Exploring Kiwiberry Dormancy and Novel Frost Protection Methods

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Introduction

No plant can grow in every climate. Plants have adopted to their native conditions through evolution, and will not grow if the climate is significantly different than the one they have adapted to. If the desired growing location for a plant has a significantly different climate than the target plant's native climate, either the environment around the plant must be modified (such as by using a greenhouse), or the plant itself must be manipulated (such as by using dormancy-breaking sprays to change the plant's natural dormancy cycle) (Grubinger 2015; McPherson et al. 2001). Horticultural traits to consider when matching plants to climate conditions include winter hardiness, preferred amount of rainfall, preferred soil type, preferred amount of light, susceptibility to heat stress, chill requirement, etc Parkes, Darbyshire, and White (2020).

Many woody perennials originating in temperate climates require a certain period of cold temperatures to successfully flower and fruit the following season (Lang 1987). This period is called dormancy, the temporary cessation of visible growth, and is generally triggered by short daylength and/or cold nighttime temperatures (Fadón et al. 2020; Liu and Sherif 2019). Characterizing the dormancy cycle of fruit crops is important because it helps determine the range of the crop, especially the southernmost boundary in the northern hemisphere.

Dormancy can be split into two phases: endodormancy, or true dormancy, and ecodormancy. Endodormancy is mediated by endogenous plant-hormones. The plant will not grow even if environmental conditions favor growth, until a certain amount of chill, known as the chill requirement, has been experienced by the plant. The chill requirement is defined as the lowest number of units of cold temperature, usually between 32 and 45 degrees Fahrenheit, needed for a plant to end endodormancy and grow again when exposed to favorable conditions (Winkler et al. 2013). The type of unit used depends on the chill model, and could be chill hours, chill units, or chill portions (Luedeling 2012). After the chilling requirement has been fulfilled, the plant shifts from endodormancy to ecodormancy, where growth is prevented only by unfavorable environmental conditions (Lang 1987; Parkes, Darbyshire, and White 2020).

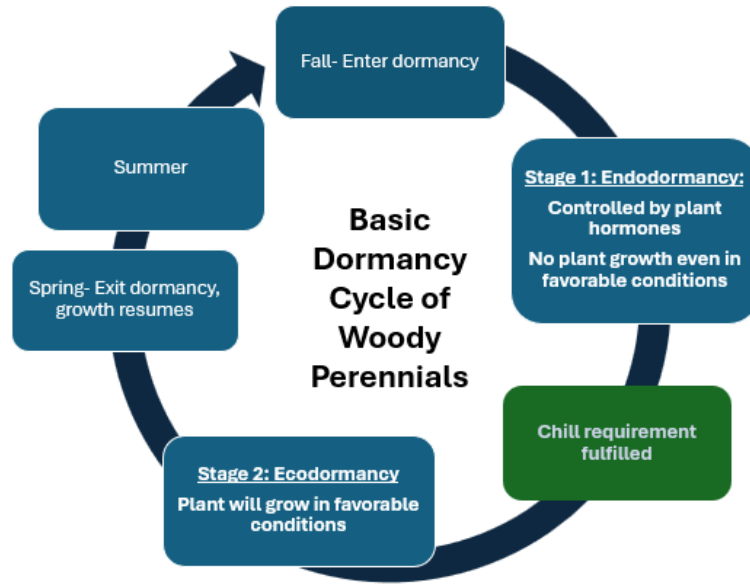Once temperatures increase in the spring, growth resumes (Fadón et al. 2020; Liu and Sherif 2019).

Figure 1. Basic Dormancy Cycle of Woody Perennials

While the exact mechanisms by which plants enter and leave endodormancy are unknown, it is understood that plant hormones, temperature, and internal carbohydrate balance may play roles (Liu and Sherif 2019; Wigge 2013; Tixier et al. 2019).

The current method to estimate endodormancy fulfillment is using chill models (Luedeling 2012). Chill models estimate the amount of chill (chill accumulation) a plant experiences by keeping track of hourly temperatures. The first chill model, known as the Chill Hours model, counts all hours with an average temperature under 45 degrees Fahrenheit as a "chill hour" (Weinberger 1950) . This model was imperfect at predicting endodormancy release, prompting the creation of the Utah model in 1974, in which any hour at 43 degrees Fahrenheit contributes one chill unit to chill accumulation, an hour below 43 degrees Fahrenheit contributes less than 1 chill-unit, and an hour above 43 degrees Fahrenheit subtracts chill units (Richardson, Seeley, and Walker 1974). However, this model failed to account for the observation that cold temperatures followed by warm temperatures appear to contribute less to chill accumulation (Fishman, Erez, and Couvillon 1987). In other words, the Utah model assumed that any hour during which the average temperature fell within the appropriate range contributes equally to chill accumulation, when in fact field experiments suggested that the adjacent hours' temperatures affect the ability of the hour preceding and/or following to contribute to chill accumulation. Improvement on the Utah model was attempted with the Dynamic model, which tries to mimic

a hypothesized physiological process of chill accumulation through a two-step procedure (Fishman, Erez, and Couvillon 1987; Egea, Egea, and Ruiz 2020; Erez and Fishman 1998). The Dynamic model assumes that dormancy completion depends on the accumulation of a dormancy breaking factor (DBF) that builds in response to certain temperature conditions. In the first step, DBF builds reversibly, increasing under low temperatures and decreasing under high temperatures. Once DBF reaches a certain level, it transforms into a stable, non-reversible unit called a "chill portion", the unit of chill accumulation for this model (Erez and Fishman 1998) .

Although the Chill Hours, Utah, and Dynamic models were initially designed for chill accumulation in peach, they have since been applied to a wide range of fruit and nut crops, including cherry, apricot, apple, pistachio, walnut, and kiwifruit (Luedeling 2012 ; Zhao et al. 2017). Chill models can be applied to Actinidia spp. based on the observation that they require chill accumulation in the winter or else require application of chemicals, such as hydrogen cyanimide, to bud, suggesting that the amount of chill required can be calculated (McPherson et al. 2001 ; Zhao et al. 2017). The success of the Dynamic model across different species suggests that it may also apply to kiwiberry (Luedeling 2012).

In studies that compared different chill models, the Dynamic model was considered generally the best at predicting the end of endodormancy, although it also is not always accurate due to the inherent difficulty of modeling a complex physiological process with only time and temperature (Luedeling 2012). To combat this uncertainty, using chill ranges (such as 20-24 CP) to account for the environmental differences in chilling requirement of the same cultivar planted on different sites instead of a definitive chill requirement (such as 20 CP) has been proposed but not widely adopted (Egea, Egea, and Ruiz 2020 ; Kaufmann and Blanke 2017).

Chill requirements vary considerably among species and cultivars. Within commercial kiwifruit (A. chinensis) using the Chill Hours model, cv. "Hayward" requires at least 950 chill hours to complete endodormancy, cv. 'Bruno' requires about 700 chill hours, and cv. 'Zesy002' requires only 400-600 chill hours (Paringatai-Hare 2023). Some accessions of hardier Actinidia species, such as A. rufa, require only 200 hours (Paringatai-Hare 2023). While the chill requirement of various Chinese cultivars of kiwiberry (A. arguta) range from 650-1,050 hours ("1st Chinese Symposium on Kiwiberry" 2019), the chill requirement of some Japanese kiwiberry varieties is only about 200 chill hours, suggesting significant diversity for the trait within the gene pool (Paringatai-Hare 2023; Phivnil et al. 2004). However, no chill requirements have been published for the only kiwiberry cultivar recommended for US producers, 'Geneva 3' (Hale 2019)

Knowing a cultivar's chill requirement is essential to determining its suitability to specific climates. If a chill requirement cannot be fulfilled reliably in a certain climate due to warm winter temperatures, the crop will not set fruit the following year (Liu and Sherif 2019). Chemical sprays may be necessary to force the end of dormancy. For example, hydrogen cyanamide is routinely used in countries such as New Zealand to combat inadaquate chilling in kiwifruit (actinidia deliciosa) (McPherson et al. 2001). Conversely, if the chill requirement is fulfilled very early in the winter season, the plant may be vulnerable to early spring temperature

fluctuations that prompt growth early in the potential frost period, leaving it open to frost damage (Debersaques et al. 2015). Knowledge of chill requirement is therefore critical to proper cultivar development, selection, and recommendation.

As the climate warms and temperature zones shift, dormancy is ending earlier due to earlier higher temperatures, leaving plants vulnerable to frost for longer periods (Winkler et al. 2013; Dmuchowski, Baczewska-Dąbrowska, and Gworek 2022) . Perennial crops require several years to reach the return on investment; if the risk of frost damage is perceived to delay return on investment too much, it may discourage growers from planting them. Cold damage is the 3rd highest cause of crop loss in the Northeast U.S. (Wolfe et al. 2018). Frost damage was one of the two most commonly cited concerns among a group of northeastern farmers who planted kiwiberry in 2023 as part of the Nor'East Kiwiberries project under the guidance of the New Hampshire Agricultural Research Station.

Current active methods to prevent frost damage, such as overhead irrigation systems and wind machines, are resource-heavy and expensive (Poling 2008; Snyder and Melo-Abreu 2005). While the chill requirements of U.S. kiwiberry varieties are undefined, their early budbreak increases susceptibility to frost damage. Kiwiberry vines often break dormancy before the last frost (Debersaques et al. 2015). Frost damage can eliminate a year's kiwiberry crop, as observed at the UNH Woodman farm in May 2022. Therefore, frost damage mitigation strategies are necessary to overcome the danger of frost to commercial kiwiberry plantings (Debersaques et al. 2015; Calvez et al. 2015).

Frost mitigation strategies are generally broken into two categories: passive and active. Passive strategies are implemented before a frost event occurs in order to avoid the use of active mitigation strategies. Some examples include: choosing a planting site where frost risk is low, planting crops and cultivars that are better adapted to withstand frost (including crops with appropriate chill requirements and cultivars that bloom later in the season), late pruning to prevent early budbreak, and using plant covers. Active methods are actions taken during a frost event to reduce frost damage, typically by manipulating plant or air temperature. Some active frost management methods include: heaters, wind machines, helicopters, surface irrigation, and sprinklers. Most growers use a combination of active and passive methods(Snyder and Melo-Abreu 2005). In a 2022 systematic review, water application via sprinkler during a frost event was the most consistently effective strategy for mitigating frost damage (Drepper et al. 2022). However, this strategy is both management and resource-intensive, requiring constant air temperature monitoring and copious amounts of freshwater. The same review noted that there has been an recent increase in studies exploring the foliar application of different biochemicals, both before and during frost events, to prevent frost damage (Drepper et al. 2022).

In areas where chill requirements are easily fulfilled, but frost risk is high, extending dormancy could be one strategy to protect kiwiberries, as plants become more vulnerable to frost after bud swell (Poling 2008). Various cultural or chemical methods might be used to manipulate a plant's dormancy cycle. Application of plant growth hormones could extend or break dormancy when applied exogenously. For example, hydrogen cyanamide is used on kiwifruit to

break plant dormancy in years in which chill requirements are not fulfilled (McPherson et al. 2001). Some evidence in the literature points to exogenous gibberellic acid as a potential dormancy extender for kiwiberry when applied during ecodormancy (Lionakis and Schwabe 1984; Zheng et al. 2018; Weaver 1959). Lionakis and Schwabe found that unrooted, partially chilled kiwifruit cuttings placed in a solution with low concentrations of gibberellic acid significantly extended dormancy (Lionakis and Schwabe 1984). Gibberellic acid was also found to significantly extend dormancy in grapes in two field experiments (Zheng et al. 2018; Weaver 1959) . Determining if gibberellic acid can protect against spring frosts by extending dormancy would reduce the risk of kiwiberry adoption for growers.

Experimental Objectives:

1. Estimate the chill requirement of 'Geneva 3' kiwiberry using the Dynamic model. If the percent budbreak significantly increases between two chill portion points, then plateaus in subsequent cuttings, then the chill requirement is likely between those two points.
2. Observe whether a delay of budbreak and/or flowering occurs after applying exogenous gibberellic acid at various concentrations onto kiwiberry laterals during ecodormancy.

Methods

Experiment 1: Determining the chill requirement of 'Geneva 3' Kiwiberry

Three blocks of two mature 'Geneva 3' kiwiberry vines each, located at the UNH Woodman Farm, Durham, NH, USA, were used. On each vine, 24 12-bud cuttings of were identified. First year laterals were preferred; however, due to the lack of renewal on some plants, first year spurs were substituted as necessary. Cuttings were randomly assigned to one of eight groups, 25, 30, 35, 40, 45, 50, 60, and 80 chill portions (CP). Each vine contributed three laterals to each treatment group.

Using the R package Chill Models (Pertille et al. 2019) and average hourly temperature data from the Woodman Farm weather station, accumulated chill portions were calculated daily starting November 2023. The start date for the model was August 1, 2023.

As each CP threshold was reached over winter 2023-24, the cuttings assigned to that group were collected by cutting between the 12th and 13th bud, and placed in quart-size mason jars with 1 inch of water. Jars were kept in the lab at room temperature (65-75 degrees Fahrenheit) under continuous light. Water was replaced every three days, and shoots were trimmed ½ cm every six days.

The top 10 buds of each cutting were assessed for budbreak every three days for four weeks. Budbreak is defined as BBCH 07, when the bud is swollen and green (green tip) (Van Labeke, Vaes, and Debersaques, n.d.).

At the end of four weeks, the percentage of buds broken out of 180 potential buds was calculated. Chill requirement was considered fulfilled when greater than 50% of buds broke in 4 weeks and the percentage of buds broken between two time points roughly plateaued (Phivnil et

al. 2004). Budbreak data was fit to a regression curve to create a model to predict chill requirement (Parkes, Darbyshire, and White 2020) .

Experiment 2: Exploring the effect of winter application of gibberllic acid on spring budbreak in 'Meader Female' and 'Ananasnaya' kiwiberry

16 mature kiwiberry vines broken into 4 blocks of four vines each at the UNH Woodman Farmwere used. There were two vines of two varieties, 'Meader Female' and 'Ananasnaya' in each block.

Within each block, one 'Meader Female' and one 'Ananasnaya' were designated as the treatment vines and the remaining two were control vines.

Within each control vine, two laterals, one facing north and one south, were marked and the number of buds per lateral were counted.

Within each treatment vine, 8 first-year laterals, 4 facing north and 4 south were chosen. Each of the four laterals on a side (north or south) were randomly assigned a treatment: control, low GA_3 concentration (10 ppm), medium GA3 concentration (100 ppm), and high GA3 concentration (1000 ppm) and marked with corresponding tags. This range was chosen based on prior studies in which gibberellic acid delayed budbreak. In Lionakis and Schwabe, 1984, a GA3 concentration of 100 ppm was found effective in delaying budbreak in kiwifruit (*A. chinensis*, cv. 'Bruno') when 40-50 cm long unrooted cuttings were stood in a solution. A further experiment testing a range of GA3 concentration on single-bud cuttings showed that concentrations of 6.25, 25, 100, 400, and 1600 ppm all significantly delayed budbreak, with 25 ppm and above preventing it completely, although 1600 ppm proved lethal to the cuttings. In a field study on grape (*Vitis vinifera* cv. Early sweet), a spray application of 10 ppm of GA3 resulted in a significant delay in bud break (Zheng et al., 2018). Number of buds on each lateral were counted.

On February 10, 2024, vines were brushed with the corresponding treatment solution of 0 ppm, 10ppm, 100 ppm, or 1000ppm GA_3. All treatments had 0.02% Tween 20. A brush application was chosen over a spray application to reduce drift between treatments.

Staring April 1, 2024, laterals were assessed every other day for budbreak (BBCH 09). When 30% of the buds on a given lateral reached BBCH 09, that was determined to be the day of budbreak for that lateral. This continued until all laterals reached budbreak on May 15, 2024.

Results

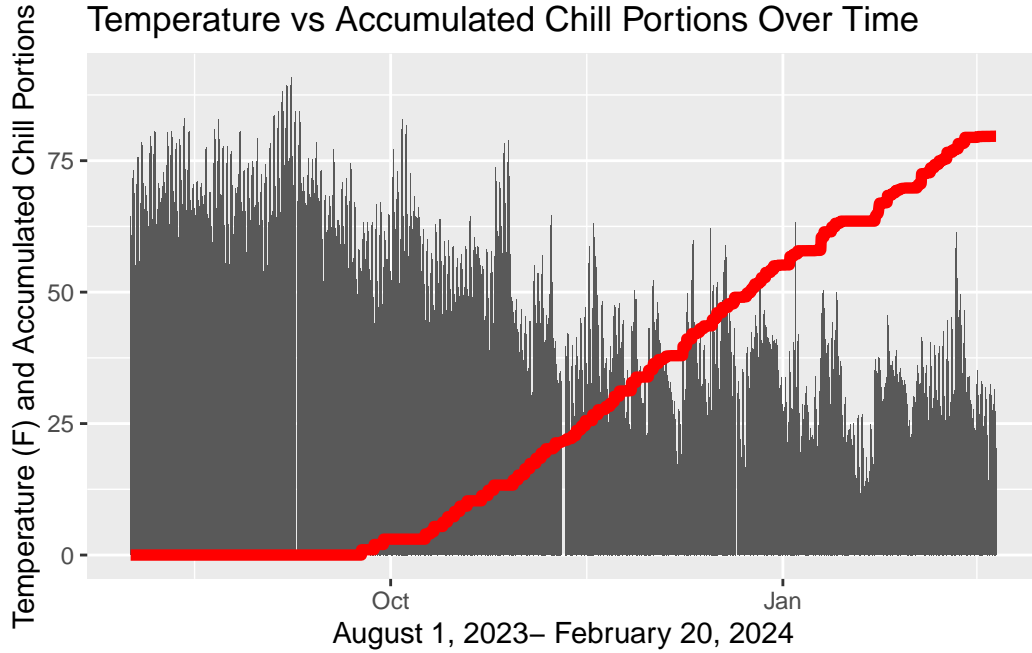***Experiment 1: Determining the chill requirement of 'Geneva 3' Kiwiberry***

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Figure 1: Average temperature over time compared to accumulated chill portions over time. The red line denotes accumulated chill portions.

Laterals were collected on November 16 for 25 CP, November 22 for 30 CP, December 1 for 35 CP, December 8 for 40 CP, December 17 for 45 CP, December 23rd for 50 CP, January 10 for 60 CP, and February 22 for 80 CP shoots . At the end of 4 weeks, there was 0%, 0%, 1.6%, 14%, 47%, 42%, 38%, and 26% of buds burst, respectively (Table 1).

Table 1: Chill Portion Data

Number of Chill Portions	Number of Buds Burst (out of 180)	Percent Budburst	Date collected
25	0	0.000	November 16
30	0	0.000	November 22
35	3	1.667	December 1
40	26	14.444	December 8
45	83	46.111	December 17
50	71	39.444	December 23
60	69	38.333	January 10
80	47	26.111	February 22

This data implies that the vines entered ecodormancy sometime between 40 and 45 CP. As

shown in Figure 2, there was a large jump in budburst between 40 and 45 CP.

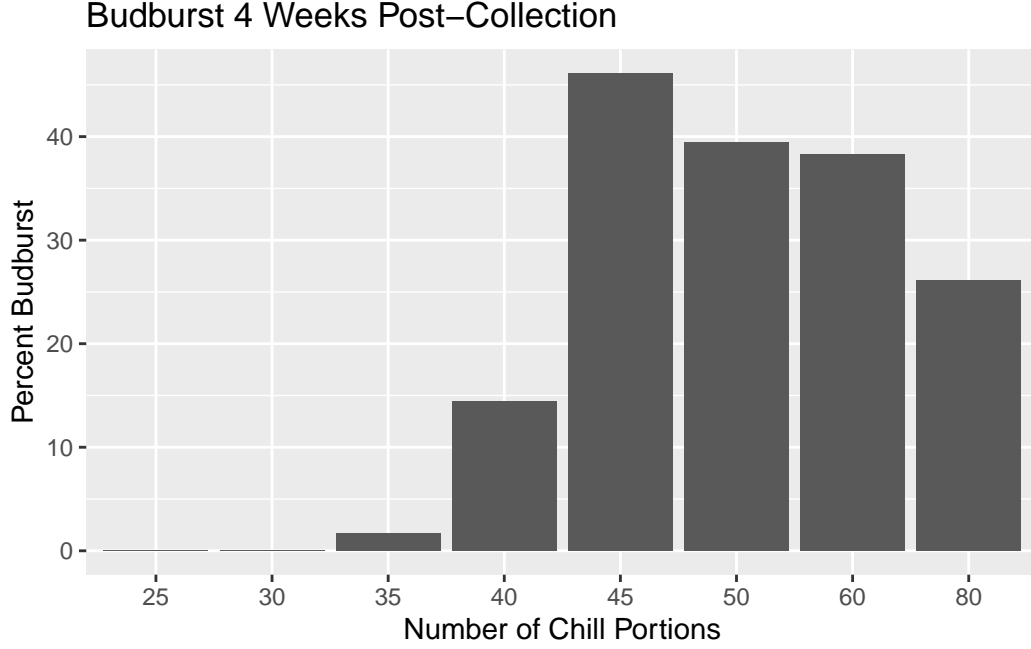### Budburst 4 Weeks Post–Collection

Figure 2: Percent Budburst of kiwiberry laterals 4 weeks post-collection. After being taken in from the field, laterals were placed in water under continuous light and assessed for budbreak after 4 weeks.

Using a logistic model, $\text{logit(P)} = B_0 + B_{\text{chill portions}} X_{\text{chill portions}}$, the effect of chill portions on budbreak of Geneva 3 kiwiberry approached the traditional limits of significance (p=0.06696). More data will be taken when the experiment is repeated during Winter 2024-25.

Experiment 2: Exploring the effect of winter application of gibberllic acid on spring budbreak in 'Meader Female' and 'Ananasnaya' kiwiberry

For experiment 2, we used a randomized complete block split-split-split experimental design, where the first split was variety ('Ananasnaya' or 'Meader Female'), the second split was aspect (whether the laterals were facing north or south, and the third split was gibberellic acid treatment (either 0, 10, 100, or 1000 ppm). Therefore, the linear model was:

Date of Budbreak= Block + Variety + Block:Variety + Aspect + Variety:Aspect + Block:Variety:Aspect + GA$_3$ Treatment + Variety: GA$_3$ Treatment + Aspect: GA$_3$ Treatment +Variety:Aspect: GA$_3$ Treatment

No interactions were found to be significant.

As shown in the ANOVA results below, there was no significant difference in the date of spring budbreak between different varieties of kiwiberry, or between laterals that faced different directions. There was also no significant difference in the date of spring budbreak between different gibberellic acid treatments.

Table 2. ANOVA: Response = Budbreak Date					
Variable	Sum Sq	DF	Mean Square	F Value	Pr (>F)
Block	68.59	3	22.86	2.494	0.2363104
Variety	51.98	1	51.98	5.67	0.09750455
Block:Variety	27.50	3	9.17		
Aspect	13.59	1	13.59	0.8363	0.3957319
Variety:Aspect	14.88	1	14.88	0.8758	0.3854739
Block:Variety:Aspect	100.52	6	16.75		
Gibberellic Acid Treatment	55.37	3	18.46	1.4061	0.25733
Variety:Gibberellic Acid Treatment	40.97	3	13.66	1.0406	0.38668
Aspect: Gibberellic Acid Treatment	9.74	3	3.25	0.2474	0.86261
Variety:Aspect:Gibberellic Acid Treatment	35.97	3	11.99	0.9135	0.44441
Residuals	459.39	35			

As shown in Figure 3, although the 1000ppm GA_3 treatment had a slight lag in budbreak, there was no significant difference in treatments.
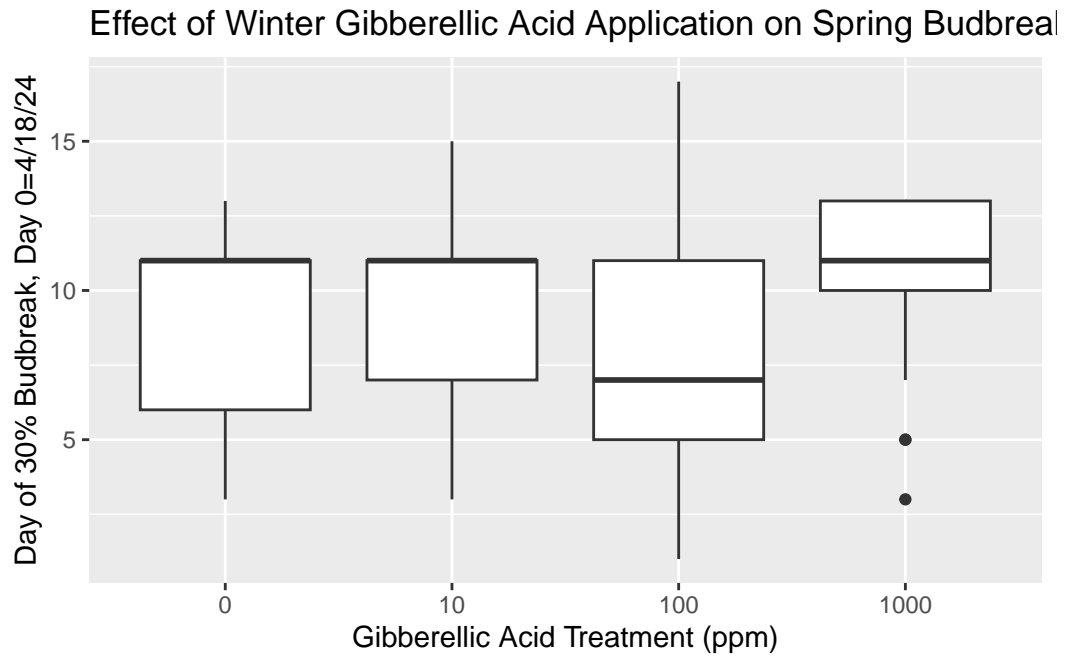
Figure 3: Effect of winter gibberellic acid application on spring budbreak. No significant differences were found between different gibberellic acid concentrations.
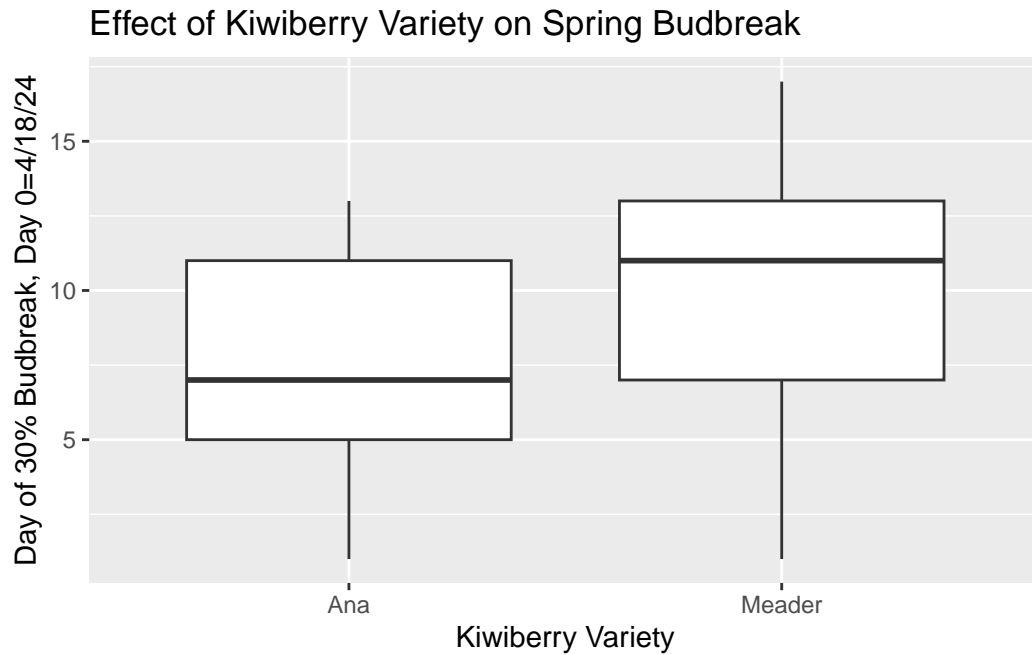
Figure 4: Effect of kiwiberry variety on spring budbreak. Although no significant difference was found between the budbreak of the two varieties, 'Meader' was on average one day later than 'Ananasnaya'

The 'Meader Female' kiwiberry variety was observed to break about one day later than the 'Ananasnaya' variety (Figure 4), but the difference was not significant.

Discussion

Locations that reached 45 Chill Portions Winter 202[...]
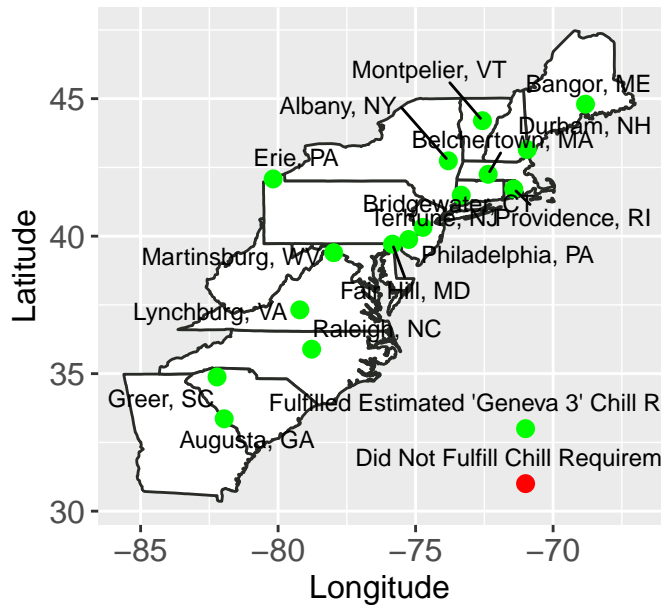
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