The Effect of Floral ReTain™ (Aminoethoxyvinylglycine, AVG) Application on Flower Longevity, Ethylene Generation, Pollen Tube Growth and Yield in Arbequina Olives (*Olea Europaea*)

By

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Abstract

Olives (*Olea europaea* L.) produce abundant flowers, but only a small subset persist as fruits. We hypothesize that the low fruit set to flower ratio is due to the short ovule longevity. Ethylene inhibition is hypothesized to prolong olive inflorescence longevity, extending the effective pollination period (EPP) and improving yield. The objective of this research is to determine whether applying exogenous aminoethoxyvinglglycine (AVG, an ethylene inhibitor) during bloom will decrease ethylene generation and increase the EPP, fruit set and yield.

A 264 ppm ReTain™ (a.i. Aminoethoxyvinylglycine) sprayed at bloom on Arbequina olives and monitored through the end of bloom significantly reduced ethylene production but did not affect visual bloom senescence or pollen tube growth, measured by pollen tube width. At harvest, fruit yield was measured, and the oil analyzed.

ReTain™ bloom application did not delay flower senescence or improve pollen tube growth. It significantly reduced ethylene generation and mildly increased fruit yield, but the yield increase was not significant. The collective results show that ReTain™ can significantly and strongly decrease ethylene generation in Arbequina olive flowers, but not extend flower longevity, improve fruit set or yield. Heavy rain, accompanied by low temperatures, during the bloom may be the reason why the flower longevity was not extended, resulting in decreased fruit set.

Introduction

History of Olive Cultivation and California Olive Production

Fossil evidence indicates Olive (*Olea europaea* L.) trees originated 20-40 million years ago in the Oligocene, in what is now the eastern Mediterranean Basin (Therios, 2009). The olive plant was first cultivated ~ 7000 years ago in the Mediterranean basin (Di Giovacchino, 2013). Olives are not native to the Americas. Spanish colonists introduced olive cultivation in present-day Peru, Chile, and Argentina in 1500s (Crosby, 2003). The Spanish missionaries brought the olives to California in 1700s (Carter, 2008). Around 1870, several small orchards with many different European varieties were cultivated for oil along the California coast, from San Diego to Sonoma, and in some foothill areas of the Sierra Nevada Mountains.

By 1885, there were 2,000 acres of oil olives planted in California. However, this olive oil industry was not competitive against seed oils or European olive oil imports. The "California Style Black Ripe" olive was developed in the early 1900s, and the California industry focused on table olives, increasing to 35,000 acres by the 1980s (Sibbett and Ferguson, 2005; Vossen, 2007). However, in the last four decades, the California table olive industry has declined. Stagnant grower prices, high hand-harvest costs and strong international competition with lower-priced imports have decreased the California table olive industry to 15,500 acres in 2020 (CDFA, 2020). During the same period, the olive oil industry increased to 20,000 acres (USDA NASS, 2020). This rapid reestablishment of a California olive oil industry was greatly facilitated

by cultivars bred to remain small, grow in intensive hedgerow orchards, crop early, and be mechanically pruned and harvested (Vossen, 2007).

For the past two decades, olive oil has been promoted as a healthier alternative to other fats and oils. American olive oil sales have increased 100% from 1991 to 2003 and continue to grow. The US ranks as fourth in world olive oil consumption (North American Olive Oil Association, 2004). This increase in olive oil consumption drove the California olive oil industry to increase domestic production, from 247,500 gallons in 1999-2000 to nearly 400,000 gallons in 2004-2005. Currently, most oil olive orchards are in San Joaquin and Sacramento valleys (USDA NASS, 2016a). Average yields for the past decade have ranged from one to six tons per acre of fresh fruit (USDA NASS, 2016a; USDA NASS, 2016b). However, while consumption is increasing and markets are strong, producers struggle to produce the 5 tons per acre and 190 to 210 gallons of oil per acre needed for economic sustainability.

Biology

The genus *Olea* in the family Oleaceae has about 35 species, but *Olea Europaea L.* is the only one that produces edible fruits. Cultivated olives are medium-size evergreen trees that reach ~ four to eight meters tall. They have dense foliage and bear fruits on the previous year's apical shoot growth. The simple, lanceolate leaves live for two to three years. The petioles are short, with each node composed of two opposite leaves. The fruit is borne on clusters in the axils of the opposing leaves (Barranco et al. 2004).

Olives grow best in Mediterranean climates with mild winters and warm, dry summers. They are cultivated primarily within the 30° to 45° north and south latitudes. Olives are cold sensitive. When actively growing, temperatures below 0°C damage shoots and kill buds. Temperatures slightly above 0°C decrease bloom. An annual mean absolute minimum temperature below -7°C demarcates the geographical range for olive production (Barranco et al., 2004). Most cultivars require winter chill of ~ 400 hours below 7°C to break dormancy and bloom. Olives are most sensitive to low and high temperatures during bloom. Flowering can be delayed at low temperatures (< 16°C), and high temperatures (> 35°C) can limit pollination and pollen tube growth (Nouri and Ferguson, 2021). The optimal temperature for olive pollen germination and pollen tube growth is 20 to 25°C (Fernandez-Escobar et al., 1983, Cuevas et al., 1994). Sufficient heat accumulation during the growing season is needed for successful fruit maturation. Full fruit development in olives required 1,225 Celsius degree days (Perez-Lopez et al., 2008).

When mature, olives are strongly apically dominant with axillary inflorescences borne on the previous year's shoot growth. Inflorescences form during the previous season on the growing-shoot leaf axils (Barranco et al., 2004). The buds are induced in early summer, initiated in the late fall, and differentiate after dormancy into perfect or imperfect flowers. (Pinney and Polito, 1990). Their reproductive or vegetative fate will be determined from mid-June to late October. The process of flower bud formation is called floral induction. The presence of fruit on one-year-old shoots inhabits floral induction on the current year's shoot growth. Thinning an olive tree within six to seven weeks of flowering increases flowering during the following year (Lavee et al., 1986). The destruction of seeds of very young fruit without destroying the

pericarp flowers can also increase the subsequent year's flowering (Stutte and Martin, 1986). Gibberellins, synthesized in the seeds of a developing fruit, were an important inhibitor of floral induction (Fernandez-Escobar et al., 1992).

Olive inflorescences are 1.5 to 4.0 cm long, with 10 to 35 flowers (Rapoport et al., 2016). The inflorescences have a paniculate structure: flowers grow on the central axis and sometimes sub-branching or secondary bearing-axes (rachillas) can occur (Figure 1). Olive flowers are actinomorphic (regular symmetry), and the corollas are composed of four white or yellowish-white petals (Barranco et al. 2004; Rapoport et al., 2016).

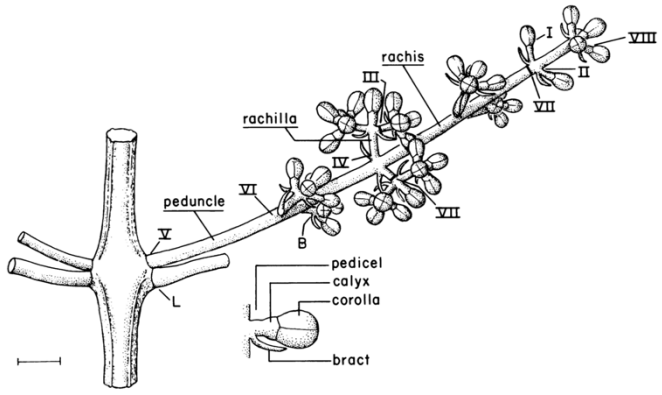

Figure 1: Morphology of olive inflorescence showing all possible patterns of branching. Scale bar - 0.5 cm (Weis et al., 1988).

Olive has an andromonoecious reproductive system, producing perfect and male flowers on an individual tree (Barranco et al. 2004). The imperfect male flower has two stamens, with relatively big, bright yellow anthers and short filaments (Figure 2). In perfect olive flowers, the female reproductive system is a pistil with bi-lobed (heart-shaped) stigma, a short white style, and a green round ovary (Barranco et al. 2004; Rapoport et al., 2016; Figure 3). The ovary has two locules or cavities, and within each are two ovules. Fruit development requires fertilization of one of the four ovules (Rapoport et al., 2016). The factors that affect the

proportion of perfect flowers include substrate competition, cultivar, and environmental conditions (Perica et al., 2001). In a normal year. About 50% (or fewer) of flowers are pistillate (Barranco et al. 2004).

Figure 2: A picture of staminate flowers in 'Arbequina' olives by Louise Ferguson. Only anthers are present.

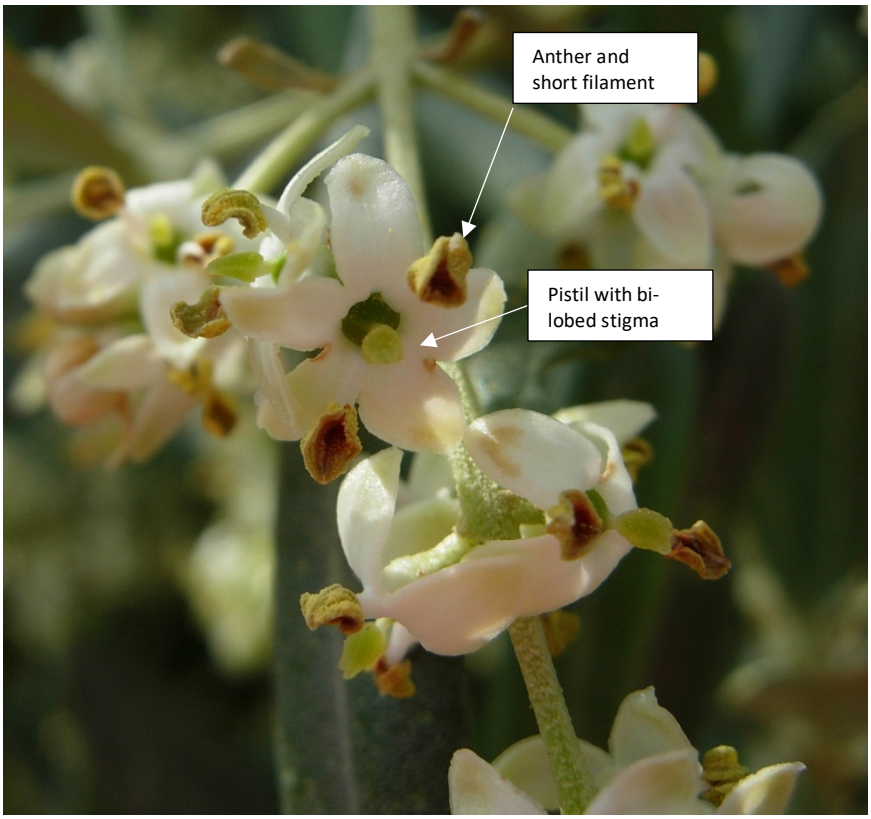

Figure 3: A picture of perfect flowers in 'Arbequina' olives by Louise Ferguson. Both anthers and pistils are present.

Pollination

Pollination and fertilization are required for fruit production. During pollination, a pollen grain lands on the stigma and germinates, producing a pollen tube, which grows through the stigmatic surface and down the style to the ovary (Barranco et al., 2004). Ovules of olives are anatropous (micropyle facing down) and during development, they orient so that the micropyle (the entrance for pollen tube of embryo sac) faces upwards (King, 1938). Like many other angiosperms, after successful pollination, one of the two olive male gametes fuses with the egg cell to become the diploid embryo. The other male gamete unites with two polar nuclei to become the triploid endosperm (Gehring and Satyaki, 2017). Only one of the four ovules is

fertilized and develops into a fruit. Vascular bundles separate the future endocarp and mesocarp (Barranco et al. 2004; Figure 4). Botanically, an olive fruit is a drupe. The mesocarp is the fleshy part of the olive and the endocarp is the stony shell that encloses the fertilized embryo.

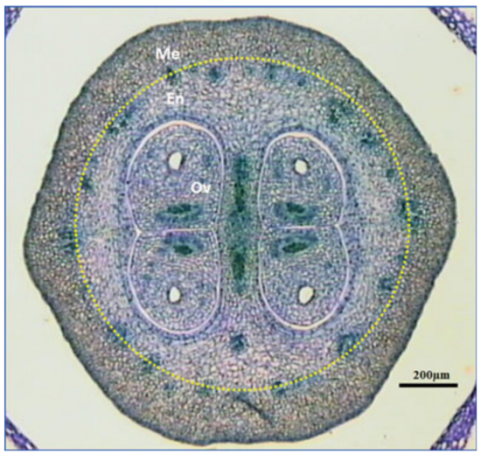

Figure 4: Microphotograph of a central transverse section of the olive ovary. The dotted yellow line denotes a ring of vascular tissues, separating the future mesocarp (Me) and endocarp (En). Each locule contains two ovules (Ov). (Rapoport et al., 2016)

Some argue olives are allogamous, meaning they require, or produce better, with cross-pollination. However, they may self-pollinate but often display self-incompatibility, particularly if temperatures are high during bloom (Diaz et al., 2006). Olive self-incompatibility is attributed to the slow growth of the pollen tubes of the same cultivar through the stigma, resulting in arrival after the ovule has degenerated (Cuevas, 1992). However, no failures in fruit set were detected in cross-pollinated Spanish olive groves, so the delay in self-pollinating cultivars might not be the reason for fruit set failure (Barranco et al., 2004). Mass abscission of young fruit and unfertilized ovaries are a result of competition for assimilates. In the 'Gordal Sevillana', cross-pollination reduces the number of shot berries and parthenocarpic fruit with little commercial value. In the 'Manzanilla de Sevilla', cross-pollination increases fruit set. However, in other

varieties such as the ‘Arbequina’, there is very little difference in the fruit set in response to cross-pollination (Suarez Garcia and Rallo, 1987).

Fruit Set

A mature olive tree produces abundant flowers, but only a small portion of them persist as fruits (Martin, 1990; Chaplin and Westwood, 1980). Anthesis (bloom) of olives lasted seven weeks, starting at bud break (BB; Kitasaki et al., 1999). Most inflorescence axis elongation happens during the third week after BB, massive bract shedding occurs the fourth week after BB, and full bloom (FB) occurs seven weeks after BB. Intense abscission of flowers and fruits happens five to seven weeks after FB (Lavee, 1986; Rallo and Fernandez-Escobar, 1985; Rallo et al., 1981). During the intense abscission period, imperfect flowers abscise before the perfect flowers and fruits, with overlap (Rapoport and Rallo, 1991).

Olive yield depends on the population of viable pistillate flowers, their pollination, fertilization, and persistence as fruits (Martin, 1990). The number of inflorescences affects final fruit set and the number of fruits within one inflorescence is consistent between years and within cultivars (Reale et al., 2006). The final olive fruit set correlates positively with the quantity of pollen during bloom (Minero et al., 1998). Ovule longevity is fundamental to flower pollination and fertilization in apples, leading the concept of an Effective Pollination Period (EPP; Williams, 1965). EPP is determined by the longevity of the ovules minus the time required for a pollen tube to grow to the ovule. Longer ovule persistence or viability could increase the EPP and, therefore, potentially fruit set in olives (Cuevas et al., 2009).

Alternate Bearing

Olive, like apple, pear, mango, and other fruit trees, are alternate bearing, meaning they produce alternating large and small crops ("on" year and "off" year; Sibbett and Ferguson, 2005). Alternate bearing does not cause harm to trees (Nouri and Ferguson, 2021), but it destabilizes management, production, and marketing (Nouri and Ferguson, 2021; Dag et al., 2010).

One hypothesis is that carbohydrate depletion during an "on" year due to fruit growth causes low fruit set in the subsequent "off" year (Sibbett and Ferguson, 2005). Olive trees placed in growth chambers under favorable conditions for growth: light, temperature, and high CO_2 concentration, had five times more non-structural carbohydrate accumulation than controls (Hackett and Hartmann, 1964). However, the olive trees in the chamber still failed to bloom or set fruit successfully. This observation suggested that the lack of photosynthates was not the primary reason for alternative bearing.

The developing fruit is an inhibiting factor for floral induction in olive. Reducing the crop load six to seven weeks after flowering resulted in increased flowering for the following year (Lavee et al., 1986). Olive fruit seed development is a strong influence on inflorescence development (Stutte and Martin, 1986). When olive fruit seeds were killed within six weeks after full bloom, leaving the bearing shoots with seedless fruits, the current year's shoot growth produced more inflorescences the following year than branches with seeded fruits. Gibberellins, synthesized in seeds of developing fruits, were an important inhibitor of floral

induction (Fernandez-Escobar et al., 1992). High GA-like (gibberellic acid-like substances) concentrations in midsummer (during embryo development period) reduce generative bud development, and the highest GA-like substance in fruits occurred in June and July (Baktir et al., 2004). These collective results suggest that gibberellins in developing seeds of fruits on one-year old growth suppress development of fruit buds on the apical current year's shoot growth, resulting in alternate bearing.

Thus, alternate bearing is due to inhibition of floral induction (Goldschmidt and Sadka, 2021), and floral induction in the current year is inhibited by fruit load from the previous year. There is a negative relationship between the current year's flowering intensity and the previous year's production (Kallsen, 2017). In 'Manzanilla de Sevilla' cultivar, 58% of the variance in the number of flowers was explained by production in the previous year. The current year's yield depends on flowering intensity, which depends on the previous year's production. This relationship was also found in pistachio. The yield of the previous-year harvest is most strongly and negatively correlated with the yield of current year (Kallsen, 2017). However, the specific mechanism of suppressing floral bud induction and development is unknown.

Ethylene and AVG

The role of ethylene in precipitating plant organ abscission, including floral organs, is clearly defined (Abeles, 1968). Ethylene production by the olive inflorescence was lowest four days before FB. It increased to a maximum seven days after FB and this peak coincided with

massive flower shedding (Kitsaki et al., 1999). Inhibition of ethylene results in longer-lived carnation flowers (Reid and Wu, 1992).

In the ethylene biosynthesis pathway, methionine is catalyzed to SAM by SAM synthetase, SAM is then converted to 1-aminocyclopropane-1-carboxylic acid (ACC) by ACC synthase, and ACC is further converted to ethylene by ACC oxidase (Yang and Hoffman, 1984). The ACC synthase is the targeted compound in limiting ethylene synthesis in tomatoes (Davies, 2010). The compound aminoethoxyvinylglycine (AVG) competitively inhibits ACC synthase activity by binding to the substrate's active site, preventing ethylene synthesis (Yu et al., 1979; Figure 5).

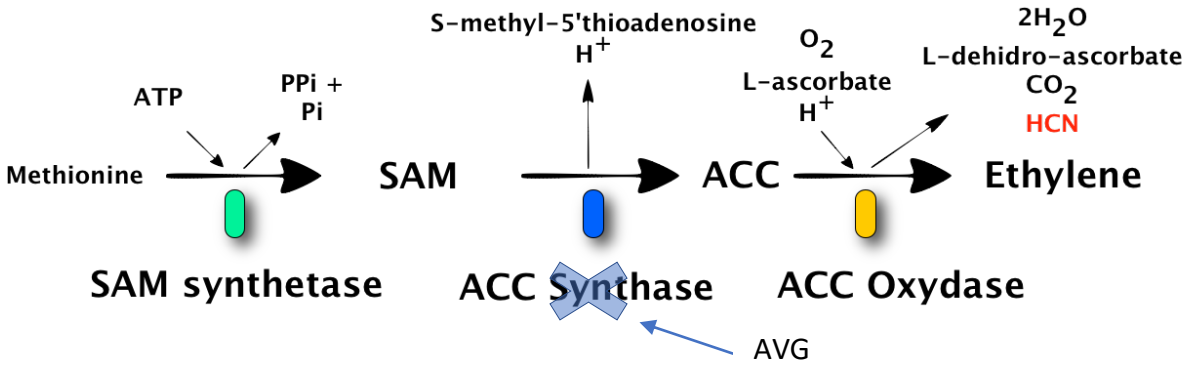

Figure 5: Ethylene synthesis pathway (Picture by UNITN-Trento). SAM stands for S-AdoMet and ACC stands for a=aminocyclopropane-1-carboxilic acid. Methionine is catalyzed to SAM by SAM synthetase, SAM is then converted to ACC by ACC synthase, and ACC is further converted to ethylene by ACC oxidase.

AVG is now used on multiple fruit crops. AVG applications to apple trees during harvest slowed ripening, effectively extending the harvest period (Ozkan et al., 2012). AVG inhibited ethylene biosynthesis, increasing peach fruit quality (Bregoli et al., 2002). Similarly, AVG (ReTain™) decreased ethylene generation and increased fruit firmness in plums postharvest (Jobling et al., 2003). AVG treatment delayed cocoa flower abscission (Aneja et al., 1999) and

decrease ethylene generation in pear flowers, increasing fruit set and yield (Carra et al., 2018, Pasa et al., 2017). Similar results were found in apples (Edgerton, 1981). Whole-tree applications of AVG on 'Regina' and 'Kordia' cherry trees significantly improved fruit set and yield (Bound et al., 2014). AVG application in walnuts increased yield because the high ethylene concentrations in female flowers caused of pistillate flower abortion (Beede and Polito, 2003).

The effect of AVG on ethylene suppression has been confirmed multiple times. For example, AVG prevented ethylene generation in rapeseed and sunflower plants (Abeles et al., 2012). AVG applications inhibited fruit ethylene production in 'Golden Supreme' apples (Yuan and Carbaugh, 2007). The primary role of AVG is inhibition of ethylene biosynthesis (Peters and Crist-Estes, 1989). Extensive research of AVG applications in walnut demonstrated that ReTain™ applied to pistillate flowers successfully decreased ethylene production and resulted in reduced flower abortion (Polito et al., 2004; Johnson et al., 2006). Subsequent orchard trials confirmed these findings. The AVG treated walnut trees produced higher yields than control trees because the AVG reduced pistillate flower abortion, improving fruit set (Beede, 2004; Johnson et al., 2006; Lemus et al., 2007a, b).

We hypothesized the short longevity of olive pistils is the primary reason for the low fruit set and yield in olives. We further hypothesized that AVG applications, as ReTain™, during bloom can potentially decrease ethylene generation by olive inflorescences, extend the pistil and ovule longevity for a longer effective pollination period, and increase yield. Therefore, we tested the ability of AVG, as ReTain™, applied at 25 to 50% bloom to decrease ethylene

production by olive flowers, extend the pistil viability, EPP, and potential successful fertilization, and increase fruit set and oil yield in Arbequina olives.

Materials and Methods

Experimental Design

The experimental site was a 12 year-old *Oleae europeae cv.* 'Arbequina' orchard located at 38.07°N, -121.21°W, farmed commercially by Lodi Farming Inc. The orchard comprises 59 rows oriented on a north-south axis with ~ 220 trees in each row. Trees are spaced at 5 feet in-row x 13 feet between rows, for 670 trees per acre. We designated this orchard as Orchard A.

A randomized complete block design was used in the experiment, with six, eight-row blocks containing two, three-row sets within a block, separated by two buffer rows. The ReTain™ treatments were applied once in every block to three contiguous rows (Figure 6). The center row of the three contiguous rows was used to collect samples and yield data.

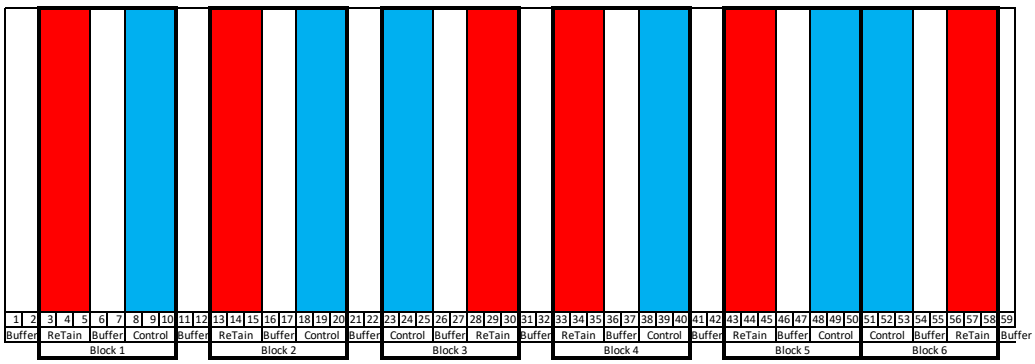

Figure 6: A map of the Orchard A, numbers indicating row numbers, and up is north. The red color represents the rows that received the ReTain™ treatment, and blue the control.

The data were analyzed using linear model procedure, ANOVA, and functional principal component analysis in RStudio (RStudio, Inc., Boston, MA, USA).

Fruit Set Potential

In 2019, to ensure the treated and control trees were equal in fruit-setting potential, the number of inflorescences, the fresh and dry weight of leaves, and the leaf area per unit shoot length were measured.

Before spraying, two trees, one each from the north and south ends of the orchard, were selected within each treatment. Two shoots per tree were collected and shoot length was measured, in addition to the number of inflorescences, the fresh and dry leaf weighs, and the leaf area.

ReTain™ Application

The treated rows were sprayed with 264 ppm ReTain™ (AVG) at 25 to 50% bloom on May 13th, 2019. Eight pouches of ReTain™ (333g per pouch, 15% a.i.) were dissolved in a 400-gal tank and applied with an airblast sprayer traveling at 2.7 m/s, with an application rate of 100 gal per acre. The control was unsprayed. The treatment calculation is shown below:

400 gal = 1514.16 L

8 pouches / tank * 333 g / pouch = 2664 g / tank = 2664 g / 400 gal = 2664 g / 1514.16 L = 1.759 g/L = 1759 ppm

1759 ppm * 15% = 264 ppm

Visual Evaluation of Flower Longevity in the Field

In 2019, to determine if ReTain™ visibly improved flower longevity, the olive flowers were evaluated for senescence in the field.

Six trees (three trees from the north end of the row and three trees from the south) in each treatment within each block were selected for visual evaluation of flower senescence. From each tree, two 12-inch uniform shoots, equal in floral load, facing two directions (east and west), and at comparable positions in the canopy, were tagged at the juncture of 1 and 2 year-old growth. These were rated daily for visible senescence using a 10-point scale. Pictures were taken daily at the same time and angle for later analysis.

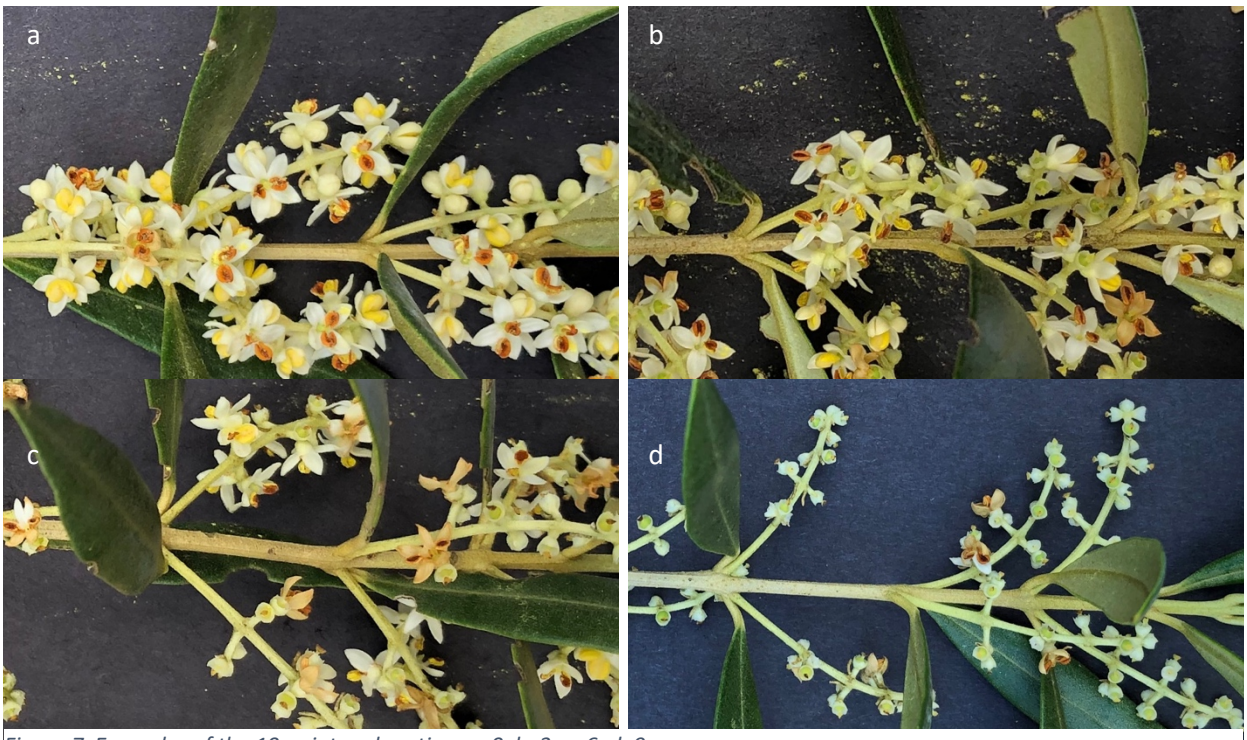

Figure 7: Examples of the 10-point scale rating. a: 0; b: 3; c: 6; d: 9

A 10-point rating scale was used to rate flower senescence visually:

0: full bloom with no noticeable senescence (Figure 7.a)

3: full bloom with a small number of brown petals (Figure 7.b)

6: full bloom with mostly brown petals and some petal drop (Figure 7.c)

9: full petal drop (Figure 7.d)

Determining Ethylene Production

In 2019, after spraying on May 13th, 2019 (day 0), sampling of ethylene evolution started and was repeated daily for 13 days, until there was no difference in ethylene generation between control and treatment trees. It rained on 10 of the 13 sampling dates.

From each treatment in each block, two trees, each from the north and south ends of the row, were selected for ethylene measurements. From each tree, two uniform shoots, similar in floral load, facing two directions (east and west), and at equal position in the canopy were collected daily for 13 days. They were placed on ice and transported to the lab immediately.

In the lab, ~ 0.6 g, or three to five inflorescences were cut from each shoot, weighed on a Mettler balance and placed in a 15 cm airtight test tube. The tubes were placed in a controlled temperature room at 20°C to equilibrate for 1 hour. After equilibration, 10 mL of air was withdrawn from each sealed tube with a 10 mL syringe and injected into Series 400, AGC

Carle Gas chromatograph for ethylene measurement. Ethylene production was expressed as μL/(kg*h).

Pollen Tube Growth

In 2019, olive inflorescence samples were collected and preserved in FAA solutions (95% ethanol, glacial acetic acid, and 37% formalin at 10:1:2 ratio). They were further dyed with Aniline Blue and observed using a fluorescence microscope. Pictures of olive ovaries were taken

(Figure 8), and pollen tube widths were measured using ImageJ. The pollen tube width was used as a measure of pollen tube growth, which reflected the effect of ReTain™.

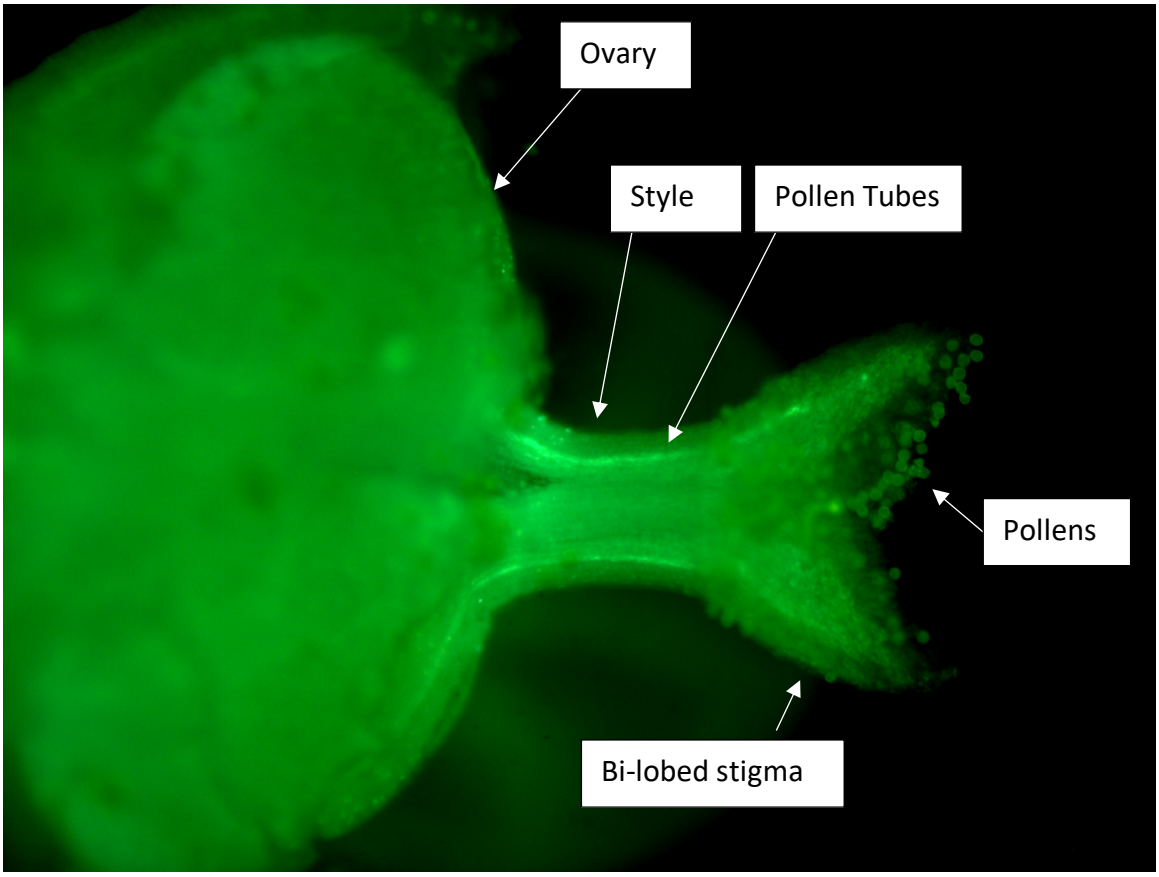

Figure 8: An example picture of olive ovary. Pollen tubes are the highlighted long lines. The style, bi-lobed stigma and pollens are also indicated in the picture.

Fruit Weight, Oil Yield and Quality

The treated and control rows were individually harvested on November 15th, 2019, by a Vinestar canopy contact parallel bow rod harvester with a single detached fruit bin traveling at a groundspeed of 1.5 mph. The weight of the detached fruit bin was determined before harvest. The fruit weight per row was then determined using a digital in-ground scale with the

bin tare entered. After weighing, a 5 kg sample was collected and put on ice for transport to the UC Olive Center Laboratory of Dr. Selina Wang at UC Davis for oil quality analyses.

Experiment in 2020

The same experiment was not repeated in 2020 due to strong alternate bearing and a lack of flowers. However, a grower trial was performed; selected rows in two orchards (Orchard B and C) were sprayed on with the same ReTain™ treatment and control, and ethylene emission was measured from inflorescences collected from the sprayed and unsprayed rows. The sprayed and control rows in Orchard B and C were harvested and yields compared on November 15th, 2020, in both orchards.

The yield from Orchard A of the 2019 experiment was measured for comparison with the yield of 2019.

Results

Fruit Set Potential

There was no significant difference in the number of inflorescences, leaf fresh weight, leaf dry weight, or leaf area per shoot length between control and treated rows in 2019 (Figure 9).

Table 1: Statistical analysis inflorescence number, leaf fresh and dry weight, and leaf area pre-treatment. The average values were similar between control and ReTain™-treated rows. The large p-values indicated non-significant differences between treated and controls pre-treatment.

Variable	Average Value		t statistics	p-value
	ReTain™-Treated	Control		
Inflorescence Number (No. per cm)	0.8	0.75	0.65	0.52
Leaf Fresh Weight (g per cm)	0.14	0.12	1.68	0.1
Leaf Dry Weight (g per cm)	0.07	0.06	1.89	0.07
Leaf Area (cm^2 per cm)	3.92	3.56	1.13	0.27

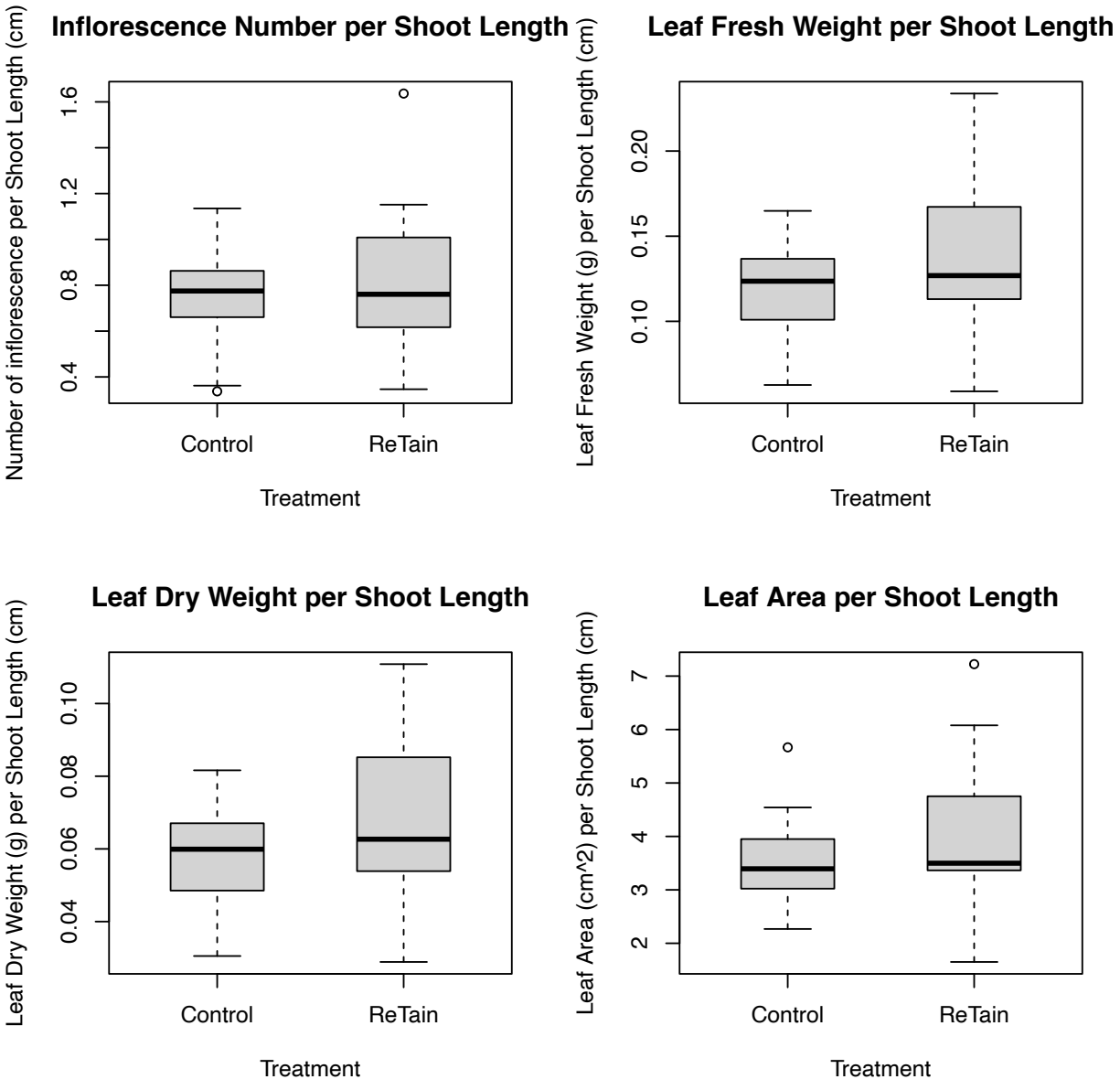

Figure 9: Potential fruit set measurements were taken on leaf area, leaf fresh and dry weight pre-treatment. 48 shoots were sampled, and ANOVA was used to determine the potential fruit set difference between the treated and control rows pre-treatment.

Visual Evaluation of Flower Longevity

In 2019, there were significant differences between treatments, blocking, directions (east and west), and position in the orchard (north and south; Figure 10). Throughout sampling,

the senescence visual ratings of the treated trees were greater than those of control trees, the rating of trees from the south end of the rows was greater than those of trees from the north end of the rows, and the rating of the east sides of the trees was greater than those of the west sides of the trees (except on Day 16). Block 1, 2, 3, and 6 had higher ratings than Block 4 and 5.

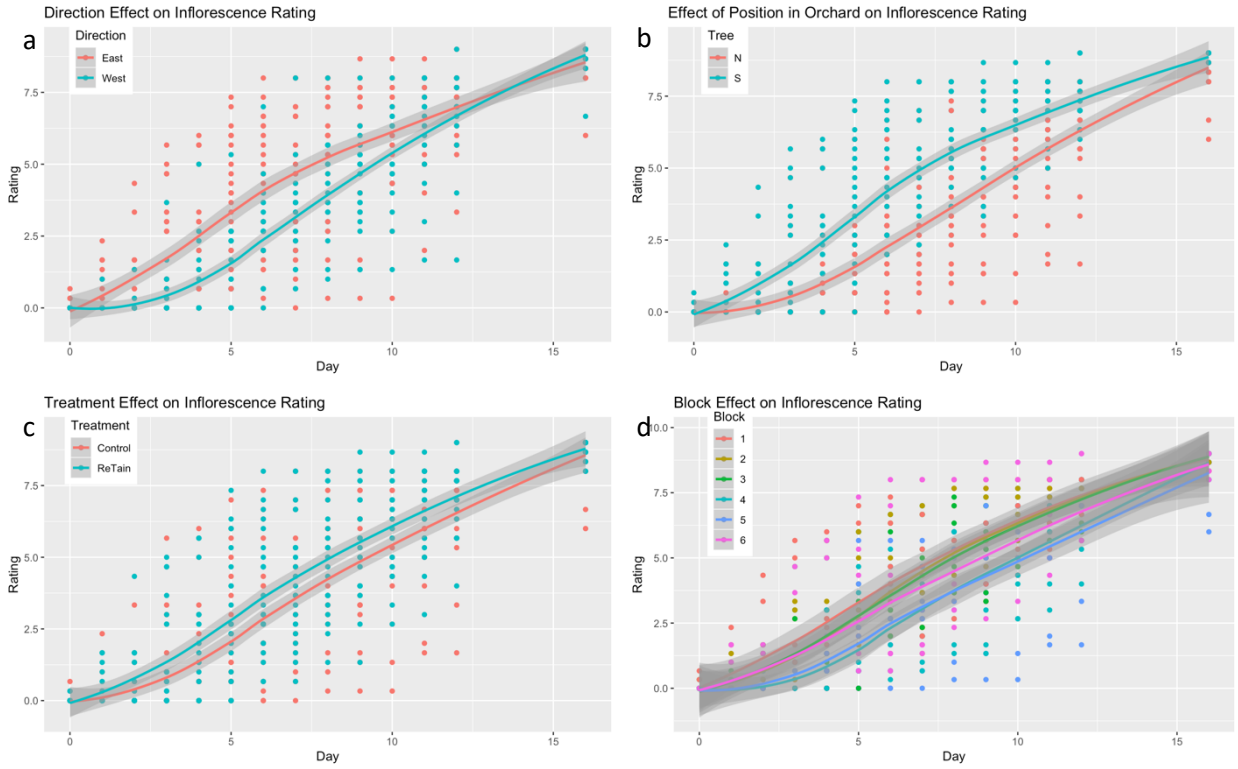

Figure 10: The effects of direction (a), position in the orchard (b), treatment (c), and blocking (d) on visual rating of flower senescence. 144 shoots were sampled daily, and ANOVA was used to determine the effects of direction, position, treatment, and blocking.

Ethylene Production

In 2019, there was a significant difference in ethylene generation between treated and control trees (Figure 11). The non-overlapping 95% confidence bands for ethylene demonstrated that the ethylene produced by treated and control trees was significantly different.

In 2020, air samples from inflorescences were collected from trees in Orchard B and C. However, no ethylene generation was detected in either treated or control flowers.

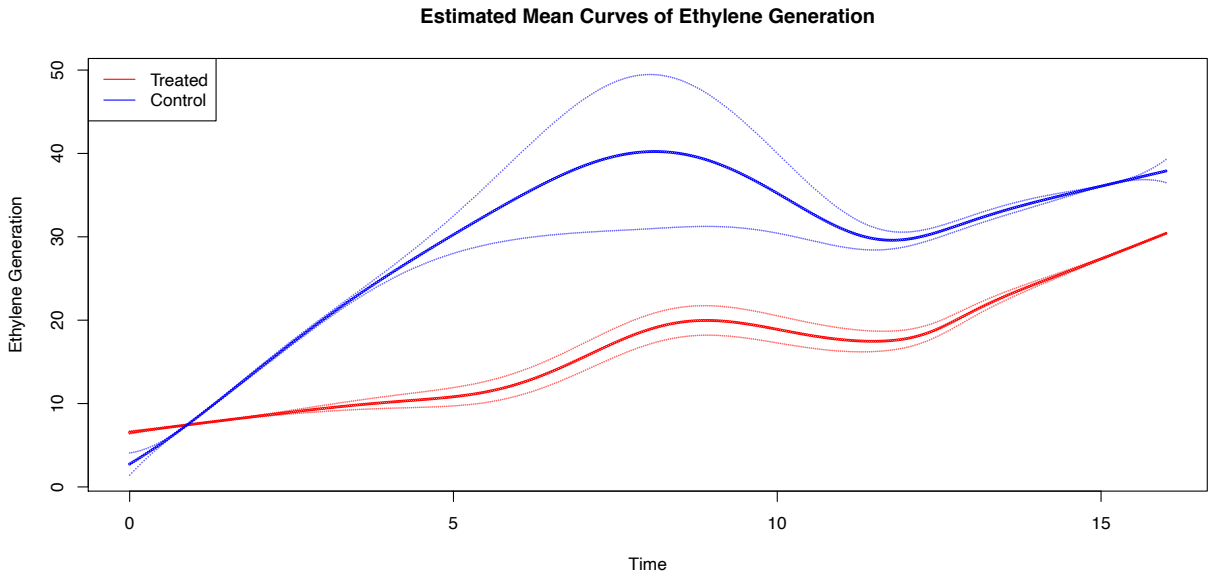

Figure 11: Ethylene production between the treated and control trees, the light blue and red curves are 95% confidence bands.

Pollen Tube Growth

One hundred and eighty ovary samples were examed for pollen tube growth in 2019. ANOVA found no significant difference between treatments or among the blocks.

Oil Quality

There were no significant differences (indicated by large p-values) in free fatty acidity, peroxide, ultraviolet absorbance (measured by K232 and K268), or 1,2-diacylglycerols between ReTain™-treated and control rows.

Table 2: Statistical analysis of treatment effect in oil quality. The average values were similar between control and ReTain™-treated rows. The large p-values indicated non-significant differences between treated and controls. Data was collected by Xueqi Li.

Variable	Average Value		t statistics	p-value
	ReTain™-Treated	Control		
Free fatty acidity (FFA) (% oleic acid)	0.14	0.13	0.91	0.39
Peroxide Value (PV) (meq O_2/kg)	2.65	2.81	0.59	0.57
K232	1.27	1.28	0.30	0.77
K268	0.08	0.08	0.00	1.00
1,2-Diacylglycerols (DAGs) (%)	95.33	96.24	1.40	0.19

Yield

In 2019, the six treated rows in Orchard A produced an average yield of 3320 lb, with a standard deviation of 408, while the 6 control rows produced an average of 2970 lb, with a standard deviation of 98. With a p-value of 0.067, the difference in yield between ReTain™ treatment and control rows was not significant when the alpha level equaled 0.05. However, it was significant when the alpha level equaled to 0.1 (Table 3).

In 2020, the six treated rows in Orchard A produced an average yield of 1160 lb, with a standard deviation of 290, while the six control rows had an average of 1185 lb, with a standard deviation of 369. The p-value was 0.9, showing no significant difference in yield between ReTain™-sprayed and unsprayed rows (Table 3). The yields across the orchards in 2019 and 2020 were also determined (Figure 12).

*Table 3: Statistical analysis of yield in 2019 and 2020. Average yields for ReTain™-treated and control rows were shown, and the standard deviation values were shown in parenthesis. * indicates a significant difference in yield under alpha level 0.1.*

	Average Yield in lbs. (Standard Deviation)			
Yield Year	ReTain™-Treated	Control	t statistics	p value
2019	3320 (408)	2968 (98)	2.051	0.0674*
2020	1162 (290)	1185 (369)	0.122	0.905

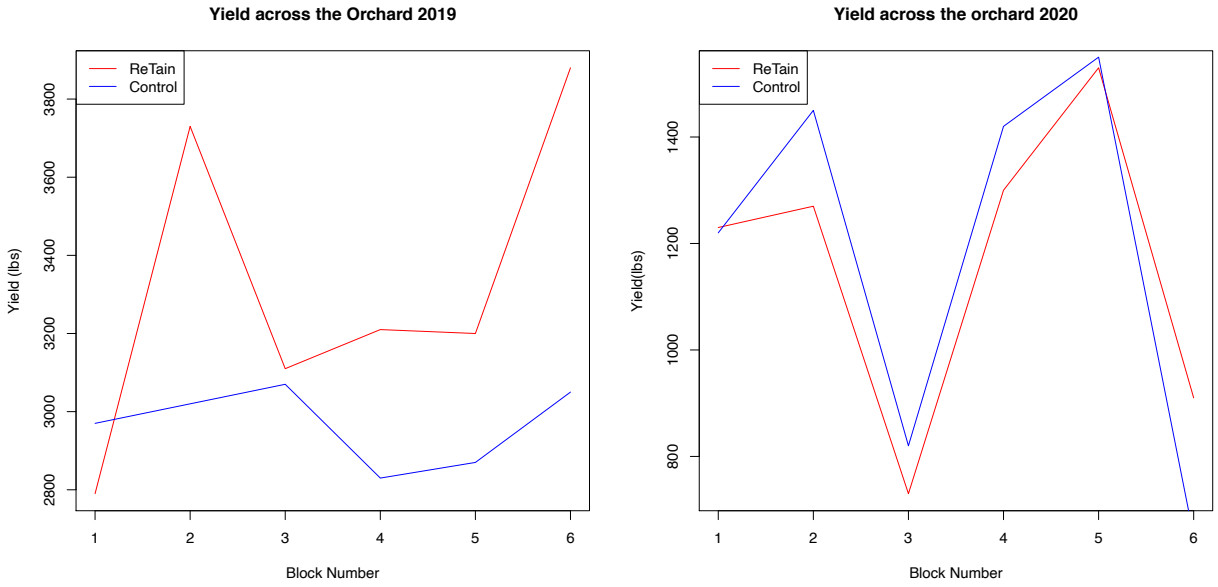

Figure 12: Yield across the orchard for 2019 and 2020. In 2019, five out of the six ReTain™ treated rows showed improved yield. However, the increase in yield was not consistent among the blocks. In 2020, when no ReTain™ was applied, there was no obvious difference in yield between the treated and control rows in the same blocks.

In 2020, the average yield per row in Orchard B was 1650 lb for ReTain™-treated trees, and 1070 lb for untreated trees. The average yield per row in Orchard C was 2110 lb for treated trees and 2220 lb for untreated trees. No statistical analysis was performed on the yield data in Orchard B or C, due to the fact that no replications were made.

Discussion

In 2019, the significant difference in ethylene generation between treated and control trees suggested that ReTain™ worked as assumed: it decreased ethylene generation. Day 0 was denoted as the ReTain™ application date. The confidence bands for ethylene generation were very narrow before day 3 and after day 12 (Figure 10). During those days, some ethylene measurements appeared to be zero and were omitted, because it is impossible to have zero ethylene generation (Abeles et al., 2012). Fewer ethylene measurements before day 3 and after day 12 resulted in narrow confidence bands. The primary finding was not affected: from day 3 to day 12, the ethylene generation from ReTain™-treated trees was significantly lower than that from control trees.

We hypothesized that delayed floral senescence was a consequence of decreased ethylene generation; however, the visual inflorescence rating data suggested the opposite. The senescence ratings of treated trees were significantly higher than the control trees, indicating ReTain™ increased the rate of flower senescence. Throughout the experiment, the first-opened flowers senesced first. The blocks on the edge of the orchard senesced earlier than blocks in the center of the orchard, east sides of the rows than the west, and the south end of the rows earlier than the north. The flowers in the row on the edge of the orchard and the ones on the south end of the rows also bloomed first. This could be potentially explained by that those flowers were at locations to receive the most heat.

The visual evaluation of inflorescence senescence was based on petal color change and petal drop. It might not indicate the ovule viability throughout the experiment. Therefore, it is possible that the petal drop was not correlated with the effective pollination period and ovule viability. Aniline blue fluorescence was argued to be an accurate method to measure ovule

senescence in olives (Stosser and Anvari, 1982; Crisosto et al., 1986; Cuevas et al., 1994; Fernandez-Escobar et al., 2008)

The findings on pollen tube growth supports this possibility. There was no significant difference between treated and control flowers in pollen tube width. This suggested both treated and control flowers were pollinated equally, even though the treated flowers senesced more rapidly than the control flowers. The non-significant difference in pollen tube width suggested that pollination an ovule viability were not affected by ReTain™.

It is possible that pollen tube width is not a good indicator of pollination. The pollen tube width is primarily a measurement of the amount of pollen deposited on the stigma that germinated and produced a pollen tube. We selected this measurement reasoning that a longer effective pollination period provides more time for pollen grains to land on the stigmas. Determining whether the pollen tube has reached the ovary might be a better indicator of successful pollination (Bradley et al., 1961). However, after reaching the ovary, the pollen tube was unrecognizable (Figure 8). The tissue in the ovary was too thick to observe under a fluorescence microscope.

In *Arabidopsis*, both ethylene-dependent and ethylene-independent pathways are required to initiate and progress through floral senescence (Bleecker and Patterson, 1997). It is possible that the floral senescence in olives is not regulated primarily by ethylene. Pollination induces a series of post-pollination developmental events, including petal senescence (Stead, 1992). Pollination-triggered senescence has multiple advantages. Once sufficient pollen has been set on the stigma, additional pollen deposition is wasteful, and excess pollen tubes may compete for nutrients. In addition, maintenance of floral structures is costly (Stead, 1992).

In 2020, the ReTain™ treatment was applied to two different orchards at the same concentration. However, no ethylene was detected from either treated or control flowers, which may be due to reduced number of flowers in an “off” year.

In 2019, heavy rain during bloom could have prolonged the flowering time. The heavy rain and low temperature combined with the spray of ReTain™, while the control rows were not sprayed, could potentially explain the earlier senescence of the treated rows. The lowest temperature during the bloom reached 8°C, while the optimal temperature for olive pollen germination and pollen tube growth is 20 to 25°C (Fernandez-Escobar et al., 1983, Cuevas et al., 1994). The low temperature during the bloom may have negatively impacted pollination in both ReTain™-treated and control trees.

In 2019, the difference in yield between ReTain™-treated and control rows was not significant at 0.05 but was significant at 0.1. However, yield fluctuated greatly in treated rows, while the yield among control rows was stable. ReTain™ strongly improved yield in five out of six rows, but the increase was not consistent and the effect of ReTain™ was not uniform among rows (Table 3). The 2020 yield data confirmed our assumption, as the harvested olives per row were similar within each block (Figure 12). This suggested that ReTain™ did have an effect on olive yield. However, this effect may have been masked by heavy rain and cold temperatures in May 2019. During the “off” year of 2020, ReTain™ seemed to have a greater effect in improving yield in the orchard with less inflorescences (Orchard B), than in the orchard with more inflorescence (Orchard C). There was no difference in any oil quality indicators between ReTain™-treated and control fruits (Table 2).

We hypothesized that a foliar ReTain™-application would depress ethylene synthesis by olive flowers, delay floral senescence (including ovule viability) and increase the effective pollination period, allowing a higher rate of successful fertilization. We monitored ethylene generation after foliar ReTain™ application, evaluated floral senescence visually and evaluated pollen tube growth to indicate pollination, weighed yield to determine whether fruit set was higher, and measured oil quality to determine whether oil quality was affected.

Foliar application of ReTain™ at 25 to 50% full bloom significantly and consistently reduced ethylene synthesis by olive flowers in an “on”, heavily-cropped year, and the yield was significantly improved under a 0.1 alpha value. However, determining whether this increased yield was caused by having a longer effective pollination period was inconclusive. The visual rating of flower senescence did not support our hypothesis: ReTain™-treated flowers senesced earlier than control flowers. As indicated by pollen tube growth, successful pollination was unaffected as the pollen tubes were unobservable after they passed down the styles. It was unclear whether ovule viability was prolonged.

This experiment was conducted only for a single year in this alternate-bearing crop, because there were insufficient flowers the second year. Repeating this trial under better conditions, such as optimal temperatures and no rain during pollination, may produce more consistent effects on yield. Also, developing a more discriminating method to measure pollen tube growth, their successful access to ovules, pollination, and perhaps pollen and ovule viability could better determine how ReTain™ produced a significant increase in yield.

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