Auxin export from proximal fruits drives arrest in competent inflorescences

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ABSTRACT

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

INTRODUCTION

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

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

RESULTS

Inflorescence arrest is not synchronous in Arabidopsis

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

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

Inflorescence arrest is a temporally-regulated process

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

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

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

Delayed inflorescence arrest in response to fruit absence occurs systemically

Contrary to this model, the results of Hensel *et al*. clearly demonstrated an extension of PI duration upon removal of secondary inflorescences, suggesting that systemic feedback from fruits can modulate the duration of individual inflorescences. We repeated this debranching treatment and confirmed that in the L*er* and Col-0 backgrounds, it does indeed extend inflorescence duration and fruit production of the PI, relative to untreated plants (**Fig. 3A,B**). Interestingly, we observed that the duration of the PI in untreated L*er* plants was longer than that in Col-0 by approximately 7-9 days (cf. **Fig. 3A** and **Fig. 2E**), suggesting there is variation in Arabidopsis ecotypes for inflorescence duration. Similarly, when we removed tertiary inflorescences from secondary

inflorescences in Col-0, we observed a small extension to the duration of secondary inflorescences, and a corresponding increase in the number of fruit they produce (**Fig. 3C**). Thus, even though the global *presence* of fruit across the plant is not sufficient to trigger arrest of individual fruitless inflorescences, the global *absence* of fruit is sufficient to extend the duration of individual fully-fruited inflorescences. Collectively, our data suggest that fruit play two distinct roles in inflorescence arrest, systemically modulating inflorescence duration, and locally driving inflorescences to undergo arrest. This likely indicates the existence of multiple fruit-derived signals that are involved in the regulation of inflorescence arrest.

Small numbers of fruit are sufficient to trigger inflorescence arrest

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

Proximal fruit are needed for temporally-competent inflorescences to arrest

These data also present a paradox: approximately 7 fertile fruit are sufficient in certain circumstances to trigger arrest, but most inflorescences produce far more than 7 fruit before arresting. Given our earlier observations of inflorescence duration (**Fig. 1A,C**) and that inflorescences on the same plant tend to arrest at approximately the same time despite individually producing different fruit numbers (**Fig. 5A,B**), these data reinforce the idea that temporally-acquired responsiveness to a fruit-derived signal is critical, rather than a threshold level of signal being reached. We therefore tested how the timing of fruit production affects inflorescence arrest. In a first experiment, we performed two treatments; 'early' plants had all flowers removed, until around 30 flowers had been produced by the PI (12-13dpb), and were then allowed to continue flowering normally. Despite producing far fewer fruit than control plants (**Fig. 5D)**, the PI of 'early' plants underwent arrest at the same time as untreated plants (approximately 21dpb) (**Fig. 5C**). This mirrored the effect seen in the dexamethasone-inducible *MS:MS1-GR* line (**Fig. 4A**). Conversely, 'late' plants were allowed to flower as normal until around 30 flowers had opened on the PI (12-13dpb); subsequently all open flowers were removed from the plant for 20 days. Despite producing the same number of fruit as 'early' plants during the first 21dbp (**Fig. 5D**), 'late' plants did not undergo timely arrest (**Fig. 5C**). However, when flower removal treatment was ended in 'late' plants at approximately 30dpb, the inflorescence was active for a further 7 days, producing around 7 fertile fruits before arresting (again consistent with the minimum fruit numbers established in **Fig 2B, 4C**). These data demonstrate that fruit are only able to trigger arrest when inflorescences have become temporally competent to arrest, at the end of their normal lifetime.

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

becomes arrest-competent, the lower the ability of those fruit to trigger arrest (**Fig 5F**); very distal fruit are completely unable to trigger arrest.

Collectively, our data suggest that inflorescence arrest is a time-dependent process, in which inflorescences become competent to arrest at a certain developmental age post-floral transition, and then undergo almost immediate arrest, as long as they receive an inhibitory signal from fruit they have recently produced. This developmental age does not directly reflect the absolute age of the inflorescence, with the relationship between developmental age and absolute age likely varying due to environmental influences or differences in growth history, and is reflected in the range of fruit numbers produced between plants.

Auxin export from fertile fruit triggers inflorescence arrest

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

Transcriptionally, the switch between activity and arrest in inflorescence meristems mirrors the switch between activity and dormancy in axillary meristems (AMs) [2]. Since this switch in AMs is controlled in part by auxin export from the AM into the stem [6,7], we hypothesised that auxin may also be a key signal in inflorescence arrest, especially given the high levels of auxin known to be produced in fruits and seeds in many species [8,9,10,11]. Previous work in Arabidopsis has identified a curve of hormone production in developing fruit, with a peak in auxin content at 6dpa [11]. To confirm whether fertilisation increases the auxin content of Arabidopsis fruit, sterile (*ms1-1*) and fertile (L*er*) fruit were sampled at 6dpa, and auxin levels were quantified using UHPLC-MS/MS. This analysis showed that auxin levels are much higher in fertile fruit (392pg/mg tissue)

than sterile fruit (16pg/mg tissue) (**Fig. 6D**), a difference further amplified by their 10-fold greater mass (**Fig. E1A**). We next ascertained whether fertile fruit indeed transport auxin into the stem, by collecting auxin exported from the pedicels of 6dpa fertile fruit from the PI. We found that individual fertile fruit export ~75pg of auxin in 21 hours, which is 7.5 fold higher than equivalent sterile fruit (**Fig. 6E**). Given that the equivalent pool of mobile auxin collected from the associated inflorescence stem is ~100-200pg [7], it is clear that a small number of fertile fruit make a very significant contribution to auxin levels in the inflorescence stem.

To directly test this model, we assessed whether exogenous application of auxin to sterile fruits could restore timely arrest of the PI. We treated sterile fruit in the *ams* mutant, which like *ms1* fails to undergo normal arrest [14], with the auxin analog NAA from 6dpa. This resulted in earlier inflorescence arrest with the PI producing ~50 fruit, compared to ~80 in mock-treated plants (**Fig. 6A**). In auxin-treated *ams* plants, arrested inflorescences have the normal 'bud cluster' morphology associated with the arrest of wild-type inflorescences (**Fig. 6C**). As expected, although auxin treatment occurred throughout flowering, it only induced arrest at the time that inflorescences normally become competent to arrest, at around 20dpa (**Fig. 6A**). When we applied NAA to the uppermost 10 sterile fruit of *ams* individuals at 20dpa (and to any fruit subsequently formed in the following 3 days), this rapidly induced a normal arrest (**Fig. 6B**) through the treatment of relatively few (~18) sterile fruit (**Fig. 6B**), consistent with the role of proximal fruit triggering inflorescence arrest only when the inflorescence is competent to do so. To rule out the possibility that auxin application to sterile fruit activates synthesis of a 'second messenger' that actually acts as an arrest signal, we performed NAA application at 23dpa to de-fruited pedicels in *ams* mutants. This treatment was completely effective at inducing timely inflorescence arrest, unlike the mock treatment, similar to the fruit application experiments (**Fig. E1B**). This shows production of a second messenger in fruit is not required for arrest, although it is possible a second messenger could still be produced in the stem.

If auxin exported from fertile fruits triggers arrest, treatments affecting the auxin transport system might be expected to inhibit the ability of fruit to export auxin, and drive arrest. To test this idea, we analysed arrest in three mutants with reduced auxin transport, namely *pin3 pin4 pin7* (*pin347*) which lacks three members of the PIN auxin efflux carrier family [15], *aux1 lax1 lax2 lax3* (*aux1 lax123*) which lacks all members of the AUX/LAX family of auxin influx carriers [16] and *smxl6 smxl7 smxl8* (*smxl678*) which has a 60% reduction in PIN1 abundance and auxin transport in the stem [17]. These mutants have some pleiotropic phenotypes, but are broadly wild-type in terms of their branching architecture [15,16,17]. Consistent with our hypothesis, two of these lines had delayed inflorescence arrest; with a clear and lengthy delay in *aux1 lax123* and *smxl678* (**Fig. E1C**). While *aux1 lax123* does reduce fruit fertility, *smxl678* mutants are normally fertile and set seed well [17], showing the effect on inflorescence arrest in this line at least is not due to reduced

fertility. We do not believe that the arrest defect in *smxl678* mutants is connected to their primary defect in strigolactone signalling, because mutants completely deficient in strigolactone signalling and synthesis arrest at essentially the same time as wild-type (**Fig. E1E**). Taken together, our data demonstrate that auxin is likely a key signal that triggers arrest in temporally-competent inflorescences.

DISCUSSION

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

We have shown that auxin exported from fruits triggers arrest in competent inflorescences. Auxin exported from dominant shoot apices is a potent but indirect inhibitor of AM activation [5], suggesting that auxin exported from fruits might act analogously to indirectly inhibit inflorescence activity. This is corroborated by data from Wuest *et al.* [3], who showed that arrested inflorescences meristems have a similar transcriptome to pre-activation AMs in Arabidopsis, supporting the idea that arrest might represent an inverse of AM/IM activation. Two major, non-mutually exclusive mechanisms have been proposed for the inhibitory effect of apical auxin on AM activation. In the 'second messenger' model, cytokinin and strigolactones are synthesised in the stem, and are transported into buds where they promote and repress AM activation, respectively. In this model, apical auxin acts by repressing cytokinin and promoting strigolactone synthesis in the stem. Conversely, in the 'canalization' model of shoot branching, it is proposed that AMs need to create a 'canalized' auxin transport link to the stem, in order to export auxin, and thus become active [7,18]. In this model, the presence of apical auxin reduces the auxin sink strength of the stem, limiting the number of AMs that can create a canalized link, and therefore grow [7,18]. Building on this model, we propose that arrest-competent inflorescence apices become inhibited and de-activated because they are out-competed for auxin sink strength in the stem by the considerable quantity of auxin exported from proximal fruit. This model in turn suggests that the arrest-competent state may be associated with a rapid loss of auxin source strength in the inflorescence apex (**Fig. 6F**). The result of losing the competition for auxin sink strength is that auxin transport connection between the apex and the stem is 'de-canalized', preventing further apical activity. It is important to note that in the canalization model it is not auxin accumulation in shoot apices that causes their growth inhibition, it is the loss (or lack) of a canalized auxin transport link in itself. In support of this model, we found that sub-apical application of the auxin transport inhibitor NPA, which completely blocks export of auxin from the PI, was sufficient to trigger regulated arrest in sterile *ams* inflorescences following the 20dpa timepoint (**Fig. E1D**).

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

MATERIALS & METHODS

Plant growth conditions

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

Plant materials

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

Phenotypic assessments

We used the following nomenclature (**Fig. S2**). The primary embryonic shoot apex gives rise to primary leaves and eventually forms the primary inflorescence. Flowering branches that form from axillary buds in the axils of primary leaves are secondary inflorescences. Secondary inflorescences formed from primary cauline leaves are cauline inflorescences (denoted C1 etc.), those from primary rosette leaves are rosette inflorescences (denoted R1 etc.). Secondary inflorescences are numbered in the order in which they activate, from the shoot apex downwards through the cauline nodes, and then into the rosette nodes. Thus, C1 is the apical-most cauline inflorescence, C2 is the second apical-most inflorescence, and so on. We have separated the numbering of the cauline and rosette nodes, such that R1 is the apical-most rosette inflorescence. Branches that form from secondary inflorescences are tertiary inflorescences, etc, and are named after the parental branching system in rootward fashion (e.g. C2.1 = uppermost tertiary branch on the second cauline inflorescence).

For the timing data in Figures 1A, 1B, 1C, 2E, 3A, 3C, 5A, 5C, 5E, E1C, E1E, S1 and S3A plants were assessed daily until visible flower buds were present at the shoot meristem. This date of floral transition was recorded, and plants were assessed daily as appropriate for inflorescence activation (scored when buds were longer than 10mm) and inflorescence arrest (scored when there were no more open flowers on the inflorescence). For fruit counts in Fig. 1D, 3B, 5B, 5D and S3B the

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

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

Micro-surgical experiments

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

IAA metabolite quantification

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

Hormone applications

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

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

Experimental design and statistics

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

Data availability

All figures in this manuscript are associated with raw data. All data will be made available upon request.

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AUTHOR CONTRIBUTIONS

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

REFERENCES

1. Khan MR, Ai XY, Zhang JZ (2014). Genetic regulation of flowering time in annual and perennial plants. *Wiley Interdisciplinary Reviews: RNA* **5**, 347-59.

2. Hensel LL, Nelson MA, Richmond TA and Bleeker AB (1994). The fate of inflorescence meristems is controlled by developing fruits in *Arabidopsis. Plant Physiol*ogy **106**, 863-876.

3. Wuest SE, Philipp MA, Guthörl D, Schmid B, Grossniklaus U (2016). Seed Production Affects Maternal Growth and Senescence in Arabidopsis. *Plant Physiology* **171**, 392-404.

4. Balanzà V, Martínez-Fernández I, Sato S, Yanofsky MF., Kaufmann K, Angenent GC, Bemer M and Ferrándiz C (2018). Genetic control of meristem arrest and life span in *Arabidopsis* by a *FRUITFULL-APETALA2* pathway. *Nature Communications* DOI: 10.1038/s41467-018-03067-5.

5. Walker CH, Bennett T (2018). Forbidden fruit: dominance relationships and the control of shoot architecture. *Annual Plant Reviews Online*, doi.org/10.1002/9781119312994.apr0640

6. Bennett T, Sieberer T, Willett B, Booker J, Luschnig C, Leyser O (2006). The Arabidopsis MAX pathway controls shoot branching by regulating auxin transport. Current Biology **16**, 553-563.

7. Prusinkiewicz P, Crawford S, Smith RS, Ljung K, Bennett T, Ongaro V, Leyser O (2009). Control of bud activation by an auxin transport switch. *Proceedings of the National Academy of Science of the USA* **106**, 17431-17436.

8. Gustafson F (1939). Auxin Distribution in Fruits and Its Significance in Fruit Development. *American Journal of Botany*, **26** 189.

9. Bangerth, F (1989). Dominance amongst fruits/sinks and the search for a correlative signal. Physiologia Plantarum **76**, 608-614.

10. Serrani J, Carrera E, Ruiz-Rivero O, Gallego-Giraldo L, Peres L, Garcia-Martinez J (2010). Inhibition of Auxin Transport from the Ovary or from the Apical Shoot Induces Parthenocarpic Fruit-Set in Tomato Mediated by Gibberellins. *Plant Physiology* **153**, 851-862.

11. Kanno, Y., Jikumaru, Y., Hanada, A., Nambara, E., Abrams, S., Kamiya, Y. and Seo, M. (2010). Comprehensive Hormone Profiling in Developing Arabidopsis Seeds: Examination of the Site of ABA Biosynthesis, ABA Transport and Hormone Interactions. *Plant and Cell Physiology* **51**, 1988-2001.

12. Feng S, Martinez C, Gusmaroli G, Wang Y, Zhou J, Wang F, Chen L, Yu L, Iglesias-Pedraz JM, Kircher S, Schäfer E, Fu X, Fan LM, Deng XW (2008). Coordinated regulation of Arabidopsis thaliana development by light and gibberellins. Nature **451**, 475-479.

13. Serrano-Mislata A, Bencivenga S, Bush M, Schiessl K, Boden S, Sablowski R. (2017.) DELLA genes restrict inflorescence meristem function independently of plant height. Nat Plants **3**, 749-754.

14. Ferguson AC, Pearce SP, Band LR, Yang C, Ferjentsikova I, King JR, Yuan Z, Zhang D, Wilson ZA (2017) Biphasic regulation of the transcription factor ABORTED MICROSPORES (AMS) is essential for tapetum and pollen development. New Phytologist **213**, 778-790.

15. Bennett T, Hines G, van Rongen M, Waldie T, Sawchuk MG, Scarpella E, Ljung K, Leyser O. (2016). Connective Auxin Transport in the Shoot Facilitates Communication between Shoot Apices. PLoS Biol **14**, e1002446.

16. Bainbridge K, Guyomarc'h S, Bayer E, Swarup R, Bennett M, Mandel T, Kuhlemeier C. (2008). Auxin influx carriers stabilize phyllotactic patterning. Genes Dev. 22, 810-823.

17. Soundappan I, Bennett T, Morffy N, Liang Y, Stanga JP, Abbas A, Leyser O, Nelson DC. (2015). SMAX1-LIKE/D53 Family Members Enable Distinct MAX2-Dependent Responses to Strigolactones and Karrikins in Arabidopsis. Plant Cell. 2015 Nov;27(11):3143-59.

18. Shinohara N, Taylor C, Leyser O (2013). Strigolactone can promote or inhibit shoot branching by triggering rapid depletion of the auxin efflux protein PIN1 from the plasma membrane. *PLoS Biology* **11**, e1001474.

19. Wilson, ZA, Morroll, SM, Dawson J and Tighe PJ (2001) The Arabidopsis MALE STERILITY1 (MS1) gene is a transcriptional regulator of male gametogenesis, with homology to the PHD-finger family of transcriptional regulators. *The Plant Journal* **28**, 27-39.

20. Ito T, Nagata N, Yoshiba Y, Ohme-Takagi M, Ma H, Shinozaki K (2007). Arabidopsis MALE STERILITY1 Encodes a PHD-Type Transcription Factor and Regulates Pollen and Tapetum Development. *The Plant Cell* **19** 3549-3562.

21. Novák O, Hényková E, Sairanen I, Kowalczyk M, Pospíšil T, Ljung K (2012). Tissue-specific profiling of the *Arabidopsis thaliana* auxin metabolome. *Plant Journal*, *72*(3), 523–536.

22. Dobrev PI, Kamínek M (2002). Fast and efficient separation of cytokinins from auxin and abscisic acid and their purification using mixed-mode solid-phase extraction. *Journal of Chromatography A* 950, 21–29.

FIGURE LEGENDS

Figure 1. Inflorescence arrest is a temporally-regulated process

(**A**) Timing of inflorescence activation and arrest across different branches. PI = primary inflorescence, C1 = secondary cauline inflorescence 1 (the uppermost on the plant) etc., R1 = secondary rosette inflorescence 1 (the uppermost rosette inflorescence). The mean time after floral transition (bolting), until the activation of each inflorescence was measured, along with the subsequent time until its arrest, for a population of Col-0 plants. The lower limit of the bar indicates the number of days after bolting that the inflorescence initiated. The upper limit of the bar indicates the end of flowering for each inflorescence. Each bar is the mean of 3-8 plants (*n*=8,8,8,3,7,8,7,6,6,4 biologically independent samples for PI-R6 respectively), since not all plants had each type of inflorescence. Any inflorescence type occurring on two or fewer plants was excluded from analysis. Error bars indicate s.e.m. Bars with the same letter are not statistically different from each other (ANOVA, Tukey HSD test).

(**B**) Mean duration, from activation to arrest, of different classes of inflorescences, in a single Col-0 plant, and across a population of Col-0 plants. The upper and lower confines of the box indicate the interquartile range, the central line indicates the median, and the whiskers represent the maximum and minimum values. For the population, *n*=8 plants. Bars with the same letter are not statistically different from each other (ANOVA, Tukey HSD test).

(**C**) Duration of the PI as an individual inflorescence, and total time from floral transition to *initial* inflorescence arrest of the whole plant (floral duration), in Col-0 plants grown in long days (16h light/8h dark) in 12 independent experiments. The upper and lower confines of the box indicate the interquartile range, the central line indicates the median, and the whiskers represent the maximum and minimum values. *n*=8-24 biologically independent samples (expts 1&12 *n*=8, expts 2&3 *n*=11, expts 4,5&9 *n*=12, expt 6 *n*=24, expt 10 *n*=10, expt 8 *n*=9, expt 10 *n*=7, expt 11 *n*=16; PI duration and floral duration bars have the same *n* value within each experiment). The upper and lower confines of the box indicate the interquartile range, the central line indicates the median, and the whiskers represent the maximum and minimum values. Bars with the same letter are not significantly different from each other (ANOVA, Tukey HSD test).

(**D**) Mean total fruit production in long day-grown Col-0 plants across 6 separate experiments when before re-flowering (light green bars) and after re-flowering ('second count', dark green bars), n=11-18 biologically independent samples (expts 2&3 *n*=11, expt 13 *n*=18, expt 14 *n*=13, expt 15 *n*=10, expt 16 *n*=12; first count and second count bars have the same *n* value within each experiment). The upper and lower confines of the box indicate the interquartile range, the central line indicates the median, and the whiskers represent the maximum and minimum values. Bars with the same letter are not significantly different from each other (ANOVA, Tukey HSD test).

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

Figure 2. Inflorescence arrest is locally regulated by fruit presence

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

(**E**) Inflorescence duration in response to local flower removal. Open flowers were removed from secondary cauline inflorescences (C1, C2, C3) every 1-2 days until 17 days post bolting (dpb), whereupon open flowers were removed daily. Inflorescence duration in secondary cauline inflorescences was significantly extended where flowers were removed (hatched light blue bars), relative to secondary cauline inflorescences in untreated plants (dark blue bars). However, the duration of primary inflorescences (which were not treated) was not different between treated (light blue) and untreated (dark blue). *n*=3-12 biologically independent samples (PI unt *n*=11, PI trt *n*=12, C1 unt *n*=11, C1 trt *n*=12, C2 unt *n*=10, C2 trt *n*=9, C3 unt *n*=6, C3 trt *n*=3). The upper and lower confines of the box indicate the interquartile range, the central line indicates the median, and the whiskers represent the maximum and minimum values. Bars with the same letter are not statistically different from each other (ANOVA, Tukey HSD test).

Figure 3. Inflorescence duration is extended by global fruit absence

(**A, B**) Effect of secondary inflorescence removal on the duration of primary inflorescences (PI) in the L*er* ecotype of Arabidopsis. In treated plants, all secondary inflorescences were removed at 7 days post bolting (dpb), and the timing of PI arrest was measured (**A**), as well as the number of flowers produced by the PI (**B**). *n*=12 biologically independent samples in all cases. The upper and lower confines of the box indicate the interquartile range, the central line indicates the median, and the whiskers represent the maximum and minimum values. Bars with the same letter are not statistically different from each other (two-tailed T-test, $p<0.001$). (**C**) Effect of tertiary inflorescence removal on the duration of secondary inflorescences in the Col-0 ecotype of Arabidopsis. In treated plants, all tertiary inflorescences were removed at 6 days post anthesis, and the daily rate of flower opening after anthesis of the first flower on the secondary inflorescence was measured until inflorescence arrest. *n*=12 biologically independent samples per treatment. The upper and lower confines of the box indicate the interquartile range, the central line indicates the median, and the whiskers represent the maximum and minimum values. Asterisks indicate statistically significant difference between the treatments (T-test with Bonferroni correction, $p<0.05$).

Figure 4. Small numbers of fruit are sufficient for local inflorescence arrest

(**A,B**) Inflorescence arrest is delayed by male sterility. Mock treated *MS1:MS1-GR ms1-1* plants are fully sterile and do not undergo timely primary inflorescence arrest, behaving the same as *ms1-1* sterile plants. However, if fertility is restored by 25μm DEX treatment at 11 and 12 days post anthesis (dpa) of the first flower on the primary inflorescence, timely inflorescence arrest occurs. n=9-12 biologically independent samples for each time point (Mock timepoints 9,11,17 days *n*=9, mock timepoints 23,30 days *n*=10, DEX timepoints 9,11,23,30 days *n*=12, DEX timepoint 17 days *n*=11), bars indicate s.e.m. Asterisks indicate significance as determined by Sidak's multiple comparisons following fitting of a mixed-effects model (**** = $p<0.0001$). (**B**) Application of DEX resulted in subsequent restoration of fertility, while mock-treated plants exhibited complete sterility. *n*=12 biologically independent samples for each treatment. The upper and lower confines of the box indicate the interquartile range, the central line indicates the median, and the whiskers represent the maximum and minimum values.

Figure 5. Proximal fruit drive arrest in competent inflorescences

(**A,B**) Effect of fruit removal on inflorescence arrest. Secondary cauline inflorescences on the same plant were subjected to four different fruit removal treatments, removing either no fruit (untreated), 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). The timing of secondary inflorescence arrest was measured (**A**), as well as the number of fruit produced by each inflorescence (**B**). *n*=12 biologically independent samples. The upper and lower confines of the box indicate the interquartile range, the central line indicates the median, and the whiskers represent the maximum and minimum values. Bars with the same letter are not statistically different from each other (ANOVA, Tukey HSD test).

(**C, D**) Effect of partial and differential fruit removal on inflorescence arrest. In 'Early' plants, open flowers were removed from the whole plant every 1-2 days until approximately 30 flowers had been produced on the primary inflorescence, following which they were allowed to flower normally. 'Late' plants were allowed to flower as normal until around 30 flowers had opened on the primary inflorescence, then all subsequently-produced flowers were removed daily until 30dpb, when the inflorescence was allowed to produce fruit again. (**C**) Shows the inflorescence duration of the PI for these different treatments. *n*=13-14 biologically independent samples (PI,A,C *n*=14, B *n*=13). The upper and lower confines of the box indicate the interquartile range, the central line indicates the median, and the whiskers represent the maximum and minimum values. Bars with the same letter are not statistically different from each other (ANOVA, Tukey HSD test). (**D**) Shows the number of flowers produced by the PI in these treatments, coloured according to whether the flower was produced before (light green) or after (dark green) treatment, or whether it was removed (grey). *n*=14 biologically independent samples, bars indicate s.e.m.

(**E**) Effect of timing of fruit production on inflorescence arrest. Secondary cauline inflorescences on the same plant were subjected to three different treatments (X,Y,Z)(see **F**). In all treatments, fruit produced up to 17 days post bolting (dpb) were removed. Treatment X inflorescences were then allowed to make fruit until arrest. Treatment Y inflorescences were allowed to set 10 fruit from 17dpb, and then were subjected to continuous flower removal until arrest. Treatment Z inflorescences were subjected to continuous flower removal until 20dpb, at which point they were allowed to set 10 fruit, before flower removal was restarted until arrest. The primary inflorescences on the same plant acted as untreated controls. The graph shows the mean time of arrest (days post bolting) for inflorescences in each of these treatments. *n*=14 biologically independent samples. The upper and lower confines of the box indicate the interquartile range, the central line indicates the median, and the whiskers represent the maximum and minimum values. Bars with the same letter are not statistically different from each other (ANOVA, Tukey HSD test).

(**F**) Diagram summarising the effects of fruit removal quantity and timing on inflorescence arrest, based on experiments in Figure 5.

Figure 6. Auxin export from fruit triggers inflorescence arrest

(**A**) Temporal production of flowers by the PI of male-sterile *ams* plants upon application of either 5mg/g NAA in lanolin, or a mock treatment consisting of lanolin and DMSO. Flower counts and lanolin treatment were performed every 2-3 days, starting from 6 days post anthesis (dpa) of the first flower on the primary inflorescence. *n*=7-12 biologically independent samples (mock days *n*=10, except for day 17 where *n*=9. NAA days 6,8,13,15,21 *n*=12, NAA days 10,17,19 *n*=11, NAA day 24 *n*=7), bars indicate s.e.m. Asterisks indicate significance as determined by Sidak's multiple comparisons following fitting of a mixed-effects model; * = <0.05; ** = <0.01; *** = 0.001; **** = 0.0001.

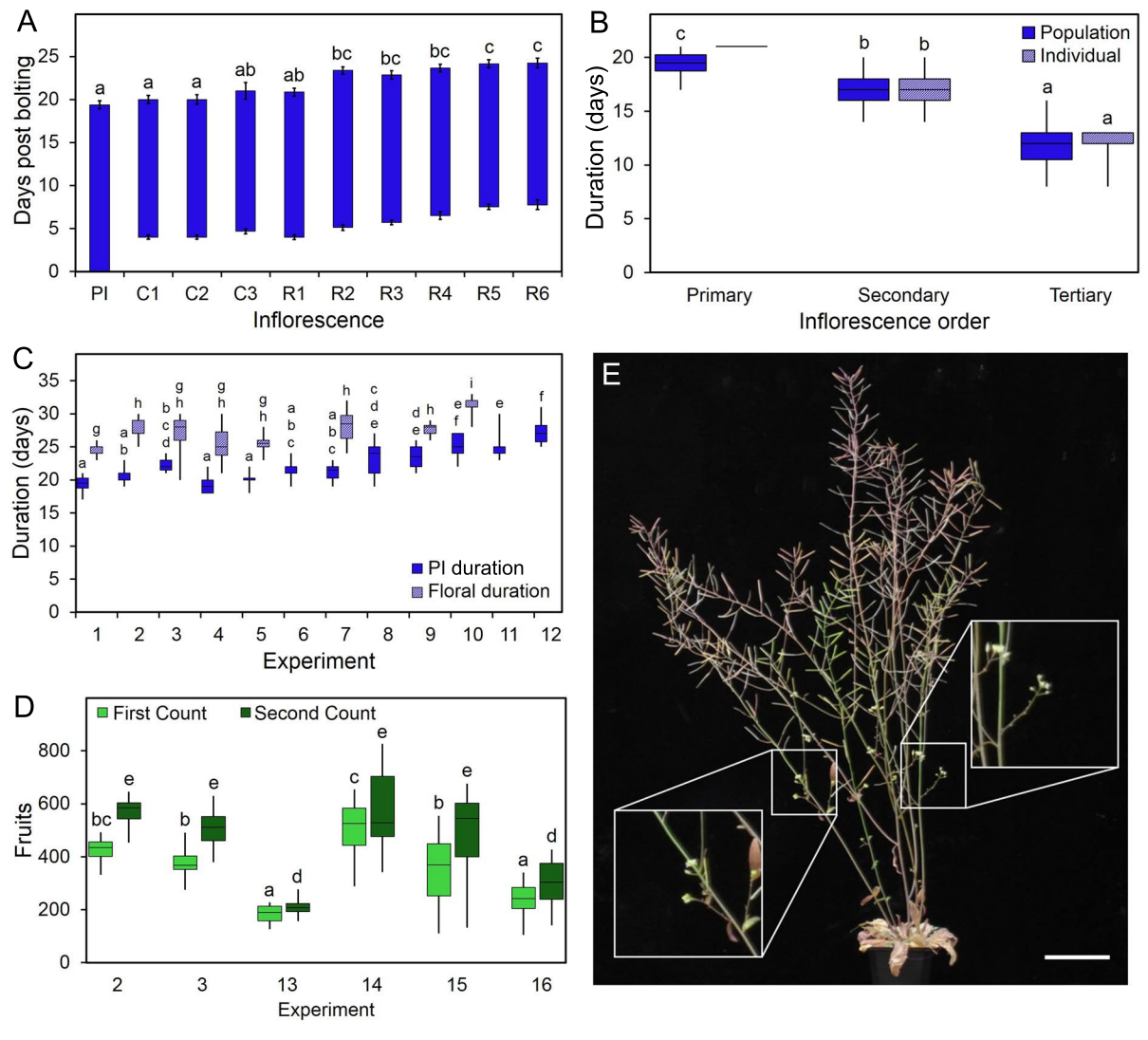
(**B**) Temporal production of flowers on the PI of male-sterile *ams* upon application of 5mg/g NAA in lanolin or mock as in (**A**). Flower counts and lanolin treatment were performed every day, starting from 20dpa. *n*=6-10 biologically independent samples (mock *n*=6, NAA *n*=10), bars indicate s.e.m.

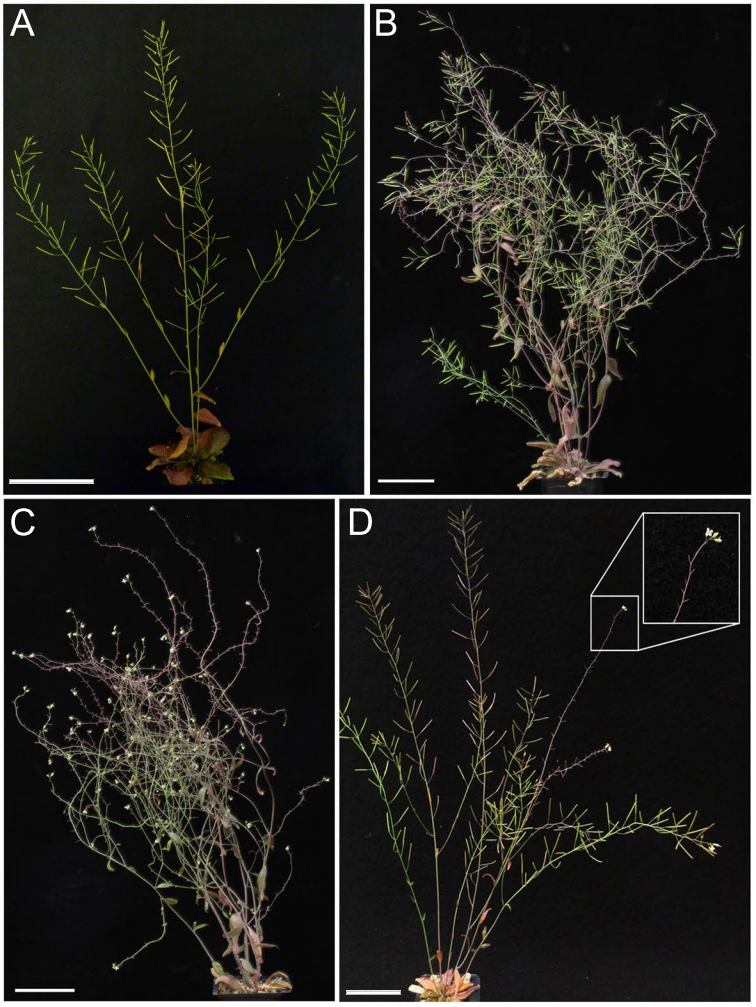
(**C**) Representative photos (3 per treatment) showing the inflorescence apices in *ams* mutants after NAA or mock treatment. NAA treated plants have arrested with a classic 'bud cluster' morphology [2], while mock-treated plants do not arrest and continue to open flowers.

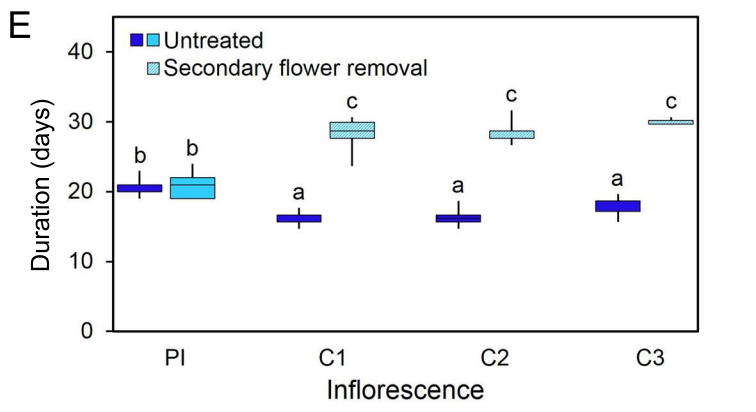
(**D**) Quantification of auxin content in 6dpa fertile (L*er*) and sterile (*ms1*) Arabidopsis fruits. *n*=5 biologically independent samples. The upper and lower confines of the box indicate the interquartile range, the central line indicates the median, and the whiskers represent the maximum and minimum values. Bars with the same letter are not statistically different from each other (two-tailed T-test, p<0.001).

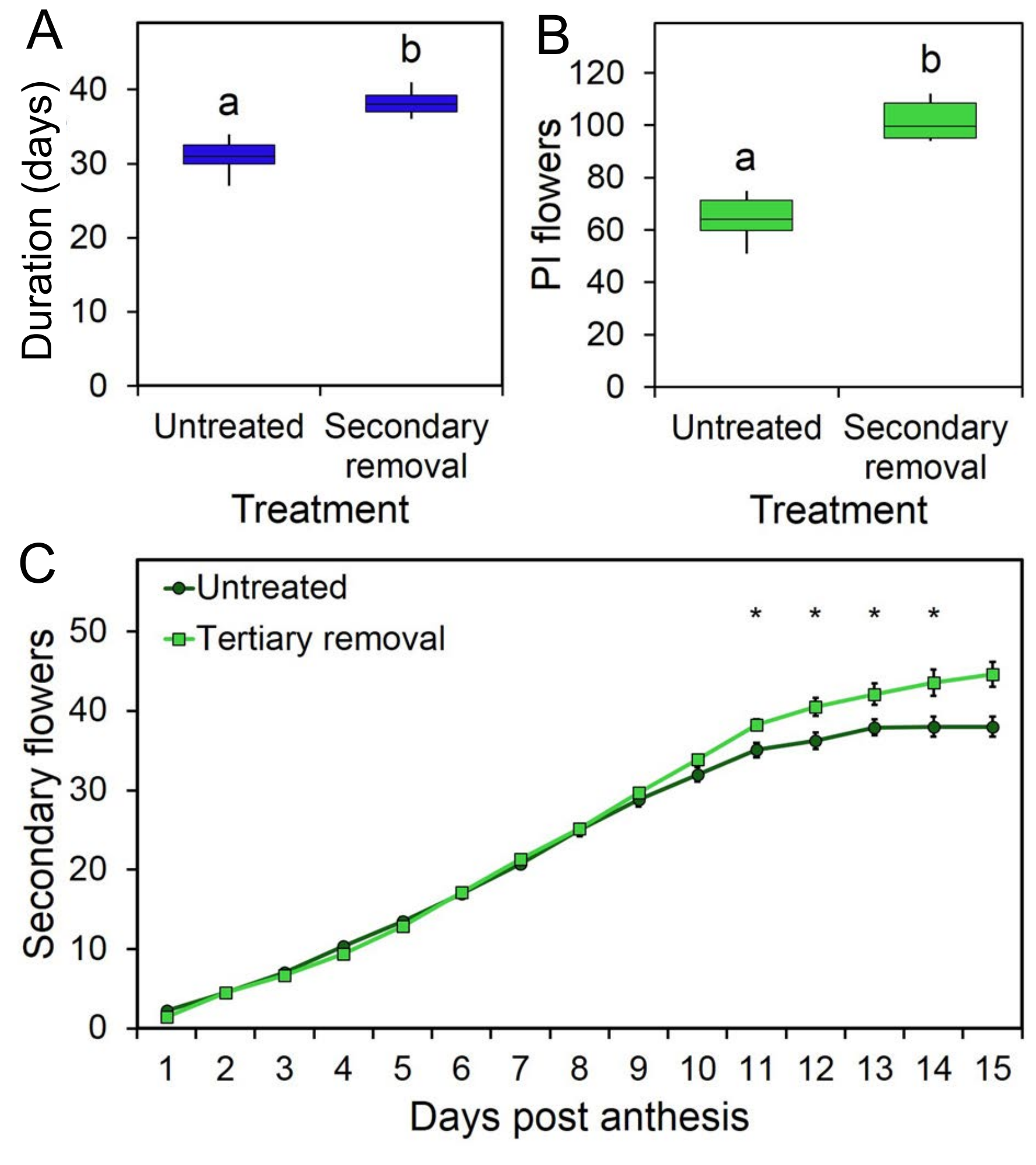
(**E**) Quantification of auxin eluted from fertile and sterile Arabidopsis fruits. *n*=5 biologically independent samples. The upper and lower confines of the box indicate the interquartile range, the central line indicates the median, and the whiskers represent the maximum and minimum values. Bars with the same letter are not statistically different from each other (two-tailed T-test, p<0.001).

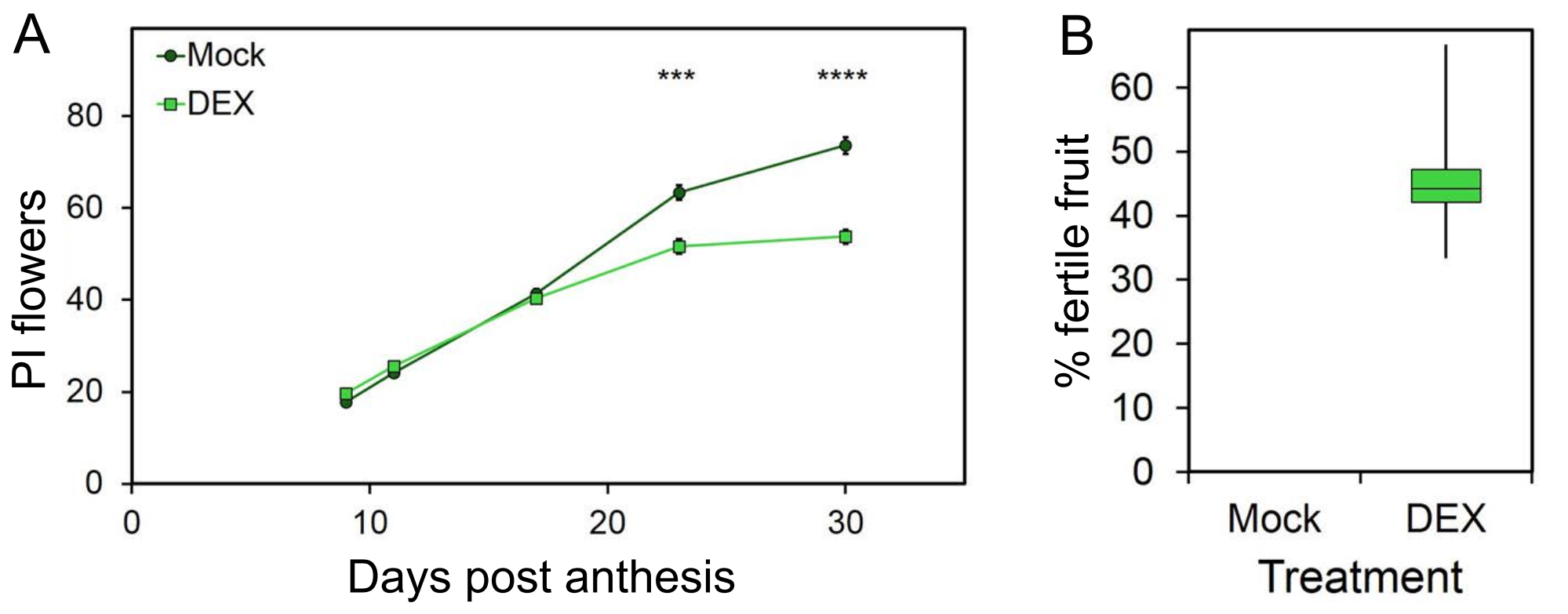
(**F**) Model for induction of inflorescence arrest. Initially, the apex can freely canalize to the polar auxin transport stream (PATS, pink). After a temporally-defined period of flowering, inflorescences reach a critical age and become capable of arrest. In the presence of ca. 6-8 fertile fruit containing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

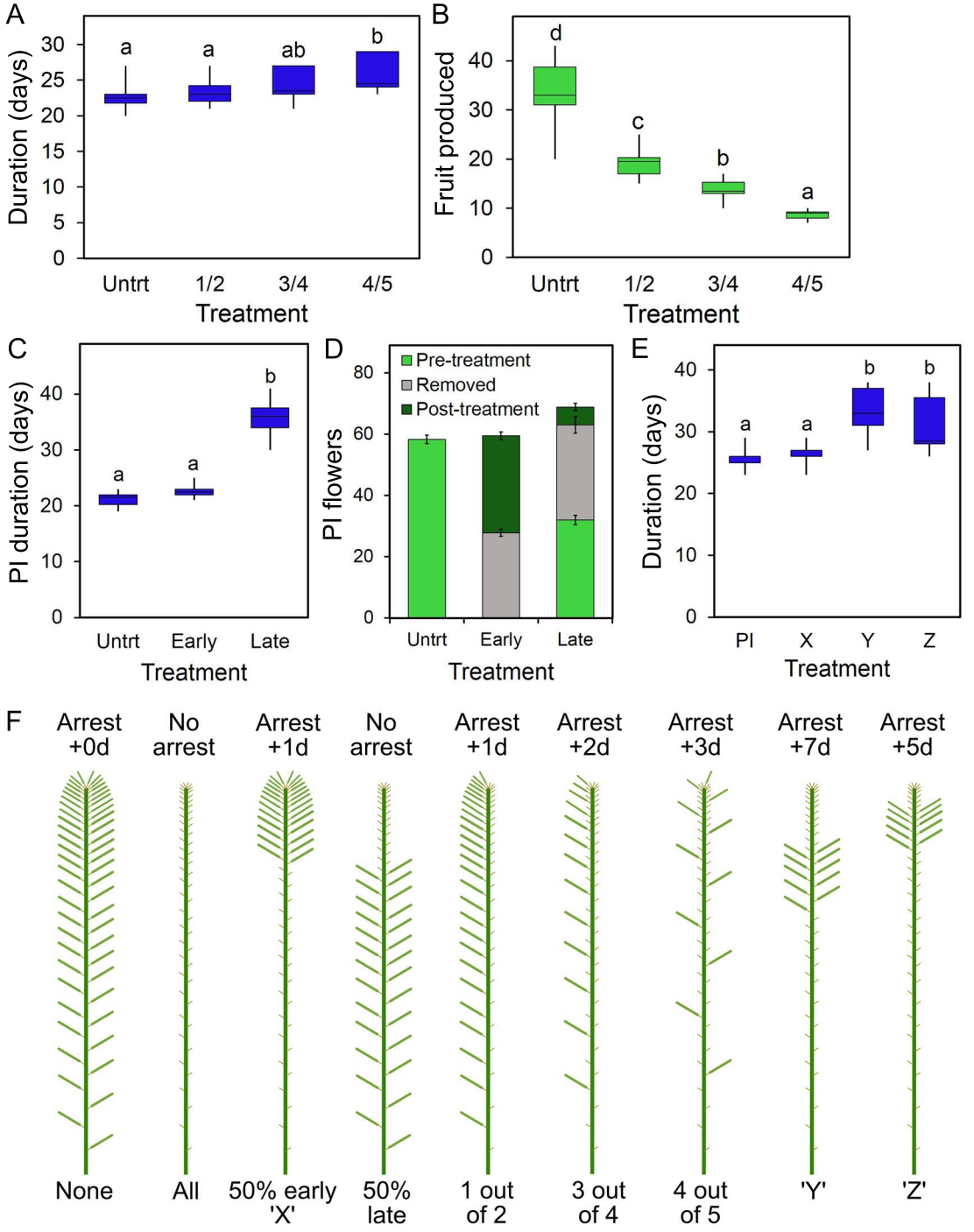

A
Days post bolting
PI
C1
C2
C3
R1
R2
R3
R4
R5
R6
Inflorescence
B
Duration (days)
Population
Individual
Primary
Secondary
Tertiary
Inflorescence order
C
Duration (days)
PI duration
Floral duration
Experiment
D
First Count
Second Count
Fruits
Experiment
E

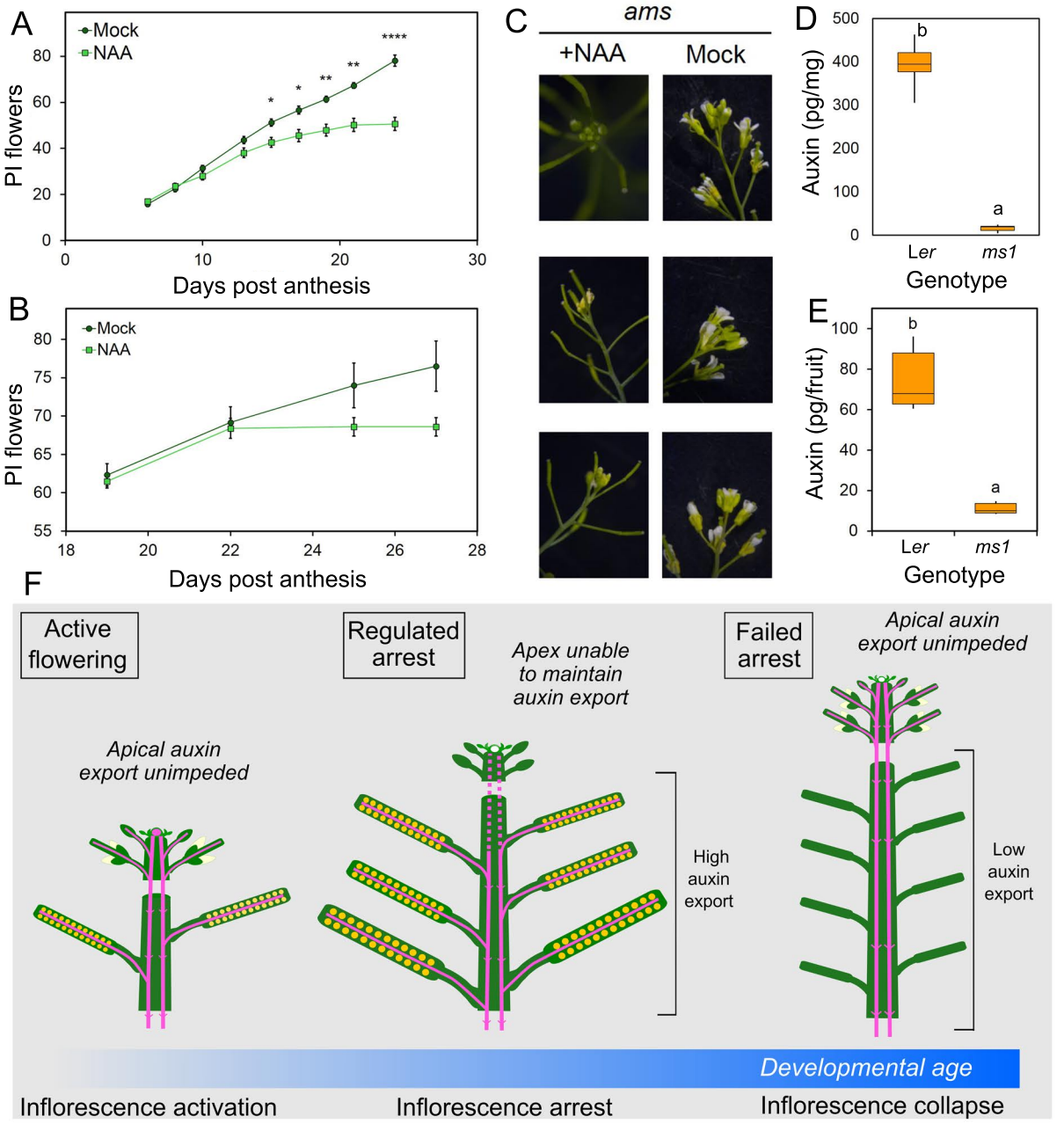

A
B
C
D

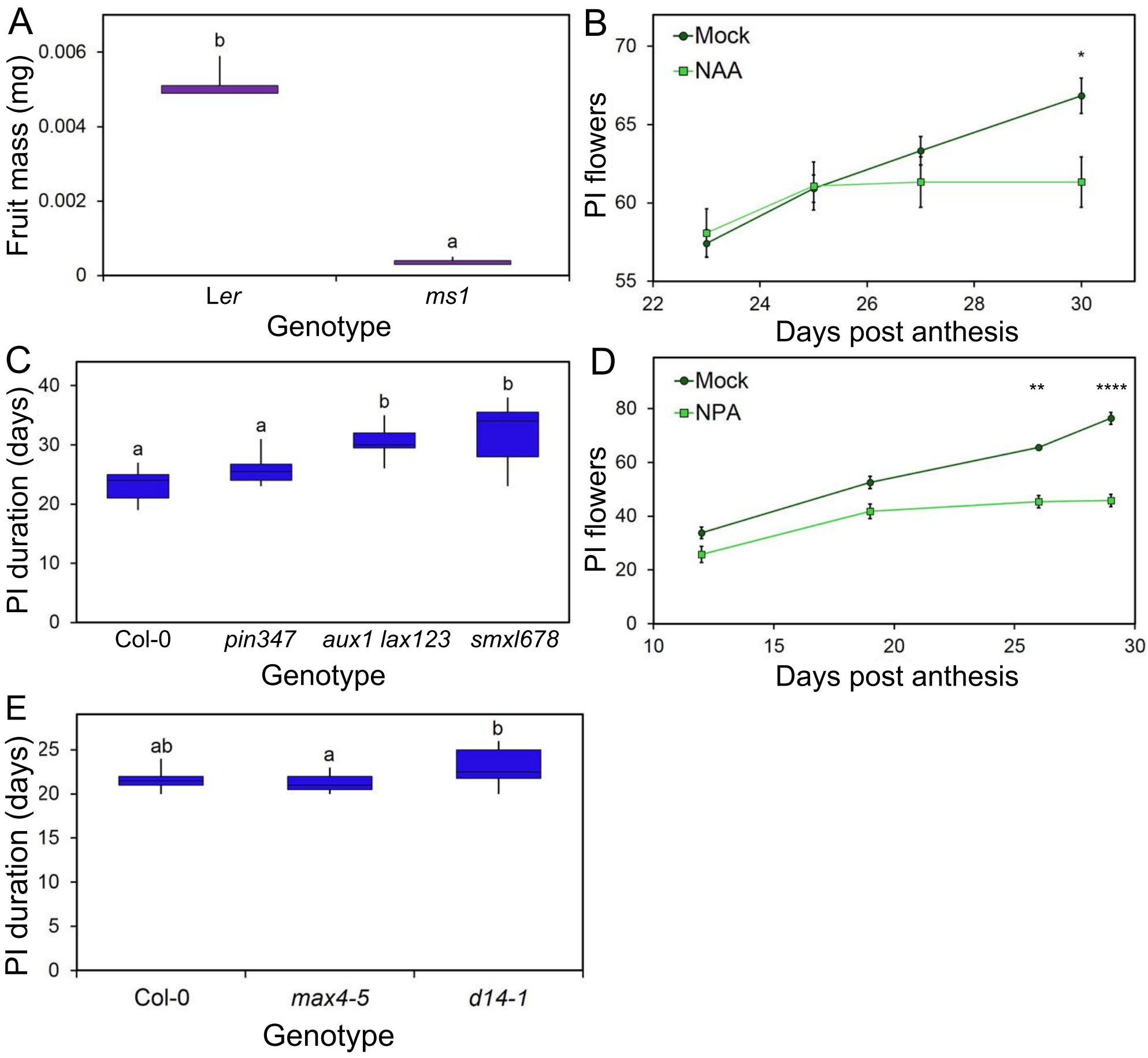

E
Untreated
Secondary flower removal
Duration (days)
0
10
20
30
40
b
b
a
c
a
c
a
c
PI
C1
C2
C3
Inflorescence


A
Duration (days)
0
10
20
30
40
a
b
Untreated
Secondary removal
Treatment
B
PI flowers
0
20
40
60
80
100
120
a
b
Untreated
Secondary removal
Treatment
C
Untreated
Tertiary removal
Secondary flowers
0
10
20
30
40
50
* * * *
1 2 3 4 5 6 7 8 9 10 11 12 13 14 15
Days post anthesis


A
Mock
DEX
***
****
PI flowers
0
20
40
60
80
0
10
20
30
Days post anthesis
B
% fertile fruit
0
10
20
30
40
50
60
Mock
DEX
Treatment


A
Duration (days)
a
a
ab
b
Untrt
1/2
3/4
4/5
Treatment
B
Fruit produced
d
c
b
a
Untrt
1/2
3/4
4/5
Treatment
C
PI duration (days)
a
a
b
Untrt
Early
Late
Treatment
D
PI flowers
Pre-treatment
Removed
Post-treatment
Untrt
Early
Late
Treatment
E
Duration (days)
a
a
b
b
PI
X
Y
Z
Treatment
F
Arrest +0d
No arrest
Arrest +1d
No arrest
Arrest +1d
Arrest +2d
Arrest +3d
Arrest +7d
Arrest +5d
None
All
50% early 'X'
50% late
1 out of 2
3 out of 4
4 out of 5
'Y'
'Z'


A
Mock
NAA
PI flowers
Days post anthesis
B
C
ams
+NAA
Mock
D
Auxin (pg/mg)
Ler
ms1
Genotype
E
Auxin (pg/fruit)
F
Active flowering
Regulated arrest
Apex unable to maintain auxin export
Failed arrest
Apical auxin export unimpeded
High auxin export
Low auxin export
Developmental age
Inflorescence activation
Inflorescence arrest
Inflorescence collapse


A
Fruit mass (mg)
0.006
0.004
0.002
0
b
a
Ler
ms1
Genotype
B
PI flowers
70
65
60
55
Mock
NAA
*
22
24
26
28
30
Days post anthesis
C
PI duration (days)
40
30
20
10
0
a
a
b
b
Col-0
pin347
aux1 lax123
smxl678
Genotype
D
PI flowers
80
60
40
20
0
Mock
NPA
**
****
10
15
20
25
30
Days post anthesis
E
PI duration (days)
25
20
15
10
5
0
ab
a
b
Col-0
max4-5
d14-1
Genotype