Understanding Crop Load and Growth Regulator Effects on Biennial Bearing in Apple Trees

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Overview

Many fruit trees including apple can show dramatic biennial (alternate-year) bearing, in which maximal flowering and fruit production alternates in a two-year, "ON-OFF" cycle (**Fig. 1**). This is a costly problem for production, due both to the need for thinning in the "ON" year and the reduction of crop in the "OFF" year. The underlying cause of this phenomenon is thought to be active suppression of floral initiation by developing fruit, possibly mediated through natural phytohormones (gibberellins, or GAs) produced in seeds. Accordingly, biennial bearing can be somewhat controlled by foliar applications of synthetic GAs in the "OFF" year. However, the biochemical pathways by which high crop load and GAs lead to suppression of flowering are completely unknown. This question is especially interesting from the point of view of fundamental science, because GAs are well known to *promote* flowering in other plant species.

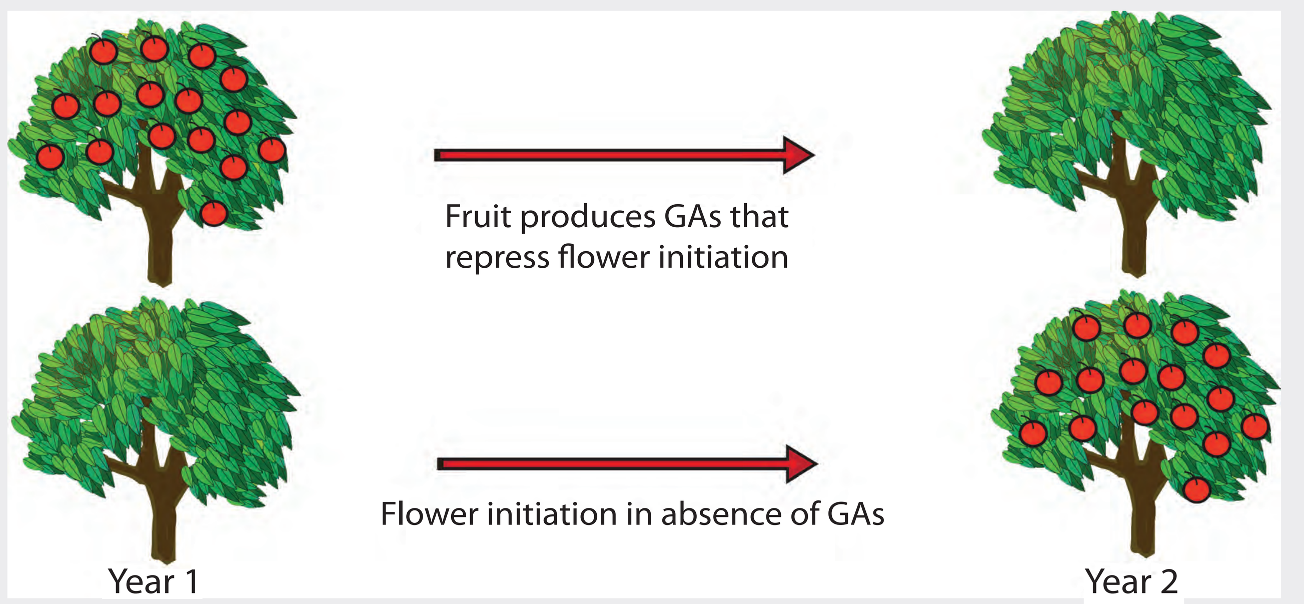

Figure 1. An illustration of the cycle of biennial bearing from one year to the next. (Upper) In trees in the "ON" year, developing fruit suppress initiation of flowers that would normally open (bloom) the following spring. (Lower) In trees in the "OFF" year, lack of fruit results in excessive floral initiation the following year.

Research Goal

The goal of this study is to identify the genetic mechanism driving biennial bearing in apple, and eventually minimalize its effects through novel production approaches, breeding strategies, and biotechnology. We hypothesize that GAs suppress flowering in apple by adaptations of genetic mechanisms that promote flowering in other plants. Consequently, we are examining the apple counterparts of genes and proteins involved in GA-mediated promotion of flowering in the research reference plant Arabidopsis (**Fig. 2**).

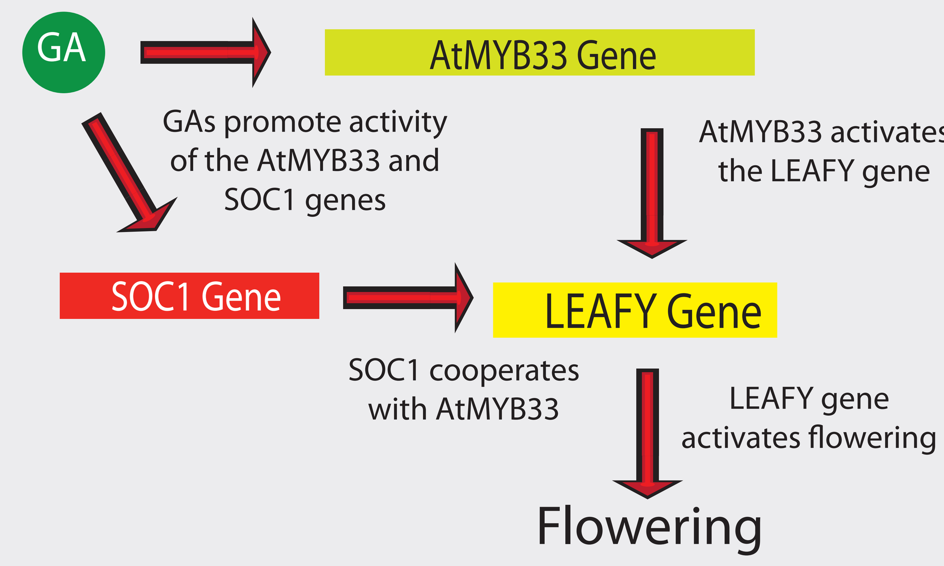

Figure 2. Illustration of the genetic pathway leading from Gibberellin (GA) to flower initiation in Arabidopsis. GAs act as a phytohormone to activate a gene called ATMYB33 and a gene called SOC1. Both of these genes cooperate to activate a third gene, called LEAFY, which is a master regulator of flowering.

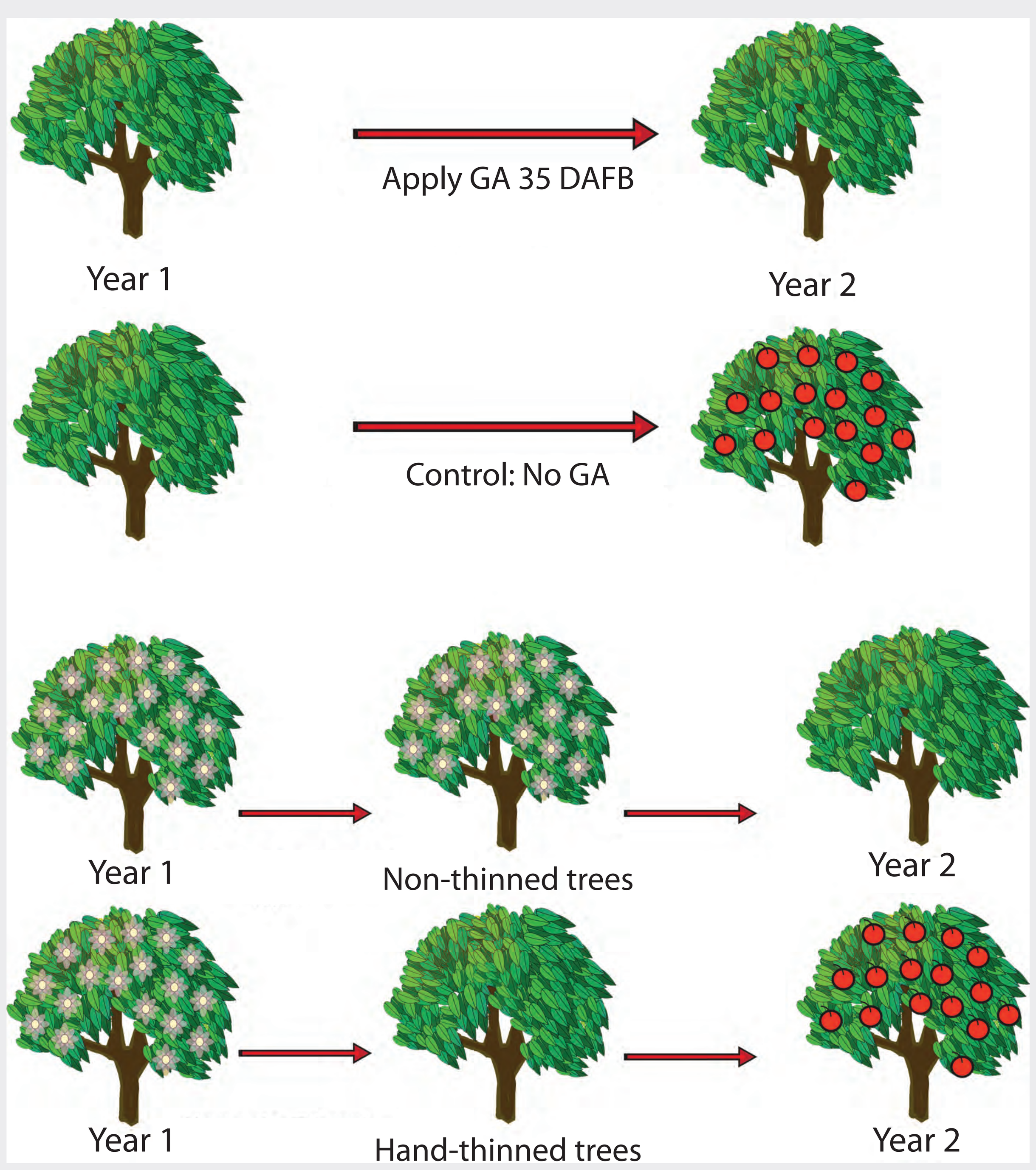

Figure 3. Experimental design for characterization of the genetic response to GA and thinning. (Top panel) Gala trees were thinned by hand at full bloom. One set of trees was subjected to foliar application of GA 35 days after full bloom (DAFB). A control set of trees was left non-treated as a control. (Lower panel) Honeycrisp trees with high flower density were selected, and one set was left non-thinned, while all all flowers were removed from the other set.

Approach

We used two apple model systems for this work (**Fig. 3**). One is cv. Honeycrisp, which exhibits a qualitative biennial bearing response. The other is cv. Gala, in which flowering can be completely suppressed by a single application of synthetic GA 35 days after flowering. We used advanced genomic techniques to characterize gene activity in the shoot apices of these trees in response to qualitative thinning or to GA application (**Fig. 4**), and identified those genes that are activated or repressed under these conditions.

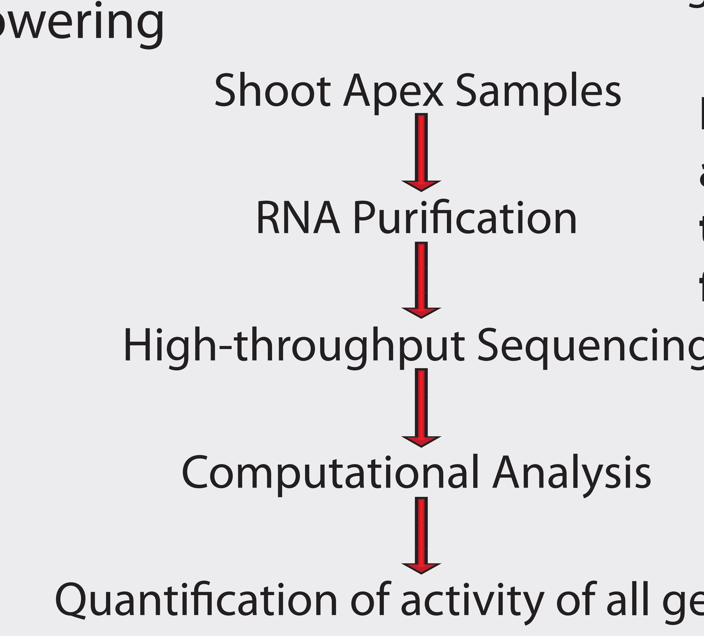

Figure 4. Flow chart of the approach to determine the gene activity from the field experiment.

Results

For Honeycrisp, hand-thinning was effective in repressing flowering the subsequent year, resulting in complete abscence of flowers (**Fig. 5**). For Gala, a single application of GA at 35 DAFB had only slight repressive activity, but was clearly distinct from the control, non-treated sets. We confirmed that the treatments of GA application and thinning did not indirectly affect flowering via alteration of the shoot architecture, through recording new shoot length and node number (**Fig 6**). We used high-throughput sequencing and computational methods to identify ~14,000 genes that were active in the shoot apices, and sequence comparisons to identify the apple LEAFY gene and three genes similar to SOC1. Analysis of proportional representation of sequences derived from these genes at various time points during the season showed that all four genes were eventually activated in shoot apices of non-thinned trees (**Fig. 7**).

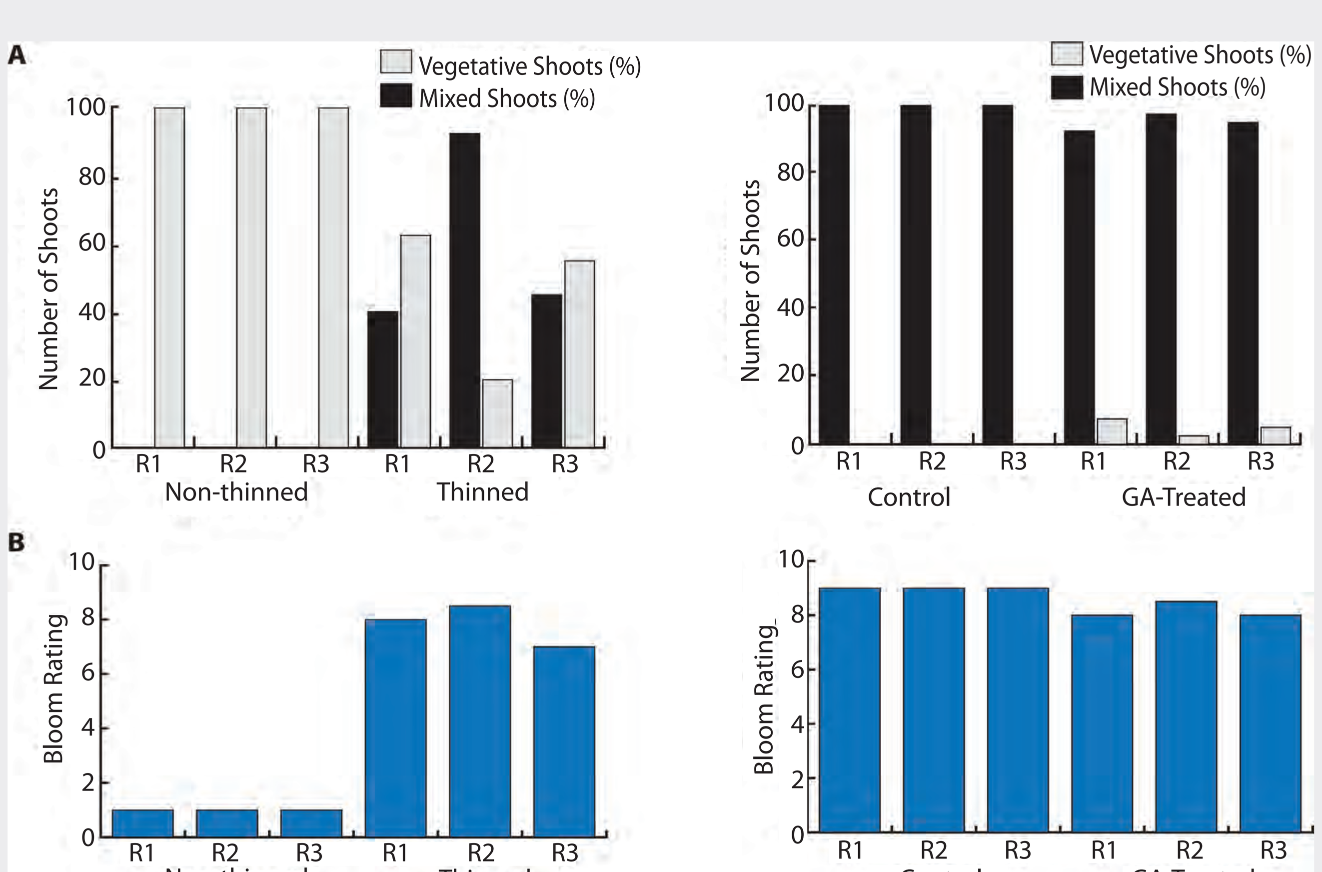

Figure 5. Assessment of return bloom in field experiements. (A) Percentage of nodes showing only vegetative shoots, or mixed vegetative/flowering shoots. (B) Visual assessment of bloom density. Rating is scaled from 0 (no flowers) to 10 (100 % bloom density). Values for each replicate tree reflect the average assessment by two independent evaluators, with a correlation coefficient R= 0.962.

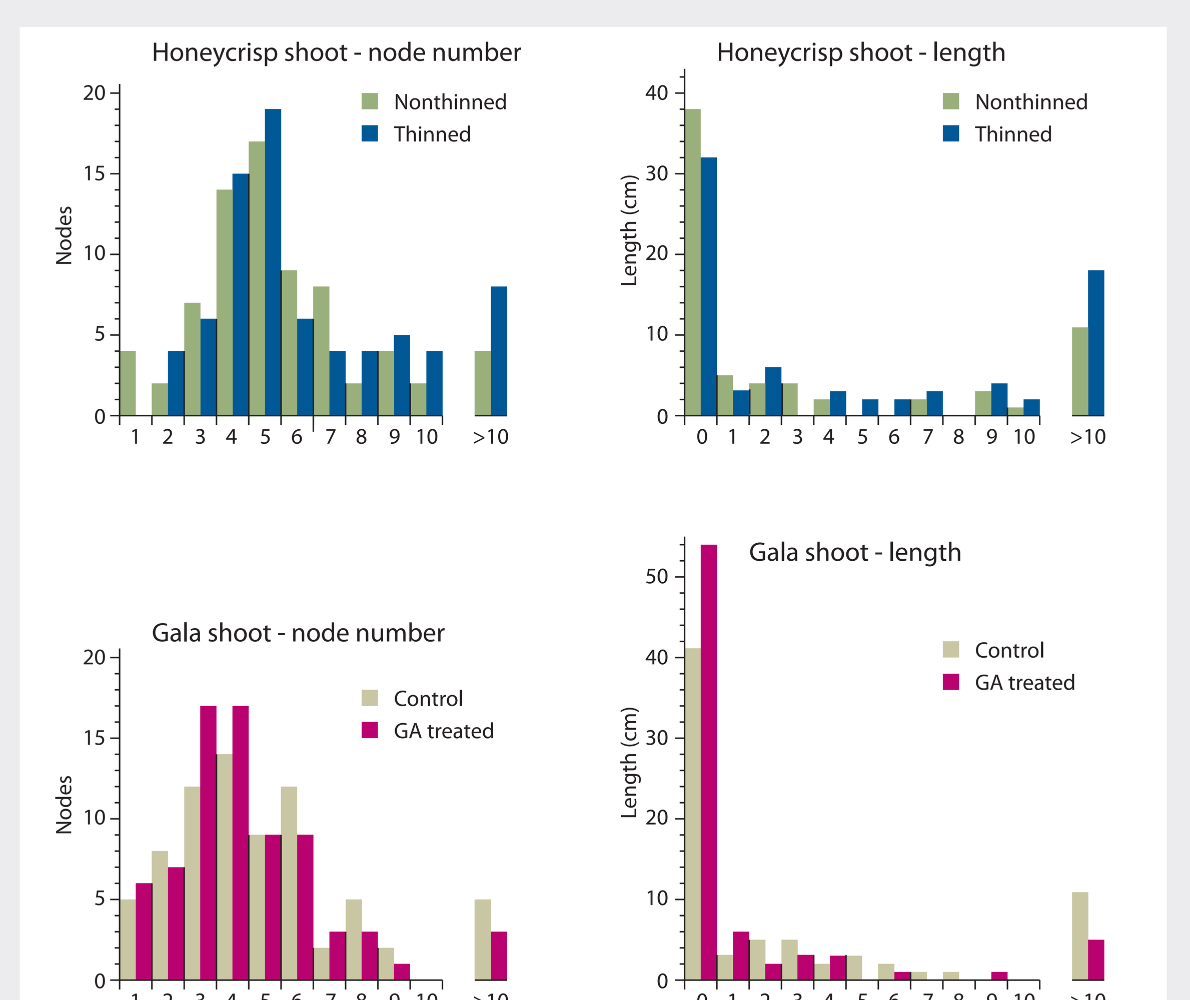

Figure 6. GA treatment or thinning did not indirectly affect flowering via alteration of shoot architecture. Shoots on treated or control trees were examined at the end of the season after treatment, and total nodes formed and length of shoot was recorded.

Conclusion

We developed two models for understanding the genetic effects of thinning or GAs on biennial bearing. For the thinned v. non-thinned Honeycrisp experiment, our data show that thinning ultimately resulted in activation of the apple counterparts of the SOC1 and LEAFY genes. These results support an intuitive crucial role for these genes in flowering in apple, and show that genetic control of flowering in repsonse to thinning is 'upstream' of both SOC1 and LEAFY. This study provides a first step to understanding how biennial bearing is endogenously controlled, which will lead to advanced in production methods, new cultivars, and biotechnological controls.

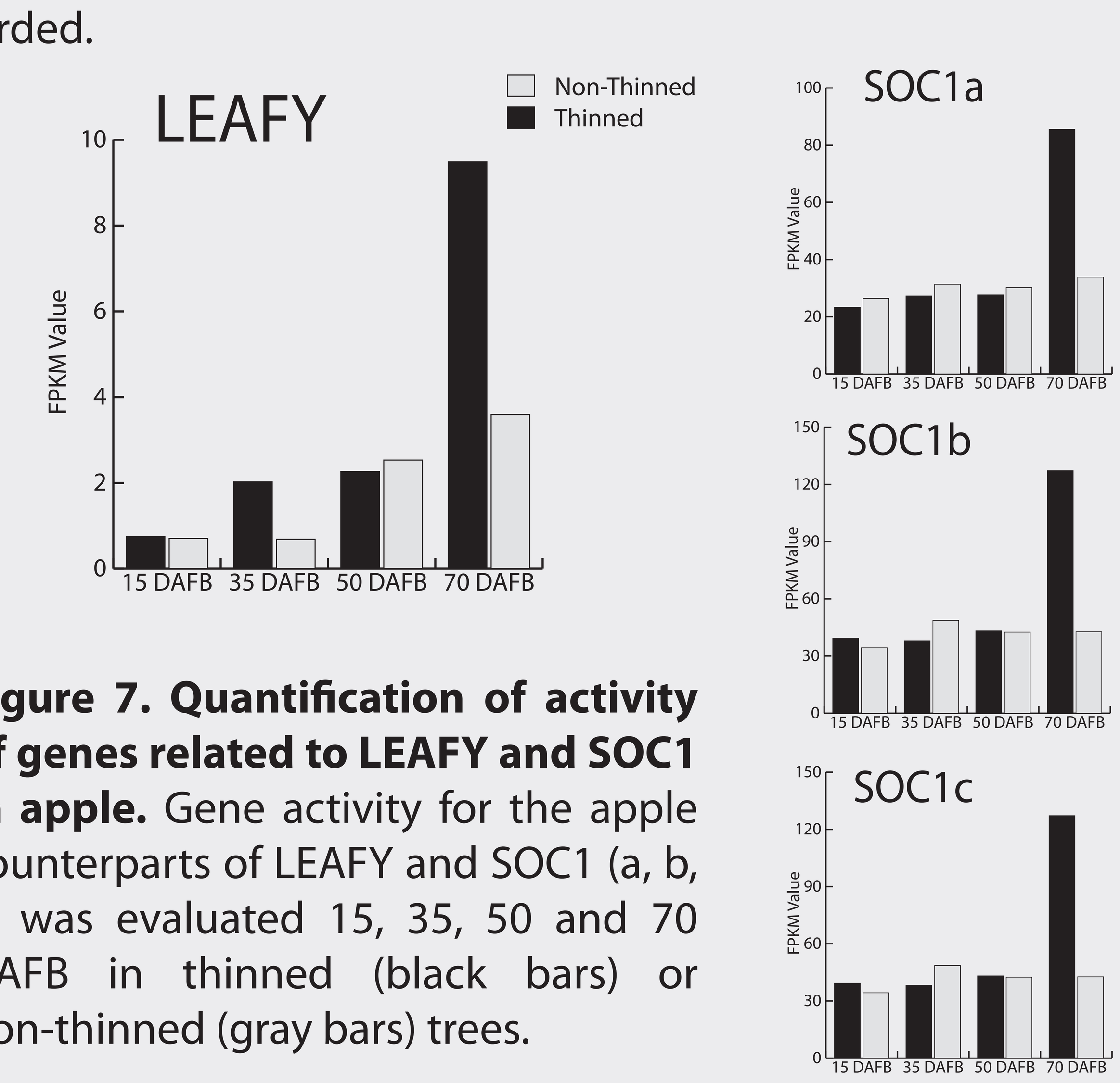

Figure 7. Quantification of activity of genes related to LEAFY and SOC1 in apple. Gene activity for the apple counterparts of LEAFY and SOC1 (a, b, c) was evaluated 15, 35, 50 and 70 DAFB in thinned (black bars) or non-thinned (gray bars) trees.

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