



# Optimization of gibberellin- and cytokinin-based programs for control of flowering and crop load in 'Honeycrisp'

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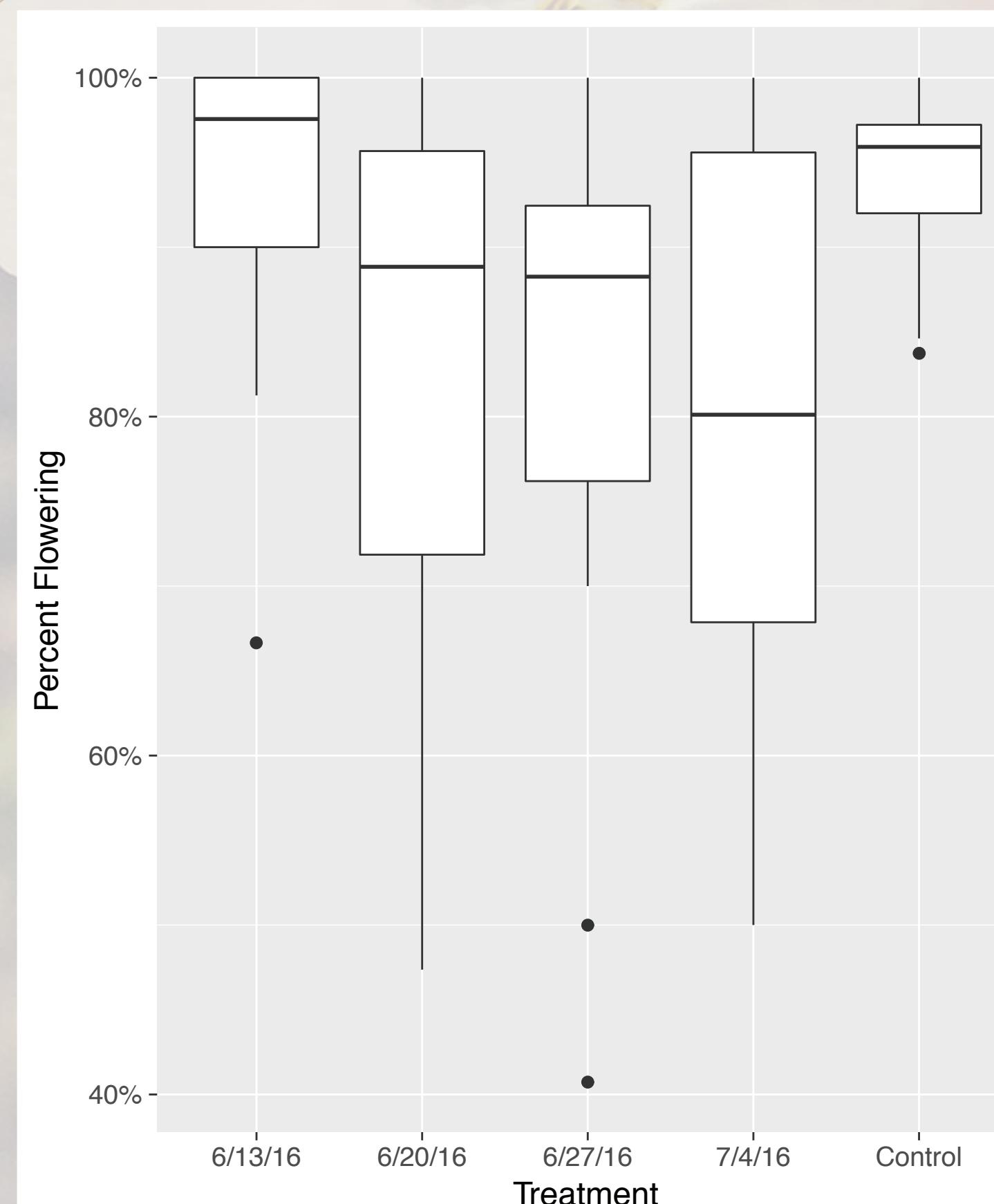
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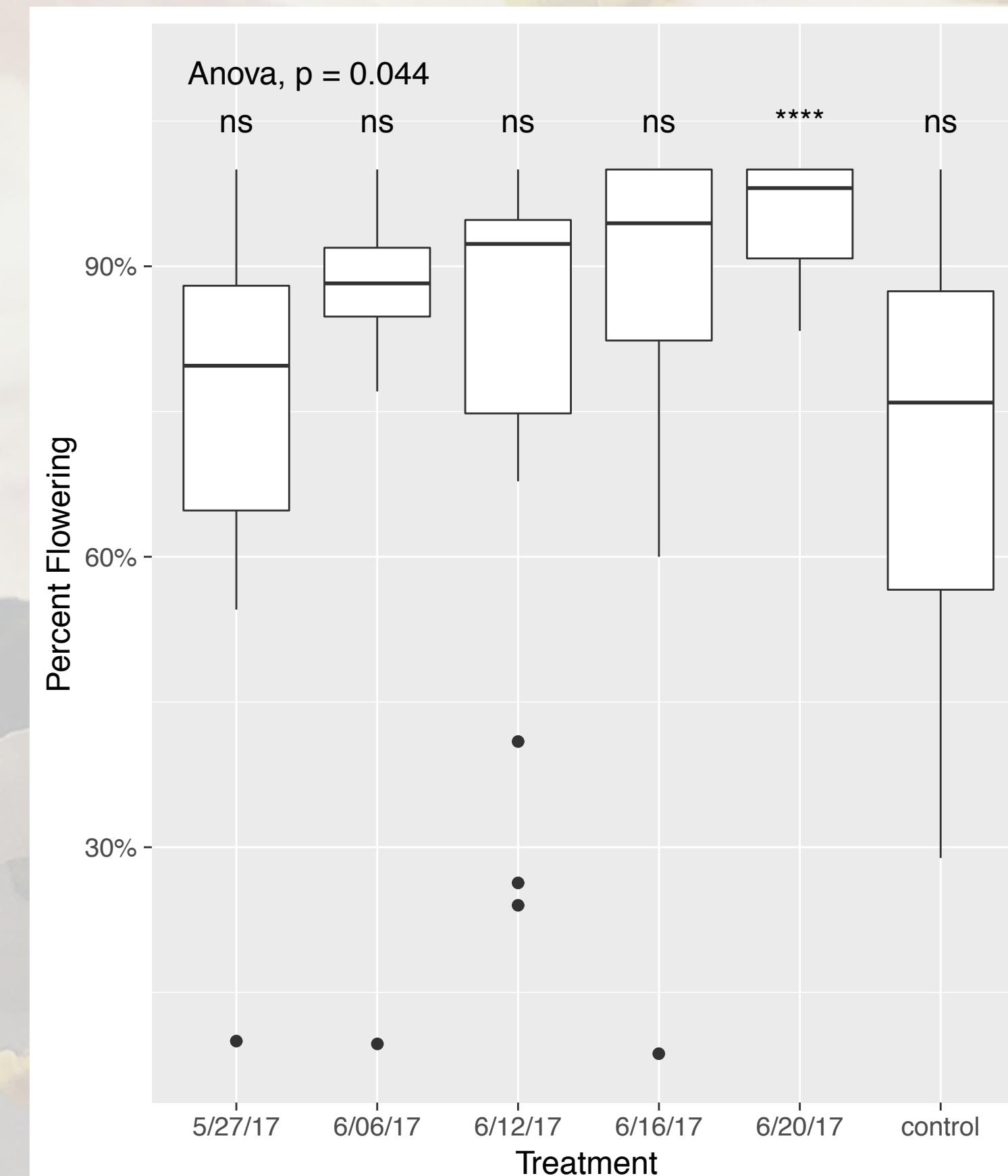


## Abstract

The plant hormones Gibberellin (GA) and Cytokinin (CK) are integral hormones that influence the flowering process in many plants and have been explored for use as plant growth regulators (PGRs) in apple production. GA has been shown to predominantly act as a repressor of flowering and promoter of vegetative growth when exogenously applied. In a similar response, CK has been shown to primarily promote lateral shoot growth through increase cell growth and differentiation, but its role in flowering is less understood. Based on their biological roles, we designed a diverse experimental spray program to assess the efficacy of both GA and CK in regulating floral initiation and floral development, respectively. Consistent with many previous studies in other cultivars, we observed repressive effects on flowering following application of GA<sub>3</sub> (ProGibb) in Honeycrisp. We documented an increasingly repressive response when applied between 36 and 57 days after full bloom (DAFB). However, the repression was not statistically significant. This repressive response was observed following the median date of floral initiation in Honeycrisp and suggests that the GA could be used to modulate late-season initiating apices. During the 2018 season, we began trialing the use of CK and an inhibitor of CK (Lovastatin) for potential influence in altering floral bud development and bloom times. The goal is to either accelerate development or slow development during the season, post-floral initiation, to potential change the date of bud break the following spring. Positive results from this work could be used to developed a method to delay bloom for the following season, which would be a useful tool to decrease the likelihood of the suffering floral injury from a spring frost.



**Figure 2.** Comparison in return flowering percentages during the 2017 season in Honeycrisp following application of GA<sub>3</sub> on selected dates during 2016 season. All trees were thinned of blossoms during the 2016 season. No significant difference in return flowering percentages was observed for any treatment when compared to the control trees.



**Figure 3.** Comparison in return flowering percentages during the 2018 season in Honeycrisp following application of GA<sub>3</sub> on selected dates during 2017 season. All trees were thinned of blossoms during the 2017 season. A significant difference was observed for that application date where the treated. Treated trees exhibited increased flowering when compared to the control trees. \*\*\*\* P<=0.0001

Product	Provide	ProGibb	Maxcel	Lovastatin
Hormone	GA <sub>4+7</sub>	GA <sub>3</sub>	Cytokinin	Cytokinin inhibitor
2016 Trial Rates (ppm)		200		
2017 Trial Rates (ppm)		200		
2018 Trial Rates (ppm)	100, 200, 500	200, 400	75, 120	12, 120

**Table 1.** PGR products used, hormone type, and treatment rates that were applied for each year of the study.

## Gibberellic Acid Trial Results

In 2016, GA<sub>3</sub> (ProGibb) was applied at a concentration of 200 ppm at four treatment dates to Honeycrisp trees. Additional work from that year documented that floral initiation occurred at a median date of ~33 DAFB in Honeycrisp. Our treatment dates began at 36 DAFB and ran through 57 DAFB, which overlapped with the latter half of the period of initiation. The application of GA<sub>3</sub> resulted in a repressive effect on the return bloom in the following year, indicating a repression of floral initiation (Fig. 1.) However, this repressive effect did not meet a statistically significant threshold. A replicated trial using GA<sub>3</sub> onto the same trees used in 2016 was conducted in 2017, but this experiment failed to replicate the results (Fig. 2). This failure to replicate a repressive effect could be attributed to increases in photosynthate accumulation during 2016 season when the trees lacked any cropload, resulting in heavy floral initiation in 2017 regardless of GA<sub>3</sub> application.

## Methods

The 2016 GA<sub>3</sub> trial was conducted at AgBioResearch's Clarksville Research Center (CRC) using trees that were over five years of age and were exhibiting full bearing habits. The trees were grafted on 'Bud9' rootstocks and grown in a trellis supported vertical-axis system. A total of 50 replicate trees were thinned of fruit once fruit set was established. Groups of three replicate trees were then assigned to either be treated with GA<sub>3</sub> at a select timepoint (see Fig. 1) or assigned as untreated controls (applied with surfactant only). During the 2018 season, we shifted to a new orchard block at the CRC that used third leaf Honeycrisp on 'Bud9' grown in a slender spindle system. These trees represent a more uniform planting and were planted in a blocked design alternating with 'Gala' every two rows, which allowed for integration of numerous trials in one orchard. All trees were thinned of blossoms or fruit within two weeks of full bloom. During this past season, we replicated the 2016 trial using GA<sub>3</sub> (400 ppm) but doubled the rate and performed an additional trial using GA<sub>4+7</sub> (500 ppm - Provide). Both trials were performed using separate complete block designs, consisting of four or five replicate trees per block with three blocks per treatment replicate. In addition, we performed select concentration applications of GA<sub>3</sub> and GA<sub>4+7</sub> to five replicate trees in the same blocked design at 42 DAFB (see Table 1). In 2018, we also began experimenting with CK and Lovastatin. We used the same blocked design as the GA trials but each CK-related chemical was applied at two different concentrations (see Table 1). Control trees were thinned of flowers and fruit and went untreated by any chemical. Applications of all PGRs occurred at two-week intervals starting two weeks after full bloom and continuing until twelve weeks after full bloom, except where noted previously. Applications were performed using a commercially available backpack spray with either one L applied per tree or until runoff was observed.

Road		Section 2		Section 3	
1	2 Weeks	2 Weeks	2 Weeks	2 Weeks	2 Weeks
2	2 Weeks	2 Weeks	2 Weeks	2 Weeks	2 Weeks
3	2 Weeks	2 Weeks	2 Weeks	2 Weeks	2 Weeks
4	2 Weeks	2 Weeks	2 Weeks	2 Weeks	2 Weeks
5	4 Weeks	4 Weeks	4 Weeks	4 Weeks	4 Weeks
6	4 Weeks	4 Weeks	4 Weeks	4 Weeks	4 Weeks
7	4 Weeks	4 Weeks	4 Weeks	4 Weeks	4 Weeks
8	4 Weeks	4 Weeks	4 Weeks	4 Weeks	4 Weeks
9	6 Weeks	6 Weeks	6 Weeks	6 Weeks	6 Weeks
10	6 Weeks	6 Weeks	6 Weeks	6 Weeks	6 Weeks
11	6 Weeks	6 Weeks	6 Weeks	6 Weeks	6 Weeks
12	6 Weeks	6 Weeks	6 Weeks	6 Weeks	6 Weeks
13	8 Weeks	8 Weeks	8 Weeks	8 Weeks	8 Weeks
14	8 Weeks	8 Weeks	8 Weeks	8 Weeks	8 Weeks
15	8 Weeks	8 Weeks	8 Weeks	8 Weeks	8 Weeks
16	8 Weeks	8 Weeks	8 Weeks	8 Weeks	8 Weeks
17	10 Weeks	10 Weeks	10 Weeks	10 Weeks	10 Weeks
18	10 Weeks	10 Weeks	10 Weeks	10 Weeks	10 Weeks
19	10 Weeks	10 Weeks	10 Weeks	10 Weeks	10 Weeks
20	10 Weeks	10 Weeks	10 Weeks	10 Weeks	10 Weeks
21	12 Weeks	12 Weeks	12 Weeks	12 Weeks	12 Weeks
22	12 Weeks	12 Weeks	12 Weeks	12 Weeks	12 Weeks
23	12 Weeks	12 Weeks	12 Weeks	12 Weeks	12 Weeks
24	12 Weeks	12 Weeks	12 Weeks	12 Weeks	12 Weeks
25	2 Weeks	12 Weeks	2 Weeks	2 Weeks	2 Weeks
26	2 Weeks	12 Weeks	2 Weeks	2 Weeks	2 Weeks
27	2 Weeks	12 Weeks	2 Weeks	2 Weeks	2 Weeks
28	2 Weeks	12 Weeks	2 Weeks	2 Weeks	2 Weeks
Road		Section 2		Section 3	
1	4 Weeks	2 Weeks	2 Weeks	2 Weeks	2 Weeks
2	4 Weeks	2 Weeks	2 Weeks	2 Weeks	2 Weeks
3	4 Weeks	2 Weeks	2 Weeks	2 Weeks	2 Weeks
4	4 Weeks	dead	2 Weeks	2 Weeks	2 Weeks
5	6 Weeks	6 Weeks	6 Weeks	6 Weeks	6 Weeks
6	6 Weeks	4 Weeks	4 Weeks	4 Weeks	4 Weeks
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9	8 Weeks	4 Weeks	4 Weeks	4 Weeks	4 Weeks
10	8 Weeks	6 Weeks	6 Weeks	6 Weeks	6 Weeks
11	8 Weeks	6 Weeks	6 Weeks	6 Weeks	6 Weeks
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26	2 Weeks	12 Weeks	2 Weeks	2 Weeks	2 Weeks
27	2 Weeks	12 Weeks	2 Weeks	2 Weeks	2 Weeks
28	2 Weeks	12 Weeks	2 Weeks	2 Weeks	2 Weeks

**Table 2.** Field design for the 2018 ongoing CK-related trials. Rates, application times (weeks after full bloom), and chemical (trade name) are displayed for each of the three replicates for each trial.

## Ongoing Cytokinin-Related Research

To evaluate if CK (Maxcel) or a CK inhibitor (Lovastatin) have an effect on altering bloom times, we conducted a large-scale field trial (see Fig. 2 for field design). We followed the same design and treatment dates at the ProGibb 400 ppm and Provide 500 ppm trials discussed in the following section about "ongoing GA trials". The only change to the experimental field design was to use four thinned trees were used instead of five trees per treatment group. Each PGR here was tested at two concentrations, for CK it was 75 and 120 ppm while Lovastatin was applied at 20 and 120 ppm. Lovastatin is a pharmaceutical typically used to inhibit cholesterol in humans and is unregistered for use in tree fruit (except when applied by a licensed researcher for experimental purposes).

## Ongoing Gibberellic Acid Research

During the 2018 growing season, we continued trialing GA<sub>3</sub> for its efficacy as a floral repressor. Due to the inconsistent results observed in the replication during the 2017 season, we have shifted into a new orchard block to eliminate potential carryover effects from the previous years' experiments. We are now conducting a more thorough trial where three replicate groups of five floral thinned trees are receiving GA<sub>3</sub> treatments over an extended treatment schedule (see Table 2). We have increased the treatments from four time points to seven, which now will encompass the entire floral initiation period, and doubled the treatment concentration from 200 ppm to 400 ppm. Treatment dates began at two weeks after full bloom (WAFB) and continued at two-week intervals until twelve WAFB. In addition, we carried out an application of GA<sub>3</sub> at a concentration of 200 ppm at six WAFB to groups of four thinned trees in a triple replicate design, to see if we can replicate the same repression observed in 2016 at that time point. We are also trialing GA<sub>4+7</sub> in a similar fashion as the GA<sub>3</sub> experiments. Instead of 400 ppm, we will be using 500 ppm for GA<sub>4+7</sub> in the same triplicate block design across the same seven time points. We also carried out 6 WAFB application of Provide at 100 and 200 ppm in the same design as the GA<sub>3</sub> 200 ppm experiment.