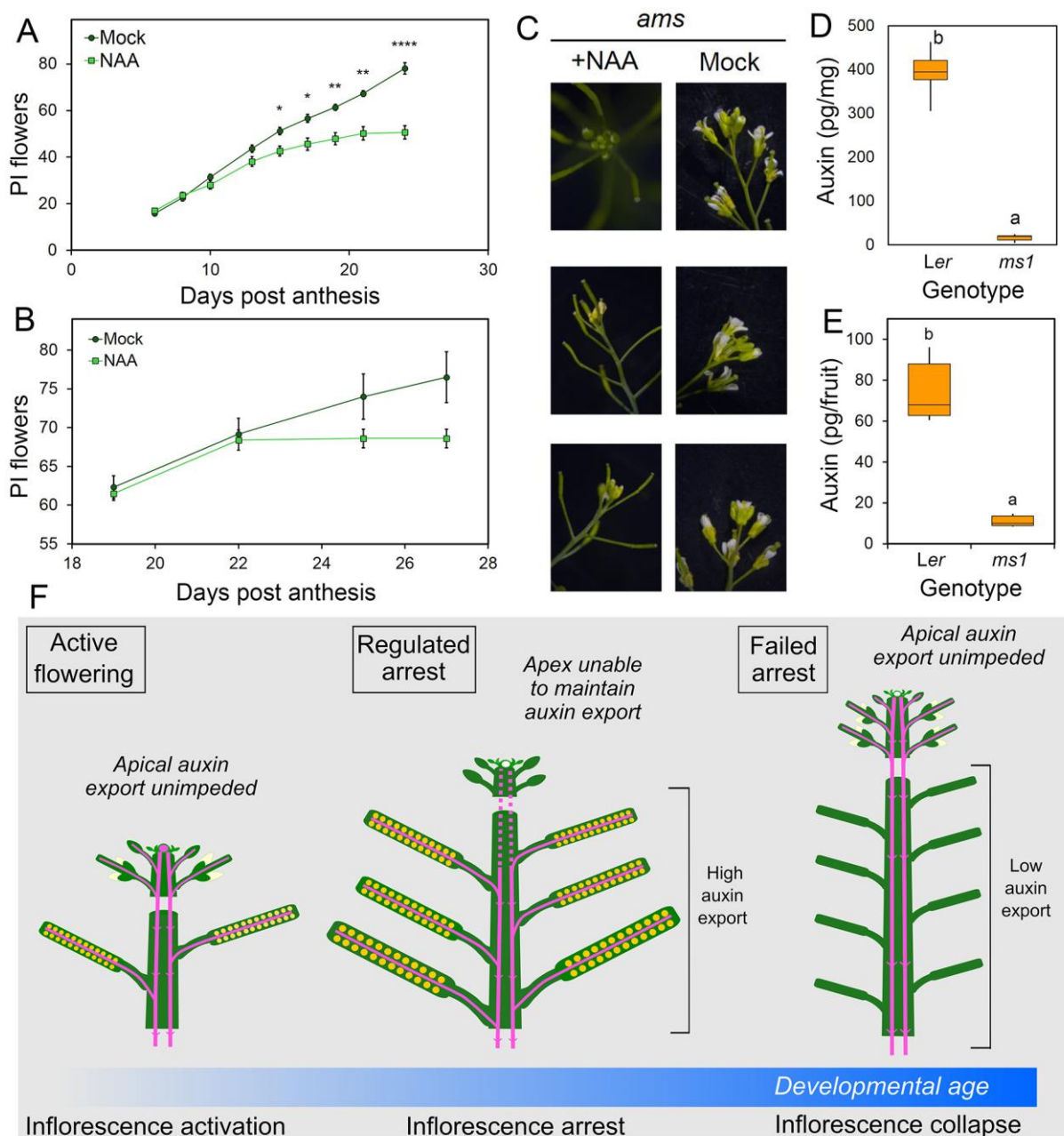


Figure 6. Auxin export from fruit triggers floral arrest

577 **A)** Temporal production of flowers by the PI of male-sterile *ams* plants upon application of either
 578 5mg/g NAA in lanolin, or a mock treatment consisting of lanolin and DMSO. Flower counts and
 579 lanolin treatment were performed every 2-3 days, starting from 6 days post anthesis (dpa) of the
 580 first flower on the primary inflorescence. n=7-12, bars indicate s.e.m. Asterisks indicate
 581 significance as determined by Sidak's multiple comparisons following fitting of a mixed-effects
 582 model; * = <0.05; ** = <0.01; *** = 0.001; **** = 0.0001.

583 **B)** Temporal production of flowers on the PI of male-sterile *ams* upon application of 5mg/g NAA in
 584 lanolin or mock as in (A). Flower counts and lanolin treatment were performed every day, starting
 585 from 20dpa. n=6-10, bars indicate s.e.m.

586 **C)** Representative photos (3 per treatment) showing the inflorescence meristem in *ams* mutants
587 after NAA or mock treatment. NAA treated plants have arrested with a classic 'bud cluster'
588 morphology [2], while mock-treated plants do not arrest and continue to open flowers.

589 **D)** Quantification of auxin content in 6dpa fertile (*Ler*) and sterile (*ms1*) Arabidopsis fruits. n=5,
590 bars indicate SD.

591 **E)** Quantification of auxin eluted from fertile and sterile Arabidopsis fruits. n=5, bars indicate SD.

592 **F)** Model for induction of floral arrest. Initially, the apex can freely canalize to the polar auxin
593 transport stream (PATS, pink). After a temporally-defined period of flowering, inflorescences reach
594 a critical age and become capable of arrest. In the presence of ca. 6-8 fertile fruit containing seed
595 (yellow circles), which actively export large quantities of auxin into the PATS, the apex is no longer
596 able to canalize to the PATS. This induces floral arrest, similar to bud dormancy. If fruit are sterile
597 (or removed), the auxin export from proximal fruit is significantly reduced. This allows the apex to
598 continue flowering beyond the point of arrest-competence, as it can still canalize to the PATS.
599 Fertilisation or auxin application at this point rapidly induces arrest. If no fertilisation occurs, the
600 meristem ultimately undergoes the terminal differentiation described by Hensel et al [2].
601