

# Rootstock and Plastic Mulch Effect on Watermelon Flowering and Fruit Maturity in a *Verticillium dahliae*-Infested Field

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**Abstract.** Separately, grafting and the use of plastic mulch can increase yield, quality, and early harvest of watermelon (*Citrullus lanatus*), especially when plants are under biotic and/or abiotic stress. A 2-year field study was conducted to evaluate the combination of four different rootstocks and two types of plastic mulch (black and clear) on date of watermelon first flowering, fruit ripening, yield, and fruit quality when plants were exposed to *Verticillium dahliae*. Seedless watermelon cv. Secretariat was grafted onto rootstocks *Lagenaria siceraria* cv. Pelop, *Benincasa hispida* cv. Round, and two interspecific hybrid squash rootstocks *Cucurbita maxima* × *C. moschata* cvs. Super Shintosa and Tetsukabuto, with nongrafted ‘Secretariat’ as the control. Fruit were harvested 0, 7, and 14 days after both the leaflet and tendril attached to the fruit pedicel were completely dry (fruit considered to be physiologically mature). The area under the disease progress curve (AUDPC) values for verticillium wilt were not different for mulch type in either year, although the overall AUDPC value was greatly reduced in the four grafted treatments (227) compared with nongrafted (743). There was no difference in days to male or female flowering due to mulch type or year, and rootstock did not affect first flowering of male flowers. Female flowering was 14 and 11 days later in 2018 and 2019, respectively, for ‘Secretariat’ grafted onto bottle gourd ‘Round’ compared with ‘Secretariat’ grafted onto ‘Tetsukabuto’. Female flowering of ‘Secretariat’ on ‘Round’ was also 7 days later compared with nongrafted ‘Secretariat’ both years. However, days to first harvest was not different with mulch or rootstock and was 92 days after transplanting (DAT) in 2018 and 114 DAT in 2019. There was no difference in yield (fruit number and weight) due to year, harvest date, or mulch, but there was a difference due to grafting. ‘Secretariat’ grafted onto ‘Super Shintosa’ had the greatest total number and weight of fruit per plant (3.7 and 14.8 kg, respectively), and nongrafted ‘Secretariat’ had the lowest (0.7 and 3.2 kg, respectively). Fruit quality attributes hollow heart formation (rating 3.2/5 on average), hard seed count (6 on average), total soluble solids (11% on average), and lycopene content were not different among mulch type, rootstock treatment, or harvest date; however, lycopene content did differ due to year (52.44 and 32.51 μg·g<sup>-1</sup> in 2018 and 2019, respectively). Flesh firmness was highest for watermelon grafted onto ‘Super Shintosa’ rootstock (6.7 N) and lowest for nongrafted watermelon (4.3 N). Overall, rootstocks reduced verticillium wilt severity and increased fruit yield whereas mulch had no effects, and 5 *V. dahliae* colony forming units (cfu)/g of soil may be the minimum level for impact on watermelon fruit yield.

Watermelon (*Citrullus lanatus*) production is limited by verticillium wilt (caused by *Verticillium dahliae*) throughout Washington State, and in western Washington where watermelon are produced on a small scale for direct marketing, production is further limited by warm summer temperatures (15 °C on average). In this environment, grafting watermelon onto disease-resistant rootstock can

be an effective management approach for verticillium wilt (Buller et al., 2013; Dabirian et al., 2017; Wimer et al., 2015). Additionally, increasing soil temperature with plastic mulch may also increase watermelon yield (Dabirian et al., 2017).

Verticillium wilt is a soilborne disease favored by moist soil and a temperature range of 21 to 27 °C (Berlanger and Powelson,

2000). The pathogen can persist in the soil or plant debris for up to 14 years in the absence of a compatible host (Bhat and Subbarao, 1999; Bruton et al., 2007). Verticillium wilt is problematic in watermelon production as the plant tends to remain symptomless until flowering and fruiting. The hyphae colonize the root cortex then invade the xylem vessels, where they spread to the aboveground part of the plant and interfere with water and nutrient uptake and transport throughout the plant (Berlanger and Powelson, 2000; Fradin and Thomma, 2006; Vallad and Subbarao, 2008). Watermelon has no known genetic resistance against *V. dahliae*, and fumigation is only somewhat effective (Pscheidt and Ocamb, 2019); thus, alternative strategies are critically needed to achieve successful disease management. Grafting watermelon plants onto disease-resistant rootstocks offers an alternative strategy, but grower adoption is contingent on information regarding rootstock disease-resistance. The most commonly used rootstocks for watermelon grafting are *Lagenaria siceraria* (bottle gourd), *Cucurbita moschata* (winter squash or pumpkin), and *C. moschata* × *C. maxima* (interspecific squash hybrid). Although watermelon grafted onto commercial cucurbit rootstocks have shown increased tolerance to verticillium wilt, no cucurbit rootstocks are known to be completely resistant to *V. dahliae* (Attavar et al., 2020; Davis et al., 2008; Paplomatas et al., 2000; Paroussi et al., 2007; Wimer et al., 2015). Yet verticillium wilt incidence was lower on grafted watermelon plants grown in artificially infested soil (Paroussi et al., 2007). Additionally, verticillium wilt severity of grafted watermelon plants was reduced in naturally infested field sites in western Washington where soil populations of *V. dahliae* were 18 to 28 cfu/g (Buller et al., 2013; Dabirian et al., 2017; Wimer et al., 2015). Increased scion health and growth was attributed to increased root mass and vigor of commercial cucurbit rootstocks and associated increased water and nutrient uptake despite pathogen infection (Davis et al., 2008; Lee, 1994; Sakata et al., 2007).

To take advantage of grafting, growers also need information regarding fruit maturity in response to rootstock–scion combination and environmental conditions. Modification of hormonal signaling in response to the rootstock–scion combination can influence time of flowering and harvest date of grafted watermelon plants (Aloni et al., 2010; Davis et al., 2008; Pulgar et al., 2000; Sakata et al., 2007; Satoh, 1996). Sakata et al. (2007) reported that watermelon grafted onto bottle gourd (*Lagenaria siceraria*) had early formation of female flowers. In contrast, another study reported a delay in flowering of up to 1 week with watermelon grafted on bottle gourd, resulting in an equal delay in fruit maturity (Davis et al., 2008). Delayed flowering was also observed for watermelon cv. Fujihikari grafted onto pumpkin (*Cucurbita maxima*), wax gourd (*Benincasa hispida*), and interspecific hybrid

squash rootstocks (*C. maxima* × *C. moschata*) compared with nongrafted treatments in the greenhouse (Yamasaki et al., 1994). In regard to fruit quality, most studies have assumed concurrent ripening of fruit from grafted and nongrafted plants, and fruit have been harvested at the same time for all treatments. Hollow heart (placental detachment from the rest of the flesh) and hard seed formation are morphological abnormalities sometimes associated with grafted triploid watermelon and may reflect rootstock–scion incompatibility or adverse environmental conditions or cultural practices (Davis et al., 2008; Lee, 1994; Yamasaki et al., 1994). Increased flesh firmness has been shown in many studies (Bruton et al., 2009; Dabirian et al., 2017; Davis and Perkins-Veazie, 2005; Devi et al., 2020; Kyriacou and Soteriou, 2015; Paroussi et al., 2007), while no change has been reported by some (Alan et al., 2018; Buller et al., 2013; Karaca et al., 2012). Similarly, for lycopene content, grafted watermelon fruit showed increased content compared with nongrafted fruit in some studies (Davis et al., 2008; Proietti et al., 2008), while there was no difference in other studies (Bruton et al., 2009; Dabirian et al., 2017; Soteriou and Kyriacou, 2014; Wimer et al., 2015). Further, a decrease in lycopene content has been reported with certain rootstock–scion combinations involving *L. siceraria* and *C. argyrosperma* (Candir et al., 2013; Davis and Perkins-Veazie, 2005). For total soluble solids (TSS), rootstock–scion combination can affect results (Davis et al., 2008; Flores et al., 2010; Proietti et al., 2008). Most studies did not find a significant effect in TSS for grafted watermelon with bottle gourd rootstocks (Alan et al., 2007; Alexopoulos et al., 2007; Candir et al., 2013; Yetisir and Sari, 2003), however, 0.5% to 1.0% reduction in TSS was reported for grafted compared with nongrafted watermelon treatments in a few studies (Bruton et al., 2009; Davis and Perkins-Veazie, 2005; Kyriacou and Soteriou, 2015; Roupael et al., 2010). Davis and Perkins-Veazie (2005) further reported that TSS was reduced only in the grafted diploid watermelon treatment. Miguel et al. (2004) and Dabirian et al. (2017) found no difference in TSS of fruit from triploid watermelon grafted onto hybrid (*C. maxima* × *C. moschata*) rootstock compared with nongrafted controls. However, elucidating effects of grafting on fruit maturity is difficult

because senescence of leaflet and tendril attached to the fruit pedicel, skin color development, and ground spot formation have not been reported.

Black plastic mulch is commonly used in the production of many vegetable crops including watermelon, to conserve soil moisture, prevent weed emergence, promote early ripening, and prevent fruit rots (Baker et al., 1998; Brown and Channell-Butcher, 1999; Ibarra-Jimenez et al., 2005). Plastic mulch warms the soil, which enhances watermelon growth and yield in areas with a warm growing season (Parmar et al., 2013; Rao et al., 2017). For example, at Mount Vernon, WA, black plastic mulch increased soil temperature by 1.8 °C at a 10-cm depth and doubled the yield of pie pumpkin compared with nonmulched soil (Ghimire et al., 2018). At the same location, Sintim et al. (2019) reported that soil temperature was 1 to 5 °C greater in black plastic mulch treatments compared with a no-mulch treatment early in the growing season, when the plant canopy had not fully developed. Later in the growing season, once the canopy had fully developed and the mulches were shaded, there was no difference in soil temperature. Clear plastic mulch has been shown to increase soil temperature by 2 to 3 °C compared with black plastic mulch in northwest Washington, especially early in the season before the crop canopy covers the mulch (Ghimire et al., 2020; Zhang et al., 2020). Dabirian et al. (2017) similarly found a higher soil temperature with clear compared with black plastic mulch treatments (30 vs. 25 °C, respectively) in Mount Vernon, WA, but there was no difference in watermelon yield between the treatments.

Watermelon is a high-value crop that is well suited for direct markets that are common in western Washington. In the northwestern United States, *V. dahliae* is present in many fields, and soil temperatures are relatively cool during the summer production season (18 to 20 °C at 10-cm depth) (Dabirian et al., 2017; Wimer et al., 2015). In this study, grafting in combination with plastic mulch was assessed for its effects on earlier watermelon fruit production, yield, and fruit quality under conditions of verticillium wilt.

## Materials and Methods

**Experimental location and design.** This study was carried out in the same field in 2018 and 2019 at Washington State University Northwestern Washington Research and Extension Center (WSU NWREC), Mount Vernon, WA (lat. 48°43'24"N, long. 122°39'09"W). The region has a warm Mediterranean climate (Peel et al., 2007), and during the summer growing season (June through September), average temperature is 15 °C, precipitation is 170 mm, and relative humidity (RH) is 80% (20-year average; AgWeatherNet, 2020). The soil type is Skagit silt loam (superactive, nonacid, mesic Fluvaquentic Endoaquepts) (U.S. Department of Agriculture Natural Resources Conservation

Service, 2019), and the field site was certified organic.

The experiment had a split-plot design with three replications of two main plot treatments and five subplot treatments, with 15 plants per subplot in both years. The main plot treatment was mulch (black and clear plastic, both 25.4 µm; Climagro, Delhi, ON, Canada). And the subplot treatment was grafting. Seedless watermelon cv. Secretariat (Sakata Seeds America, Inc., Morgan Hill, CA) was grafted onto four types of commercially available rootstocks: *Lagenaria siceraria* cv. Pelop (Rijk Zwaan, Salinas, CA), *Benincasa hispida* cv. Round (Kitazawa seed co., Oakland, CA), and two interspecific hybrid squash (*C. maxima* × *C. moschata*), cv. Super Shintosa (Syngenta Seeds, Minneapolis, MN), and Tetsukubato (American Takii, Salinas, CA). Nongrafted 'Secretariat' was the control. Beds were 3 m center to center, and spacing was 0.9 m between plants in a single row. The study included a border row on each side both years, plus border plants at the ends of all experiment rows.

**Plant and field preparation.** All plants were grown at WSU Mount Vernon NWREC, and grafting was with the one-cotyledon method following procedures developed for this site (Devi et al., 2020; Miles et al., 2016). Both years, the field was fertilized with 105 kg·ha<sup>-1</sup> of nitrogen, 30 kg·ha<sup>-1</sup> of phosphorus, and 70 kg·ha<sup>-1</sup> of potassium (NutriRich 8N–0.8P–3.3K; Stutzman Environmental Products, Canby, OR). In 2018, supplemental sulfur was applied in the form of sulfate (12% sulfur, Garden Pearls Gypsum; Columbia River Carbonates, Woodland, WA) at the rate of 20 kg·ha<sup>-1</sup> based on soil test recommendations. Both years, fertilizer was applied using a drop spreader over bed centers, then beds were shaped (Rain-Flo 2600; Rain-Flo Irrigation, East Earl, PA), and drip tape (T-Tape model 508-08-340, 20-cm emitter spacing, 4.3 L·min<sup>-1</sup> per 100 m; Rivulis San Diego, CA) was simultaneously laid on the center of each bed and covered with plastic mulch. Beds were 15 cm tall and 90 cm wide. In 2018, all plants were transplanted to the field site on 11 June, and in 2019, transplanting was done on 21 May. Pollenizer 'Wild Card Plus' (Sakata Seeds America, Inc., Morgan Hill, CA) was planted at every third plant on the opposite side of each row both years.

**Soil assays for *V. dahliae*.** Soil samples were collected using a systematic sampling method in an X pattern before transplanting to estimate *V. dahliae* soil population. Samples were collected on 8 May 2018 and 23 Apr. 2019, and also at the end of the growing season on 11 Nov. 2018 and 24 Oct. 2019. At each sampling time, six soil cores, each measuring 4.5 cm diameter and 15 cm deep, were collected. Each soil core was broken into pieces and placed in a paper tray and allowed to air dry in a greenhouse for 1 week. From each of the six dried soil samples, a 1.5-g subsample was placed in a sterile mortar and ground by firmly rotating a sterile pestle in a full circle 10 times, and 1 g was transferred to a salt shaker. To avoid cross

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contamination, mortars, pestles, and salt shakers were sterilized with 70% ethanol between the processing of samples. Each subsample then was carefully dispersed uniformly over 10 plates of Sorensen's NP-10 agar medium (Goud and Termorshuizen, 2003) following the method of Butterfield and DeVay (1977). The plates were then incubated in the dark at ambient temperature ( $\approx 25$  °C) for 4 weeks. The surface of the plates was rinsed under running tap water, and the number of cfu per gram of dry soil was estimated using a dissecting microscope at 40 $\times$  magnification. The colonies of *V. dahliae* were identified by their morphology as described by Goud et al. (2003).

### Data Collection

**Environmental conditions.** Meteorological data were collected throughout the growing season (May through September) by the WSU AgWeatherNet system located 0.5 km from the field site. Mean, minimum, and maximum temperatures; average RH; total solar radiation; and total precipitation were recorded daily during the growing season each year. Soil temperature and volumetric water content were measured every 30 min using data loggers (Hobo Onset, Bourne, MA). Temperature and moisture probes (S-TMB-M002 and S-SMC-M005 respectively; Onset Computer, Corp., Bourne, MA) were placed in the center of both main plots in the second replicate, at 10 cm depth under the plastic mulch and centered between two plants in the center of the bed.

**Verticillium assessments.** Plants were visually observed for characteristic symptoms of verticillium wilt (chlorosis, necrosis, and wilting) both years, and a severity rating was recorded for all plants in each subplot every week from the onset of foliar symptoms. Ratings occurred 58, 65, 72, 79, and 85 DAT, with the first rating on 8 Aug. and the last rating on 4 Sept. in both years. Percent severity was recorded on a per plant basis and the average per plot was calculated on each measurement date. At the end of the field study each year, AUDPC values were calculated from the severity ratings for each treatment to compare disease development among the treatments (Shaner and Finney, 1977) using the formula:

$$\text{AUDPC} = \sum_{i=1}^n \left[ \frac{Y_{i+1} + Y_i}{2} \right] [X_{i+1} - X_i]$$

in which,  $Y_i$  = disease severity (per unit) at the  $i^{\text{th}}$  observation,  $X_i$  = time (days) at the  $i^{\text{th}}$  observation, and  $n$  = total number of observations.

**Flowering and harvest date.** The emergence of the first fully open male and female flower on each plant in each plot was monitored between 9:00 AM and 11:00 AM 3 d per week (Monday, Wednesday, and Friday) both years. The first flower date was recorded for each plant, and the average was calculated for each plot. Fruit maturity was monitored in each plot 3 d per week (Monday, Wednesday,

and Friday) for several weeks leading up to first harvest. Each fruit was tagged, and the date recorded when both the leaflet and tendril attached to the fruit pedicel were completely dry (fruit considered to be physiologically mature) (Georgia Vegetable Team, 2000). At least three fruit were harvested per plot for each maturity date: 0, 7, and 14 d after the leaflet and tendril were dry.

**Fruit yield and quality.** For each harvest date both years, the number and weight of harvested fruit were recorded for each plant per plot and total yield was calculated for each treatment. Three representative fruit per plot were randomly selected at each harvest date to assess fruit internal ripeness characteristics (fruit quality). Each fruit was cut in half longitudinally, and each half was rated for hollow heart based on a 0 to 5 scale, where 0 = no visible cracking, marketable; 1 = <6 mm cracking in one direction, marketable; 2 = 6 to 13 mm cracking in a single or multiple directions, marketable; 3 = 13 to 25 mm cracking in one or multiple directions, not marketable; 4 = 25 to 38 mm cracking in multiple directions, not marketable; and 5 = >38 mm cracking with center cavity of fruit exposed, not marketable. Each fruit was then quartered, and the number of black hard seeds were counted for the exposed fruit surfaces in all four quarters of each fruit. From one quarter, a 25-cm<sup>3</sup> sample was taken from the center and flesh firmness (reported as Newton, N) was measured to a depth of 1 cm with a penetrometer (FR-5120, range of the gauge 0.05–196.10 N; QA Supplies LLC, Norfolk, VA) equipped with a 4-mm diameter cylindrical blunt-end tip mounted on a drill-press. Juice from the sample was squeezed onto a digital refractometer (MISCO, Cleveland, OH) to determine total soluble solids content (% measured as °Brix).

For lycopene analysis, 50 cm<sup>3</sup> was removed from the center of each fruit, and held in plastic bags at  $-20$  °C until used for the measurement. Total lycopene was measured in 2018 from frozen samples at the Plants for Human Health Institute, North Carolina State University, Kannapolis, NC, following the method of Davis et al. (2003). Frozen samples were shipped overnight with dry ice. Samples were thawed, placed in a vial and ground using a cell and tissue homogenizer (Geno/Grinder; Spex SamplePrep, Metuchen, NJ). Five grams of the ground puree was diluted with 15 mL of distilled water (wt/vol), and tubes were vortexed for 1 min. The mixture was then placed in a 20-mL glass cuvette and the absorbance (A) recorded at 560 and 700 nm using a colorimeter (Ultra-Scan PRO Spectrophotometer; Hunter Associate Laboratory Inc., Reston, VA). Total lycopene was calculated using the following formula:

$$\begin{aligned} \text{Total lycopene } (\mu\text{g}\cdot\text{g}^{-1}) \\ = (A560 - A700)28 \times 4, \end{aligned}$$

where A560 represents the lycopene peak, A700 represents light scatter, 4 is the dilution factor, and 28 is the slope.

In 2019, total lycopene content was determined by spectrophotometer at WSU NWREC following the method of Buller et al. (2013), modified from Nagata and Yamashita (1992). In the modified method, 2:3 acetone:hexane solution was prepared 1 d before the analysis and kept in the refrigerator (4 °C), whereas in the original procedure, the solution was used immediately. The tubes were covered using aluminium foil in the modified procedure, whereas in the original method, tubes were not covered. After adding cold acetone:hexane solution to watermelon samples on the day of analysis in the modified procedure, the tubes were kept in the freezer ( $-20$  °C) for 1 h, whereas in the original method, the analysis was done immediately. The following is a summary of the method used in the current study. Samples were removed from the freezer and kept at room temperature (23 °C) for 10 min, then homogenized in a blender (Magic Bullet; Homeland Housewares, Pacoima, CA). Three 1-g puree subsamples were placed in separate plastic centrifuge tubes wrapped in aluminium foil, and 16 mL of a cold high-performance liquid chromatography grade 2:3 acetone:hexane solution was added to each tube. The tubes were agitated by hand for 1 min for thorough mixing, then returned back to the freezer at  $-20$  °C for 1.5 h. Tubes were removed from the freezer and left to rest for 5 min to separate the hexane layer from the rest of the mixture. Three mL of the hexane layer was placed in a glass cuvette and absorbance determined with a spectrophotometer (ultraviolet-VIS spectrophotometer ultraviolet-1280; Shimadzu Scientific Instruments, Inc., Columbia, MD). Absorbance at 453, 505, 645, and 663 nm was recorded and total lycopene was calculated using the following formula:

$$\begin{aligned} \text{Lycopene } (\mu\text{g}/\text{g FW sample}) \\ = [(-0.0458 \times A663) + (0.204 \times A645) \\ + (0.372 \times A505) - (0.0806 \times A453)] \\ \times [10/0.1042], \end{aligned}$$

where 10/0.1042 is the extinction coefficient for lycopene in hexane [adapted for  $\mu\text{g}/\text{g}$  sample fresh weight (FW); in the original method the formula was for mg/100 mL of extract].

**Statistical analysis.** All data were analyzed using JMP software (Version 14.0.0 for Windows; SAS Institute, Cary, NC). The main plot and subplot treatments were explanatory variables, and flowering, fruit yield, fruit quality, and verticillium wilt severity were response variables. Data for all parameters were tested for normality using Shapiro-Wilk test and analyzed using analysis of variance. When significant effects were detected, means were discriminated using Tukey's honestly significant difference at significance level  $P < 0.05$ . AUDPC values in 2018 and 2019 did not satisfy normality assumptions and were analyzed using nonparametric Wilcoxon test.

### Results

**Environmental and soil conditions.** Environmental conditions were similar both years.

The average air temperature was 16 °C during the 2018 growing season (June through September) and 15 °C during the 2019 growing season (May through September) (Table 1). Average minimum temperature

was 10.5 °C, and average maximum temperature was 21.5 °C for the 2 years. Average daily RH was 75% in 2018 and 64% in 2019. Although total precipitation was 78 mm in 2018 and 234 mm in 2019, 140 mm of

rainfall fell on 9 Sept. 2019, 3 d before harvest. Total solar radiation was 2207 MJ·m<sup>-2</sup> in 2018 and 2018 MJ·m<sup>-2</sup> in 2019. Average soil temperature at 10-cm depth under black plastic mulch was 25 °C, which was 3 °C lower than under clear plastic mulch (28 °C), and both were 7 to 10 °C greater than the 2-year average bare ground soil temperature (18.5 °C). Average soil moisture under clear plastic mulch (0.21 cm<sup>3</sup>·cm<sup>-3</sup>) was slightly lower throughout the growing season than under black plastic mulch (0.24 cm<sup>3</sup>·cm<sup>-3</sup>) (Table 1).

*Verticillium assessments.* In 2018, *V. dahliae* soil population density was 5 cfu/g at transplanting and 10 cfu/g after harvest. In 2019, the soil population of *V. dahliae* was 15 cfu/g at the time of transplanting and 21 cfu/g after harvest. There was no significant difference in AUDPC value due to mulch treatment either year ( $P = 0.15$  and  $P = 0.09$ , respectively) (Table 2). In both years, the overall AUDPC value for verticillium wilt was more than 3 times greater for nongrafted ‘Secretariat’ watermelon (683 and 802 in 2018 and 2019, respectively) than for the four grafted treatments (on average 217 and 236 in 2018 and 2019, respectively) ( $P < 0.0001$  both years) (Table 2). There was also no interaction between mulch treatment and grafting ( $P = 0.66$ ), nor between year, mulch, and grafting treatment ( $P = 0.78$ ) (Table 3).

*Flowering and harvest date.* Male flowers first appeared 58 DAT on average for all treatments in both 2018 and 2019, and was not different for mulch ( $P = 0.30$ ), grafting treatment ( $P = 0.80$ ), or year ( $P = 0.64$ ) (Table 2). Appearance of first female flower was unaffected by mulch treatment ( $P = 1.00$ ) or year ( $P = 0.80$ ). Grafting treatment did affect female flowering ( $P = 0.02$ ), and on average was earliest for ‘Secretariat’ grafted onto ‘Tetsukabuto’ (49 DAT), followed by ‘Super Shintosa’, ‘Pelop’, and nongrafted (55 DAT), and latest for ‘Secretariat’ grafted to ‘Round’ (61 DAT) (Table 2). There were no interactions among mulch, grafting, and year for male or female flowering (Table 3).

Table 1. Environmental and soil conditions during the growing season at Mount Vernon, WA, in 2018 and 2019.

Environmental parameters <sup>z</sup>	2018 <sup>y</sup>		2019	
Average daily air temperature (°C)	16		15	
Average daily max air temperature (°C)	22		21	
Average daily min air temperature (°C)	10		11	
Total solar radiation (MJ·m <sup>-2</sup> )	2207		2018	
Relative humidity (%)	75		64	
Total rainfall (mm)	78		234 <sup>x</sup>	
Wind speed (kph)	5.3		5.4	
	Black <sup>w</sup>	Clear	Black	Clear
Soil temperature (°C) <sup>v</sup>	28	31	23	25
Volumetric water content (cm <sup>3</sup> ·cm <sup>-3</sup> ) <sup>v</sup>	0.22	0.19	0.25	0.23

<sup>z</sup>Data from Washington State University Ag WeatherNet Station located ≈0.5 km from the field site.

<sup>y</sup>2018 growing season was 11 June to 11 Sept. 2019 growing season was 21 May to 12 Sept.

<sup>x</sup>On 9 Sept. 2019 there was 140 mm of rainfall, 3 d before harvest.

<sup>w</sup>Black and clear plastic mulch both years was 25.4 μm (Climagro, Delhi, ON, Canada).

<sup>v</sup>Measured every 30 min at 10 cm depth with data loggers (Hobo Onset, Bourne, MA) and averaged for the growing season.

Table 2. Area under the disease progress curve (AUDPC) values for severity of verticillium wilt in 2018 and 2019, and days after transplanting (DAT) for appearance of first male and female flower (years combined) of watermelon grown with black and clear plastic mulch (grafted and nongrafted combined) and for grafting treatments at Mount Vernon, WA.

Treatment <sup>z</sup>	AUDPC value <sup>y</sup>		First flowering (DAT)	
	2018	2019	Male	Female
Black mulch	445	467	60	55
Clear mulch	345	387	59	55
<i>P</i> value	0.15	0.09	0.80	1.00
Secretariat (control)	683 a <sup>x</sup>	802 a	59	54 bc
S/Tetsukabuto	183 b	202 b	58	49 c
S/Super Shintosa	187 b	220 b	56	56 b
S/Pelop	198 b	200 b	57	55 abc
S/Round	298 b	323 b	60	61 a
<i>P</i> value	<0.0001	<0.0001	0.60	0.02

<sup>z</sup>Black and clear plastic mulch both years was 25.4 μm (Climagro, Delhi, ON, Canada). All grafted plants had watermelon cv. Secretariat (S) as the scion and are denoted as scion/rootstock.

<sup>y</sup>Disease severity was recorded as the percent of the plot canopy displaying verticillium wilt visual symptoms (chlorosis, necrosis, and wilting); each year AUDPC values were calculated based on four rating dates, from the onset of disease symptoms and each week thereafter.

<sup>x</sup>Means with the same letter within a column are not significantly different at  $P < 0.05$ ; means were discriminated using Tukey’s honestly significant difference.

Table 3. Interactions (*P* values) from analysis of variance of the main factors “mulch,” “grafting,” “day of harvest,” and “year” for the parameters measured for grafted and nongrafted watermelon in 2018 and 2019.

	AUDPC value <sup>z</sup>	First female flowering		Days to first harvest			
		Male	Female				
Mulch × grafting	0.66	0.22	0.45	0.59			
Mulch × year	0.27	0.73	0.25	0.08			
Grafting × year	0.41	0.69	0.39	0.16			
Mulch × grafting × year	0.78	0.61	0.06	0.09			
	Number of fruit	Fruit weight (kg)	Hollow heart (1–5) <sup>y</sup>	Number of hard seed	Firmness (N)	TSS (%)	Lycopene (μg·g <sup>-1</sup> )
Mulch × grafting	0.61	0.22	0.62	0.52	0.09	0.28	0.34
Mulch × harvest	0.88	0.46	0.32	0.28	0.11	0.06	0.20
Grafting × harvest	0.09	0.07	0.27	0.69	0.48	0.71	0.52
Mulch × grafting × harvest × year	0.12	0.30	0.53	0.07	0.08	0.56	0.08

<sup>z</sup>Disease severity was recorded as the percent of the plot canopy displaying verticillium wilt visual symptoms (chlorosis, necrosis, and wilting); each year area under the disease progress curve (AUDPC) values were calculated based on four rating dates, from the onset of disease symptoms and each week thereafter.

<sup>y</sup>Hollow heart rating on a 0 to 5 scale, where 0 = no visible cracking; 1 = <6 mm cracking in one direction, marketable; 2 = 6 to 13 mm cracking in a single or multiple directions, marketable; 3 = 13 to 25 mm cracking in one or multiple directions, not marketable; 4 = 25 to 38 mm cracking in multiple directions, not marketable; and 5 = >38 mm cracking with center cavity of fruit exposed, not marketable.

Days to first harvest (0 d), when both the leaflet and tendril attached to the fruit pedicel were completely dry, was on 11 Sept. 2018 (92 DAT) and 12 Sept. 2019 (114 DAT), and did not differ due to mulch or grafting treatments ( $P = 0.78$  and  $P = 0.45$ , respectively). There were no interactions among year, mulch, and grafting (Table 3). Second harvest (7 d) was at 99 DAT in 2018 and 121 DAT in 2019, and third harvest (14 d) was at 106 DAT in 2018 and 128 DAT in 2019.

**Fruit yield and quality.** There were no differences in average number of fruit and

fruit weight due to year, harvest date, or mulch ( $P \geq 0.24$ ) (Table 4), nor were there any interactions (Table 3). In contrast, grafting treatment affected total fruit number and weight in both years ( $P < 0.0001$  and  $P = 0.002$ , respectively) (Table 5). The average total number of fruit per plant over all three harvest dates was 2.1 and ranged from 0.7 (nongrafted ‘Secretariat’) to 3.7 (‘Secretariat’ grafted onto ‘Super Shintosa’ and ‘Tetsukabuto’). The average total fruit weight over all three harvest dates was 9.4 kg per plant and ranged from 3.2 kg (nongrafted ‘Secretariat’), to 5.3 kg (‘Secretariat’ grafted onto ‘Round’), to 14.8 kg (‘Secretariat’ grafted onto ‘Super Shintosa’).

For fruit quality characteristics measured, no differences were found for year except for lycopene, and there were no interactions among treatment, harvest date, and year (Table 3). Although lycopene content differed by year ( $P < 0.0001$ ), there were no differences due to harvest date ( $P = 0.48$  and  $P = 0.70$ , respectively), mulch ( $P = 0.92$  and  $P = 0.41$ , respectively), or grafting treatment ( $P = 0.99$  and  $P = 0.39$ , respectively) either year. The overall mean lycopene content in 2018 was  $52.44 \mu\text{g}\cdot\text{g}^{-1}$  and was  $32.51 \mu\text{g}\cdot\text{g}^{-1}$  in 2019 (Table 6), a 38% difference. Hollow heart occurred on all harvest dates with no differences due to mulch ( $P = 0.42$ ) or grafting treatment ( $P = 0.33$ ). Overall average hollow heart rating was 3.2 (range 13–25 mm cracking) ( $P = 0.82$ ) (Table 6). The number of hard seeds due to harvest date ( $P = 0.53$ ), mulch ( $P = 0.61$ ), and grafting treatment ( $P = 0.23$ ) was not different, with an overall average number of six hard seeds per fruit (Table 6). Watermelon fruit firmness did

not differ due to harvest date ( $P = 0.07$ ) or mulch treatment ( $P = 0.45$ ) but was different due to grafting treatment ( $P < 0.0001$ ) in both years. Watermelon grafted onto ‘Super Shintosa’ rootstock had the firmest flesh (6.7 N) and nongrafted watermelon had the lowest firmness (4.3 N), while the other treatments were intermediate (5.7 N on average) (Table 5). TSS was similar both years (11.2% on average) and did not differ due to harvest date ( $P = 0.09$ ), mulch ( $P = 0.32$ ), or grafting treatment ( $P = 0.08$ ) (Table 6).

## Discussion and Conclusions

In this study, the severity of verticillium wilt was unaffected by use of black or clear plastic mulch. These results differ from those of Dabirian et al. (2017), who used the same field and found that plants grown with black plastic mulch had AUDPC values  $\approx 2$  times greater for verticillium wilt than plants grown with clear plastic mulch. In both studies, the average soil temperature under black plastic mulch (25 °C) was 3 to 5 °C lower than under clear plastic mulch (28 to 30 °C). This differences in disease severity among studies could be due to differences in *V. dahliae* soil populations in each study. For example, at the time of planting, *V. dahliae* soil populations was 5 to 15 cfu/g in the current study and was 28 cfu/g in the study by Dabirian et al. (2017). Other studies also reported that verticillium wilt and fusarium wilt (caused by *Fusarium oxysporum*) on cucumber (*Cucumis sativus*), tomato (*Solanum lycopersicum*), and strawberry (*Fragaria ananassa*) can be successfully managed by using clear plastic mulch (Ashworth and Gaona, 1982).

Verticillium wilt severity was 3.5 times less on grafted than on nongrafted ‘Secretariat’ plants, and rootstocks ‘Super Shintosa’, ‘Tetsukabuto’, ‘Pelop’, and ‘Round’ were equally effective in this study (227 AUDPC value on average). These results are similar to previous studies at the same experimental site where different watermelon scion cultivars (Crisp n Sweet, Sugar Baby, and TriX Palomar) grafted with different rootstocks cultivars (Emphasis, Just, Strongtosa, Super Shintosa, Tetsukabuto) also exhibited less disease than nongrafted watermelon plants (Buller et al., 2013; Dabirian et al., 2017; Wimer et al., 2015). Similarly, in Thessaloniki, Greece, Paroussi et al. (2007) reported that ‘Crimson Sweet’ grafted on ‘Mamouth’, ‘Dako’, and ‘Calago’ plants inoculated with  $10^6$  *V. dahliae* conidia per milliliter had infection of 20% to 37% compared with 87% infection for nongrafted plants. In contrast, Attavar et al. (2020) found no difference in disease severity between nongrafted ‘Secretariat’ and ‘Secretariat’ grafted onto ‘Tetsukabuto’ and 14 cucurbit germplasm accessions when *V. dahliae* population was 7.8 cfu/g soil and plants were inoculated with 104 cfu of *V. dahliae* microsclerotia at transplanting. Although it is not possible to directly compare inoculum levels between the two studies, the colony-forming unit count may underestimate the number of viable

Table 4. Average watermelon fruit number and weight (kilograms) per plant for watermelon cv. Secretariat combined for nongrafted and grafted (rootstock cvs. Tetsukabuto, Super Shintosa, Pelop, and Round) treatments, at three harvest dates (0, 7, and 14 d after physiological maturity; both the leaflet and tendril attached to the fruit pedicel were completely dry) and two plastic mulch types (black and clear) at Mount Vernon, WA, in 2018 and 2019.

Treatment	Number of fruit		Fruit wt (kg)	
	2018	2019	2018	2019
0 d	2.2	1.8	11.3	9.1
7 d	2.1	1.8	12.2	11.7
14 d	1.9	2.4	10.5	7.4
<i>P</i> value	0.32	0.25	0.56	0.24
Black <sup>z</sup>	2.3	2.1	11.4	10.3
Clear	2.2	1.9	10.6	8.5
<i>P</i> value	0.75	0.55	0.45	0.37

<sup>z</sup>Black and clear plastic mulch both years was 25.4  $\mu\text{m}$  (Climagro, Delhi, ON, Canada).

Table 5. Average fruit number and weight (kilograms) per plant and fruit firmness (Newton, N) of watermelon cv. Secretariat nongrafted and grafted onto four rootstock (cvs. Tetsukabuto, Super Shintosa, Pelop, and Round at Mount Vernon, WA, in 2018 and 2019 (years combined).

Treatment <sup>z</sup>	Number of fruit	Fruit wt (kg)	Fruit firmness (N)
Secretariat (control)	0.7 b <sup>y</sup>	3.2 c	4.3 c
S/Tetsukabuto	3.7 a	13.1 ab	6.1 ab
S/Super Shintosa	3.7 a	14.8 a	6.7 a
S/Pelop	1.2 b	10.6 b	5.7 b
S/Round	1.3 b	5.3 c	5.2 b
<i>P</i> value	<0.0001	0.002	<0.0001

<sup>z</sup>All grafted plants had watermelon cv. Secretariat (S) as the scion and are denoted as scion–rootstock.

<sup>y</sup>Means with the same letter within a column are not significantly different at  $P < 0.05$ ; means were discriminated using Tukey’s honestly significant difference.

Table 6. Mean lycopene ( $\mu\text{g}\cdot\text{g}^{-1}$ ), hollow heart rating, hard seed count, fruit firmness (Newton, N), and total soluble solids (TSS, %) per watermelon fruit at three harvest dates, 0, 7, and 14 d after physiological maturity (both the leaflet and tendril attached to the fruit pedicel were completely dry) at Mount Vernon, WA, in 2018 and 2019 (years combined).

Harvest	Lycopene ( $\mu\text{g}\cdot\text{g}^{-1}$ )		Hollow heart (1–5) <sup>y</sup>	Number of hard seed	Fruit firmness (N)	TSS (%)
	2018 <sup>z</sup>	2019				
0 d	51.70	33.10	4.0	6	6.2	11.30
7 d	52.50	31.80	2.8	4	7.0	10.98
14 d	53.13	32.63	3.0	8	6.8	11.22
<i>P</i> value	0.49	0.70	0.82	0.45	0.07	0.09

<sup>z</sup>Lycopene analysis method in 2018 was (Davis et al., 2003) and in 2019 was modified from Nagata and Yamashita (1992).

<sup>y</sup>Hollow heart rating on a 0 to 5 scale, where 0 = no visible cracking; 1 = <6 mm cracking in one direction, marketable; 2 = 6 to 13 mm cracking in a single or multiple directions, marketable; 3 = 13 to 25 mm cracking in one or multiple directions, not marketable; 4 = 25 to 38 mm cracking in multiple directions, not marketable; and 5 = >38 mm cracking with center cavity of fruit exposed, not marketable.

conidia by 50% (Jaronski and Liebmann, 2000).

Grafting 'Secretariat' onto 'Round' (bottle gourd) tended to delay female flowering by 7 d compared with nongrafted plants, and the delay was 11 to 14 d compared with grafting with 'Tetsukabuto' (interspecific hybrid squash). A similar result for interspecific hybrid squash rootstock was reported by Yamasaki et al. (1994). In contrast, Sakata et al. (2007) found earlier female flowering occurred when watermelon was grafted to bottle gourd rootstock. The differences in flower initiation response could be due to hormonal signaling combined with environmental affects that occur during graft union formation. For example, cytokinin is higher in grafted plants than nongrafted plants and could potentially have an effect on flowering time (Aloni et al., 2010; Kyriacou et al., 2017).

In the current study fruit from all treatments reached physiological maturity (senescence of leaflet and tendril attached to the fruit pedicel) at the same time in both years. A similar result was found in a study in Hermiston, OR, where first flowering was delayed 2 d for 'Secretariat' grafted with 'Shintosa Camelforce' and 'Tetsukabuto', but fruit reached physiological maturity at the same time as nongrafted plants (Devi et al., 2020). Although Attavar et al. (2020) reported that grafted treatments tended to flower at the same time as nongrafted 'Secretariat', a comparison of days to first harvest could not be made due to premature death of nongrafted plants from verticillium wilt. For nonclimacteric fruit like watermelon, fruit maturity at harvest has a major impact on fruit quality characteristics (Kader, 2008; Kyriacou et al., 2017). Reliable external indicators of fruit physiological maturity are needed to harvest fruit when quality is optimal, and further studies are needed to determine whether these external indicators apply to fruit of nongrafted as well as grafted plants. Further studies are also needed to assess more watermelon and rootstock cultivar combinations in a diversity of environments to test the reliability of these external maturity indicators.

Grafting can increase watermelon yield due to soilborne-disease resistance (Davis et al., 2008; King et al., 2008; Kyriacou et al., 2017). In the current study, a 3.4-fold increase in fruit weight per plant was achieved using grafted plants (Table 1) when *V. dahliae* soil population was 5 to 15 cfu/g at the time of planting. Dabirian et al. (2017) found a 1.8-fold increase in fruit weight per plant when *V. dahliae* soil population was 28 cfu/g at the time of planting and scion-rootstock combinations were 'TriX Palomar' grafted with 'Super Shintosa', 'Tetsukabuto', and 'Just'. Similarly, Wimer et al. (2015) reported a 2.2-fold increase in fruit weight per plant when *V. dahliae* population was 18.0 cfu/g soil of at the time of planting and 'Sugar Baby' was grafted with 'Tetsukabuto'. Further, Paroussi et al. (2007) reported that yield of 'Crimson Sweet' grafted to

'Mamouth' rootstock had a 2.9-fold increase in weight per plant following plant inoculation with  $10^6$  conidia of *V. dahliae* per milliliter. In contrast, at field sites in eastern Washington where the *V. dahliae* soil population density was low (1 to 5 cfu/g at the time of planting), fruit yield was similar for grafted and nongrafted treatments (Dabirian et al., 2017; Wimer et al., 2015). Although the threshold for watermelon is not known, these results indicate that grafting can increase watermelon productivity when *V. dahliae* soil population is above 5 *V. dahliae* cfu/g of soil.

Quality of grafted fruit must be equal to or better than that of nongrafted watermelon to ensure market acceptability (Bruton et al., 2009; Colla et al., 2010; Proietti et al., 2008). Although some studies have found grafting to have a negative impact on TSS and lycopene (Alexopoulos et al., 2007; Davis et al., 2008; Lee and Oda, 2003; López-Galarza et al., 2004), the current study did not show any differences between grafted and nongrafted watermelon fruit. Difference in lycopene content due to year in this study was likely due to the two methods that were used for the analysis each year. The potential cause may be the grinding of the sample in 2018, which results in small particles that aid lycopene extraction. Flesh firmness in the current study was greater for grafted 'Secretariat' than for the nongrafted treatment, as has been reported in numerous studies with other watermelon scions (Bruton et al., 2009; Buller et al., 2013; Dabirian et al., 2017; Davis and Perkins-Veazie, 2005; Devi et al., 2020; Kyriacou and Soteriou, 2015; Wimer et al., 2015; Yetisir et al., 2003). Increased flesh firmness in grafted watermelon could be due to higher cell density (Fallik and Ziv, 2020; Soteriou et al., 2017) and is a positive enhancement of fruit quality because firm fruit have an extended shelf life (Bruton et al., 2009; Saftner et al., 2006). However, in the current study, the overall hollow heart formation was high (13–25 mm cracking) for all treatments in both years, which is considered unmarketable. Although there is no definitive cause of hollow heart formation, studies suggest that multiple factors such as watermelon genetics, pollination/pollen viability, flowering time, and environmental stress such as cold night temperature can cause this physiological disorder, especially in seedless watermelon in the United States (Guan, 2018; Johnson, 2014, 2015). At the study site, night temperature was 10.5 °C on average, which could account for the high incidence of hollow heart.

In conclusion, plastic mulch type (black and clear) did not have an impact on verticillium wilt severity when *V. dahliae* soil population was 5 to 15 cfu/g, nor was there an effect on fruit yield or quality. Grafting did decrease disease severity and increase fruit yield compared with the nongrafted treatment under this *V. dahliae* soil population density, and 5 *V. dahliae* cfu/g of soil may be the minimum level for impact on watermelon fruit yield. Further research is needed to

better understand the yield potential of watermelon under different *V. dahliae* soil populations under different climatic conditions. Grafting increased fruit firmness but did not alter other fruit quality attributes. However, fruit maturity indices are needed to compare results across studies that have different scion and rootstock cultivar combinations and varying environmental conditions. Consistent with other studies, grafted watermelon has the potential to increase watermelon yield in the United States when there is disease pressure from *V. dahliae*.

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