Grafting to Interspecific Rootstocks Increases Fruit Size and Yield of Cantaloupe, *Cucumis melo*

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Introduction

Grafting of the Cucurbitaceae crop family originated in Japan during the 1920s (Edelstein 2017). Growers began grafting cantaloupe melons (*Cucumis melo*) in 1931 for potential yield increases and disease resistance. Since then, grafting of greenhouse melons has become a standard growing practice in many countries. Documented benefits of grafting include enhanced plant vigor, disease resistance, low temperature tolerance and improved nutrient and water absorption (Davis et al. 2008). The adoption of melon grafting has been slow in the United States. This is likely due to cantaloupe crops being primarily field grown in Southeastern US states where melon growing conditions are ideal. Conversely, farmers in New England have had limited success growing this potentially high value crop primarily due to the abundance of soil-borne pathogens and cooler temperatures (Ohletz & Loy, 2020).

While much research has been conducted on the usefulness of grafting melons within greenhouse systems (Rouphael, C. 2012), few studies have focused on field conditions of the Northeast United States. Grafted plants require additional labor, knowledge, cost, and more intensive care of individual plants than are required for non-grafted plants. Yet, the practice may be worthwhile due to the potentially dramatic yield increases within less optimal growing conditions.

Under sub-optimal melon growing conditions, grafting has been shown to boost productivity (Okimura et al. 1986; Yetisir and Sari 2004). The mechanism by which the interspecific rootstocks promote growth of the melon scion remains unclear. Researchers in the Loy lab had observed increased vegetative growth of grafted plants but had not previously quantified this effect. Other researchers have documented physiological effects of grafting including increased nutrient absorption under abiotic stressors such as soil salinity and low temperatures (Pulgar et al., 2000; Nie and Chen, 2000) as well as enhanced resistance to soil borne pathogens (Davis et al. 2008). Ohletz and Loy (2020) have found that the rootstock used, *Cucurbita maxima x Cucurbita moschata,* is able to withstand a wider range of temperatures than non-grafted plants. Additionally, grafting may increase cytokinin production, a plant hormone synthesized primarily in the roots known to influence vegetative growth (Kato and Lou, 1989). Interspecific rootstocks are likely to produce a larger more robust root system which can support a larger plant canopy than non-grafted melon plants (Bertucci et al., 2018). Melon scions grafted to interspecific rootstocks with a larger, more robust plant canopy may be able to sustain a larger fruit load than non-grafted plants.

More knowledge on the effects of grafting is essential before farmers adopt this potentially beneficial practice. Growers need a better understanding of the costs vs. benefits of grafting and how grafting may affect their yields, fruit size, and fruit quality. The objectives of this research study were to compare vegetative growth between grafted and non-grafted melon plants and to quantify the effects of grafting on yield, fruit size, and soluble solids content among three commercial varieties of cantaloupe.

Materials and Methods

Plant Materials. The interspecific rootstock used was an unreleased F1 rootstock *Cucurbita maxima x Cucurbita moschata*, named IS 1349, created through Dr. Brent Loy's Cucurbit breeding program at the University of New Hampshire and the New Hampshire Agriculture Experiment Station. Three F1 scion cultivars were compared: 'True Love' (High Mowing Seed Company, Wolcott VT), 'Sugar Rush' and 'Sugar Cube' (Seneca Vegetable Research, Geneva NY). Previous researchers in the Loy lab confirmed the grafting compatibility of rootstock and scion cultivars. The scion seeds were sown on 5 May 2019. Both the rootstock and non-grafted plants were sown on 7 May 2019. All seeds were planted in 50-cell plug trays containing soilless growing media (Pro-Mix BX). The seeds were put in a Conviron PGR15 growing chamber to promote germination and kept under a 16-hour photoperiod at 25°C during the lighted period and 22°C while dark.

Grafting technique. Grafting was performed at the Macfarlane Research Greenhouses in Durham, NH on 15 May 2019, using the One Cotyledon Grafting (OCG) method. Grafting was performed after both the scion seedlings and rootstock seedlings displayed their first true leaf. The OCG method was achieved by using a razor blade to remove one of the rootstock's cotyledons with a 45° cut, while also removing its apical and lateral meristematic tissue. The scion seedlings were cut approximately 1.5 cm below the cotyledon (Ohletz & Loy, 2020). The rootstock and scion were then fused using plastic grafting clips. The grafted seedlings were placed in a Conviron PGR15 growing chamber to heal. The seedlings were kept under a 16-hour photoperiod with florescent lighting at ~250 µmol $m^{-2}s^{-1}$ PPF. The chamber temperature was set to 25°C during the lighted period and 22°C while dark. Clear dome lids were put over the trays and hand misted as needed in order to maintain a relative humidity of ~95%. After three days, the clear lids were removed for two hours at a time to begin plant acclimation. Four days after grafting, the plants were removed from the healing chamber and kept in a temperature-controlled greenhouse (25°C day and 22°C night) with natural sunlight for two weeks until transplanting.

Experimental site description. The field experiment took place at Kingman Horticultural Research Farm in Madbury, New Hampshire from June 2019 to September 2019. All plants were transplanted on 3 June 2019, three weeks after grafting. The soil type of the research plot is Charlton fine sandy loam. Nitrogen and potassium were applied to the field at a rate of 100.9 kg/ha during bed preparations. Soil tests showed that phosphorous levels were already sufficient. Plants were grown in raised black plastic covered beds (0.61 m width x 24.4 m long x 0.15 m high) with drip irrigation. Standard cultivation practices were used to control pests and disease.

Data collection. The number of leaves, both on the main stem and in total, as well as the number of lateral branches on every plant were counted weekly and recorded throughout the month of June. Fertilized flowers were tagged with their pollination dates. Ripe fruits were harvested from the research plot three times per week, when their stems easily slipped from the fruit. Each fruit was then weighed. The soluble solids content (SSC) for every harvested melon was recorded by taking two core samples and squeezing a small sample of the fruit's juice on a refractometer (RHB-32 Handheld Refractometer, Westover Scientific) then averaging the two samples. Observations on netting and any superficial quality issues were also recorded.

Experimental Design and Analysis. The field study was organized in a split plot design with four replicates of grafted vs. non-grafted as the main plot and variety as sub-plots. This experimental design was chosen to limit competition effects of the more vigorous grafted plants. Each block included either 7 grafted plants or 9 non-grafted plants. Within each block there was 0.5 m spacing between individual non-grafted plants and 0.75 m spacing between individual grafted plants. The additional space provided to grafted plant was due to previous findings that grafting may increase vegetative vigor (Tarchoum et al. 2005; Yang et al. 2006). A spacing of 2.74 m was used in between replicate blocks. Guard rows were placed at both outer rows of the field to limit bias from additional space and sunlight. Data were analyzed using the statistical software, JMP Pro 13 (SAS Institute, Cary NC). Analyses of variance were conducted to reveal the impacts of grafting and scion variety on yield, fruit size, SSC, and vegetative growth parameters. Interactions between the cultivars and grafting were also analyzed and wherever a P value <0.05 was obtained, Tukey's HSD was performed to sort out treatment effects.

Results and Discussion

Vegetative growth. Grafting increased vegetative growth in all three scion varieties. Two weeks after transplanting on June 18, all three varieties had approximately 90% more leaves on the grafted plants compared to non-grafted (Table 3). This was likely due to the interspecific rootstock having a larger, more vigorous root system which was able to uptake more water and nutrients, however, it could also be due to the increased space provided to grafted plants. Grafted plants of both 'Sugar Rush' and 'Sugar Cube' produced higher numbers of lateral branches than their non-grafted counterparts throughout the month of June. Grafted 'True Love' plants displayed more lateral branches than non-grafted plants on June 18, however, by June 28 the two did not differ (Table 3). This may have been caused by the grafted rootstock's improved tolerance of the cool soil temperatures experienced during early June. This finding helped confirm previous field observations of the larger, more robust canopy observed on grafted melon plants compared to non-grafted plants.

Fruit Size. Variety and grafting interacted to affect fruit size (Table 2). While the variety played a role in fruit size, grafting increased the average weight of all three varieties. True Love had the largest sized fruit while Sugar Cube had the smallest and Sugar Rush was in the middle. The weight per fruit when grafted increased on average by 40.9% in True Love melons, 25.8% in Sugar Cube melons and 17.6% in Sugar Rush melons (Table 1). Research conducted by Lee and Oda (2003), Condoruso et al. (2012) and Verzara et al. (2014) noted that grafting may affect fruit size differently among varying melon cultivars. These findings are important for growers seeking to obtain melons in a finely defined size class.

Yield. Grafting increased yields in all three scion melon varieties. Sugar Cube yields had an impressive 123% increase in total yield. The increase in yield was likely enabled by the enhanced photosynthetic capacity of the larger plant canopy observed on the grafted plants. The plot yields were converted into yield in kilograms per hectare. On average, non-grafted Sugar Cube plants produced 4,889 kg/ha, whereas grafted plants produced 10,919 kg/ha. In Sugar Rush, non-grafted plants yielded 6,519 kg/ha compared to 8,904 kg/ha when grafted. Non-grafted True Love plants produced 6,845 kg/ha whereas grafted plants yielded 9,052 kg/ha (Table 1). These yield increases are even more impressive since grafting blocks contained two less plants than non-grafted blocks. Reducing the number of plants per hectare is a way for growers to offset the increased costs of growing grafted melon plants.

Fruit Quality. Soluble solids content (SSC) was used as a measure of fruit quality, due to its high correlation with the approximate sugar level of fruit flesh. The commercial standard for SSC within cantaloupes is a minimum of 9 ºBrix to be marketable, and values of 11 ºBrix and higher are considered gourmet (Suslow, 1997). SSC differed among the three melon scion varieties. (Table 1). True Love had the lowest level of soluble solids at 11 ºBrix whereas Sugar Rush had an average of 13 ºBrix and Sugar Cube with 14.8 ºBrix in non-grafted melons and 13.3 ºBrix in grafted melons. True Love melons had significantly lower SSC levels than Sugar Cube and Sugar Rush, however, the majority of the fruit harvested still had higher than 10 ºBrix. Both grafting and variety interacted to affect SSC. Grafting decreased SSC in Sugar Cube melons but did not affect it in True Love or Sugar Rush. The decrease in SSC was most likely caused by the more than doubled fruit load of the grafted plants. Despite this slight decrease, SSC levels of the grafted Sugar Cube melons still exceeded market standards.

Other studies have found changes in fruit flesh including decrease in firmness, vitrescence, and changes in taste as a result of grafting (Németh et al., 2020). Some grafted Sugar Cube melons displayed slight vitrescence of the fruit flesh whereas none of the non-grafted melon exhibited this trait. This was likely due to the variety Sugar Cube having a lower flesh firmness than the other two cultivars used. Adding calcium to the soil may help minimize this problem (Johnstone et al., 2008). Other studies have noted undesirable changes in the taste of melons when grafted (Rouphael et al., 2010), however, no taste changes were observed in this experiment.

Conclusion

The application of grafting to melon cultivation within the Northeast region of the United States is promising for disease and cold tolerance as well as increased yields. Grafting increased the vegetative growth in three commercial varieties of melon. Grafting also increased yields and fruit size, though in our study, the additional plant spacing used with grafted plants could have impacted this. Additional work is needed to verify this effect. No major changes in quality of the three varieties were observed. Additional studies are needed to focus on the potential season expansion, disease mitigation and fruit quality effects for different varieties.

Acknowledgements

This study was funded by the Weeks Day Fellowship grant and supported by the New Hampshire Agricultural Experimental Station. We are grateful to Dr. Becky Sideman for reviewing our manuscript. Special thanks and acknowledgement to the late Dr. Brent Loy for creating the interspecific rootstock and directing this research prior to his passing.

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Table 1. Average weight per fruit (kg), yield per 3.25m² plot (kg)*, yield per hectares (kg) and soluble solids content (ºBrix) of three commercial varieties when grafted to *Cucurbita maxima x Cucurbita moschata* vs. non-grafted. Plants were grown and evaluated at Kingman Research Farm in Madbury, NH during the summer of 2019.

Variety and Treatment	Average weight per fruit (kg)	Average yield per plot (kg)	Average yield per hectares (kg)	Average soluble solids content (°Brix)
Sugar Cube- GR[z]	1.22 c[y]	73.7 a	10,919	13.3 b
Sugar Cube- NG	0.97 d	33.0 c	4,889	14.8 a
Sugar Rush- GR	1.60 b	60.1 ab	8,904	13.3 b
Sugar Rush- NG	1.36 c	44.0 c	6,519	13.8 b
True Love- GR	2.55 a	61.1 a	9,052	11.0 c
True Love- NG	1.81 b	46.2 bc	6,845	11.1 c

*all plots were identical in size; grafted plots contained 7 plants whereas non-grafted plants contained 9.
[z] GR symbolizes grafted and NG non-grafted.
[y] Values within the same columns that share a letter in common do not differ at $p<0.05$ according to Tukey's HSD.

Table 2. ANOVA p-values from data analysis of fruit size (kg), yield per plot (kg) and soluble solids content (SSC). P-values <0.05 are significant. There were no significant differences between replicates. P values for the error term Grafting*rep were all > 0.05.

	Fruit size	Yield per plot	SSC
Variety	<0.0001	0.7448	<0.0001
Grafting vs. non-grafting	0.0013	0.0003	0.0259
Variety * grafting vs. non-grafting	0.0002	0.0296	0.0087

Table 3. Average number of leaves and laterals per plant for three commercial varieties when grafted to *Cucurbita maxima x Cucurbita moschata* vs. non-grafted on two separate dates, June 18th and 28th, 2019. Plants were grown and observed at Kingman Research Farm in Madbury, NH during the summer of 2019.

Variety and treatment	Leaves per plant		Laterals per plant	
	6/18	6/28	6/18	6/28
Sugar Cube- GR[z]	9.53 a[y]	21.83 c	3.03 a	3.81 c
Sugar Cube- NG	5.05 b	11.80 d	1.17 b	2.27 e
Sugar Rush- GR	9.67 a	20.65 c	2.91 a	4.13 c
Sugar Rush- NG	5.10 b	12.60 d	1.59 b	3.07 cd
True Love- GR	9.52 a	20.58 c	2.84 a	3.55 cd
True Love- NG	5.04 b	12.15 d	1.59 b	2.66 de

[z] GR symbolizes grafted and NG non-grafted.
[y] Within a column, values that share a letter in common do not differ at p<0.05 according to Tukey's HSD.

Table 4. ANOVA p-values from JMP data analysis of average number of leaves and laterals on 18 June 2019 and 28 June 2019. P-values <0.05 are significant. There were no significant differences between replicates. P values for the error term Grafting*rep were all > 0.05.

	Leaves June 18	Leaves June 28	Laterals June 18	Laterals June 28
Variety	0.9529	0.8153	0.7345	0.0079
Grafting vs. non-grafting	<0.0001	0.0001	0.0151	0.0215
Variety * grafting vs. non-grafting	0.9894	0.3587	0.2757	0.1781