Does Position in Cantaloupe (*Cucumis melo* L.) Fruit Affect Seed Quality?

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Abstract

Effects of the location of seed development inside cantaloupe (*Cucumis melo* L.) fruit on seed germination percentage and vigor was compared for two Western Shipper cantaloupe cultivars ('Ropey King' and 'Expedition'). Mean time to germination (MTG), as $\Sigma(N_iT_i)/\Sigma(N_i)$ where N_i is the number of newly germinated seeds at time T_i after imbibition was calculated as a measure of seed vigor. Fruit was grown in Woodland, CA, in a randomized complete block design, consisting of 4 blocks (i.e., replicates). Melons were harvested at the full slip stage of maturity and were measured and sliced into six equal sections (blossom end top, blossom end bottom, middle top, middle bottom, stem end top, and stem end bottom). Harvested seed was equally divided for germination testing without drying immediately after harvest (no seed storage, NSS), and after 6 months of seed storage (6MSS) of dried seeds. 6MSS was stored in a cold temperature-controlled (3.33°C, 35% relative humidity) refrigerator in sealed containers for six months, so moisture content did not fluctuate. Both cultivars showed improved germination percentage after 6MSS. Moreover, the 6MSS of both cultivars resulted in significantly higher ($P \leq 0.05$) germination percentages in the stem end and middle sections of both cultivars. In contrast, MTG increased after 6MSS compared to NSS possibly because of differences in hydration. However, after 6MSS seeds from the stem end and middle fruit sections of both cultivars germinated faster ($P \leq 0.05$) compared to seed from the blossom end indicating the most vigorous seeds developed in those sections.

Introduction

Cantaloupe (*Cucumis melo*) is a warm season, frost sensitive annual, netted melon whose center of origin is believed to be Northeast Africa where it was domesticated as early as 2400 BC (Welbaum, 2015). The name, cantaloupe, was derived from the Italian city, or a castle located in the city of Cantaluppi. As a member of the Cucurbitaceae family, cantaloupe cannot tolerate frost; therefore, cantaloupe production is seasonal, meaning production is largely limited to warmer times of the year. However, cantaloupe is now being traded worldwide and consumed all twelve months of the year. In 2011, a total of 25.5 million metric tonnes of cantaloupe were produced worldwide with China, Turkey, Iran, and the United States leading the world in production (Welbaum, 2015). Cantaloupes are an important world food crop, mostly eaten fresh or possibly in a salad. Production of cantaloupe fruit is an exact science that starts from flat, oval shaped seeds and leads to a delightful world-renowned fruit.

Cantaloupe fruit production starts with a healthy and vigorous seed that must germinate. The quality of cantaloupe seed is determined by two factors: germination percentage, a measure of viability, and vigor, how well seeds germinate particularly under adverse conditions. Testing seedlings is crucial for determining seed quality. A germination test is a standard evaluation technique to determine if essential structures are in place to produce normal plant life (McDonald, 1993). In other words, germination is the beginning of life as the plant emerges from the seed and develops into a plant. Seed vigor is complex and can be defined in several ways. More specifically, vigor is a well-balanced sum of seed genetic attributes that favor rapid and uniform seedling growth (McDonald, 1993).

Each cantaloupe contains roughly 300 to 500 seeds nestled inside a seed cavity in the center of a fruit. These seeds are oriented laterally within the fruit ranging from the blossom end to the

stem end as well as the top and bottom half of the fruit. While many studies have discovered how to improve seedling germination and vigor with treatments, little to no research has analyzed if seed position within fruit impacts overall seed germination and vigor (Basra et al., 2007). Previous research has not determined if seeds within a single fruit vary in germination and vigor.

Seedling vigor, which is closely correlated with the speed at which seeds germinate, is a good predictor of germination performance under field conditions (Welbaum, 2015). Therefore, the purpose of this quantitative research study was to determine if the location of the seed within the cantaloupe fruit or the fruit size affects seed quality.

Review of Literature

Cantaloupe fruit production starts with a healthy growing plant that utilizes 80 to 150 pounds per acre of nitrogen, 40 to 200 pounds per acre of P_2O_5, and smaller amounts of other micro and macro nutrients (Hartz et al., 2008). These nutrients play a key role in plant growth and development (Welbaum, 2015). Cantaloupe plants have two separate kinds of flowers, male (staminate) flowers, and perfect flowers that contain both male and female flower parts. This type of sex expression is termed andromonoecious. Cantaloupe flowers are open for only one day before senescing (Mussen & Thorp, 1997). Cantaloupe pollen is very sticky so even though anthers and stigmas on perfect flowers are only millimeters apart, the transfer must occur via insects. Pollen transfer from anthers to stigmas of perfect flowers largely depends upon honeybees, which are the most efficient pollinators (Mussen & Thorp, 1997). Adequate amounts of honey bees must be within 600 feet of the cantaloupe crop, as greater travel distances have shown to decrease honey bee activity (Hartz et al., 2008). Approximately ten to fifteen bees to flower interactions are required for optimal flower pollination. Pollen must be evenly distributed among all three lobes of

the stigma for successful fruit set to occur. Individual pollen grains must germinate and grow through the style and fertilize at least 300 ovules scattered among all three carpels for the fruit to set and for fruit develop to be uniform. After ovule fertilization and fruit set, pressure-driven flow moves photoassimilates produced in leaves (i.e., source) to developing fruit and seed tissues (i.e., sink) via the phloem. There are no direct phloem connections with the seeds. After transport, photoassimilates are unloaded from the phloem and move apoplastically until they are reabsorbed by developing seed tissues (Zhang et al., 2007). These seeds fill a cavity within the fruit lined in an oval shaped pattern starting from the stem end all the way to the blossom end. Important characteristics of melon seed production are stage of maturity at harvest, fruit harvest from a seed field, seed extraction, seed cleaning, seed drying, and seed storage to encourage after-ripening. Indications of seed quality include: 1) germinability, 2) seed vigor, and 3) seed size.

Germination

Germination testing is a standard evaluation technique to determine if essential structures are in place to produce normal plant life and rates seeds as normal, abnormal or dead (Elias et al., 2012; McDonald, 1993). In the US, standardized testing produces, many on paper towels or blotters, have been developed by the Association of Official Seed Analysts and are recognized by the US government to test seed germinability (AOSA, 1995). These tests are performed by private and government laboratories to ensure seeds sold commercially conform to state and federal laws that govern commercial trade.

The objective of seed vigor testing is to determine how well living seeds germinate particularly under adverse conditions (Elias et al., 2012). Seed vigor tests are not required by state and federal seed laws that govern the commercial sale of seeds but are invaluable to growers and

seed companies to assess the quality of their seeds, particularly to determine how well they may perform.

Cantaloupe seeds contain a thin membranous endosperm with associated callose deposition on its outer surface that completely encases the embryo. The mature endosperm creates a semipermeable barrier between the embryo and seed coat that allows water uptake but prevents the escape of solutes. This is believed to be an adaptive mechanism to reduce electrolyte leakage that would attract disease causing microbes (Welbaum and Bradford, 1990). Osmotic swelling occurs when apoplastic solutes trapped inside the endosperm attract water (Yim & Bradford, 1998). When swelling is sufficient to split the seed coat, the condition is called osmotic distention or "fishmouthing" (Welbaum and Bradford, 1990). Such seeds are either severely damaged or dead and should not be planted.

The embryo consists of an embryonic axis and two large cotyledons that provide storage of nutrient reserves for the seed and become photosynthetic after germination. Germination occurs when the radicle emerges and develops in the root system (Nerson & Edelstein, 2005) (Figure 1). The hypocotyl expands after radicle emergence and the seed coat is extracted by catching on a small hook structure at the base of the hypocotyl that holds the testa so the cotyledons can emerge.

Germination is a fundamental process where various requirements (moisture, plant hormones, temperature, etc.) must be met for success. More specifically, mRNA and proteins are stored within the seed and are ready to be activated when conditions allow radicle emergence (Bentsink et al., 2018). Proteins and hormones are derived from the mother plant and the conditions that existed during embryo development. Additionally, Galland and Rajjou (2014) notes how important abscisic and gibberellic acids (growth hormones) are in the initial few hours of germination. These hormones are regulated in ways that tell the seed to germinate or not (Bentsink et al., 2018). Indolacetic acid and ethylene are hormones that drive radicle and hypocotyl elongation during early seedling growth.

Figure 1. Germinated cantaloupe seeds

Cantaloupe seed germination and early growth was found to be boosted using a growth regulator, salicylic acid. Salicylic acid use has proven to increase plant ion uptake and transport, photosynthesis, stomatal closure, and membrane permeability (Basra et al., 2007). After soaking cantaloupe seed in different valued solutions of salicylic acid, seeds showed enhanced germination and earlier seedling emergence (Basra et al., 2007). Furthermore, seedling emergence showed higher levels of germination uniformity, or decreased days to germinate, which is likely due to seed priming. Germination can also be aided by a process called seed priming, which is a controlled hydration process, a low external water potential, which therefore limits hydration allowing key metabolic processes to proceed but not radicle emergence. Priming is followed by redrying so

seeds can be planted using normal procedures and equipment. However, when primed seeds are reimbibed they germinate faster compared to nonprimed seeds. Studies have shown that the final days seeds spend in mature fruit have the same effects on germination performance as seed priming. This is because the water potential inside fruit tissue is very similar to an osmotic priming solution, so in-fruit seed maturation is *in situ* priming. However, seed priming had a negative effect on seed longevity in storage, particularly under adverse storage conditions. Seed priming is commonly used to increase the rate, percentage, and uniformity of cantaloupe seed germination (Nascimento, 2003). Seed priming can make immature seeds harvested prematurely perform like more mature seeds. Seed priming proved to be an effective means for increasing seed germination, even at different seedling temperatures (Nascimento, 2003). While Nascimento (2003) found that seed priming increased seed germination and aided seed vigor, fruit size was not investigated in this study. Once germination is achieved, seed vigor becomes a key aspect for future plant development.

Seed vigor is defined as a seed's ability to germinate under adverse conditions. It wasn't until 1950 that the International Seed Testing Association (ISTA), began accepting the difference between germination and vigor, and therefore saw its importance (Marcos, 2015). During the 1960's when seed physiology was better understood, Woodstock (1976), urged seed vigor to become a more standard physiological seed testing procedure.

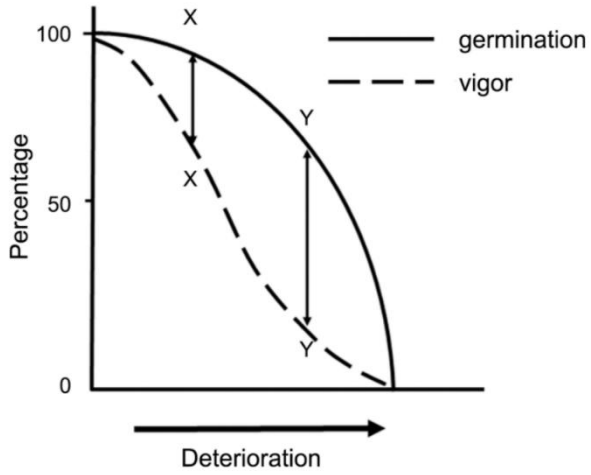

Figure 2. The relationship between germination percentage and vigor deterioration. Reproduced from Marcos (2015) under CC-BY-NC.

Seed vigor testing was aimed to provide accurate differences in physiological potential amongst seeds (Marcos, 2015). Figure 2 shows the relationship between germination and vigor with points "X" and "Y". With "X" representing a seed lot that has a higher germination percentage and vigor. Whilst "Y" seed lot is experiencing more deterioration, with lower seed vigor and germination percentages. Seed vigor can be tested in several ways (e.g., cold test, accelerated aging test, seedling growth rate, etc.) (Elias et al., 2012; Grabe, 1976).

It has been noted that the speed of germination is a good indicator of overall seed vigor (Welbaum, 1998). In other words, higher quality seeds germinate faster than lower quality seeds. Lack of germination speed can be tied to environmental conditions that existed during the time the seed was developed (Delouche & Caldwell 1960). For example, drought or disease have been known to cause less vigorous seeds (Delouche & Caldwell 1960).

Seed priming, shown to improve seed germination, also has shown to improve cantaloupe seedling vigor measured by the average number of days to germinate (MGR) (Nascimento, 2003). Seeds of two different cultivars of cantaloupe were stored at 43°C and 100% relative humidity (RH) at varying lengths of time. Longer storage times created more adverse conditions for germination, therefore decreasing germination percentages along with vigor. However, seed priming, after storage, proved to increase the germination rate and vigor of seedlings which had been exposed to longer storage times (i.e., harsher conditions) (Nascimento, 2003). This result can be accredited to the seed priming hydration process initiating the seed's natural repair mechanisms (Burgass & Powell, 1984). Therefore, aged, damaged seeds, can be repaired by seed priming (Nascimento, 2003).

Nascimento's (2003) benefits of seed priming are contradicted, as the longevity of primed seeds in storage is reduced, especially when compared to seeds that are not primed (Hussain et al.,

2015). For example, Schwember & Bradford (2005) found that primed lettuce seeds, under high temperatures and moisture, were prone to reduced longevity when compared to non-primed seeds. Additionally, primed sweet corn resulted in poor seedling growth and germination after being stored at 25 degrees Celsius when compared with non-primed seed (Chiu et al., 2002). Conclusively, Hussain et al., (2015) found similar results with rice, as non-primed seed again outperformed primed seed in germination and viability factors.

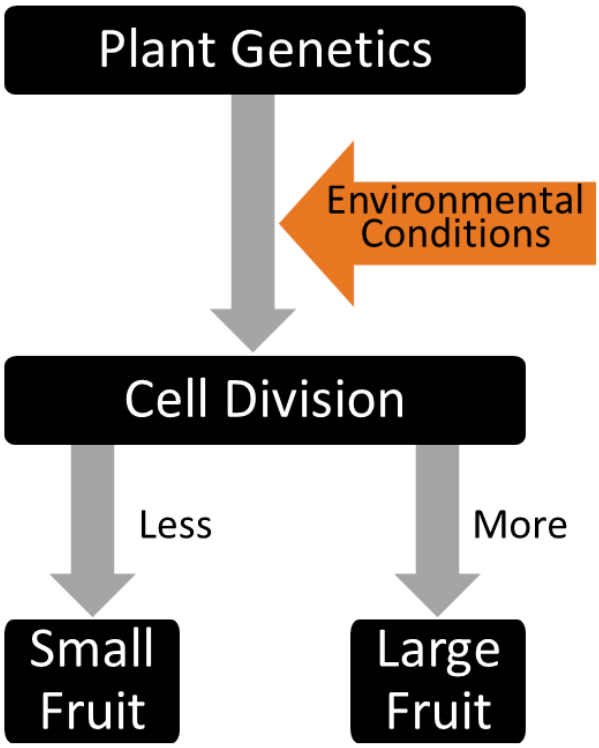

Figure 3. The process of plant physiology that determines fruit size. Figure adapted from Higashi et al. (1999).

Fruit Size

Cantaloupe fruit shape and size are largely tied to the outcomes of plant physiology and genetics (Figure 3). The plant's genetics plus environmental factors will determine the phenotype. Fruit development can be divided into three phases (Acquaah, 2009). The three phases are: phase I forms meristematic tissue and flower development which occurs before pollination; phase II ensues during rapid cell division; and phase III arises as cell enlargement takes place (Gillaspy et al., 1993). Phase II was discovered to be the critical phase for determination of overall fruit size and shape (Higashi et al., 1999). A cantaloupe's fruit source-sink relationship, or where the net photosynthetic rate and the rate at which photoassimilates remobilize from source tissues, is also a critical factor in cell division (Higashi et al., 1999 & Smith et al., 2018). During phase II,

environmental conditions, such as temperature, largely indicate if the plant has more, or less cell division. The more cell division, the larger the fruit, and visa versa. Higashi et al (1999), discovered this while testing two cantaloupe genotypes that produced different sized fruits based upon environmental factors. Considering all these factors, fruit size and shape can be predicted by measuring cell division during phase II. However, Higashi et al (1999), did not expand this study to draw conclusions between fruit size and seed quality (vigor and germination).

Cantaloupe, a fruit of increasing popularity, requires many nutritional inputs to produce viable seed. Cantaloupe seed germination is a delicate process that can be aided with pre-planting treatments that increase seedling emergence uniformity. Seed vigor is a measure of how well germinable seeds perform, particularly when planted under adverse environmental conditions. Additionally, while overall fruit size can be predicted, it has not been directly correlated with seed vigor and germinability. Therefore, the goal of this research is to investigate if the location of the seed within the cantaloupe fruit and fruit size impact seedling germinability and vigor.

Western Shipper cantaloupe cultivars maturity can be characterized by the "slip" stage that describes fruit separation "slip" from the vine as ripening progresses (Welbaum, 2015). For locally located markets, cantaloupes are harvested at half "slip" which is when the fruit is half to one quarter attached to the vine. However, for distant markets, cantaloupes will be harvest preslip to ensure quality while shipping (Welbaum, 2015). Western Shippers typically have a range of 40 to 60 days after anthesis (DAA) (Welbaum, 2015).

Material & Methods

Plot Location and Design

Cantaloupe was chosen due to availability of land, growing economic importance, and ease of production. Cantaloupe growth and production took place in Woodland, CA (Yolo County, Figure 4), during the 2020 growing season (March to August) at Pacific Star Gardens. Pacific Star Gardens is a family-owned organic farm that offers a "pick your own" business style offering a variety of farm fresh goods (eggs, strawberries, cantaloupe, watermelon, etc.). Woodland, located in the northern portion of the San Joaquin Valley of California, offers ideal cantaloupe growing conditions with temperatures averaging 90 to 95 degrees Fahrenheit during the summer months (https://www.cityofwoodland.org). Two commercially common F1 (hybrid) western shipper cantaloupe cultivars ('Ropey King' and 'Expedition') were directly sown into the soil, at a depth of four inches on May 26, 2020 (Hartz et al., 2008). Seeds were evenly spaced apart, at six inches, with three seeds per hole. The

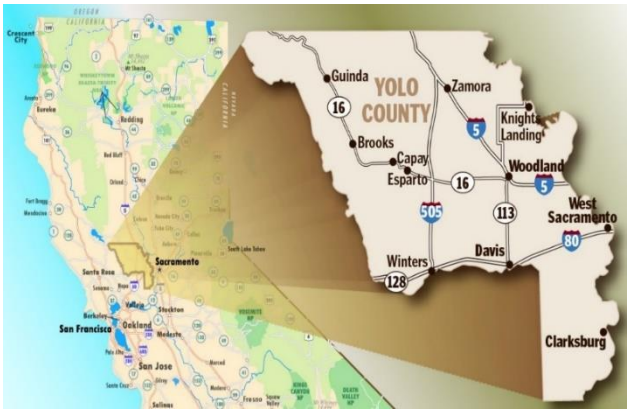

Figure 4. California map indicating Woodland, CA in relation to Yolo County and the state of California. Image from Visit Yolo County

experimental design was a randomized complete block design, consisting of 4 plots or blocks (i.e., replicates) that were blocked by cultivar. Trial plots were ten feet long by thirty inches, randomized along a single bed to ensure identical growing practices were used (fertilization, irrigation). The field contained 18 rows (roughly 2 acres) all containing honeydew or western shipper melons

Plot = 10′ x 30″

N

Row 1	Cantaloupe–Honeydew	Cantaloupe–Honeydew	Cantaloupe–Honeydew	Cantaloupe–Honeydew	Cantaloupe–Honeydew	Cantaloupe–Honeydew	Cantaloupe–Honeydew
Row 2	Cantaloupe–Western Shipper	Cantaloupe–Western Shipper	Cantaloupe–Western Shipper	Cantaloupe–Western Shipper	Cantaloupe–Western Shipper	Cantaloupe–Western Shipper	Cantaloupe–Western Shipper
Row 3	Cantaloupe–Western Shipper	Plot 1 'Expedition'	Plot 2 'Ropey King'	Plot 3 'Expedition'	Plot 4 'Ropey King'	Cantaloupe–Western Shipper	Cantaloupe–Western Shipper
Row 4	Cantaloupe–Western Shipper	Cantaloupe–Western Shipper	Cantaloupe–Western Shipper	Cantaloupe–Western Shipper	Cantaloupe–Western Shipper	Cantaloupe–Western Shipper	Cantaloupe–Western Shipper

Figure 5. Diagram of the randomized complete block design experiment (shaded boxes) within the larger field.

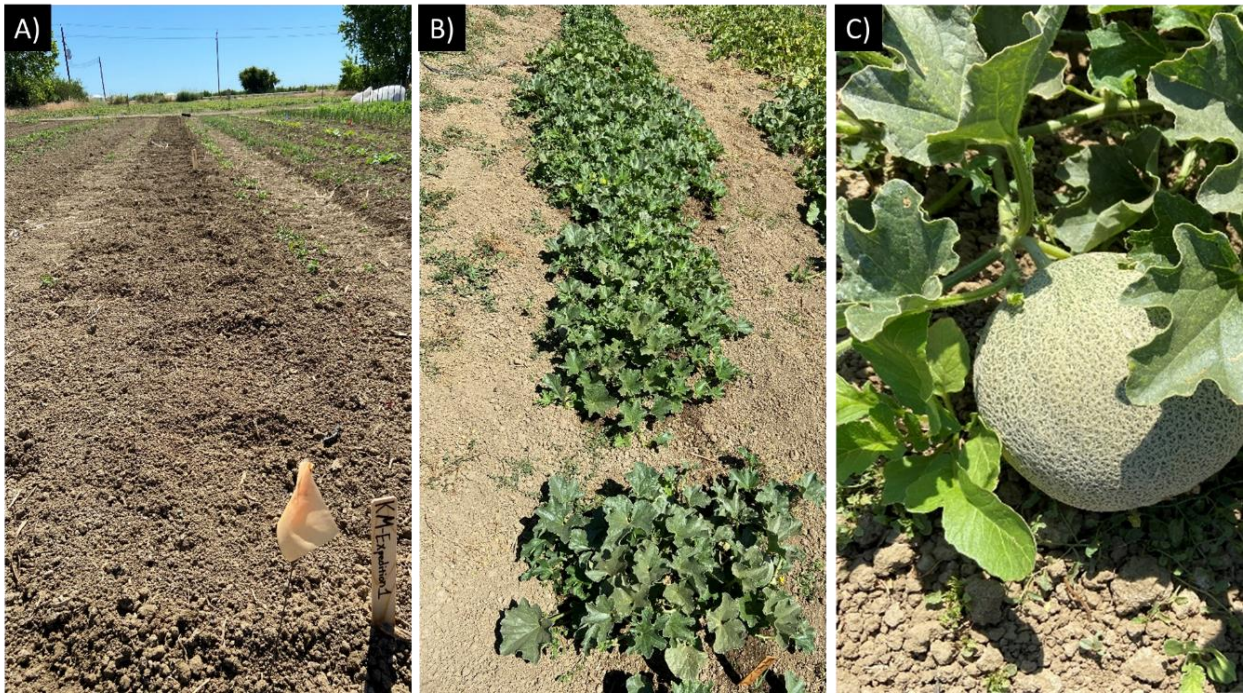

Figure 6. Experimental cantaloupe growth stages. A) Plots at time of sowing. B). Plots at time of anthesis. C) Representative image of a growing cantaloupe fruit

(Figure 5). Duplicate plants were thinned (removed) two weeks after planting to reduce plant competition resulting in each cultivar having two replicates of identical plots each containing ten healthy growing plants. Plots were grown on bare soil, 30-inch beds with commercially standard irrigation, fertilization, and pest management protocols (Hartz et al., 2008) (Figure 6).

Plot management

Cantaloupe plots were grown and cultivated for 79 days, after the time of sowing. Plots were irrigated every other day via drip irrigation. Fertilizer was applied on a bi-weekly basis via fertigation. Hand hoeing was completed weekly. To ensure proper DAA were recorded: Perfect flowers were tagged each morning, with colored ribbon tape, to ensure accurate tracking of development. Ground squirrel damage became an issue and was rectified via smoke bombs and fencing.

Data Collection

Three melons were harvested, on August 18, 2020, from the crown set per plot (total of 12) at the same state of development and day. Fruits were harvested at full maturity, half-slip stage, based upon days after anthesis (DAA). Cantaloupe fruit were first analyzed based on weight,

dimensions (length, width, and circumference), and overall size (small or large) (Table 1). Size is an objective judgement when comparing melons measurements and weights.

Table 1: Table showing data points collected

Cultivar	Plot	Melon	Size	Weight	Height	Length	Circumference
Expedition	1	1					
		2					
		3					
	2	1					
		2					
		3					
Ropey King	1	1					
		2					
		3					
	2	1					
		2					
		3					

The harvested cantaloupe fruit were weighed and measured for length (side to side) and width (top to bottom) to determine six equal sections (blossom end top, blossom end bottom, middle top, middle bottom, stem end top, and stem end bottom) (Table 2, Figure 7, and Figure 8).

Table 2: Table showing the 6 sections for fruit

	Ropey King 1	Rokey King 2	Ropey King 3	Expedition 1	Expedition 2	Expedition 3
Stem End - Top						
Stem End - Bottom						
Middle - Top						
Middle - Bottom						
Blossom End - Top						
Blossom End - Bottom						

The ground spot, or the lighter colored spot, ultimately determined which side of the melon is considered the bottom. Three melons from each plot, for a total of 12 melons (6 per cultivar), were sampled. Melons were divided into groups based upon size (large or small). This will determine if fruit size also impacts overall seed vigor and germination. Seeds were extracted from

each section of the fruit and analyzed by plot and cultivar. Seeds were hand washed for identical durations until seed mucilage was removed (Welbaum, 2015).

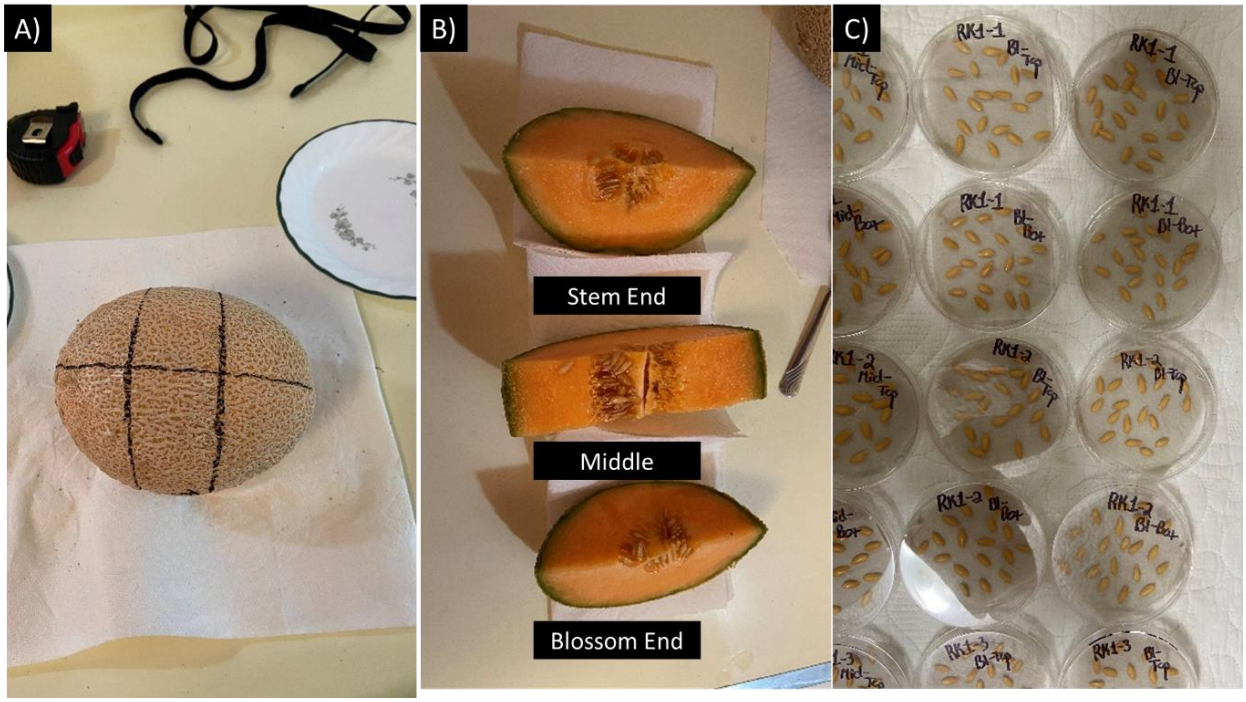

Figure 7. Melon data collection process. A) Measured lines marking fruit section to be cut. B) Cut Fruit Sections C) Seeds in germination dishes.

Figure 8. Harvested A) 'Ropey King' and B) 'Expedition' melons.

The 6MSS collected seeds were identically air-column dried to the same moisture content (5%). The NSS were tested immediately and therefore were not dried (dehydrated). Seeds were germination tested on germination blotters using Association of Official Seed Analysts (AOSA) testing procedures (Figure 7). Half of the collected seeds were tested immediately after harvest. This seed lot will be called no seed storage (NSS). The other half were stored in a cold temperature-controlled (3.33 degrees C, 35% RH) container for six months in sealed containers, so moisture content will not fluctuate. Following storage this half of seeds were also subject to an identical germination test procedure. This seed lot were called six-month seed storage (6MSS). Germination testing at two separate time points will determine differences in germination and vigor based upon length of storage.

Germination testing was conducted at 20°C, which follows AOSA testing standards (AOSA, 1995). Two replicates of each fruit section for all 12 melons were conducted, for a total of 144 germination tests per time point. Testing consisted of 20 seeds (five rows of four seeds) for each fruit section for a total of 40 seeds per fruit section and 280 seeds for each fruit. Seeds were placed equidistant apart in a closed petri dish (9 cm in diameter) on white germination blotter paper hydrated with 12 ml of water. Each dish represented one repetition. To measure seed vigor, the speed of radicle emergence was compared. Germination was recorded twice daily and germinated seedlings were removed from each dish after scoring. Radicle emergence was used as the criteria for germination and records were kept from the start of imbibition, scoring seeds in each dish. No additional water was added to the dish after planting and the petri dish was sealed with Parafilm and remain closed, except when removing germinated seedling, to minimize water loss during the experiment. Germination data was collected until no new germination occurred for three consecutive days. This germination test was be repeated for the 6MSS.

Data Analysis

Germination percentage and the mean time to germination (vigor) are two different parameters used separately to assess seeds. Germination percentage was calculated based on the number of seeds out of 100 that germinated and was used as a measure of seed viability (whether a seed is alive or dead). Germination rate (vigor) is a measure of how fast living seeds in a population are able to germinate and thus is a measure of speed with units of time (days or hours). Seed vigor was calculated using the mean time to germination (MTG), as $\Sigma(N_iT_i)/\Sigma(N_i)$ where N_i is the number of newly germinated seeds at time T_i after imbibition. MTG is commonly used as an indicator of seed vigor because high vigor seeds germinate faster than low vigor seeds and therefore MTG was used as an indicator of seed vigor in this study. Germination rate is an inverse of MTG, as germination rate is measured in units of seeds per day while MTG is the units in days that it would take for an average seed in a population to germinate.

Tukey's Honest Significant Difference tests were used to determine statistical significance for differences in seed germination percentage and MTG when comparing melon cultivars, seed sections, and melon size. P-values of ≤ 0.05 were considered to be significantly different for all analyses. The results (Figure 9) presented are the means of the 6 melons for each cultivar and the variation between the melons is represented by standard error of the mean (SEM) bars**.**

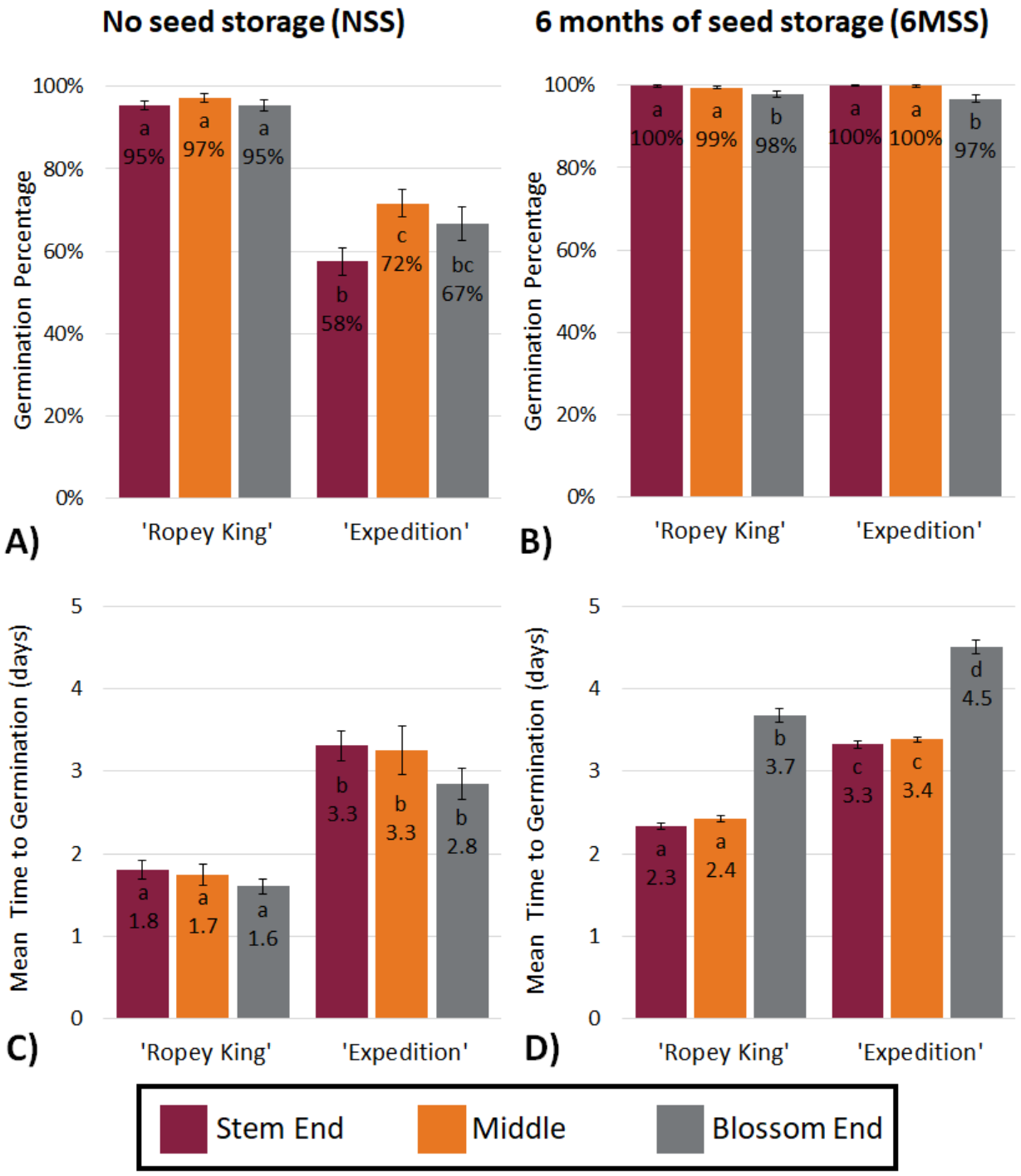

Figure 9. Comparison of seed germination percentage and vigor (in days) results for no seed storage (NSS) (**A** and **C**) and 6 month seed storage (6MSS) (**B** and **D**) seed germination percentages. Error bars represent standard error of the mean.

Seed Germination Percentage

Overall, both 'Ropey King' and 'Expedition' showed higher germination percentages after seed storage of 6 months (6MSS) (Figure 9A-B). 'Ropey King' averaged 95.6% germination NSS, compared to 99% with 6MSS. 'Expedition' resulted in a 65.6% germination percent average for NSS and 99% 6MSS. Therefore, these results support the common practice of seed storage post-harvest to encourage afterripening. Interestingly 'Ropey King' resulted in an acceptable average germination percentage (95.6%) without 6 month's storage. On the other hand, 'Expedition' demonstrated the importance of seed storage, increasing its average germination percentage by 33.4%.

When comparing germination percentages from the different fruit sections (stem end, middle, blossom end), there were no significant differences observed for 'Ropey King' NSS (Figure 9A). In contrast, for Expedition' NSS seeds from the middle section germinated to a significantly higher percentage (72%; $P \leq 0.05$) compared to seeds from the stem end. However, in Expedition' NSS germination percentage of seeds from the blossom end (67%) were not significantly different ($P \geq 0.05$) from either the middle or stem end (58%). The 6MSS, amongst both cultivars, showed significantly higher germination percentages (2-3% more; $P \leq 0.05$) in the stem end and middle sections compared to the blossom end (Figure 9B).

MTG

The MTG of both cultivars increased after 6 months of storage (Figure 9C-D). Prior to seed storage, 'Ropey King' averaged 1.7 days for MTG, compared to 2.8 days after storage. For 'Expedition', 3.1 days were averaged before storage compared to 3.7 days after storage.

When comparing fruit sections (stem end, middle, blossom end), with NSS conditions both 'Ropey King' and 'Expedition' resulted in no significant differences ($P \geq 0.05$) (Figure 9C).

However, 6MSS resulted in both cultivars having significantly higher ($P \leq 0.05$) MTG in seeds from the blossom end (Figure 9D). For 'Ropey King', the blossom end was 1.35 days slower compared to the average of the stem end and middle. For 'Expedition' similar results were found with the blossom end being 1.15 days slower compared to the average of 3.35 for the stem end and middle (Figure 9D). These results are likely due to the NSS lots not being dehydrated, giving them a head start compared to the 6MSS.

Melon Size

Interestingly, melon size was not a significant factor ($P \geq 0.05$) in determining differences in seed germination percentage or MTG.

Discussion

The 6MSS of both 'Ropey King' and 'Expedition' resulted in higher germination percentages compared to the NSS. However, due to the lack of dehydration process, the NSS is difficult to compare to the 6MSS. Seed storage is an essentially common practice that preserves the viability of seeds (Delouche, 1968). Historically, seeds were stored in cool places solely to preserve them for the sequential growing season (Justice & Bass, 1978). Early studies in the 1800's suggested that storing seeds improved its quality (germination) (Justice & Bass, 1978). However, storing seeds does not improve seed quality, but rather, it preserves the quality of the seeds (Hong et al., 1996). Once seeds are harvested the deterioration process begins, thus storage is used to discontinue the deterioration process. If seeds are stored at ambient temperatures with high relative humidity, deterioration is quickened. Thus, supporting this research's results of high quality (germination) compared to seeds that were not stored. This could be a result of after ripening. After ripening is likely related to hormone levels in the seed that change in storage or oxidative reactions that affect gene expression (Zainal et al., 2019). Hydrated seeds likely have a big advantage and

reduces the MTG because they have a head start compared to dry seeds. The cold storage may have limited the amount of after ripening as well. Melon seeds have an interesting characteristic that they germinate better under mild water stress than in pure water. This sensitivity to high water potentials may have come into play in this data.

Further results, for both cultivars, showed increased germination percentages in the stem end and middle sections and higher MTG for the blossom end. Studies on squash, a crop in the same family as cantaloupe, have observed improvement of offspring quality suggesting that the rate of pollen-tube growth in the style is positively correlated with the rate of seedling growth in the next generation (Jóhannsson & Stephenson, 1997). Experiments with *Dianthus chinensis* demonstrated that when pollen tubes had to develop a longer distance through the style, the offspring had increased MTG (Mulcahy & Mulcahy, 1975). Winsor et al., (1987) found that in zucchini (*Cucurbita pepo)*, the amount of pollen load (low, medium, or high) had a direct effect on the number of seeds (fertilized ovaries) inside the fruit. A zucchini fruits progeny (seeds) was more vigorous under intense pollen-tube competition (high pollen loads) compared to conditions under little or no pollen competition (low pollen loads) (Winsor et al., 1987). In other words, zucchini can improve its average seed quality by selectively aborting fruits on the basis of seed number. Additionally, zucchini seed vigor was improved by larger pollen loads (Quesada et al., 1993). The pollen load (small or large) deposited on the stigma has a significant impact on the vigor of the plant's progeny (Quesada et al., 1993). Furthermore, Susko & Lovett-Doust (1999) suggest that in *Alliaria petiolate* (Brassicaceae), seeds located near the stem end of fruits were an important factor in seed quality. Stem-end located seeds were more mature and developed when compared to distantly located ovules (blossom end). Seed development, therefore, is crucial for the overall quality (germination and vigor) of seeds. Cucumber (*Cucumis staivus* L.) and

cantaloupe showed increased germination and vigor when seeds were fully developed from the ovule, compared to those that weren't (Jing et al., 2000 & Mann & Robinson 1950). These studies suggests that faster growing pollen tubes, with large pollen loads, reach ovules in the stem end first, followed by the middle and blossom end sections. This faster growth gives seeds in the frontward locations the advantage of being fertilized first, therefore becoming sinks earlier in development and attract photoassimilates first. This supports this research in that seeds located in the blossom end of the fruit, yielded slower MTG.

Seeds located in the blossom end resulted in less vigorous and decreased germination, after 6 months of storage, which could indicate that pollen grain growth and growing length alone doesn't determine MTG of developing seeds. This could be directly related to source-sink strength. Source, or the location where sugars are produced in the plant (leaf) and sink, the areas that consume the sugars for growth (fruit). A source-sink relationship is crucial for forming the framework of how plants assimilate nutrients (Patrick, 1993). In Cucurbitaceae crops, stress, like drought stress and insufficient pollen load, causes the ovules at the blossom end of the fruit to abort before seeds develop at the stem end (Rashidi & Seyfi, 2007). The stem end should receive a greater allocation of resources (sugars) because this tissue and these ovules are closer to the source, so the photoassimilates have a shorter distance to travel before they are unloaded from the phloem (Valantin et al., 1998). In other words, ovules developing near the stem end have the first opportunity to use photoassimilates, so if they are all used at one end of the fruit when resources were limiting, there would be not enough left at the other end (blossom end) so the ovules will abort.

Pollen competition is also one of the primary drivers for cryptic self-incompatibility favoring outcrossed pollen for fertilization. Faster pollen tube growth rate in *Dalechampia*

scandens results in reduced inbreeding depression in mixed-mating systems due to intense pollen competition after self-pollination (Armbruster et al., 1995). However, in a cross-pollination situation (Example: 'Ropey King' successfully pollinating a 'Expedition' female flower) from the other cantaloupe cultivar, research suggests that no significant advantage is obtained compared to a selfed flower (Winsor et al., 1987). Thus, supporting that outcrossing in this experiment would have no impact on the number of fertile seeds during harvest.

Results yielded lower MTG, in 'Ropey King', in the NSS compared to the 6MSS. Vertucci & Ross (1993) point out that seeds must be dehydrated to an acceptable level for proper storage, around three to seven percent. After storage, seeds must then regain adequate moisture, from the surrounding environment (soil, etc.) to complete inhibition. Inhibition, or the process of dry seeds obtaining moisture to activate enzymes, is a fairly quick process that is required for the seed to germinate (Welbaum et al., 1990). With this being said, the NSS were not dried (dehydrated) and therefore required less time for inhibition and therefore germination, explaining why NSS germinated faster with a lower MTG. The 6MSS set was dehydrated before storage, therefore this seed set could have resulted in slower MTG due to more time required for inhibition. Future studies should dehydrate seeds to properly assess this characteristic.

Unfortunately, fruit size did not affect the MTG and germination percentages of the seeds in the section. Research has shown a relationship between seed numbers within the melon and fruit size. However, this research showed that fruit size did not affect seed quality.

Additionally, the three top sections (blossom end top, middle top, stem end top) showed no significant difference amongst themselves, therefore the data was averaged. The bottom three sections (blossom end bottom, middle bottom, stem end bottom) followed the same pattern and were also averaged.

The research is the starting point for a larger scale project with more data points. Practically, this research can be directed to vegetable seed companies, who are always looking for ways to improve hybrid germination and reduce MTG. The stem end and middle sections average 2% higher germination percent and averaged 2 days faster mean time to germination but only after 6-month storage. During harvest, vegetable seed companies could separate the blossom end seed into a lower quality section, selling it for lower quality prices.

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Appendices

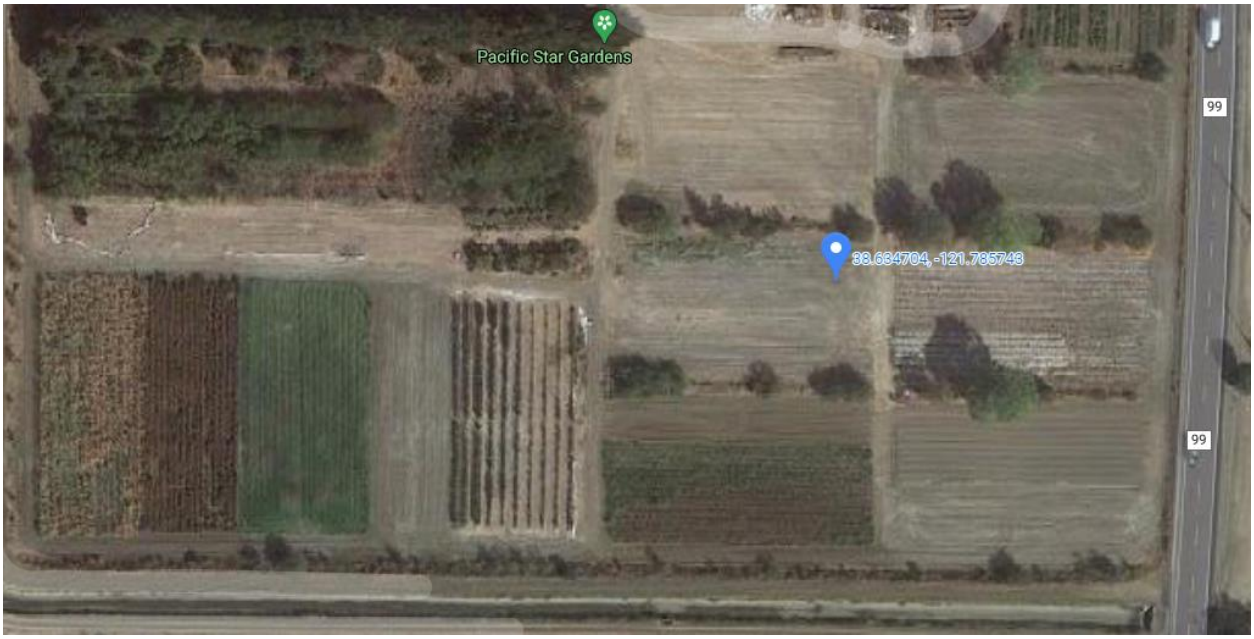

Figure 1: GPS location of melon trials at Pacific Star Gardens (Google Maps).

Figure 2: A) Checking fertilized flowers. B) Plot management practices (hoeing)

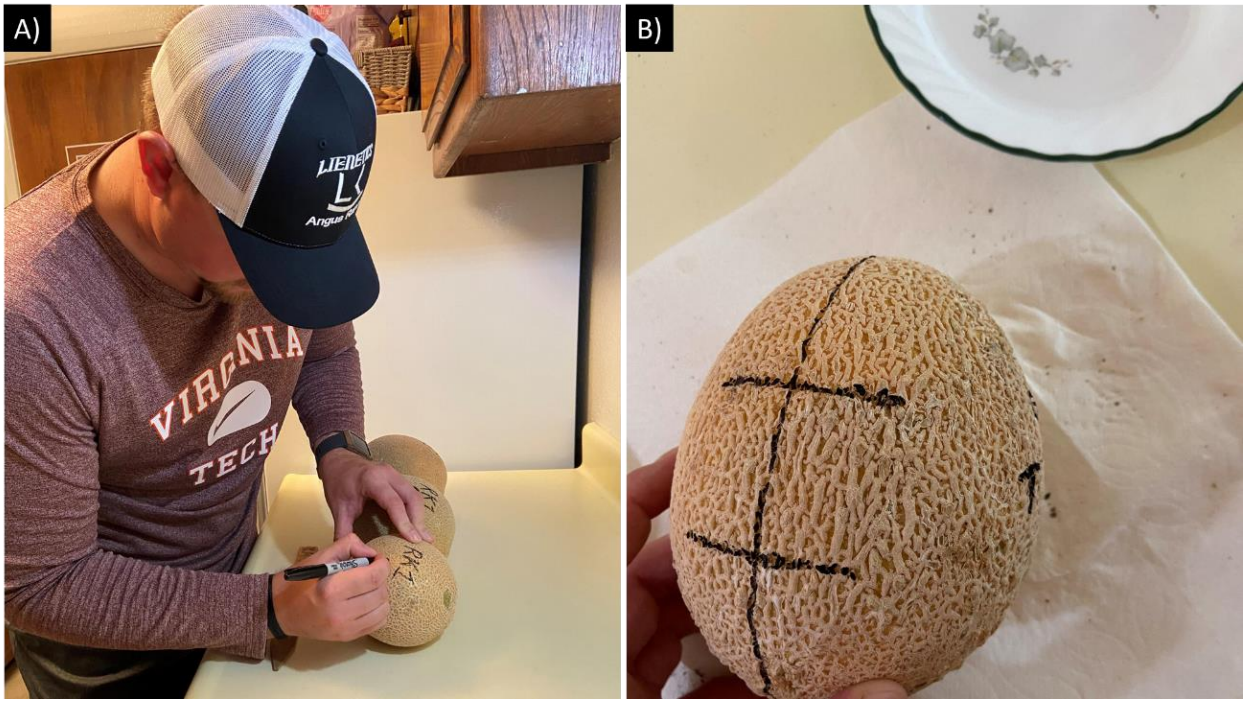

Figure 3: A) Labeling fruit. B) Measuring and marking fruit