Investigating Kiwiberry Fruit Quality and Germplasm Suitability

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Abstract

Recent years have seen an increase in consumer demand and interest for a diversity of kiwifruit products (Harker et al. 2007; Jaeger et al. 2003; Latocha and Jankowski 2011). As market trends for local produce and consumer interest in diversifying food options increase in the United States, the opportunity to grow local fruit with novel characteristics becomes desirable. This interest has spurred investigation into kiwiberry as a new cold-hardy crop for Minnesota. The name kiwiberry is used to differentiate the species *Actinidia arguta* and *A. kolomikta*, which are two berry-sized, pubescence free, cold-hardy kiwifruit, from the fuzzy kiwifruit common in the marketplace *A. chinensis* (Hastings, 2018; Huang, 2016). *Actinidia arguta* and *A. kolomikta* are of particular interest for production in Minnesota as they have been reported to have relative ranges of cold-hardiness appropriate for cultivation in USDA zone 4 (-20 °F to -30 °F) (Lin et al. 202; Sun et al. 2020). Though there is increasing interest in these species, research is still needed to examine suitability of kiwiberry as specialty crops in Minnesota. This research utilized the established kiwiberry germplasm at the University of Minnesota (UMN) containing approximately 306 accessions of *A. arguta, A. kolomikta,* and their hybrids to address the following objectives: 1) determine consumer willingness-to-pay for kiwiberry; 2) improve curation of North American kiwiberry collections; 3) determine the impacts of harvest timing on fruit quality and storability for accessions of two kiwiberry species (*A. arguta* and *A. kolomikta*); and 4) examine whether the climacteric nature of kiwiberry allows for reduced spotted-wing drosophila (SWD) infestation pressure at harvest (as a function of harvest date or fruit

firmness) and the subsequent impacts of infestation on fruit quality of two kiwiberry species (*A. arguta* and *A. kolomikta*) in the Upper Midwest.

Consumers were willing to pay comparable prices for kiwiberries as other berries based on a second-price auction examining four kiwiberry accessions. A total of 262 kiwiberry accessions along with 106 National Plant Germplasm System (NPGS) accessions were fingerprinted using single nucleotide polymorphisms (SNPs) generated with genotyping-by-sequencing (GBS). Identity of 97 accessions were confirmed, of which 36 were previously unknown, and redundant accessions called. Fruit quality traits were observed for eleven known accessions over three years and optimal harvest timing for production was determined for each accession. Harvesting firm fruit was shown to reduce the infestation rates of spotted-wing drosophila (SWD) on fruits. Implications of this work are 1) identification of consumer segments interested in purchasing kiwiberry; 2) improved curation of UMN kiwiberry germplasm for more informed future breeding; 3) recommendations of harvest timing balancing fruit quality and storage; and 4) leveraging the climacteric nature of kiwiberry to reduce SWD infestation.

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Introduction

Botany: Actinidia spp.

The genus *Actinidia* contains multiple species with approximately 54 species designations (Li et al. 2007). *Actinidia* species usually have a base chromosome number of 29 and include diploids, tetraploids, hexaploids, octoploids, and decaploids with no clear delineation between ploidy and species (Ferguson and Huang 2007). The cold-hardy species *A. arguta* (Siebold & Zucc.) Planch. ex Miq. and *A. kolomikta* (Rupr. et Maxim.) Maxim. present in the University of Minnesota (UMN) germplasm have been found to be tetraploid and diploid, respectively (Start et al. 2007). Similar ploidy levels in these species have been shown in wild collections throughout native ranges (Asakura and Hoshino 2016). However, polyploidy is often associated with greater cold hardiness and distributions of ploidies in Japan showed that higher polyploid individuals (hexaploids) were found in colder northern regions while lower ploidies (diploids) were found in warmer regions (Kataoka et al. 2010). Though this holds true in many cases, diploid *A. kolomikta* has been shown to have cold-hardiness greater than many tetraploid *A. arguta* individuals as the native ranges for these species overlap.

A. arguta has the most extensive geographical range which extends from Eastern Russia down into central and southern China and Japan (Huang 2016). *A. kolomikta* shares a range similar to *A. arguta*, but is absent in south-eastern China. Both species exhibit cold hardiness with *A. arguta* surviving temperatures to as low as -30 °C (-22 °F) (Williams et al. 2002; Cui et al. 2002) and *A. kolomikta* withstanding temperatures to as low as -40 °C (-40 °F) (Debersaques et al. 2014; Marosz 2009; Strik 2005).

Actinidia species are cryptic dioecious plants with male plants having flowers with only staminate organs present and female plants have flowers with pistillate organs along with staminate organs that produce nonviable pollen to attract pollinators (Kawagoe and Suzuki 2004). Both a staminate and pistillate vine must be present to produce fruit and adequate pollination of pistillate flowers is important for fruit set and appropriate fruit sizing (Pinto et al. 2018). One staminate vine for every six to ten pistillate vines is usually recommended (Strik 2005). Berries are produced either singly or on axillary cymes by actinomorphic, hypogynous pentamerous flowers (Latocha et al. 2021). Staminate vines tend to flower more profusely and longer than pistillate vines though overlapping stages of flowering occur on a single plant throughout this time. The berries produced are roughly the size of a table grape (1 to 30 g), but size between kiwiberry species can vary with *A. kolomikta* having smaller fruit weight compared to *A. arguta*. Kiwiberries have characteristically smooth skins that distinguishes them from kiwifruit which has fruit with corky peridermal skin (Macnee et al. 2019). Fruit color for kiwiberry accessions in the UMN germplasm is primarily green, but a range of both skin and flesh colors including red, orange, and purple are present in native regions (Crowhurst et al. 2008).

In addition to providing a novel eating experience, kiwiberries have excellent health benefits (Latocha 2017). When compared to equal weights of other fruits, kiwiberry has a higher vitamin C content typically between 7 and 18 mg per 100g fresh weight (Leontowicz et al. 2016; Nishiyama et al. 2004). Kiwiberry has also been reported to have high presence of antioxidants like lutein which is a strong antioxidant that is

correlated with reduction to eye diseases like macular degeneration (Datson and Ferguson 2011; Debersaques et al.2014; Wojdyło and Nowicka 2019; Wojdyło et al. 2017).

Background on Actinidia production

Actinidia fruits were consumed as a forage crop by ancient Chinese peoples. Western cultivation began with *A. chinensis* var. *deliciosa* in New Zealand in 1904 and expanded to become a global market by the late 1960s. Production of kiwifruit up until 1970 consisted almost exclusively of the cultivar 'Hayward', which is a green-fleshed kiwifruit capable of prolonged cold storage. Simultaneous to global marketing of 'Hayward' in the 1960s and 1970s, research began in China exploring the golden kiwifruit or *A. chinensis* var. *chinensis*. However, it was once again a cultivar from New Zealand that commercially launched this species in the global market. Prior to the turn of the century, 'Hort16A', a yellow-fleshed *A. chinensis* var. *chinensis*, started to enter production in New Zealand. Until 2010, 'Hayward' and 'Hort16A' were the top two globally produced kiwifruit cultivars. Then in 2011, 'Hort16A' plantings began to lose production due to infection by *Pseudomonas syringae* pv. *Actinidiae* (Psa). Psa, a bacterial canker, destroyed plantings of the susceptible 'Hort16A' leading to the first major declines in the global kiwifruit market. However, the industry quickly responded with 'Gold3' (*A. chinensis* var. *chinensis* 'Zesy002') which is currently marketed as Zespri® SunGold® Kiwifruit.

Though literature on global kiwifruit sales excludes kiwiberry production, production and market trends described above show that the market is interested in new fruit characteristics such as yellow-fleshed fruits with tropical flavors (Jaeger et al. 2003). Such trends could provide opportunities for development of fruits in the *A. arguta* and *A.*

kolomikta species which provide unique flavors in a hairless fruit for an easy eating experience. Currently *A. arguta* is being investigated and cultivated as the next product from the *Actinidia* genus by researchers in New Zealand, China, the U.S., and many countries in Europe (Latocha et al. 2017).

Current production of kiwiberry in the U.S. is less than a total of 80 hectares in Oregon (Hurst Berry Farm International) and Pennsylvania (Kiwiberry Organics, Co) (Latocha et al. 2017). The cultivar *A. arguta* 'Ananasnaya' is thought to be the most widely planted kiwiberry cultivar in the United States (Strik and Hummer, 2006). Oregon State University (OSU), the University of New Hampshire (UNH), and the UMN have been working to develop kiwiberry as a new horticultural crop. Ongoing research at these institutions is focused on cultivar trialing to identify cultivars, or plants selected for desirable traits and usually clonally propagated, suitable for each region. *Actinidia arguta* cultivars available to growers within the U.S. in addition to 'Ananasnaya' include, but are not limited to, 'Geneva 3', 'Natasha', and 'Tatyana'. Additionally, though not in large scale production currently and with limited available, there are *A. kolomikta* cultivars available on the market including 'Krupnopladnaya', 'Sentyabraskaya', and 'Aromatnaya'. These *A. kolomikta* cultivars originate from Northwoods Nursery in Molalla, Oregon who imported them from the Vavilov Institute Agricultural Experiment Station in Leningrad in 1986 (Koller, 1990).

Consumer interest

Kiwifruit has been experiencing consistent economic gains as an industry since the 1970s with the exception around the turn of the century when Psa decimated golden kiwifruit plantings (Ferguson 2016). However, the industry quickly responded with a

resistant cultivar and gains have been seen as these plantings mature. With the introduction of golden kiwifruit on the market, consumer interest in this novel fruit was evident (Harker et al. 2006; Jaeger and Harker 2006). In other fruit crops, breeders have recently developed and commercialized unique products like Cotton Candy® grapes or Driscoll's Rosé Strawberries® which have unique flavors integrated in familiar fruits. Kiwiberry fits this trend by providing a novel eating experience for an already familiar fruit.

Another trend of the past decade has been a push for local production of goods which have been found to increase consumer willingness-to-pay (WTP) for products (Fan et al. 2019). Consumers are becoming more conscious of where their food comes from and how their purchases might affect local businesses. This trend can be seen in the rise in the number of local farmers markets in the past decade. A study of consumers at farmers markets in the Twin Cities found that they were most interested in local foods for the quality and freshness (Yue and Tong 2009). With adequate cold hardiness for production in Minnesota, kiwiberry offers growers an exotic seeming product grown locally. However, kiwiberry is relatively uncommon for many consumers throughout the United States and consumer acceptability and WTP is unknown.

Germplasm resources

In order to explore kiwiberry as a novel crop, resources such as diverse germplasm are invaluable for breeding efforts. The USDA's National Plant Germplasm System (NPGS) has records of 196 *A. arguta* and 74 *A. kolomikta* accessions, but nearly 80% are listed historic records or unavailable (Latocha et al. 2021). There are limitations in place on importation to the U.S. of new kiwiberry material due to phytosanitary

restrictions for Psa. These restrictions place germplasm curation as a priority for future breeding efforts. Previous work by Melo et al. (2017) examined the USDA NPGS, UNH, and some of the UMN kiwiberry germplasms and identified genotypically unique and redundant accessions. Approximately 53% of *A. kolomikta* and 41% of *A. arguta* were redundant within these collections (Melo et al. 2017). These redundancies call attention to the importance of genomic curation of germplasms.

The UMN kiwiberry germplasm (44°86'N, -93°64'W) at the Horticultural Research Center (HRC) has been assembled since the 1980s largely thanks to the efforts of volunteer curator, Dr. Robert Guthrie (Guthrie et al. 2006). The HRC kiwiberry germplasm consists of two plantings which Start et al. (2007) examined using flow cytometry to identify the ploidy of 61 accessions in the UMN germplasm. Start et al. (2007) found that all *A. kolomikta* accessions are diploid and most *A. arguta* are tetraploid in these plantings. An understanding of ploidy in the germplasm is important to inform appropriate breeding and cross planning. However, accessions have been added and removed since then and field labels sometimes do not match previous records. This might convolute results for determination of unique individuals based on observational data alone requiring the use of genomics platforms like genotyping-by-sequencing (GBS) to improve current UMN kiwiberry curation.

Harvest maturity and fruit quality

Harvest date and postharvest handling are important for *Actinidia* fruit production because maturity at harvest and how they are stored impacts fruit qualities such as flavor (McMath et al. 1991; Gilbert et al. 1996), aroma (Young and Paterson, 1985), firmness (Fisk et al. 2008;), sugar and acid content (Krupa et al. 2011), and nutrient composition

(Fisk et al. 2006; Latocha et al. 2014; Rassam and Laing, 2005). Though most of this research has been done in *A. chinensis* var *deliciosa* and *A. chinensis* var *chinensis*, a growing body of knowledge has examined *A. arguta* and *A. kolomikta* cultivars for harvest and fruit quality.

Actinidia species have climacteric fruit which means that once fruit are picked from the vine, they continue to ripen in storage in response to ethylene (Kim et al. 2020). This postharvest ripening is key for kiwiberry production since vine ripened fruit will be soft and prone to damage from harvest handling and thus allows for production of marketable fruit. However, since *Actinidia* do not display external signs of ripening such as skin color changes before vine ripeness, it is important to inspect internal indicators. The first indicator to examine is a darkening of the seed coat. *Actinidia* seeds first develop with a white or tan seed coat that then darkens to a deep brown or black color close to maturity (Hastings and Hale 2019). Harvesting prior to seeds darkening results in fruit that unevenly ripen in storage or develop off flavors. Due to within- and between-vine fruit maturity variability, multiple vines in a planting should be sampled before deciding to harvest (Tiyayon and Strik 2003a, 2003b). The number of vines to sample depends on how large the planting is, but typically at least five vines per acre should be examined (Hastings and Hale 2019). Once vines have been selected, five berries should be harvested per vine from across the vine canopy. If seeds are dark brown or black, then the kiwiberry has reached early physiological maturity indicating the appropriate time for more reliable harvest indicators.

The most common means of ensuring harvest readiness is by examining the sugar content of the fruit by examining the soluble solids content (SSC) of fruit juice. A

refractometer is used to estimate SSC in a juice sample based on light refraction in the solution. Consumer likeability of *A. arguta* fruit increased as SSC increased between 10 and 17.5 °Brix (Hunter et al. 2020). Harvest recommendations based on *A. arguta* 'Ananasnaya', 'Cheongsan', and 'Geneva 3' have found that harvesting at 8 °Brix improved postharvest fruit quality compared to harvesting at lower SSC (Fisk et al. 2006a; Han et al. 2019; Mumford et al. 2023). Chesoniene et al. (2004) examined *A. kolomikta* harvest maturity and reported that between 9 and 10 °Brix might be appropriate. Overall, SSC does provide an easy threshold for field evaluation of harvest timing, but it can vary depending on weather events. Though there are recommendations on harvest timing and the resulting impacts on fruit quality for *A. arguta* cultivars, these cultivars are unreliable for cultivation in Minnesota where winter temperatures have resulted in severe winter injury. Additionally, previously examined *A. kolomikta* cultivars differ from available cultivars in the U.S. Overall, there is a gap in current literature regarding harvest timing and fruit quality of genotypes appropriate in Minnesota.

Pests impacting fruit quality

Spotted-wing drosophila (SWD), *Drosophila suzukii* Matsumura, is an economically important introduced pest of fruit in the US. Females have serrated ovipositors that enable SWD to infest a wide range of hosts that have soft or thin skin prior to ripening. Fruit quality traits, including pH and soluble solids content (SSC), have been examined in relation to their impacts on SWD host suitability (Bellamy et al. 2013). Skin thickness has been positively associated with reduced infestation levels (Pelton et al. 2017; Steffan et al. 2013). Additionally, studies have shown that fruit susceptibility to oviposition increases as fruit firmness decreases (Gullickson et al. 2023; Ioriatti et al.

2015; Kinjo et al. 2013; Wohner et al. 2021). This relationship between firmness and oviposition could be exploited for cultural control of SWD on climacteric fruit. However, there is limited literature examining SWD on kiwiberry (Wannemuehler and Tran 2021), and no reports of subsequent impacts of SWD infestation on fruit quality of kiwiberry.

Dissertation objectives

The research objectives of this dissertation are as follows: determine consumer willingness-to-pay for kiwiberry (Chapter 1); improve curation of North American kiwiberry collections (Chapter 2); determine the impacts of harvest timing on fruit quality and storability for accessions of two kiwiberry species (*A. arguta* and *A. kolomikta*) (Chapter 3); and examine whether the climacteric nature of kiwiberry allows for reduced SWD infestation pressure at harvest (as a function of harvest date or fruit firmness) and the subsequent impacts of infestation on fruit quality of two kiwiberry species (*A. arguta* and *A. kolomikta*) in the Upper Midwest (Chapter 4). This work was conducted to enhance current understanding of kiwiberry germplasm curation and identify future resources for breeding new cultivars. Implications of this work are 1) identification of consumer segments interested in kiwiberry; 2) improved curation of UMN kiwiberry germplasm for more informed future breeding; 3) recommendations of harvest timing balancing fruit quality and storage; and 4) leveraging the climacteric nature of kiwiberry to reduce SWD infestation.

Chapter 1: Consumer preferences for kiwiberries: Implications of experimental auctions

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Abstract

In today's global market, developing novel products is important for staying competitive. The development of horticultural products often manifests as new cultivars. Gauging consumer interest is an important step in cultivar development as it is a resource intensive process. The present study uses an experimental auction to measure consumers' willingness-to-pay (WTP) for novel fruit referred to as kiwiberry (*Actinidia arguta* and *A. kolomikta)* and explore consumer segmentation for their preferences. Mean WTP for 6-oz packages of kiwiberries ranged from $1.63 to $2.19 depending on species and cultivar. Four groups of consumers were identified in relation to their WTP in kiwiberries. Using survey data, socio-demographic variables such as age, education, and neophobic attitudes were significantly different between Mixed Kiwiberry Price Premium and Kiwiberry Discounting groups. This study's findings suggest that consumers with variety-seeking tendencies have a WTP for kiwiberries that is comparable to other berries and proposes marketing strategies for these groups.

Introduction

Global production and new cultivar introductions have resulted in increased availability of diverse fruits and berries in the market. Ultimately, consumers drive the demand for food innovation as consumers have different preferences for food products (Drescher et al. 2008; Lee and Brown 1989) with certain consumer segments showing greater interests in health (Asioli et al. 2017) or product innovation (Nazzaro et al. 2019). In the horticultural market, demand for innovation results in the development of new cultivars with improved fruit quality (Iezzoni et al. 2015; Jaeger et al. 2003; Weil 1998). Every year new fruit cultivars are introduced offering improved production, better pest resistance, or superior taste. Cultivar success has been shown to depend on grower, processor, and consumer acceptance, and breeding efforts to meet these innovation demands often take years with considerable resource requirements. An example of one successful product innovation and diversification with years of development in the horticulture market is the kiwifruit (*Actinidia chinensis*).

The fuzzy kiwifruits familiar in the U.S. marketplace are typically the green-fleshed *Actinidia chinensis* var. *deliciosa* 'Hayward'. Green kiwifruit was introduced into the United States in the late 1970s making these fruits relatively recent introductions. 'Hayward' was developed in the 1930s and remains the predominant cultivar of kiwifruit produced globally (Ferguson 2016). However, the demand for innovation spurred exploration into the over 60 identified species in the genus *Actinidia* for opportunities for improvement and development of novel kiwifruit cultivars. One instance of such successful innovation is the golden-fleshed 'Gold3' kiwifruit belonging to *Actinidia chinensis* var. *chinensis*. Golden kiwifruit was introduced in the 1990s and has grown to

compete with green kiwifruit (Ferguson 2016; Patterson et al. 2003). In an effort to further meet the demand for new fruit cultivars, further *Actinidia* species are being cultivated for production.

Actinidia arguta and *A. kolomikta* are two *Actinidia* species with growing interest in the market (Cossio et al. 2015; Latocha et al. 2017). These species are often referred to as kiwiberry and present novel fruit qualities like smooth, edible, green skin and smaller size compared to kiwifruit. In addition to providing a novel eating experience, nutritional qualities of *A. arguta* have been reported (Latocha et al. 2015; Pinto et al. 2020; Wojdyło and Nowicka 2019; Wojdyło et al. 2017). Studies examining chemical composition of kiwiberry fruits reported: high levels of vitamin C (Leontowicz et al. 2016; Nishiyama et al. 2004), high levels of polyphenols relating to antioxidant activity (Wojdyło and Nowicka 2019; Wojdyło et al. 2017), and the presence of various minerals beneficial for human health (Jin et al. 2014; Latocha 2017). Nutritional substances such as vitamins, minerals, and phenolic compounds like those found in *A. arguta* are associated with health benefits from consuming fruit (Beattie et al. 2005; Paredes-López et al. 2010; Pinto et al. 2020). Diets diverse in fruits and vegetables have been linked to increased intake of nutrients and improved health (Herforth et al. 2019; Lachat et al. 2018). The novel eating experience and nutritional profile of kiwiberries indicate that this crop necessitates further consideration for crop development to bring diverse, nutritious food opportunities to market.

Currently, *A. arguta* ‘Ananasnaya’ is one of the most widely planted kiwiberry cultivars in the United States (Fisk et al. 2006) and is widely planted throughout global production regions along with ‘Weiki’ and ‘Geneva’ (Latocha et al. 2017). *A. arguta* has

been favored for production because it typically produces larger fruit (approximately 10-12 g) with a range of flavors. However, production of *A. arguta* in cold climates results in challenges as extreme winter and low spring temperatures often result in yield loss. Hence, despite its smaller fruit size (approximately 3-5 g), *A. kolomikta* is another species that should be investigated for production value due to its improved cold-hardiness. However, the kiwiberry is relatively uncommon throughout the United States and consumer acceptability is currently unknown.

From the perspective of a new product, understanding consumer willingness-to-pay (WTP) is a critical step in marketing kiwiberry. Consumer studies have examined *A. arguta* fruit qualities, such as sweetness and flavor, and their impacts on consumer preferences in New Zealand (Hunter et al. 2020) and Belgium (Vanhonacker and Debersaques 2017). Hunter et al. (2020) indicated a favorable increase in kiwiberry-liking rating as soluble solids content (a proxy for sweetness) increased. Similarly, Vanhonacker and Debersaques (2017) found that after tasting fruit, 85% of participants rated the flavor of kiwiberry as favorable. While fruit quality attributes such as flavor is an important influence for repeat purchasing (Gilbert et al. 1996), studies have suggested that the cost of a product, rather than sensory qualities influencing taste, impacts first time consumer purchasing habits (Jaeger and Harker 2006; Steptoe et al. 1995). Pricing was indicated as a possible concern by Vanhonacker and Debersaques (2017) in Belgium as only 18% of participants were willing to pay €2.50 (US$2.64) for kiwiberry. This is why consumer WTP should be investigated to better market new and novel fruit.

One method for estimating WTP is with experimental auctions which obtain realistic estimations of WTP by implementing nonhypothetical consequences such as an

exchange of real goods for real money (Lusk 2003). In experimental auctions, participants with the highest bid wins but pays an amount of the *n*th-price (2^{nd}, 3^{rd}, etc.). Vickrey (1961) showed that in such an auction it is a weakly dominant strategy for participants to bid their true WTP. An experimental sealed-bid auction aims to reduce participants' probability of over bidding and under bidding as other participants' bids are unknown which incentivizes truthful bidding (Lusk and Hudson 2004; Yue et al. 2010). If a participant bids lower than their true willingness-to-pay, they run the risk of missing out on a profitable transaction. Conversely, if a participant bids higher, they risk paying more than their real willingness-to-pay. Advantages of experimental auctions include utility in group settings, placing participants in an active market, and eliciting WTP estimations for each participant. However, there are limitations to the use of experimental auctions which require participants to estimate product values without external inputs like posted prices; may be impacted by participant preferences at the time of evaluation; and are more costly than hypothetical approaches such as conjoint analysis (Lusk et al. 2004; Yue et al. 2010). Experimental auctions have been used to estimate consumers' value of many agricultural products (e.g., Alfnes and Rickertsen 2003; Fan et al. 2019; Froehlich et al. 2009; Gallardo et al. 2018; Hayes et al. 1996; Jaeger and Harker 2006; Lund et al. 2006; McAdams et al. 2013; Short et al. 2018; Tegene et al. 2003; Umberger and Feuz 2004; Yue et al. 2009, 2011, 2016).

Previous studies have explored the importance of the relationship between kiwifruit fruit quality and consumer preference (Ball et al. 1998; Crisosto and Crisosto 2001; Gilbert et al. 1996; Harker et al. 2006, 2009, 2019; Hunter et al. 2020; Jaeger et al. 2003, 2006, 2011; Latocha and Janjowski 2011; Marsh et al. 2006) and a few studies

have investigated WTP for kiwifruit (Jaeger and Harker 2006) and kiwiberry (Vanhonacker and Debersaques 2017). Currently, WTP for kiwiberry has been estimated using survey methods (Vanhonacker and Debersaques 2017). The primary objective of this research was to determine consumer WTP and explore consumer segmentation in terms of preferences for novel kiwiberries and other berries. This research provides important marketing implications on the target market(s) and insights on pricing strategies for kiwiberries.

Materials and Methods

Products

The products in the experiment are four types of kiwiberries: 'Geneva3', 'Passion Popper', 'Krupnopladnaya', and Nahodka Seedling 6. 'Geneva3' and 'Passion Popper' are both cultivars of *A. arguta,* while 'Krupnopladnaya' and Nahodka Seedling 6 are a cultivar and seedling (respectively) of *A. kolomikta.* To compare consumer preferences for kiwiberries and their close substitutes in the market, we also included four other types of berries: strawberry (*Fragaria* x *ananassa*), raspberry (*Rubus idaeus*), blackberry (*Rubus fruticosus*), and blueberry (*Vaccinium corymbosum*). Strawberries, raspberries, blackberries, and blueberries all met the requirements for USDA grade 1 being uniform, well colored, well developed, not overripe, and free from damage or disease. A random sampling of twenty-five berries at point of consumption were juiced for examination of soluble solids content (SSC) and titratable acidity (TA). Mean SSC was 6.72 °Brix (strawberry), 8.34 °Brix (raspberry), 10.56 °Brix (blueberry), and 6.48 °Brix (blackberry). Mean TA was 0.7 g/100ml (strawberry), 1.47 g/100ml (raspberry), 0.66

g/100ml (blueberry), and 0.76 g/100ml (blackberry). The kiwiberries were grown by the researcher's lab and the market substitute berries were provided by a third-party vendor with each fruit type consisting of a single cultivar. Fruit samples for tasting were prepared for auction by washing fruit and then placing a single berry in a 1 oz clear plastic cup with a lid. Each fruit type was given a randomized three-digit numeric code that corresponded to a clamshell package of the fruit on a display table for participants. Fruit samples were then arranged in a randomized order at participant stations along with an attribute rating booklet, bidding sheet, and survey. Fruit packages for auction bidding were displayed in 6 oz clear plastic clamshells with the exception of strawberry which was packaged in a 1 lb clear plastic clamshell to reflect common market packaging for each fruit type.

The timing for the experiment was chosen as a midpoint between the kiwiberry species *A. kolomikta* and *A. arguta* which ripen in August and September, respectively, in Minnesota. Ripeness was determined by observing seed coloration (with black indicating near ripeness), estimated percent softening of fruit on the vine, and degree Brix which serves as a proxy for percent sugar in the fruit juice. Firm fruit from the *A. kolomikta* genotypes 'Krupnopladnaya' and Nahodka Seedling 6 were harvested on August 20th with a sampling of 5 berries averaging 7.8 °Brix and 6.9 °Brix at harvest, respectively. Fruits were stored in low-flow plastic clam shells in an ethylene-free 4 °C cooler. Fruits from *A. arguta* 'Geneva3' and 'Passion Popper' were selectively harvested for early softening fruits on September 5th averaging 7.5 °Brix and 8.3 °Brix at harvest, respectively. SSC and TA were additionally sampled at point of consumption using a random sample of five fruit. Mean SSC for kiwiberry samples were 13.2 °Brix

(Krupnopladnaya), 10.4 °Brix (Nahodka Seedling), 13.8 °Brix (Passion Popper), and 15.1 °Brix (Geneva3). Mean TA for kiwiberry samples were 1.57 g/100ml (Krupnopladnaya), 1.57 g/100ml (Nahodka Seedling), 1.24 g/100ml (Passion Popper), and 1.26 g/100ml (Geneva).

Experimental procedure

In past decades, experimental auctions have been used to value a wide variety of food quality attributes (see, e.g., Hobbs et al. 2005; Jaeger and Harker 2005; Brown et al. 2005; Alfnes and Rickertsen 2003). A second-price sealed-bid auction is an auction where bidders submit sealed bids, the price is set to the second highest bid, and the winner is the participant with the highest bid. The second-price auction is a popular mechanism because it is theoretically demand revealing, it is easy to explain, and its market-clearing price is endogenous. Experimental auction methods from Yue et al. (2016) and Short et al. (2018) were used as a framework for this study.

The experiment was conducted at the University of Minnesota, St. Paul with a total of 96 participants. Participants were recruited by placing advertisements in local newspapers and on social media. To be eligible for the experiment, participants had to be 18 years of age and purchase groceries. Of the 105 individuals who registered, 98 attended, of which 96 provided complete information to be included in the study. Participants were scheduled for one of five sessions that took place over two days with approximately 20 participants scheduled per session. Experiment sessions were held in a conference room. Auction booklets were provided to participants to follow the experimental procedure and randomized fruit cups were setup for each participant to taste the fruits. Fruits in packages were displayed on a table for participants to examine.

The experiment began by leading participants through a detailed explanation and practice examples of second-price auctions to ensure participants' understanding. This included how the identification of a product winner would occur. Participants were then instructed to taste the eight fruits and fill out an attribute rating sheet for each fruit as they tasted. The presentation order of fruits for participants was randomly arranged between sessions to prevent potential ordering effect on tasting results. After tasting and rating all the fruits, participants were asked to silently examine corresponding labeled packages of each fruit on display at the back of the room. They were then asked to complete a prepared bid sheet that listed each fruit sampled. Participants were not allowed to communicate with each other during the bidding process.

After all participants' bid sheets were collected, researchers sorted the bids from highest to lowest for each fruit and identified the winner(s) for each. The binding product for each participant was drawn. Only when a participant won their binding product were they required to purchase the product. After submitting bid sheet, participants completed a survey asking questions about their demographics, shopping behaviors, and likelihood of trying new foods. Demographics included, but was not limited to, questions on age, gender, income, relationship status, and education level. Questions for shopping behavior focused on frequented shopping location. Finally, participants were provided with statements such as "I purchase new, different, or innovative food" or "I seldom taste or experiment with new, different, or innovative foods" and asked to indicate their agreement with the statement (1 being Strongly disagree; 7 being Strongly agree). Table 1 shows the summary statistics for demographic questions. Table 3 shows the summary statistics of the shopping location and habit questions. After completing these questions,

participants who did not win were paid $30 to compensate for their time and winners received their respective fruit package and $30 minus the market price of the fruit they won.

Data for participants were compiled and analyzed. All bids were adjusted to reflect 6oz package prices for comparison between all berries. To investigate participants' heterogeneous preferences for kiwiberries, we compared their bids for the two species of kiwiberries to their bids for other berries henceforth referring to strawberry, raspberry, blueberry, and blackberry. Specifically, we compared the bids for each kiwiberry to the mean bids for other berries. Participants were grouped into different groups based on if they bid higher for one of the two kiwiberry species (*A. arguta* Price Premium or *A. kolomikta* Price Premium), a combination of both species (Mixed Kiwiberry Price Premium), or neither (Kiwiberry Discounting) compared to the average bid of the other berries. Analysis of variances (ANOVAs) were used to determine significant differences in fruit attribute ratings, demographics, and shopping habits across the groups.

Results

Table 1 shows the summary statistics of sociodemographic backgrounds for the 96 auction participants. The average age of participants was in the category of 51 and 60 years old. Approximately 71% of participants identified as female. Thirty-six percent of participants reported education of some college or below; 36.46% had a college diploma or some graduate school; 27.08% reported having a graduate degree or above. The average annual income was between $50,000 and $65,000. Fifty percent of participants held either a part- or full-time job, while the remaining participants were either students,

retired, or unemployed. Forty-six percent of participants were either in a relationship or married, and the average household size was two people with most participants reporting having no children under 12 years old in the household (89.52%). Most participants (81.25%) reported not having membership in environmental groups.

Eight percent of participants reported consuming berries once a month or less. Thirteen percent (13.54%) of participants consume berries multiple times a month and 18.75% reported eating berries once a week. Approximately fifty-eight percent of participants reported eating berries multiple times a week (45.83%) or daily (13.54%).

Figure 1 uses histograms to display the distribution of bids for 6-ounce package of berries. The berries in the top row (other berries instead of kiwiberries) display normal distributions of bids from approximately $0.00 to $5.99 with a mean bid of approximately $1.12 to $2.48 depending on the berry. The bids for kiwiberries have a broader spread with both *Actinidia* species having two slight peaks based on the observed density line. *A. kolomikta* is skewed towards the lower bid values. Bids for *A. arguta* range from $0.00 to $5.99 with mean bids of $2.00 and $2.19 for 'Geneva3' and 'Passion Popper', respectively (Table 2). The bids for *A. kolomikta* range from $0.00 to $4.99 with a mean bid of $1.63 and $1.65 for 'Krupnopladnaya' and Nahodka Seedling, respectively.

Figure 2 expands further on the observed distribution of kiwiberry bids by graphing the difference between participants' bids for kiwiberry species and the average bid for other berries. The horizontal axis of Figure 2 is the bid difference between *A. arguta* and other berries and the vertical axis is the bid difference between *A. kolomikta* and other berries. Using Figure 2, participants were grouped into one of four groups: *A.*

arguta Price Premium, *A. kolomikta* Price Premium, Mixed Kiwiberry Price Premium, or Kiwiberry Discounting. The upper right quadrant shows that some participants had higher bids for both kiwiberry species compared to other berries (Mixed Kiwiberry Price Premium). The lower right quadrant shows participants with higher bids for one or both of the *A. arguta,* but not *A. kolomikta* (*A. arguta* Price Premium). The lower left quadrant shows participants with lower bids for kiwiberries compared to other berries (Kiwiberry Discounting). Interestingly, the group for those who bid high for one or both of the *A. kolomikta* is somewhat obscured as participants tend to favor only one not both resulting in low *A. kolomikta* average bids (*A. kolomikta* Price Premium).

The differences between groups for kiwiberry bids are more apparent in Figure 3 which shows boxplots by kiwiberry cultivar and kiwiberry price premium group. The bids of Kiwiberry Discounting group are lower than all other groups for all kiwiberries while the Mixed Kiwiberry Price Premium group has bids comparable or higher than the other kiwiberry price premium groups. Figure 3 further confirms that the *A. arguta* Price Premium group bids higher for *A. arguta* than *A. kolomikta,* and vice versa for the *A. kolomikta* Price Premium group. Table 2 further displays each groups mean bid, standard deviation, and a pairwise comparison of all fruit bids between groups. The pairwise comparisons indicate these groups' WTP significantly differed for kiwiberries, raspberry, and strawberry. Blackberry and blueberry showed no difference between groups. Raspberry and strawberry bids were significantly different ($p < 0.10$) between the *A. arguta* Price Premium and Kiwiberry Discounting groups (Table 2), but not for the Mixed Kiwiberry Price Premium and *A. kolomikta* Price Premium. Bid differences between groups for the kiwiberry cultivars had mixed results, but for all kiwiberries the

means between Mixed Kiwiberry Price Premium and Kiwiberry Discounting groups remained significantly different at $p < 0.10$ (Table 2). For 'Geneva3' and 'Passion Popper', the *A. arguta* Price Premium groups bids significantly differed from the Kiwiberry Discounting group at $p < 0.01$. The highest mean bid for kiwiberry was $2.88 for 'Passion Popper' from the *A arguta* Price Premium group.

To identify profiles for these groups, participants' socio-demographic and purchasing habit variables were compared among groups. Table 3 shows the mean, standard deviation of these variables, and ANOVA results. Age and education were the only two socio-demographic variables that demonstrated statistically significant differences among groups. Though age is not significant across all groups ($p = 0.109$), a pairwise comparison between the groups indicates that the Mixed Kiwiberry Price Premium group (the mean age was in the 41-50 years old category) was significantly different from the Kiwiberry Discounting group (the mean age was in the 51-60 years old category). Education was highly significantly different between *A. arguta* Price Premium and both *A. kolomikta* Price Premium and Kiwiberry Discounting groups. The mean education level for *A. arguta* Price Premium group was college diploma, while the mean education levels for *A. kolomikta* Price Premium and Kiwiberry Discounting groups were high school diploma to some college.

Purchasing habits differed among groups in terms of participants grocery purchasing stores and their attitude toward new products. For stores, cooperatives were visited by the Mixed Kiwiberry Price Premium group more frequently than the Kiwiberry Discounting group. Other stores such as convenience stores, high-end retailers, supermarkets, and roadside markets had no significant differences between any of the

groups. In terms of attitudes, the Kiwiberry Discounting group had a higher rating for negative purchasing attitudes (i.e., seldom taste and experiment with new foods; afraid of eating foods not tasted before; reluctant to try foreign or new foods) than all other groups with a significantly higher rating than the *A. arguta* Price Premium and Mixed Kiwiberry Price Premium groups. *A. arguta* Price Premium and Mixed Kiwiberry Price Premium showed positive attitudes for interest in new foods or variety-seeking attitudes.

Further differences between groups were observed when examining participant ratings of fruit quality attributes (Table 4). The first panel of Table 4 reports mean and standard deviation of attribute ratings for all kiwiberry cultivars for each group. For seven out of the twelve attributes, the Mixed Kiwiberry Price Premium group differed significantly from the Kiwiberry Discounting group with the former having a higher rating for all attributes. The *A. arguta* and *A. kolomikta* Price Premium ratings were between the Mixed and Discounting groups. The second panel of Table 4 shows the mean attribute ratings for other berries for each group. There were no significant differences noted between groups for any of the attribute ratings of other berries. However, other berries' attributes are rated higher than kiwiberries' attributes.

In addition to comparing between groups, within group differences were examined for attribute ratings between kiwiberries and other berries. For the Kiwiberry Discounting group, all mean ratings were significantly different between kiwiberries and other berries. For the three kiwiberry price premium groups, sweetness and tartness were not significantly different at a p-value of 0.10. Additionally, for the Mixed Kiwiberry Price Premium group, the overall flavor was not significantly different at 10% between kiwiberry and other berries. Overall flavor was significant at $p < 0.10$ for the *A. arguta*

Price Premium and *A. kolomikta* Price Premium groups. Texture was significantly different at $p < 0.01$ for all groups with all groups rating other berries texture higher than kiwiberry texture.

Discussion and Conclusions

Early steps in developing a product for the market include estimating consumer WTP and identifying interested consumer groups. Understanding consumer preferences and WTP for the new product can inform producers, suppliers, and marketers how to price the product and select the target markets of the new product. The results from our experimental auction suggest that the mean WTP for kiwiberry ranges from $0.76 to $2.88 for a 6 oz package. However, WTP varies across groups with a majority of participants willing to pay similar amounts for kiwiberry as for other berries. Furthermore, differences in consumer socio-demographics may influence WTP.

Our results indicate that WTP for kiwiberry might be lower for older, lower degree holding, and food neophobic individuals. Participants in the Kiwiberry Discounting group were significantly different in terms of age being higher and education being lower from certain kiwiberry price premium groups. Additionally, the Kiwiberry Discounting group had significant neophobic responses to questions regarding new or unfamiliar foods. Marketing strategies for this group should address potential underlying causes of neophobic attitudes associated with food safety concerns of unfamiliar foods such as health and safety (Onwezen and Bartels 2011; Raudenbush and Frank 1999). Product information for intangible features such as labeling for production location,

product safety, and promoting product health benefits could better market novel fruit products to concerned consumers by reducing perceived risks (Nazzaro et al. 2019).

Meanwhile, marketing strategies for the variety seeking groups, which includes the *A. arguta* Price Premium, *A. kolomikta* Price Premium, and Mixed Kiwiberry Price Premium groups, should consider promoting the novelty of kiwiberries in terms of convenience and flavor. Promoting novel characteristics might be effective for consumers interested in kiwiberry because it promotes the uniqueness of the fruit eating experience for groups that value new experiences. When considering where to market kiwiberries for the kiwiberry price premium groups, most shopping locations are ubiquitous among the groups. However, supermarkets, discount stores, and cooperatives are locations for marketers to consider as these were the most frequently reported shopping locations for the Mixed Kiwiberry Price Premium group.

The attribute ratings of the studied berries can be used by breeders and producers to inform breeding goals or production methods to improve fruit quality. Visual fruit quality attributes were the highest rated kiwiberry attributes within kiwiberry price premium groups indicating participants like the appearance of the fruit. Eating experience attributes (texture and firmness) for kiwiberries more frequently received low scores across the groups. Texture and firmness deterioration could have occurred as *A. kolomikta* berries were harvested prior to the auction to ensure adequate fruit availability. Texture can be impacted by harvest timing, storage conditions, or differences in cultivar and each component could be targets for improvement (Fisk et al. 2006). Though texture and firmness were low rated attributes, all kiwiberry attributes were consistently rated lower than other berries. This could in part be due to participants' biased familiarity with

the other berries which might increase their ratings. Additionally, the fruit provided for the other berries was of exceptionally high quality with larger, uniform fruit that might be superior to berries typically available for purchase. Despite these possible factors impacting attribute ratings, fruit qualities related to eating experience and taste remain targets for breeders.

Although kiwiberry is a novel product with relatively small production in the U.S., most participants in this study indicated a WTP for kiwiberry that was comparable with other berries. However, the results show differences in WTP between *A. arguta* and *A. kolomikta* cultivars with *A. arguta* 'Passion Popper' having a higher average WTP for participants in the kiwiberry price premium groups. (Though later genomic results revealed that 'Passion Popper' and 'Geneva3' are likely the same individual.) The underlying neophobic attitudes for participants disliking kiwiberries warrants further investigation to determine where the unfavorable attitudes arise and if unfavorable attitudes stem from unfamiliarity of the product or other concerns. Overall, there are opportunities for future development and improvement of kiwiberries with consumers ready to buy.

Tables

Table 1. Summary statistics (mean, standard deviation, percent of sample) of the 96 participants in the kiwiberry experimental auctions (N = 96).

	Percent of Sample
Age	
1 = 18-30 years old	25%
2 = 31-40 years old	8.33%
3 = 41-50 years old	2.08%
4 = 51-60 years old	18.75%
5 = 61-70 years old	30.21%
6 = >70 years old	15.62%
Mean (S.D.)	3.68 (1.86)
Gender	
0 = Female	70.83%
1 = Male	29.17%
Educational Level	
1 = Some high school education	0%
2 = Earned a high school diploma	5.21%
3 = Some college education	31.25%
4 = Earned a college diploma	26.04%
5 = Some graduate school education	10.42%
6 = Earned a graduate degree	27.08%
Mean (S.D.)	4.23 (1.29)
Income	
1 = <$15,000	9.47%
2 = $15,001 - $25,000	15.79%
3 = $25,001 - $35,000	11.58%
4 = $35,001 - $50,000	13.68%
5 = $50,001 - $65,000	4.21%
6 = $65,001 - $80,000	13.68%
7 = $80,001 - $100,000	13.68%
8 = $100,001 - $150,000	10.53%
9 = >$150,000	7.37%
Mean (S.D.)	4.80 (2.52)
Employment Status	
0 = Retired/Unemployed/Student	50.00%
1 = Full-/Part-time Employed	50.00%
Mean (S.D.)	0.50 (0.50)
Relationship status	

0 = No relationship	37.50%
1 = In a relationship	62.50%
Mean (S.D.)	0.62 (0.49)
Presence of children under 12 at home	
No	89.58%
Yes	10.42%
Mean (S.D.)	0.10 (0.31)
Household size	
Mean (S.D.)	1.98 (1.01)
Environmental group membership	
No	81.25%
Yes	18.75%
Mean (S.D.)	0.19 (0.39)
Frequency of berry consumption	
1 = Daily	13.54%
2 = Multiple times a week	45.83%
3 = Once a week	18.75%
4 = Multiple times a month	13.54%
5 = Once a month	6.25%
6 = Less than once a month	1.04%
7 = Never	1.04%
Mean (S.D.)	2.60 (1.23)

Table 2. Mean willingness-to-pay ($) and standard deviation (SD) for all berries for each kiwiberry groups determined by the difference between WTP for kiwiberries and other berries are presented.

Fruit Bids	All participants		Groups								ANOVA
			A. arguta Price Premium		*A. kolomikta* Price Premium		Mixed Kiwiberry Price Premium		Kiwiberry Discounting		
	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD	p-value
Blackberry	2.12	1.05	2.11	1.26	2.34	0.76	2.21	1.11	1.94	0.86	0.700
Blueberry	2.48	1.13	2.79	1.31	2.55	1.06	2.55	1.14	2.13	0.95	0.220
Raspberry[c]	2.47	0.94	2.82	1.04	2.43	0.98	2.51	0.92	2.15	0.8	0.094
Strawberry[c]	1.12	0.46	1.32	0.43	1.14	0.46	1.17	0.48	0.91	0.4	0.017
Geneva3[c,d,f]	2.00	1.26	2.68	1.11	1.43	0.77	2.48	1.19	0.93	0.71	<0.001
Passion Popper[a,c,d,f]	2.19	1.34	2.88	1.02	1.23	0.91	2.85	1.20	0.97	0.68	<0.001
Krupnopladnaya[b,f]	1.63	1.11	1.36	0.89	1.66	0.96	2.32	1.06	0.85	0.74	<0.001
Nahodka Seedling[b,e,f]	1.65	1.24	1.12	0.70	2.16	0.70	2.46	1.30	0.76	0.66	<0.001

[a]Means of *A. arguta* Price Premium significantly different from *A. kolomikta* Price Premium at $p < 0.10$;
[b]Means of *A. arguta* Price Premium significantly different from Mixed Kiwiberry Price Premium at $p < 0.10$;
[c]Means of *A. arguta* Price Premium significantly different from Kiwiberry Discounting at $p < 0.10$;
[d]Means of *A. kolomikta* Price Premium significantly different from Mixed Kiwiberry Price Premium at $p < 0.10$;
[e]Means of *A. kolomikta* Price Premium significantly different from Kiwiberry Discounting at $p < 0.10$;
[f]Means of Mixed Kiwiberry Price Premium significantly different from Kiwiberry Discounting at $p < 0.10$.

Table 3. Mean and standard deviation (SD) for socio-demographic, purchasing store, and attitude variables shown by kiwiberry groups (Groups are determined by the difference between WTP for kiwiberries and other berries).

	A. arguta Price Premium		*A. kolomikta* Price Premium		Mixed Kiwiberry Price Premium		Kiwiberry Discounting		ANOVA
Sociodemographic	Mean	SD	Mean	SD	Mean	SD	Mean	SD	p-value
Age (yr) [f]	52.71	18.11	53.71	17.94	46.58	19.85	57.89	17.36	0.108
Gender	0.19	0.39	0.43	0.50	0.27	0.45	0.36	0.48	0.522
Education (yr)[a,c,f]	17.24	1.81	14.86	1.08	16.40	2.11	15.07	2.16	0.001
Income ($)	59,286	34,903	57,143	49,821	63,875	45,482	65,000	45,562	0.951
Household members	1.86	0.71	2.00	1.33	2.08	1.06	1.93	1.00	0.870
Purchasing location and attitudes									
Mass merchandiser	1.29	0.45	1.43	0.5	1.23	0.42	1.29	0.45	0.720
Cooperative[f]	1.24	0.43	1.00	0.00	1.32	0.47	1.07	0.26	0.037
High end retail	1.14	0.35	1.29	0.46	1.25	0.43	1.32	0.47	0.560
Supermarket	1.52	0.50	1.29	0.46	1.55	0.50	1.68	0.47	0.280
Convenience store	1.05	0.21	1.00	0.00	1.05	0.22	1.07	0.26	0.900
Discount store	1.38	0.49	1.71	0.46	1.50	0.50	1.68	0.47	0.140
Roadside	1.29	0.45	1.57	0.50	1.27	0.45	1.32	0.47	0.480
Negative purchasing[c,f]	-0.30	0.81	0.07	0.99	-0.16	0.87	0.44	0.90	0.019

[a]Means of *A. arguta* Price Premium significantly different from *A. kolomikta* Price Premium at $p < 0.10$ or better

[c]Means of *A. arguta* Price Premium significantly different from Kiwiberry Discounting at $p < 0.10$ or better

[f]Means of Mixed Kiwiberry Price Premium significantly different from Kiwiberry Discounting at $p < 0.10$ or better

Table 4. Mean and standard deviation (SD) for 12 attribute ratings (N = 96 for each attribute), broken into kiwiberry and other fruits by kiwiberry group with between and within group significance shown.

	A. arguta Price Premium		*A. kolomikta* Price Premium		Mixed Kiwiberry Price Premium		Kiwiberry Discounting		ANOVA
Attribute rating: kiwiberries	Mean	SD	Mean	SD	Mean	SD	Mean	SD	p-value
Overall liking[f]	4.97	1.24	4.64	1.61	5.47	0.91	4.34	1.35	0.002
Overall appearance[f]	5.12	1.13	4.64	1.74	5.40	0.78	4.71	1.24	0.059
Overall flavor[f]	4.90	1.10	4.68	1.61	5.36	0.85	4.28	1.28	0.002
Shape[f]	5.44	1.18	5.62	1.06	5.61	0.92	4.88	1.16	0.046
Color	5.51	1.16	5.11	1.55	5.69	0.77	5.14	1.10	0.150
Size	5.32	1.15	5.00	1.68	5.67	1.03	5.00	1.28	0.120
Cosmetic defect	4.70	1.15	4.12	1.61	5.14	1.13	4.64	1.30	0.150
Sweetness[f]	4.76	1.24	4.68	1.31	5.22	0.97	4.38	1.21	0.030
Tartness[f]	4.71	1.30	4.79	0.80	5.03	1.08	4.31	1.06	0.087
Juiciness[f]	5.01	1.24	4.54	1.58	5.38	0.89	4.54	1.31	0.026
Texture[f]	4.26	1.39	4.46	1.41	5.07	1.03	4.04	1.45	0.009
Firmness[f]	4.20	1.41	4.04	1.67	4.86	0.92	4.02	1.51	0.039
Attribute rating: other berries									
Overall liking	5.91	0.93	6.00	1.18	5.91	0.67	5.92	0.91	0.990
Overall appearance	6.49	0.67	6.36	1.28	6.41	0.58	6.42	0.60	0.970
Overall flavor	5.50	0.96	6.04	0.64	5.59	0.77	5.63	1.18	0.640
Shape	6.56	0.62	6.75	0.38	6.47	0.63	6.40	0.70	0.580
Color	6.63	0.45	6.75	0.29	6.54	0.56	6.46	0.68	0.560
Size	6.29	1.11	7.00	0.00	6.31	0.74	6.19	0.88	0.230

Cosmetic defect	5.61	1.19	5.61	1.34	5.64	1.10	5.54	1.23	0.980
Sweetness	5.27	1.05	5.21	1.21	5.37	1.01	5.35	1.35	0.980
Tartness	5.18	1.32	4.86	1.14	5.15	1.03	5.18	1.29	0.930
Juiciness	5.97	0.81	6.00	0.92	5.78	0.89	5.72	1.17	0.780
Texture	5.95	0.98	6.50	0.52	5.83	0.86	5.73	1.08	0.320
Firmness	6.11	0.89	6.14	1.24	5.96	0.82	5.81	1.01	0.680
The p-values of ANOVA to compare the attribute ratings of kiwiberries and other berries within each group									
Overall liking	0.010		0.097		0.017		<0.001		
Overall appearance	<0.001		0.058		<0.001		<0.001		
Overall flavor	0.078		0.060		0.210		<0.001		
Shape	<0.001		0.023		<0.001		<0.001		
Color	<0.001		0.017		<0.001		<0.001		
Size	0.008		0.014		0.003		<0.001		
Cosmetic defect	0.018		0.097		0.051		0.011		
Sweetness	0.160		0.440		0.530		0.007		
Tartness	0.260		0.910		0.590		0.008		
Juiciness	0.006		0.056		0.050		0.001		
Texture	<0.001		0.008		0.001		<0.001		
Firmness	<0.001		0.020		<0.001		<0.001		
[f]Means of Mixed Kiwiberry Price Premium significantly different from Kiwiberry Discounting at $p < 0.10$									

Figures

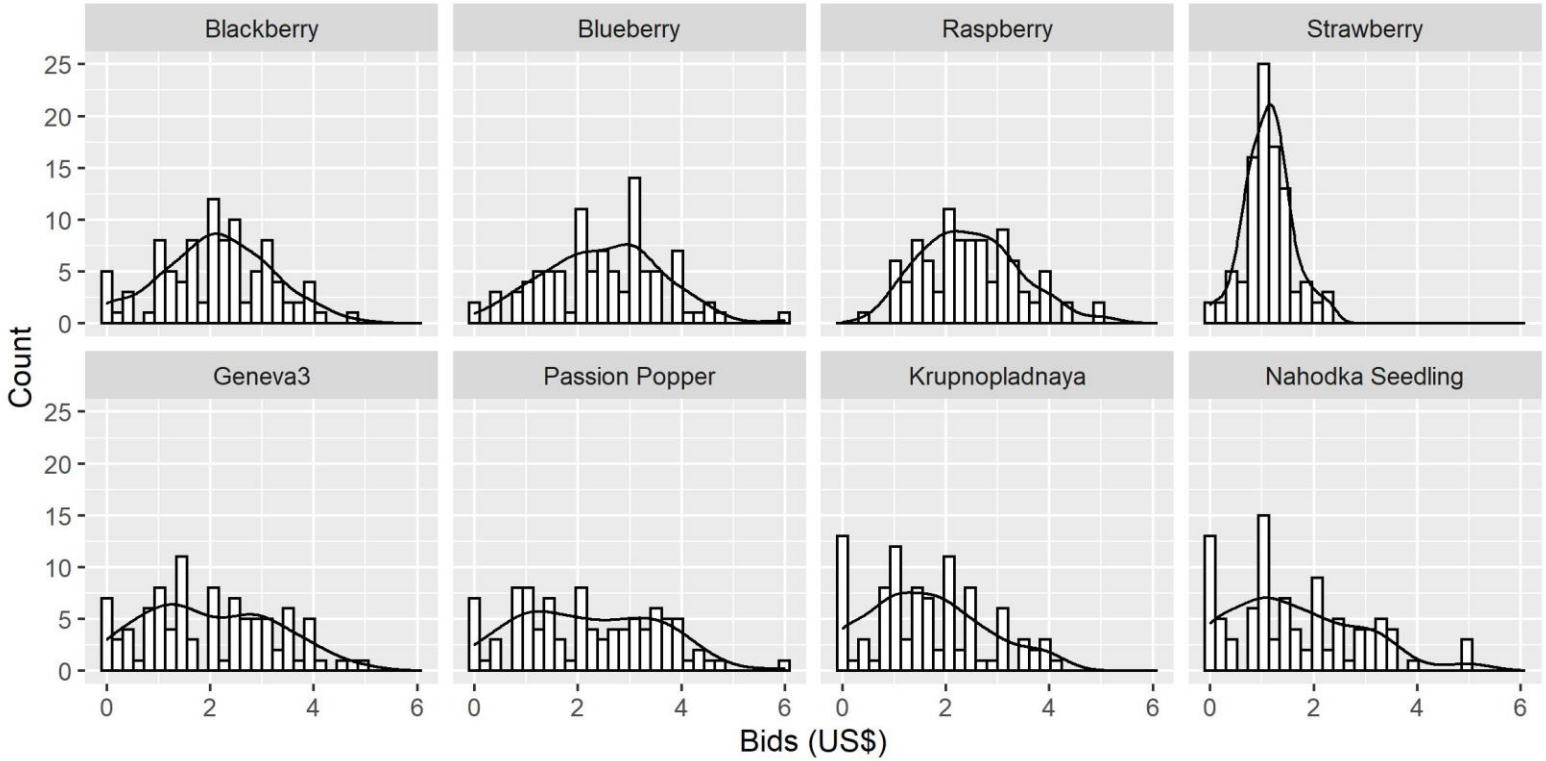

Figure 1. Histograms of bids for 6-ounce berry samples (N = 96 for each sample) with distribution curves overlaid. Strawberry bids have been adjusted to reflect bid amounts per 6-ounce package rather than the 1-pound package presented at the auction. Bids for other berries (blackberry, blueberry, raspberry, and strawberry) are shown on the top row. Bids for *A. arguta* ('Geneva3' and 'Passion Popper') and *A. kolomikta* ('Krupnopladnaya' and Nahodka Seedling) are shown on the bottom row.

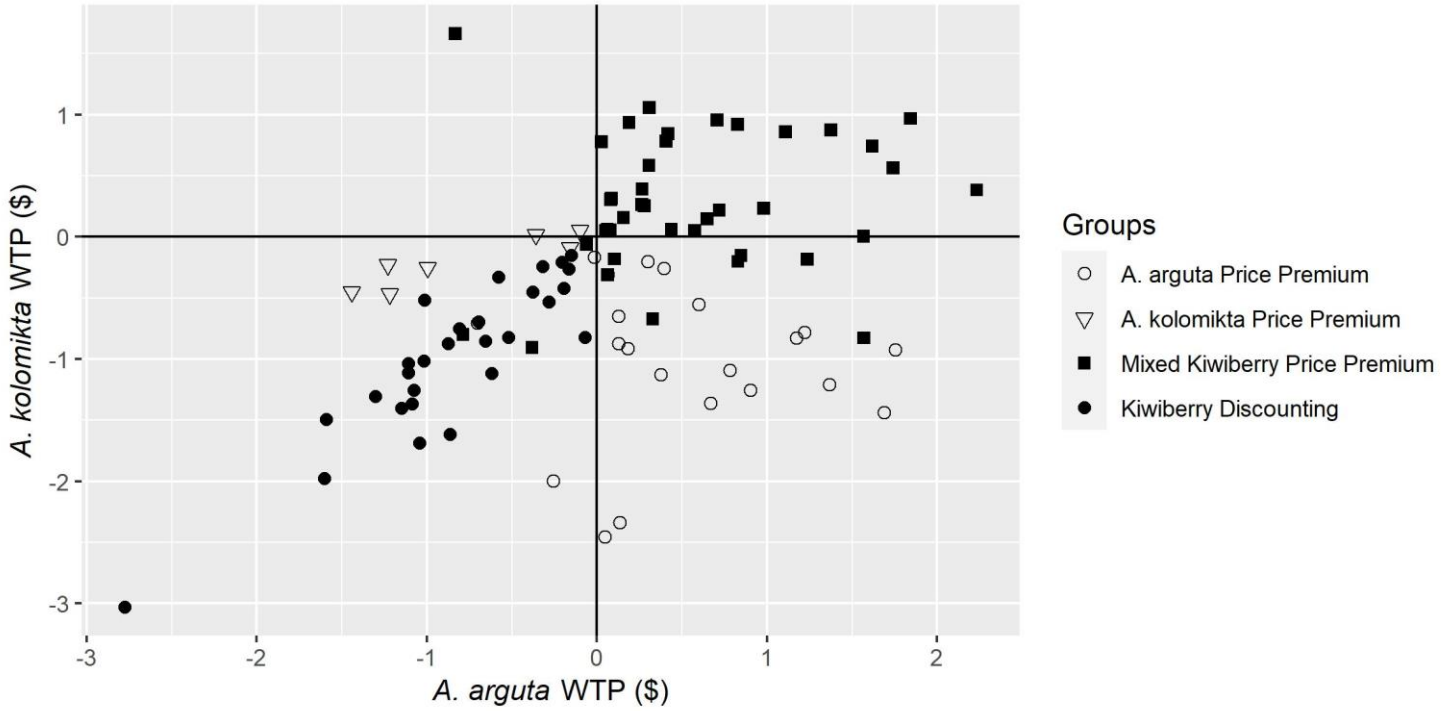

Figure 2. Scatter plot of the distribution of the differences between willingness-to-pay (WTP) for kiwiberry species and WTP for other berries for each participant. The difference between WTP for A. arguta and other berries is graphed along the x-axis, and the difference between WTP for A. kolomikta and other berries along the y-axis. Participants are labeled based on their WTP for kiwiberry cultivars and fall into one of four groups: *A. arguta* Price Premium, *A. kolomikta* Price Premium, Mixed Kiwiberry Price Premium, and Kiwiberry Discounting.

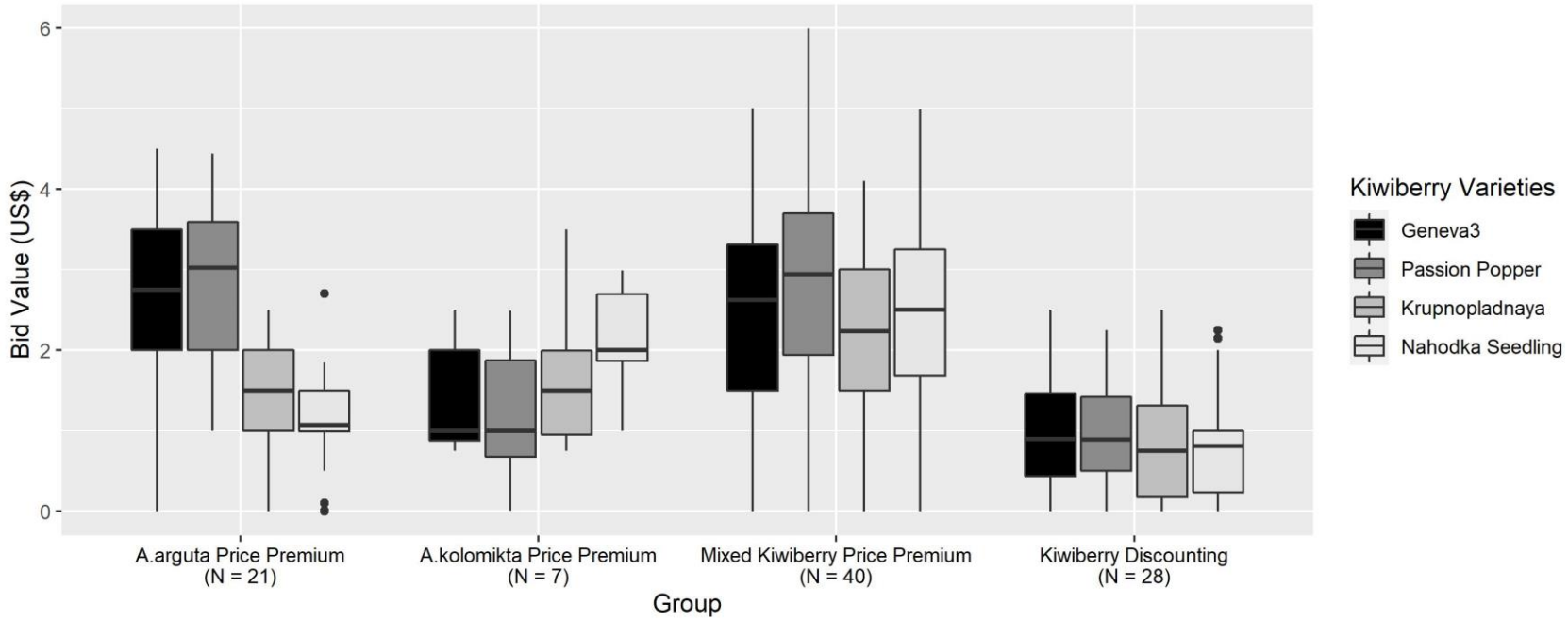

Figure 3. Standard boxplot of bids for 6-ounce kiwiberry samples, separated into groups (*A. arguta* Price Premium, *A. kolomikta* Price Premium, Mixed Kiwiberry Price Premium, Kiwiberry Discounting) based on WTP for kiwiberry cultivars ('Geneva3', 'Passion Popper', 'Krupnopladnaya', Nahodka Seedling). Within each group, boxes represent the interquartile range of bids for each kiwiberry cultivar with the black line denoting median bid.

Chapter 2: Genomic informed curation of North American kiwiberry germplasm

Introduction

The genus *Actinidia* is composed of approximately 76 identified species native to regions of eastern Asia (Ferguson and Huang, 2007). Most global production relies on two taxa: *A. chinensis* var. *deliciosa* (green kiwifruit) and *A. chinensis* var. *chinensis* (golden kiwifruit). Development of genomic resources in *Actinidia* have mostly focused on *A. chinensis*. The first sequenced genome for this genus was the diploid *A. chinensis* 'Hongyang' [2n = 2x = 58] draft genome, which was completed in 2013 and updated in 2019 (Huang et al. 2013; Wu et al. 2019). Additionally, draft genomes of *A. chinensis* 'Red5' have been constructed (Pilkington et al. 2018; Tang et al. 2019; Yao et al. 2022). In recent decades, interest has increased for cold-hardy *Actinidia* species, such as kiwiberry, to expand production into cold climates.

The term kiwiberry refers to smooth, berry sized fruits in the genus *Actinidia* and typically is used in reference to *A. arguta* and *A. kolomikta* fruits. In addition to kiwiberry, these fruits are known by many common names such as hardy kiwi, arctic kiwi, grape kiwi, baby kiwi, cocktail kiwi, bower vine, and taro vine. These cold-hardy, fuzz-free fruits combine a unique eating experience with healthy benefits including high contents of vitamin C and antioxidants such as lutein (Latocha 2017; Leontowicz et al. 2016; Wojdyło and Nowicka 2019). Other *Actinidia* species, such as *A. polygama* and *A. rufa,* are also sometimes referred to as kiwiberry. *Actinidia polygama* has unpalatable fruit, but interesting foliage provides value for breeding for landscape usage. *Actinidia*

rufa has green fruit skin with fine brown pubescence and fruit sizes larger than *A. arguta* and *A. kolomikta* (Huang 2016). The fruit and ornamental qualities of these species have drawn interest in kiwiberry as a novel fruit crop for growers in cold climates.

Actinidia arguta and *A. kolomikta* were introduced to the U.S. primarily in the 19th and 20th centuries. These species have been used as landscape plants and, more recently, for commercial fruit production. Importation to the U.S. has been banned or severely restricted in the 21st century due to phytosanitary restrictions attempting to control a global outbreak of *Pseudomonas syringae* pv. *Actinidiae* (Psa). Pedigrees of kiwiberry cultivars are difficult to validate due to several factors including: reclassification, renaming, and mislabeling. For example, one of the most popularly grown cultivars of *A. arguta* is 'Ananasnaya' (Fisk et al. 2006) but, the *A. arguta* 'Ananasnaya' is different phenotypically and genotypically from the *A. kolomikta* 'Ananasnaya' that was originally bred by I.V. Michurin in Russia in 1924 (Melo et al. 2017; Michurin 1949). Years later an *A. arguta* × *A. kolomikta* hybrid was named 'Ananas de Mitchourine' also differing from the *A. arguta* cultivar 'Ananasnaya' that finally arrived in the United States in 1972 (Melo et al. 2017). 'Ananasnaya' is only one example of mislabeling or renaming of kiwiberry cultivars. In the United States National Plant Germplasm System (NPGS), as many as 130 accessions of *A. arguta* have previously been listed along with 28 *A. kolomikta* accessions (Melo et al. 2017). Melo et al. (2016) used a genotyping-by-sequencing (GBS) approach to generate single nucleotide polymorphisms (SNPs) using mock reference genomes to fingerprint kiwiberry accessions in the USDA NPGS as well as cultivars on the market. Melo et al. (2017) identified 60 unique *A. arguta* accessions (out of 130 accessions) and 13 unique *A.*

kolomikta accessions (out of 28 accessions). This quantification of accession redundancy by Melo et al. emphasizes the need for fingerprinting kiwiberry collections to resolve identity and create uniform nomenclature to improve curation across the prominent germplasm collections. Previous studies have used similar SNP-based approaches to curate germplasm in crops such as avocado (Kuhn et al. 2019b), melon (Wang et al. 2021), mango (Kuhn et al. 2019a), olive (Islam et al. 2021), and pecan (Bentley et al. 2019). These studies emphasize the need for a clear understanding of pedigree, cultivars, and genetic relatedness for applying modern breeding technologies.

The University of Minnesota (UMN) has maintained a kiwiberry germplasm collection at the Horticultural Research Center (HRC) since the 1980s, largely through the efforts of volunteer curator, Dr. Robert Guthrie (Guthrie et al. 2006; Guthrie and Thole 2021). Guthrie obtained *Actinidia* accessions from various collectors in the U.S. and provided samples to the USDA NPGS. Additionally, Guthrie collaborated with the University of New Hampshire (UNH) kiwiberry program which helped resolve some of the incongruities between germplasm collections at the UNH, NPGS and the UMN (Melo et al. 2016). Kiwiberry has been a backyard fruit in the Northeast since the 1870s which provided opportunities for the UNH to gather materials and develop a breeding program in 2013 (Hastings 2015).

Previous research at the UMN identified the ploidy of select accessions present in 2007 (Start et al. 2007), but accessions have been added or removed since then. In addition, the relationship to material obtained from or contributed to the USDA NPGS is uncertain. The present study aims to use the GBS-SNP-CROP pipeline (Melo et al. 2016) and genomic resources to fingerprint the materials of the UMN and USDA NPGS

kiwiberry germplasm to verify cultivars, understand genetic diversity, and validate genetic relatedness (e.g., hybrids). The goal of this research was to improve the curation of North American kiwiberry collections held at the HRC and the NPGS.

Materials and Methods

Germplasm collection

A total of 368 accession samples were collected from the HRC and NPGS in 2022. Plant tissue was collected from the HRC kiwiberry germplasm located in Victoria, MN (44°86'N, -93°64'W). The germplasm is separated into two plots with incomplete accession replication between the plots. From these two plots, a total of 143 tetraploid *A. arguta* accessions [expected 2n = 4x = 116] and 96 diploid *A. kolomikta* accessions [expected 2n = 2x = 58] were collected (Tables 1-4). Previous studies examined UMN germplasm ploidy and all *A. kolomikta* were reported as diploid while *A. arguta* were reported as tetraploid (Start et al. 2007). Further passport information was assembled based on phenotype records from the 2019 to 2022 seasons and field labels. Tissue from UMN germplasm was collected into envelopes placed on dry ice, transported to the lab, lyophilized for 48 hours, and stored.

In addition to UMN germplasm, leaf tissue was requested from the USDA NPGS in Corvallis, OR. A total of 77 *A. arguta* accessions and 20 *A. kolomikta* accessions were sampled (Table 1b). Tissue from the USDA NPGS was collected directly into 96 well plates and lyophilized before shipping to the UMN. The combined samples from the UMN and NPGS collections were extracted and sequenced together and genotype information is collectively referred to as the UMN dataset.

DNA extraction and genotyping-by-sequencing (GBS)

Approximately 30 mg of lyophilized tissue for each sample was placed in a 1.2 ml Qiagen collection microtube on a 96 well plate. Plates were then submitted to the University of Minnesota Genomics Center (UMGC) for DNA extraction and sequencing. DNA was extracted using a Qiagen DNEasy Plant Mini Kit and 100 ng of extracted DNA was multiplexed into GBS libraries using a two-restriction enzyme protocol (*PstI-MspI).* The libraries were sequenced using 150 bp paired-end reads on a single lane of Illumina NovaSeq 6000 platform at the UMGC (Illumina, San Diego, CA).

Bioinformatic analysis and genotype identification

The UMGC provided demultiplexed FASTQ files, which were processed using version 2.0 of the GBS-SNP-Calling Reference Optional Pipeline (GBS-SNP-CROP; https://github.com/halelab/GBS-SNP-CROP) (Melo et al. 2016). The first two steps of the pipeline were skipped because demultiplexed reads were provided. Sequence analysis and genotype calling were conducted following default pipeline parameters with two exceptions. Samtools view parameters were changed to retain samples with a minimum mapping quality (MAPQ) of 30. The second change was the use of the *A. chinensis* V3.0 reference genome for alignment rather than creating mock reference genomes (Wu et al. 2019).

In addition to examining samples collected from the UMN and NPGS, previous samples examined in Melo et al. (2017) were included (Table 1a) to confirm identities across three collections. Melo et al. provided demultiplexed paired-end files for 191 accessions of *A. arguta*, *A. kolomikta,* and *A. polygama* from their study. The Melo et al.

samples were processed using the same pipeline as outlined for UMN samples. This genotype information is collectively referred to as the UNH dataset.

Downstream analysis

GBS-SNP-CROP produces merged SNP files in formats compatible for downstream analyses in R v 4.0.5 (R Core Team, 2021). R packages were used for genotype evaluation and accession redundancy calls. The function *daisy* from the cluster package (Maechler et al. 2022) was used to generate a Gower's dissimilarity matrix to assess accession dissimilarity of bi-allelic SNPs based on genotypic state data (i.e., heterozygous = 0; homozygous = 0.5; heterozygous = 1). Based on previous studies, minimum GD values of 0.0024 (*A. arguta*) and 0.0036 (*A. kolomikta*) were used to call genotypic redundancy in the UMN dataset (Melo et al. 2017).

The variant call format (VCF) file was imported into R for population analyses. *K*-means clustering was carried out using the R package *cluster* (Maechler et al. 2022). PCA was carried out with the package *adegenet* (Jombart 2008). Both analyses were performed on the combined datasets to identify populations in the germplasm. Identified populations from *k*-means clustering were used to subset the VCF file, and PCAs were run to examine further population structure and distribution of accessions from different datasets. Once populations were confirmed, UPGMA (complete) cladograms were created using the *hclust* function in the *stats* package (R Core Team 2021).

Results

Identified SNPs and species clustering

A total of 4,458 high-quality SNPs (MAF = 0.072) were called for the species of the combined UMN and UNH datasets. The optimal number of clusters for *k*-means was determined by silhouette, sum of squares, and gap statistic which identified four clusters to be present (Figure 1).

Combined GBS dataset PCA clusters by species

Data compatibility between UMN and UNH samples was determined by running a PCA on the combined set of SNPs and color-coding samples based on UMN or UNH dataset (Figure 2). Samples did not cluster by dataset, but were intermixed (Figure 2) indicating the two datasets could be considered together for other analyses. This suggests that differences between datasets such as differences in technologies, service providers, or across years were not main components affecting structure of samples. Further investigation found that species were clustering and driving the separation with approximately 68.25% of variation explained by PC1 and PC2 (Figure 3). The species clusters were as expected given previous research and pedigree information with additional evidence from newly sequenced UMN samples. Records from the UMN collection indicated the presence of crosses between *A. kolomikta* and *A. polygama,* along with these crosses back-crossed to *A. kolomikta.* Figure 3 shows separate clusters of *A. kolomikta* and *A. polygama* with the hybrid offspring clustering between the two species, as expected. In addition to these three groups, two other clusters are present representing *A. arguta* samples in one and *A. kolomikta* × *A. rufa* hybrids in the other, as expected.

Species level PCA shows separation by dataset for A. arguta

Samples per species were split out and analyzed further. The PCA examining the *A. arguta* GBS samples from both the UMN and UNH datasets indicates that the samples are clustering based on dataset (Figure 4). The variation explained by both PCs was low (16.65%), but there were two distinct clusters. Further analysis of the allele depth plotted against PC2 which separated the *A. arguta* datasets indicating an association between PC2 and allele depth (Figure 5). A PCA of the UMN and UNH *A. kolomikta* samples did not indicate the species separating based on dataset with 22.26% of the variance explained by PC1 and PC2 (Figure 6). No sub-populations of *A. kolomikta* were identified for the combined datasets based on *k*-means clustering which is confirmed by the lack of clustering on the PCA plot (Figure 6).

UMN species sub-populations

Actinidia arguta samples in the UMN dataset displayed low separation based on PCA (Figure 7). *K*-means analysis was conducted to explore *A. arguta* sub-population structure which indicated that between three and five populations may be present (Figure 8). *Actinidia kolomikta* sub-population structure was examined in the UMN dataset via PCA and *k*-means, and no sub-populations could be decisively identified for *A. kolomikta* alone as was true with analysis of the combined UMN and UNH datasets (Figure 9). The hierarchical clustering of the UMN dataset shows three sub-populations for *A. arguta* and single populations for each of *A. polygama*, *A. kolomikta*, *A. kolomikta* × *A. polygama*, and *A. kolomikta* × *A. rufa*, as expected from PCA and *k*-means analyses (Figure 10).

Redundant accessions identified

A cladogram of *A. arguta* cultivars from the UNH dataset shows three groups which clustered similar individuals as the UMN dataset (Figure 11). A cladogram of *A. arguta* cultivars from the UNH dataset separated the three clusters identified from *k*-means and the UMN cladogram into five groups (Figure 11). Of the 83 named accessions from the NPGS and UMN germplasms, 44 unique *A. arguta* accessions were identified (Table 1). Identity of the UMN samples was evaluated using the NPGS collection as reference (Table 2). An example of identity confirmation would be 'Dumbarton Oaks' (UMN) matching with 'Dumbarton Oaks' (NPGS – PI 617135) (Table 1). Comparing across UMN and UNH datasets, redundant accessions calls can be further confirmed such as the UMN dataset clustering of 'Geneva 1' (NPGS – PI 667886), 'Kuchta' (NPGS – PI 617127), 'Geneva 3' (UMN – C001), and Dave's Fave (UMN – F001) which matches the clustering of these samples in the cladogram developed using the UNH dataset (Table 1). However, some samples vary such as 'Ken's Red' -S23 and 74-55-S22 which clustered together from the UMN data, but not with their NPGS counterparts.

The *A. kolomikta* cladogram showed similar clustering of redundant individuals, but redundant calls between institutions were not always clustered together (Figure 12). One example is the redundant individuals for 'Leningradskaya Pozdnaya' which clustered between GBS datasets and within each collection (Figure 12 and Table 3). Other clusters grouped as one regardless of original dataset such as the cluster for 'Nahodka' (PI 617120) and 'Pautske' (PI 667978) (Figure 12). A majority of *A. kolomikta* samples between the two datasets showed similar calls of redundancy. The exception to this uniformity was 'Oluyckos' from the UMN dataset which clustered

together with the USDA NPGS ‘Oluyckos’ sample but neither clustered with ‘Sentyabraskaya’ samples as observed in the UNH dataset (Figure 12). Of the 33 named *A. kolomikta* accessions in the UMN dataset, only 11 unique named accessions were identified (Table 3). Identity of 20 out of 75 previously unidentified *A. kolomikta* accessions were resolved based on GD and hierarchical clustering (Table 4).

Based on a recorded group of UMN accessions, a set of expected *A. kolomikta* sibling accessions were found to have a mean Gower’s dissimilarity coefficient of 0.0184 (Table 5). Additionally, three accessions labeled as members of this offspring population were found to match different named cultivar accessions at dissimilarity values less than 0.0058 (Table 5).

Discussion

GBS data are invaluable for assessing a population for which no species-specific genome is currently available. However, as seen in the separation of the UMN and UNH *A. arguta* datasets, comparison across datasets might not be directly applicable. Differences in DNA extraction, random nature of enzyme cut locations across the genome, read depth per individual, and filtering of SNPs based on missingness (larger or more divergent populations have more missingness) are all possible sources of ascertainment bias that might lead to samples clustering. A comparison of the total allele depth with the principal component (PC2 - 6.58%) separating datasets confirmed that for this study the differences in read depth, estimated by total allele depth, between the datasets accounted for a portion of variation preventing dataset integration for *A. arguta*. However, though read depth appears to be a factor, other possible sources of

ascertainment bias cannot be ruled out and may have accounted for additional differences between datasets. Differences in genotype between 20x and 60x read depths are to be expected for an autotetraploid species since read depths in the hundreds are needed to predict polymorphic alleles in polyploids (Wang et al. 2022). Thus, differences in read depth for a tetraploid will result in datasets not easily combined since three possible heterozygous genotypes are possible at any biallelic locus. The likely increased number of heterozygous genotypes in polyploids requires greater read depths to identify genotypes with 90% probability confidence (Wang et al. 2022). Compounding this issue is that data for the autotetraploid *A. arguta* were aligned using a haploid reference genome of a different species, which can introduce noise from unphased haplotypes. Despite these possible causes of error, information regarding identity was obtained and examined within the two separate datasets.

Kiwiberry accessions are costly to maintain in germplasm collections, and maintaining redundant individuals is a drain on labor and space resources. Based on the presented results, approximately 55 unique named accessions of 116 previously recorded accessions have been identified and support the previous examination of the USDA NPGS kiwiberry collection (Melo et al. 2017). The USDA NPGS could remove redundant individuals that clustered together within these two datasets after phenotypic observation to confirm expected redundant accessions.

The presented results are important for the UMN kiwiberry germplasm curation. Results confirmed the presence of populations of *A. arguta, A. kolomikta,* and *A. polygama* in addition to hybrids between *A. kolomikta, A. polygama,* and *A. rufa.* Start et al. (2007) previously examined the UMN germplasm and recorded the presence of 61

accessions belonging to *A. arguta* and *A. kolomikta*. However, due to missing labels only 17 labeled accessions remained in the field that were previously recorded. The results indicate that only approximately five of the unaccounted accessions may remain in the germplasm along with likely duplicates [i.e., '74-8' (CACT 81), '74-8' (UMN-S10), Unknown (UMN-S12)] (Tables 2 and 4). Identification of duplicates should be confirmed with phenotypic observations before removal of individuals. Though similar redundant accessions were identified in both the UMN dataset and the UNH dataset, the possibilities for erroneous identity calls remains. The example of 'Oluyckos' emphasizes the possibility of erroneous calls in that 'Oluyckos' clustered with 'Sentyabraskaya' in the UNH dataset, but clustered independently in the UMN dataset. However, a majority of the named accessions between the two datasets displayed similar clusters indicating that they have a very high likelihood of being redundant accessions. In addition to identifying redundant accessions, these results indicate the presence of unique offspring in the UMN germplasm. Approximately 92 unknown *A. arguta* accessions were found to be unique individuals and 21 previously unknown *A. kolomikta* and its hybrid accessions were confirmed to be unique.

Though fingerprinting of accessions and comparing accessions between SNP datasets was the goal of this study, future examination could consider further methods to explore this SNP data. Combining GBS data with phenotypic observations of bud break or flower timing could lead to identification of alleles associated with early flowering. Additionally, future use of this SNP data should consider using methods specific for polyploids to assess *A. arguta* accessions.

Conclusion

With limited ability to import new *Actinidia* material into the U.S. due to a nationwide importation ban to prevent the spread of the bacterial disease *Pseudomonas syringae* pv. *Actinidiae* (Psa), existing kiwiberry germplasm must be catalogued and preserved for future utilization. Results of this study confirm most previous results of redundant accessions and identifies novel, unique accessions. Future breeding and germplasm collection curation will be better informed by identifying unique accessions for evaluation and redundant accessions for removal.

Tables

See attached document

Figures

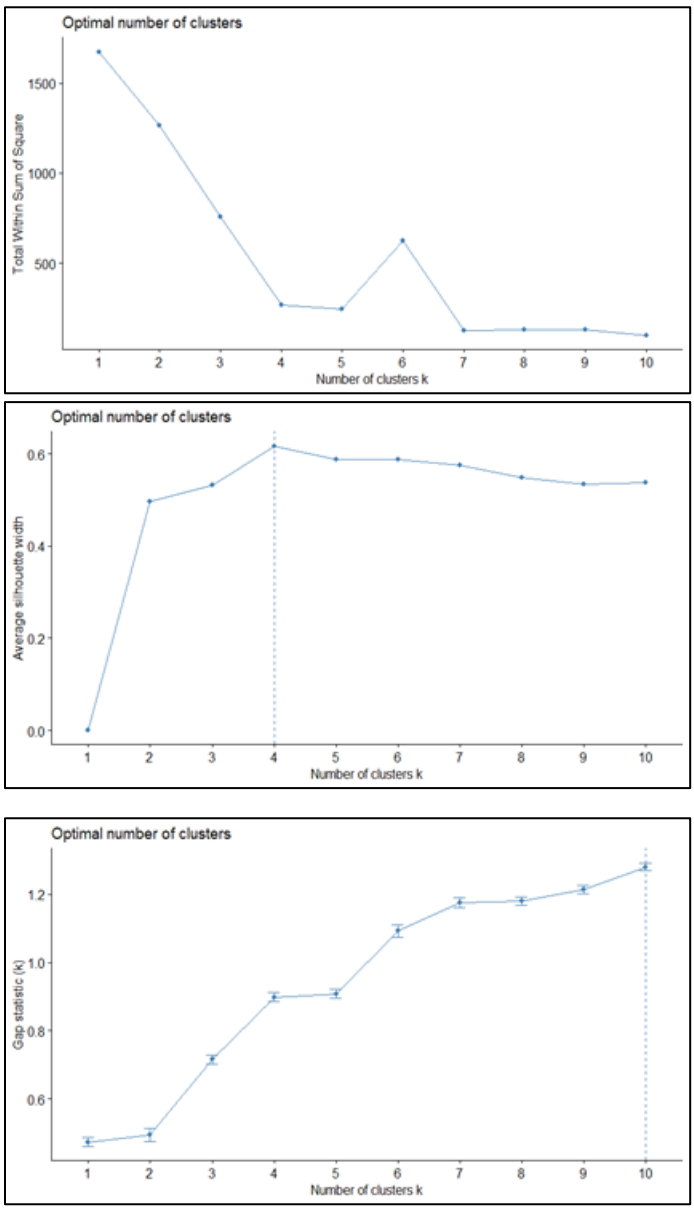

Figure 1. Visualizations of k-means clustering for combined University of Minnesota and University of New Hampshire *Actinidia* SNP datasets. Sum of squares (A), silhouette (B), and gap statistic (C) procedures indicate that four clusters are the optimal minimum number of clusters to apportion variation.

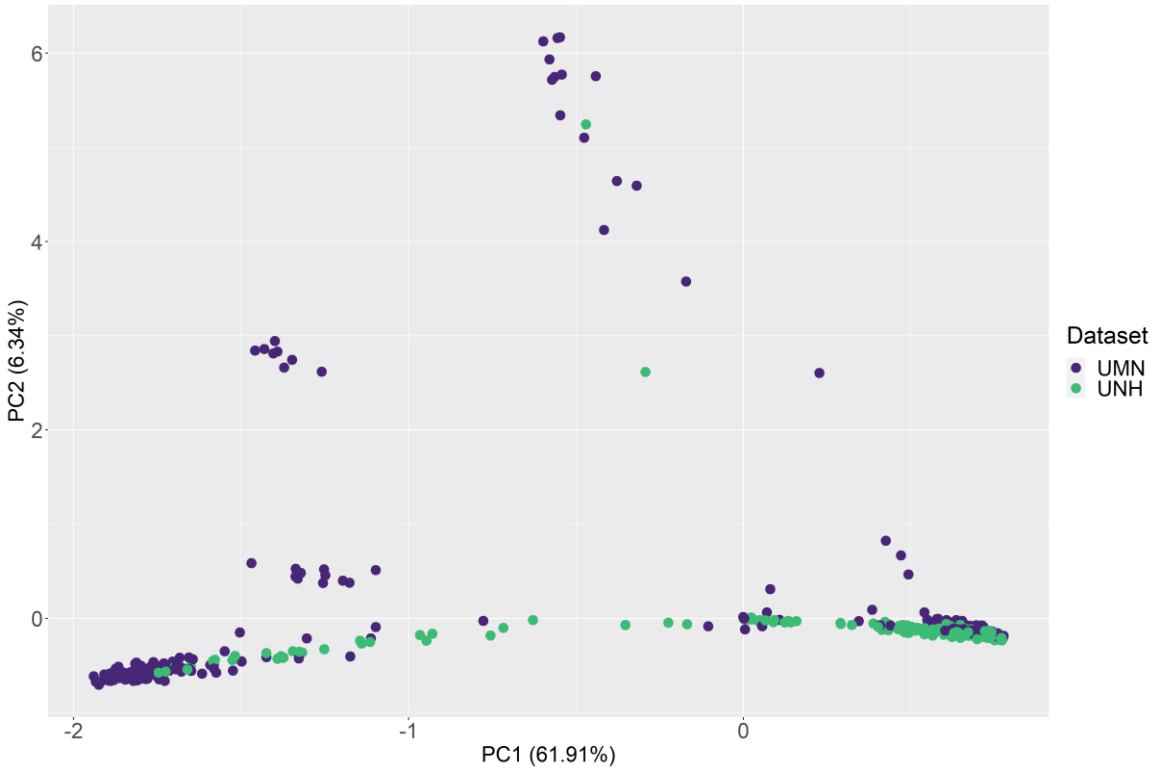

Figure 2. PCA of the combined University of Minnesota (UMN) or the University of New Hampshire (UNH) *Actinidia* SNP datasets with approximately 68.25% of variation explained. Accessions are colored according to dataset of origin.

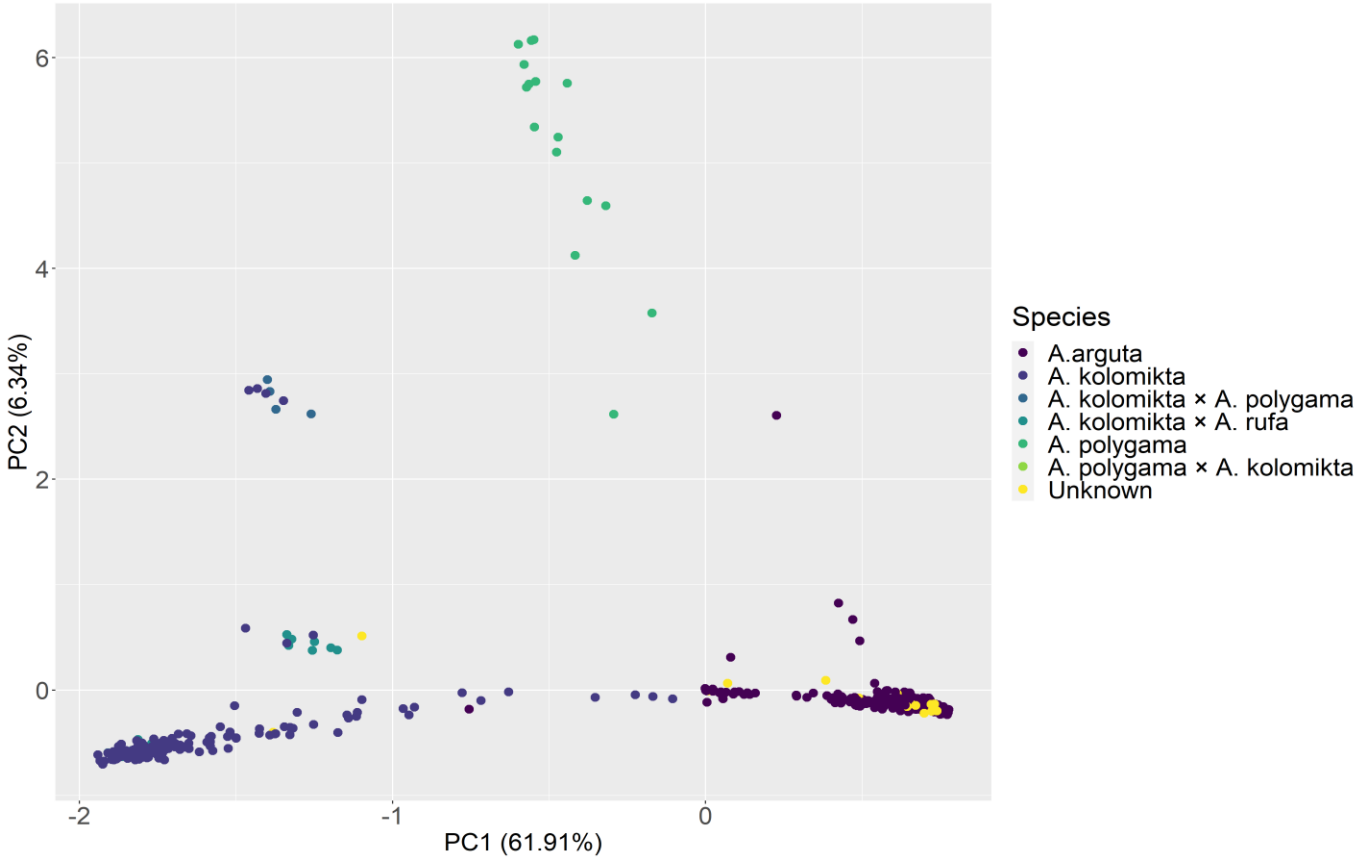

Figure 3. PCA of the combined University of Minnesota (UMN) or the University of New Hampshire (UNH) *Actinidia* SNP datasets with approximately 68.25% of variation explained. Accessions are colored according to species if known.

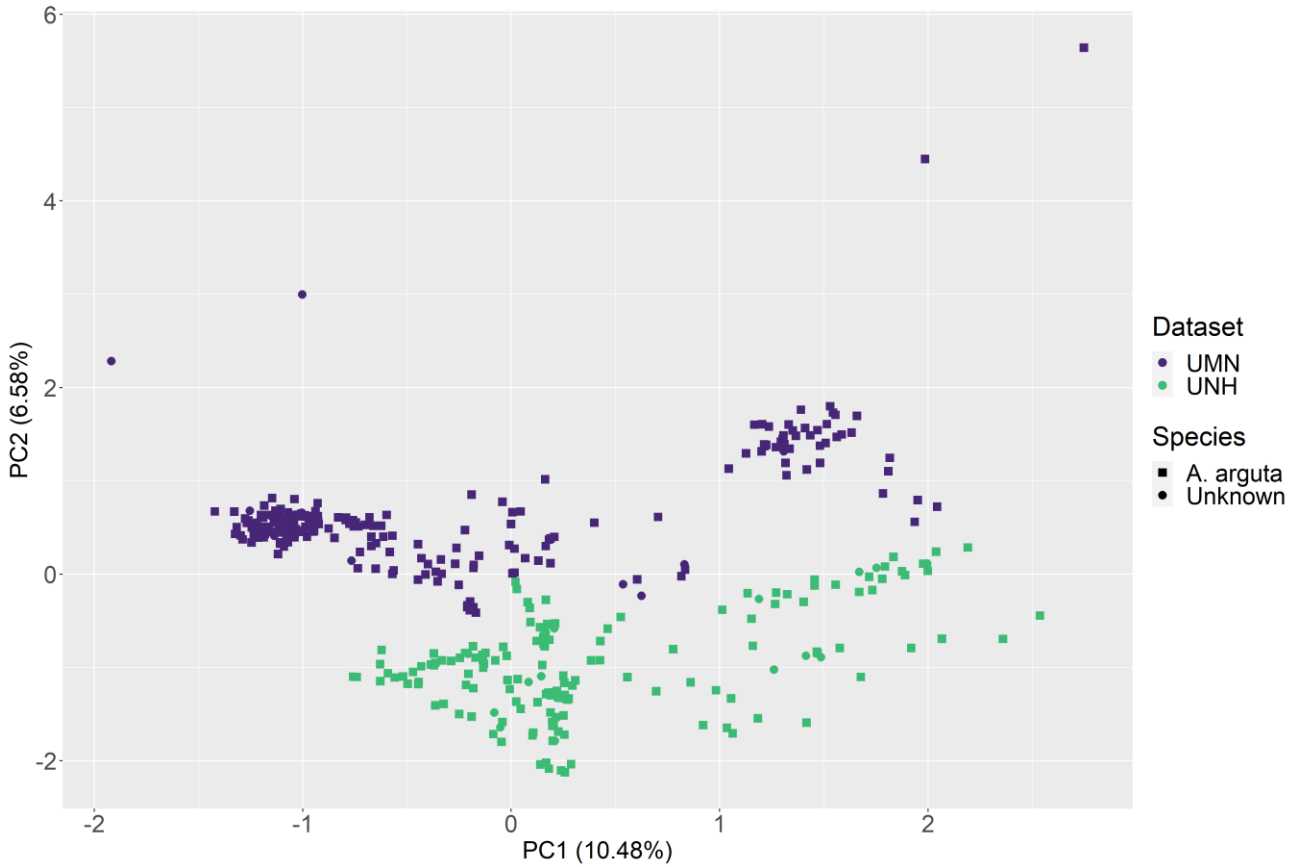

Figure 4. PCA which explains 16.61% of variation for *A. arguta* accessions colored by the dataset of origin. Datasets are either the University of Minnesota (UMN) or the University of New Hampshire (UNH).

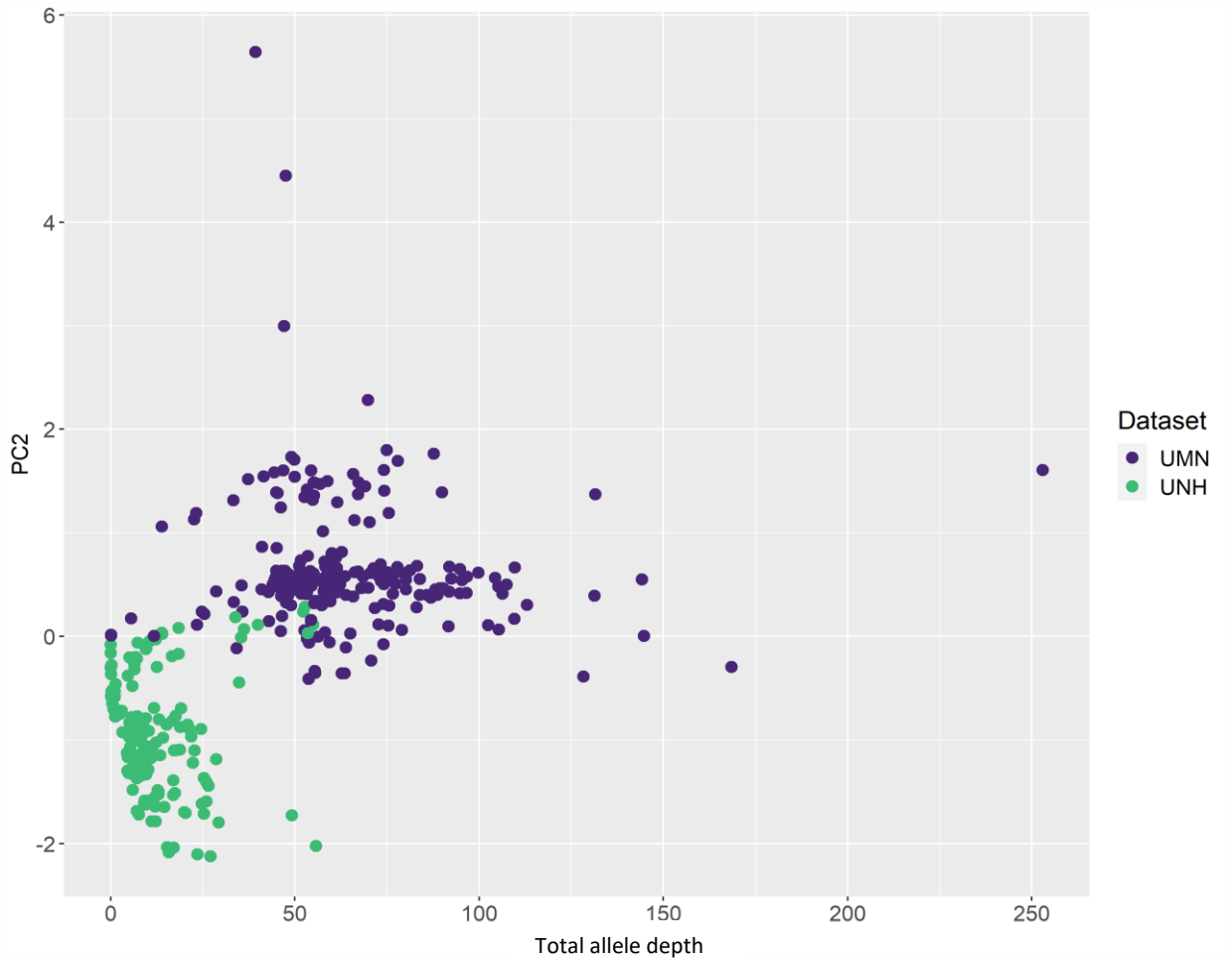

Figure 5. Total allele depth graphed against principal component 2 (PC2) from the PCA examining *A. arguta* accessions colored by dataset of origin (R^2 = 0.33). Datasets are either the University of Minnesota (UMN) or the University of New Hampshire (UNH).

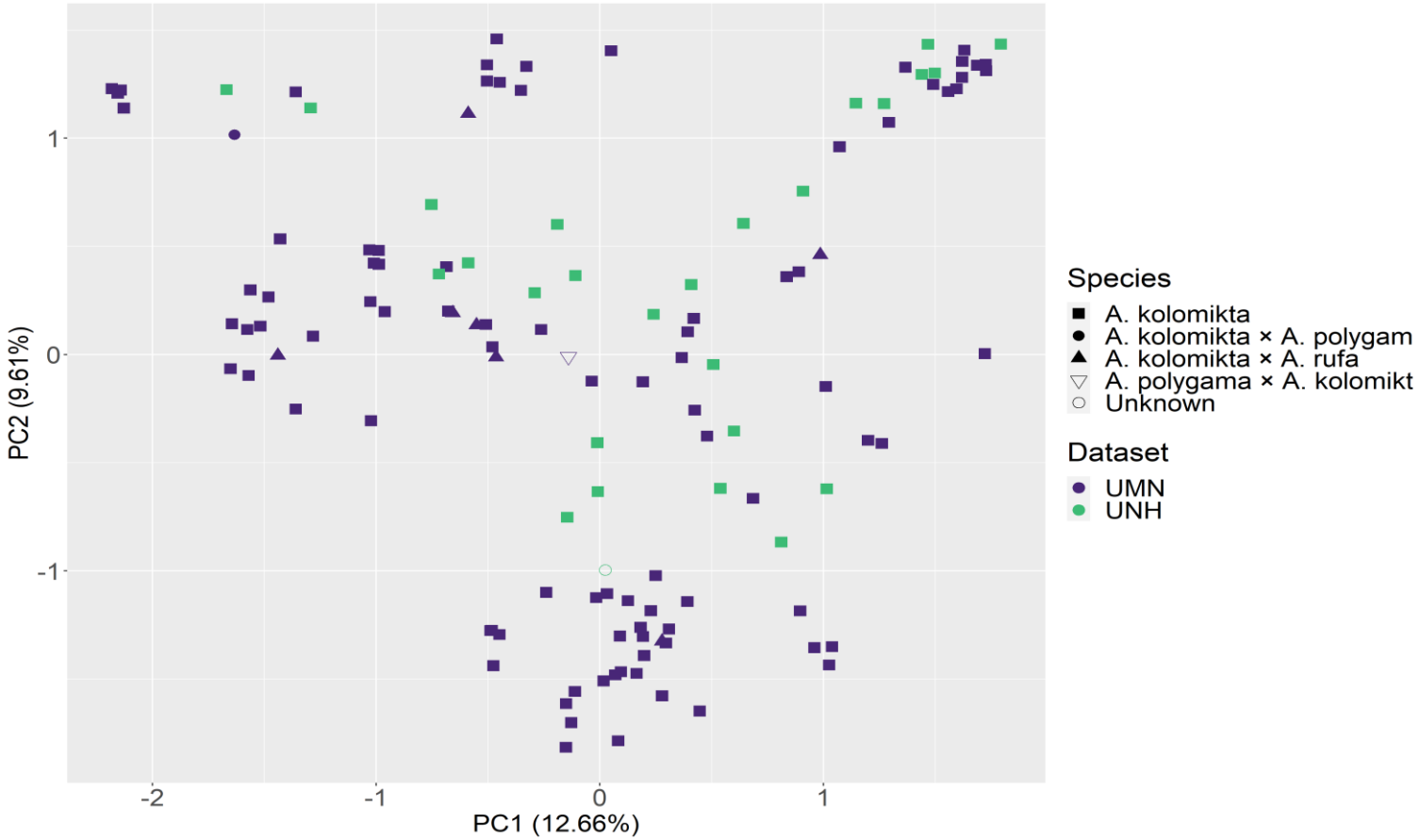

Figure 6. A PCA of all *A. kolomikta* accessions and hybrid accessions colored by the dataset of origin. Datasets are either the University of Minnesota (UMN) or the University of New Hampshire (UNH).

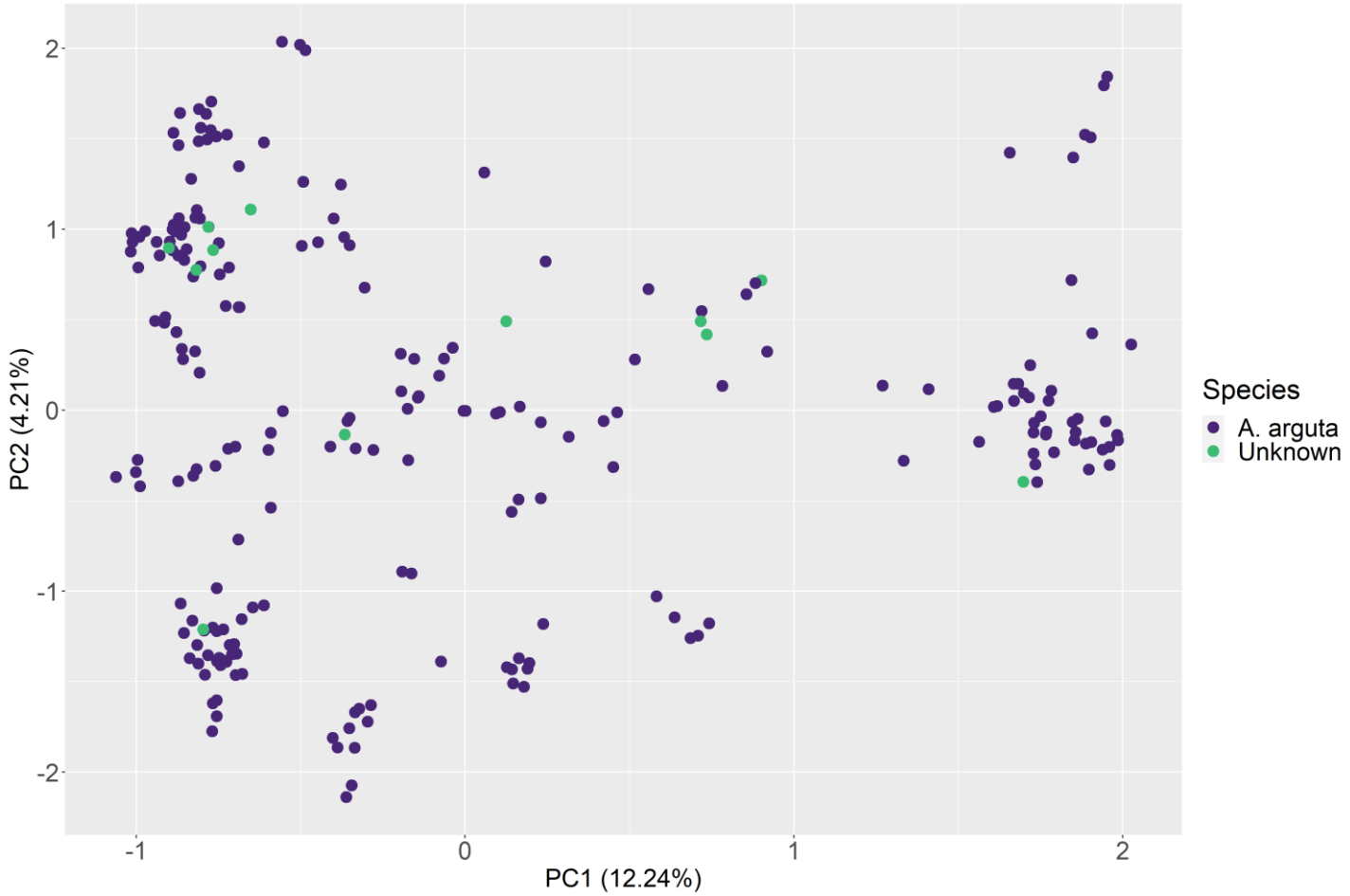

Figure 7. A PCA of *A. arguta* accessions in the SNP dataset from the University of Minnesota (UMN).

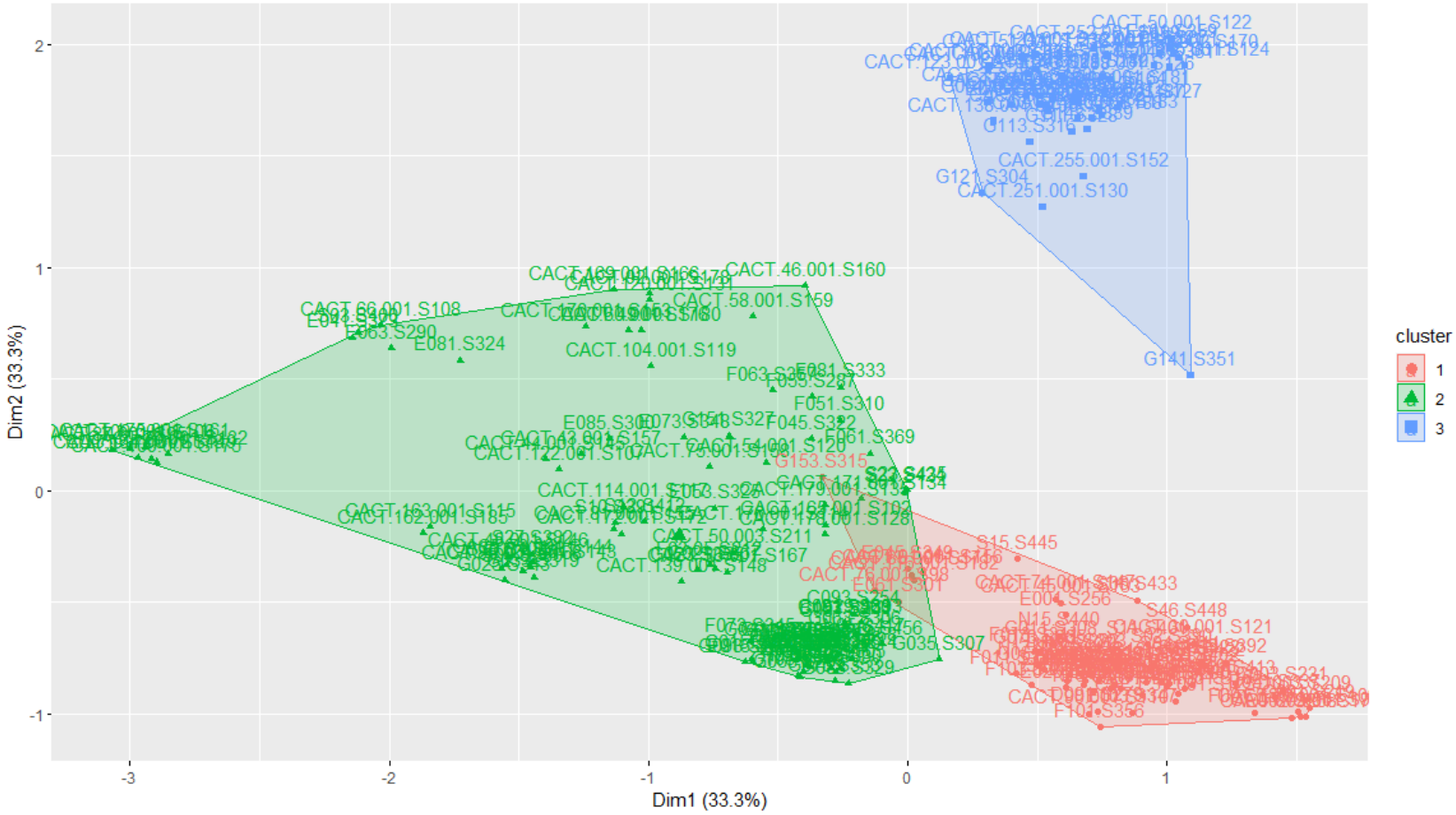

Figure 8. Visualization of clusters based on k-means scores for *A. arguta* accessions from the University of Minnesota SNP dataset. Cluster 3 consists mainly of accessions expected to originate from northern Asia.

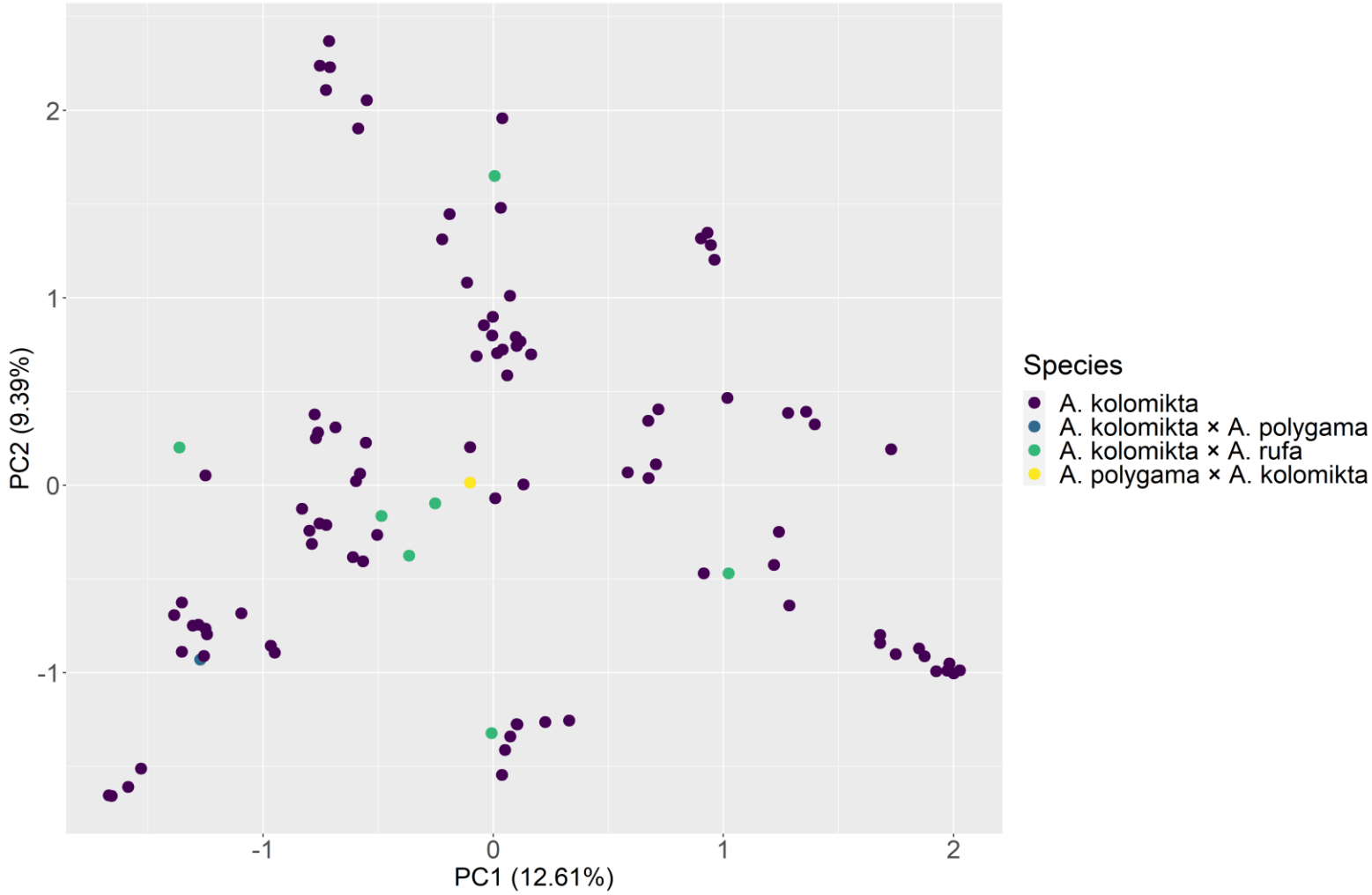

Figure 9. A PCA of *A. kolomikta* accessions using the SNP dataset from the University of Minnesota examining sub-population presence.

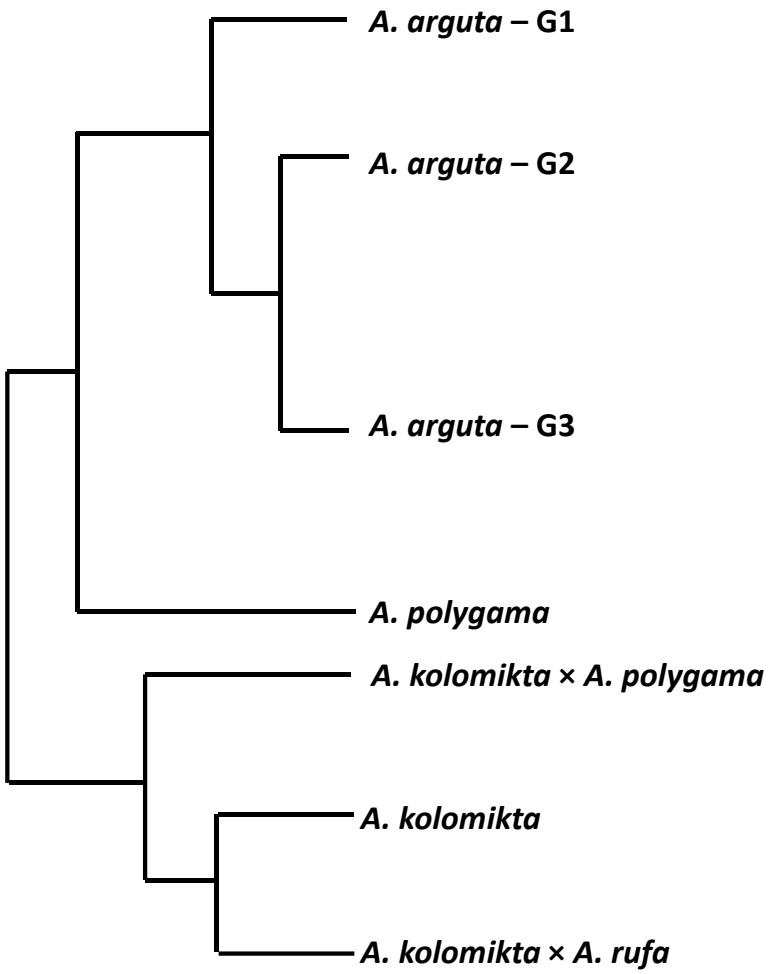

Figure 10. A cladogram based on Gower's dissimilarity coefficient showing the population structure of accessions from the University of Minnesota germplasm and USDA NPGS and confirmed with *k*-means clustering.

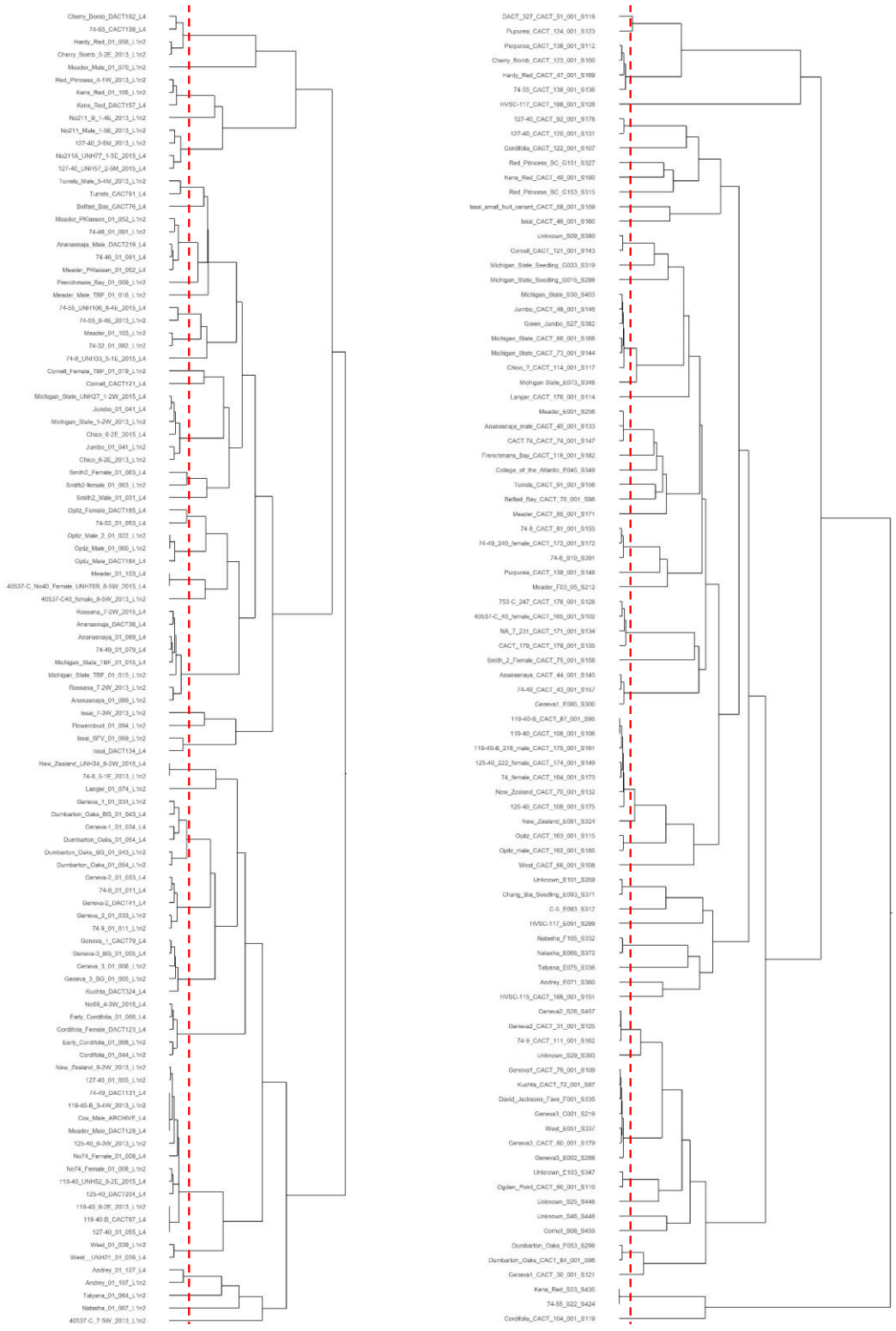

Figure 11. Cladograms of *A. arguta* named accessions based on Gower's dissimilarity coefficient (GD) for SNP datasets from the University of New Hampshire (left) and the University of Minnesota (right). Dotted line indicates GD of less than 0.0024 used to call redundant accessions (Melo et al. 2017).

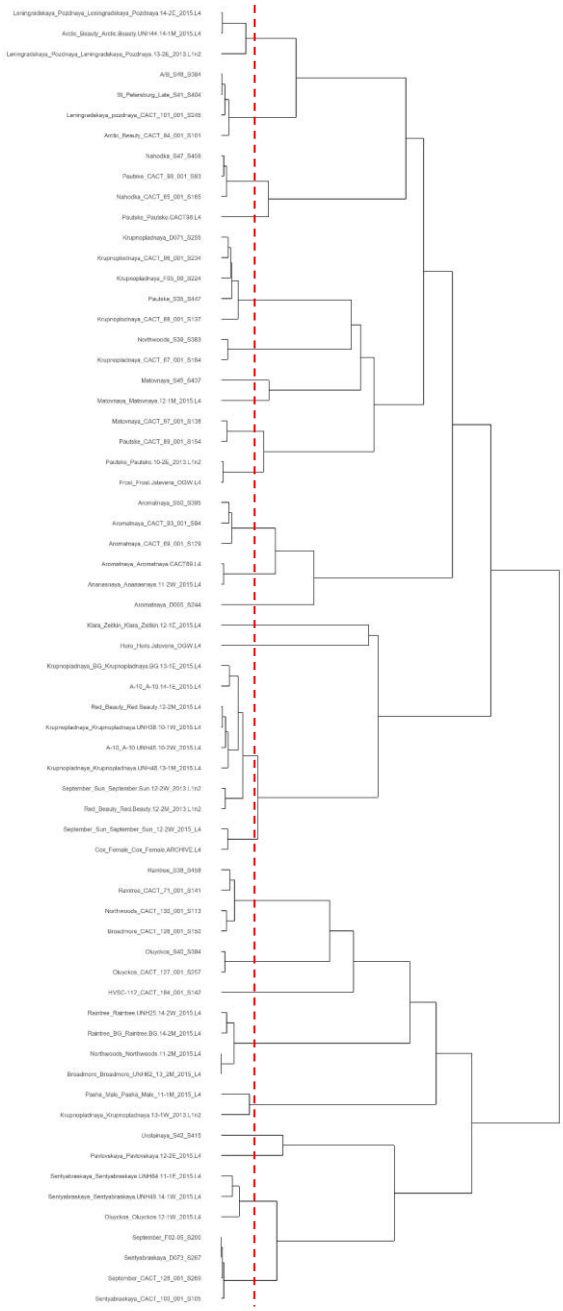

Figure 12. Cladogram of *A. kolomikta* named accessions based on Gower's dissimilarity coefficient (GD) for SNP datasets from the University of New Hampshire (UNH) and the University of Minnesota (UMN). Dotted line indicates GD of less than 0.0046 used to call redundant accessions (Melo et al. 2017).

Chapter 3: Evaluation of fruit quality traits at harvest and after cold storage in a kiwiberry germplasm collection

Abstract

Kiwiberry, a specialty crop that is gaining interest in Minnesota, combines familiar flavors with a novel smooth-skinned fruit for consumers. *Actinidia arguta* Planch and *A. kolomikta* Maxim. are two cold-hardy species in the kiwifruit genus that are being examined for production in the state. *Actinidia arguta* has been cultivated in other U.S. regions; however, appropriate times for production fruit harvest, meaning physiologically mature fruit that have not softened, for accessions within these species remains uncertain for Minnesota growers. Fruits from eleven accessions were harvested multiple times throughout the 2019, 2020, and 2021 growing seasons. At each harvest, fruits were collected for initial fruit quality analysis and the remaining fruits were put into storage at 2 °C. Each week for six weeks, postharvest fruit quality metrics were conducted on 5-fruit samples per cultivar. Fruit quality metrics examined include titratable acidity (TA), soluble solids content (SSC), firmness, and storage life. Thresholds for consumer acceptability were determined for each fruit quality metric and favorable harvest windows were identified for each cultivar. Averaged across seasons, the highest fruit quality (TA between 1.3-2.5 mg/ml and SSC > 12°Brix) and longest storability were achieved in harvest windows throughout August for *A. kolomikta* accessions and in mid-September for *A. arguta* accessions. These findings provide Minnesota kiwiberry growers with harvest windows to achieve improved fruit quality and storability.

Introduction

Cultivation of green kiwifruit (*Actinidia chinensis* var. *deliciosa*) began in New Zealand in the 1950s with the cultivar Hayward rising to prominence in the industry where it remains. The success of 'Hayward' in global markets prompted exploration into other species in the genus *Actinidia* resulting in the introduction of golden kiwifruit (*Actinidia chinensis* var. *chinensis*). Both kiwifruits have been successful in global markets by satisfying demands for nutritious, novel eating experiences. One limitation for U.S. kiwifruit production is the requirement for winter temperatures above -18 °C (0 °F), limiting production to primarily Southern and Western regions of the U.S. The cold-sensitivity of kiwifruit provides an opportunity for further development and marketing of cold-hardy *Actinidia* species.

Growers are exploring kiwiberry, the term commonly applied to the fruit of the species *Actinidia arguta*, and occasionally *Actinidia kolomikta*, for production in regions with winter temperatures below -18 °C. In addition to cold hardiness, kiwiberries offer a unique eating experience with smooth, edible skin and smaller size similar to table grapes (*Vitis* spp.) (2g to 25g). The fruit is aromatic with flavors described as pineapple, melon, banana, grassy, rhubarb, blackcurrant, and tropical fruit (Matich et al. 2003; Williams et al. 2002). Previous studies have examined the nutritional attributes of kiwiberries and have reported 7.12 mg and 18.78 mg of vitamin C per 100 g fresh fruit weight (Leontowicz et al. 2016; Nishiyama et al. 2004), the presence of polyphenols relating to antioxidant activity (Wojdyło and Nowicka 2019; Wojdyło et al. 2017), and the presence of various minerals beneficial for human health (Jin et al. 2014; Latocha et al. 2017). Additionally, both *A. arguta* and *A. kolomikta* are considered cold hardy and have been

reported to survive temperatures as low as -30 to -35 °C (Latocha et al. 2017; Sun et al. 2020) and -40 °C (Chat 1995; Marosz 2009; Paulauskiene et al. 2020), respectively. The nutritional profile and cold hardiness make kiwiberry an attractive opportunity for specialty crop production in the Upper Midwest.

Kiwiberries have several storage and postharvest challenges. Kiwiberry, like kiwifruit, is usually harvested while firm but at physiological maturity (8 °Brix) to ensure ripening of fruit after cold storage (Fisk et al. 2006). Unlike kiwifruit, which can have a storage life of up to six months (Mastromatteo et al. 2011), kiwiberries typically have a storage life between one and two months and are prone to dehydration (Debersaques and Mekers 1987; Fisk et al. 2008). Previous studies have investigated harvest timing for improving storage life by identifying harvest indicators and examining fruit quality traits such as soluble solids content (SSC), firmness, and titratable acidity (TA) (Chesoniene et al. 2004; da Silva et al. 2020; Fisk et al. 2006a, 2006b; Han et al. 2019; Kim et al. 2018b; Lee et al. 2015; Lin et al. 2022; Oh et al. 2017; Park et al. 2022). These studies have reported that typically firmness and TA decrease and SSC increases with later harvests and after cold storage. Additionally, postharvest studies examined impacts of packaging (Fisk et al. 2008), storage temperature (Kim et al. 2020), pre-storage treatments (Krupa et al. 2022b; Stefanik et al. 2017), controlled atmosphere (Latocha et al. 2014; Krupa et al. 2022a), and fruit coatings (Fisk et al. 2008) on storability of kiwiberry. Low vent packaging reduced weight loss of *A. arguta* ‘Ananasnaya’ and, when paired with fruit coatings, storage life was extended up to 14 weeks (Fisk et al. 2008). Treatments with 1-methylcyclopropene (1-MCP) at harvest improved fruit firmness after storage (Krupa et al. 2022b; Stefanik et al. 2017). Controlled atmosphere conditions with low oxygen

improved kiwiberry storage life (Latocha et al. 2014; Krupa et al. 2022a, 2022b; Stefanik et al. 2017).

Studies for harvest timing have primarily examined *A. arguta* cultivars as it is currently the most widely planted kiwiberry species with many cultivars in production (Latocha et al. 2021). However, *A. kolomikta* is of interest in the Upper Midwest due to reported cold hardiness greater than many *A. arguta* cultivars (Chesoniene 2000; Paulauskiene et al. 2020; Pranckietis et al. 2009). The objective of this study was to determine the impacts of harvest timing on fruit quality for accessions of two kiwiberry species (*A. arguta* and *A. kolomikta*).

Materials and Methods

Plant material

A total of 50 accessions from the germplasm were sampled from 2018 to 2021 at the University of Minnesota's Horticultural Research Center (HRC) in Victoria, MN (44°86'N, -93°64'W). Kiwiberry plantings include two training systems, a pergola and T-bar, and were managed with low inputs which included supplemental watering upon wilting, no fertilizer, fungicide, or herbicide applications, pesticide application for Japanese beetle (*Popillia japonica*), and early summer pruning. In January 2019, winter temperatures reached a minimum of -37.8 °C (-36 °F) which resulted in winter injury of many *A. arguta* accessions based on dissection of plant tissues. Winter injury and year-to-year fruit set variation prevented repeated harvest of many accessions each year, but a subset of 10 accessions were consistently sampled across years in addition to approximately 30 accessions varying from year to year.

Harvest and storage

2019 and 2020

Harvest sampling began with destructive sampling of fruits to determine maturity. Fruits were cut to visually inspect seed coat color. A white seed coat indicated underripe fruits, while a black or dark brown seed coat indicated fruits were mature enough for inspection of soluble solids content (SSC), a proxy for total sugar in fruit juices. SSC between 6 °Brix and 8 °Brix was used as the target range for harvest, but values approaching 8 °Brix were considered the preferred value at which to begin harvest. For each plant, three to four harvests were conducted, depending on fruit availability, with a minimum of 35 fruits harvested at each timepoint. Five fruits were evaluated for fruit quality at harvest and the remaining fruits were rinsed and stored.

Stored fruits were placed in 6 oz low-flow plastic clamshell containers commonly used for other berry crops like raspberry (*Rubus idaeus*). The smaller fruit size of *A. kolomikta* accessions enabled the fruits to be stored in a single container. *A. arguta* fruits were placed in two containers due to larger fruit sizes. All fruits were kept in a cooler without other produce to reduce amounts of external ethylene. No ethylene scrubbers, controlled atmosphere, or fruit coating applications were implemented. Fruits were stored at 2 ± 1 °C (36 ± 2 °F) for up to six weeks. Each week, a random sampling of 5 fruits per accession was removed from storage and allowed to ripen at room temperature (22.2 °C / 72 °F) for four days. After ripening at room temperature, fruits were evaluated for postharvest fruit quality.

2021

In 2021, ten accessions were harvested at two time points after fruit measured 7-8 °Brix with a minimum of 130 fruits per harvest per accession (Table 1). Harvests in 2021 used the same procedures to identify appropriate timing through use of SSC and seed coat color. At the time of harvest, 20 fruits per accession were evaluated for initial fruit quality while remaining fruits were rinsed with deionized water and placed in storage.

In 2021, harvested fruits were separated into six packages with 20 fruits each at the time of harvest with care taken to ensure variation in fruit size was randomly distributed throughout the packages. Fruits were then placed in low-flow plastic clamshells containers and clamshells were placed in plastic storage bins. Bins were stored in a separate cooler from other produce to minimize exogenous ethylene and kept at 2 ± 1 °C (36 ± 2 °F) for up to six weeks. Each postharvest week, a clamshell containing 20 fruits was removed and allowed to ripen at room temperature for four days before evaluating fruit quality. The number of fruits per postharvest sampling was increased to 20 to account for variability in fruit ripeness observed in previous years.

Fruit quality evaluation

For initial harvest sampling and postharvest fruit sampling, fruit were allowed to acclimate to room temperature (22 °C/ 72 °F) in cardboard scintillation vial trays for a minimum of 24 hours before collecting phenotype data. Phenotypic data were collected for the following traits: tactile firmness (rating), weight (g), mechanical firmness (N), titratable acidity (mg/mL), pH, and soluble solids content (°Brix).

Tactile firmness (TF) was recorded on a 1 to 5 scale that includes human perception of firmness in addition to visual cues including fruit color change and transparency of skin primarily in *A. kolomikta* fruit. Scoring followed these criteria: firm

fruit with no softening and no skin transparency (1), slight softening to fruit and 25% or less transparency (2); notable softening but fruit retains integrity with approximately 26% to 50% skin transparency (3); fruit easily compressed but maintained flesh integrity with 51% to 75% skin transparency (4); and fruit was easily compressed and lost flesh integrity (5). TF ratings for each fruit were taken by two independent observers and then averaged.

Fresh weight was recorded in grams. Mechanical firmness was then recorded using a 5 mm penetrometer probe to puncture fruit 0.5 cm on the Mohr Digi-test penetrometer and texture analyzer, hereafter referred to as MDT (Mohr 2000). Fruits were then cut in half longitudinally with the punctured and unpunctured halves separated. Pooled samples of 5 fruit halves were collected in 50 mL conical vials with all punctured halves together and unpunctured halves in a separate conical vial. The unpunctured halves were immediately submersed in liquid nitrogen and then placed in a -80 °C freezer for examination of soluble oxalate. The punctured halves were homogenized for fruit chemistry analyses. The conical vial with the fruit was weighed and the vial weight was subtracted to determine weight of the fruit. Fruit was placed in a Magic Bullet blender with double the weight of deionized water (1 g fruit per 2 mL water). The fruit flesh was completely homogenized and then the mixture was poured back into the 50 mL conical vial. Conical vials were then weighed again in preparation for centrifuge use. Samples were spun for 10 minutes at 4,000 rpm. The supernatant was then collected using a pipette with care to not disturb the sediment or floating foam. The supernatant was placed in a 20 mL scintillation vial and stored in a -80 °C freezer for later analysis.

For juice chemistry, vials were removed from the freezer and allowed to thaw. Titratable acidity was measured on two technical replicates from each vial using an automated titrator (T50A Mettler Toledo, Columbus, OH) which records the final volume of NaOH necessary to neutralize the total acid in the juice. Titratable acidity is reported as citric acid equivalents per liter. The pH of the juice sample was measured using a pH probe (DG 115-SC, Mettler Toledo, Columbus, OH). Finally, the SSC was measure with a digital handheld refractometer (PAL-1 3810, Atago Co. Ltd., Tokyo, Japan; ±0.2 °Brix) and recorded as °Brix which is a proxy for juice sugar content.

Data analysis

Prior to statistical analysis, means were calculated for each sample of five fruits for weight, MDT, and TF. Data was then combined with SSC and TA, and all years of data (2019-2021) were compiled for a complete fruit quality dataset. Samples with missing values were removed before statistical analyses were performed using r v. 4.0.5 (R core team 2021). Each fruit quality was examined with a histogram to ensure normal distribution. ANOVAs were used to assess fruit quality with the following linear model used for all postharvest and harvest timepoints: $Y_{\text{fruit quality trait}} = \beta_{\text{accession}} + \beta_{\text{year}} + \beta_{\text{year} \times \text{accession}} + \beta_{\text{harvest} \times \text{postharvest}} + \beta_{\text{year} \times \text{accession} \times \text{harvest}} + \beta_{\text{year} \times \text{accession} \times \text{postharvest}} + \beta_{\text{residual error}}$. Observations for all fruit qualities at postharvest week three were subset for comparison to reduce variation and examine impacts of harvest. The following linear model was used for the postharvest three dataset: $Y_{\text{fruit quality trait}} = \beta_{\text{accession}} + \beta_{\text{year}} + \beta_{\text{year} \times \text{accession}} + \beta_{\text{year} \times \text{accession} \times \text{harvest}} + \beta_{\text{residual error}}$.

Results

Sources of trait variation

Year, accession, year × accession interaction, and harvest × postharvest interaction had significant effects on all fruit quality traits examined in this study ($p < 0.001$). The three-way interaction of year × accession × harvest had a significant effect on TF ($p < 0.05$). Additionally, the interaction of year × accession × postharvest had significant effects ($p < 0.001$) on TF, MDT, and TA. Figure 1 illustrates the proportions of variation explained by each factor for each mean fruit quality trait across all sample years, harvests, and postharvest timepoints. Accession accounted for the largest proportion of variation for all quality traits except TF and MDT. Interactions among factors including harvest × postharvest and year × accession × postharvest accounted for most variation for TF and MDT. When examining all harvest fruit sample means at a single postharvest timepoint, accession accounted for a significant proportion of the variation for all fruit quality traits (Figure 2). Interactions among factors, specifically year × accession and year × accession × harvest, accounted for most of the remaining variation within a postharvest timepoint. ANOVA of postharvest week three fruit sample means indicated that all fruit quality traits significantly differed ($p <0.05$) based on year and/or accession. Weight and MDT varied significantly ($p < 0.01$) based on year × accession, and only weight was found to vary significantly ($p < 0.05$) due to year x accession x harvest.

Among and within species trait variation across storage weeks

Fruit quality trait means across all accessions and harvests are shown by species for six postharvest evaluation weeks in Figure 3. Significant differences between species

were observed for SSC, TA, SAR, and weight (Figure 3). In general, SSC increased across storage weeks for both species (Figures 3 and 4). Mean SSC for *A. arguta* ranged from 10.61 to 20.35 °Brix at harvest and 10.88 to 21.94 °Brix after three weeks in storage with significant differences among *A. arguta* accessions (Figures 3 and 4). *A. arguta* accessions E065 and E075 had higher SSC values compared to *A. arguta* accession E093 (Figure 4 and Table 2). Mean SSC for *A. kolomikta* ranged from 6.55 to 15.79 °Brix at harvest and 9.30 to 16.80 °Brix after three weeks in storage with significant differences being observed among *A. kolomikta* accessions (Figures 3 and 4). Of the *A. kolomikta* accessions, D013 and D031 generally had the highest SSC levels (Figure 4).

Mean TA for both species generally decreased in storage (Figures 3 and 4). Mean TA ranged from 1.00 to 2.12 g/100ml at harvest for *A. arguta* and 0.88 to 1.71 g/100ml after three weeks in storage. *A. arguta* accession E075 had lower mean TA, between 0.88 and 1.10 g/100ml, than other *A. arguta* accessions (Figures 3 and 4). Mean TA ranged from 1.26 and 2.30 g/100ml at harvest and 1.01 and 2.07 g/100ml for *A. kolomikta*. *A. kolomikta* accessions D013 and D031 had relatively high mean TA levels, between 1.01 and 2.30 g/100ml, compared to other *A. kolomikta* accessions (Figure 4).

Mean SAR increased in storage for both species with higher SAR for *A. arguta* compared to *A. kolomikta* means (Figure 3). Harvest mean SAR for *A. arguta* accessions was between 9.07 and 14.86, and increased to 10.32 and 21.06 after six weeks in storage. Mean SAR for *A. kolomikta* was between 4.25 and 10.54 at harvest, and increased to between 7.20 and 15.11 (Figure 4). Lower SAR in *A. kolomikta* includes the accessions D013 and D031 as both measured high for SSC and TA resulting in SAR values comparable to other accessions.

Mean fruit weights for both species decreased in storage with *A. arguta* accessions having mean fruit weights between 7.5 and 13 g and *A. kolomikta* accessions between 2.2 and 3.8 g. Significant variation for fruit weight among accessions within a species was not observed (Figure 4). Mean TF ratings were lower for postharvest samples of *A. arguta* compared to *A. kolomikta* within each year (Figure 3).

Within species, mean TF ratings among accessions did not vary significantly (Figure 4). Mean MDT values at harvest were higher for *A. kolomikta* compared to *A. arguta*, but mean storage values were not significantly different (Figure 3). After postharvest week two, both species mean MDT values plateaued between 0.88 and 3.06 N. (Figure 3). Mean MDT values for *A. arguta* varied at harvest between years, but not between accessions within a year. Mean MDT values for *A. arguta* in 2021 were higher compared to previous years (Figure 4). Mean MDT values varied between *A. kolomikta* accessions within years. *A. kolomikta* accessions D013, S37, and S47 had higher mean MDT values than other *A. kolomikta* accessions (Figure 4). Table 2 reports the fruit quality trait means for harvest, postharvest week three, and postharvest week 6 shown in Figure 4. Additionally, the mean soluble oxalate content is reported and indicates *A. kolomikta* had between 1.38 and 5.91 mg/100g fresh weight while *A. arguta* ranged between 0.14 and 0.54 mg/100g fresh weight.

Harvest effects on among and within species trait variation after three weeks of storage

Fruit quality trait means for each species three weeks after harvest are displayed in Figure 5. Mean SSC for *A. arguta* accessions varied depending on the harvest and year with 2019 and 2021 showing within harvest differences (Figure 5). In contrast, mean SSC values were similar across harvest weeks for *A. kolomikta* in all years (Figure 5). For *A.*

arguta accessions E065 and E093, mean SSC was higher for later harvest dates in 2019 and 2021. In 2020, most *A. kolomikta* harvests met the minimum threshold at postharvest week three, while in 2021 all harvests appear to meet the minimum threshold at this timepoint and remained relatively constant across harvest dates. *A. kolomikta* accessions varied for SSC with accessions D013 and D031 having high average SSC values and other accessions (S35, S45, S47) having SSC values that were frequently lower than the minimum threshold (Figure 6 and Table 3). S37 only displays one harvest due to limited fruit availability in 2021, but the harvest met the minimum SSC threshold.

Mean TA values did not exhibit a consistent pattern across species, years, and harvests (Figures 5 and 6). Variation for mean TA values was observed among accessions within a species three weeks postharvest (Figure 6). For *A. arguta* accessions E065 and E093, mean TA increased with later harvest dates (Figure 6). TA was relatively constant across harvests for *A. kolomikta* accessions in 2020 and 2021; however, variation was observed for *A. kolomikta* accessions in 2019 with increases across harvests observed for S37 and decreases across harvest observed for D031 (Figure 6). Within species, mean SAR remained constant, but variation was observed for individual accessions (Figure 5). Accessions displayed constant SAR values across harvests in 2020 and 2021 except for C025 and S35 in 2020 which increased between harvest two and three. Mean SAR for 2019 was variable across harvests for both *A. arguta* and *A. kolomikta* accessions (Figure 6). *A. arguta* accession E075 had the highest SAR, between 14.21 and 15.15, across all years for all accessions at postharvest week three.

Within a species and year, mean fruit weight did not typically vary across harvest dates (Figure 5). Mean *A. arguta* fruit weight was greater than mean *A. kolomikta* fruit

weight in all years and harvests after three weeks in storage (Figure 5). For *A. kolomikta* accessions S37, S40, S45 and S47, fruit weight was relatively constant across harvests in 2020 and 2021 but increased in 2019 (Figure 6). *A. kolomikta* accessions C025 and C013 demonstrated variation for weight at postharvest evaluation week three (Figure 6).

TF and MDT values were inversely related (Figure 5). MDT and TF exhibited relatively constant values for accessions within each year (Figure 6). *A. arguta* demonstrated variation in the MDT values in 2021 with MDT values decreasing from harvest one to harvest two, but remaining above previous years values (Figure 5). TF increased slightly for E075 across harvests but remained relatively constant for the other *A. arguta* accessions. No trends were observed within or between species for mean pH (Figure 5).

Discussion

Results from this study will enable the development of guidelines for determining appropriate harvest timing of kiwiberry (*Actinidia* species) in northern climates like Minnesota and provide phenotype information to inform parent selection for future breeding. Determining harvest timing is important for the introduction of novel crops because harvest maturity stage impacts important fruit quality traits including TA, SSC, and firmness of kiwiberry (Fisk et al. 2006).

Sources of observed variation among kiwiberry samples:

Multiple sources of variation that impacted fruit quality traits in this study were identified. Year, accession, harvest timing, and storage significantly impacted fruit quality traits across three seasons. When postharvest timepoint was fixed, the effects of

year, accession, and harvest timing on fruit quality traits became more prominent. For example, accessions accounted for over 75% of variation for weight throughout storage. The large proportion of variation accounted for by accession was not surprising as fruit size varies between species and accessions within species. Because the accessions were sampled as single, non-replicated plants, variation due to spatial factors in the orchard were confounded with genetic variation. Another factor that might have contributed to experimental variation was missing data. Missing data are common challenges in horticultural experiments. To mitigate the effects of missing data in this study, experiments were repeated across years with replicated fruit samples within year.

Fruit weight

A. arguta fruit often weighed more than A. kolomikta fruit

Differences in fruit weight were observed between *A. arguta* accessions and *A. kolomikta* accessions. As expected, fruit of *A. arguta* accessions generally had larger fruit weights compared to fruit of *A. kolomikta* (Figure 3). However, with each subsequent year, mean fruit weight decreased compared to previous years across all storage weeks for both species (Figure 3) and across accessions (Figure 4). Lower fruit weights could have been due to management of the vines (e.g., intense shoot removal to renew vines after winter injury, irrigation challenges, etc.). Compared to *A. kolomikta,* larger fruit weights for *A. arguta* may provide quicker return on investment for growers as larger fruits allow more efficient harvesting and potentially greater yields.

Fruit weight decreased in storage

In 2021, containers of fruit were weighed before and after storage to determine if mean fruit weights decrease in storage. Dehydration in storage and shipping is a known

challenge for kiwiberry (Strik 2005). While sampling bias (e.g., unconscious evaluation of larger fruit in early evaluations) might have impacted weights across storage evaluation periods, fruit weights were shown to decrease due to dehydration in storage. Container weights decreased by approximately 10% to water loss after two weeks in cold storage. Minimal decreases in fruit weights were observed after two weeks in cold storage suggesting most water loss occurred early in storage. Dehydration was observed across years and accessions; therefore, methods to mitigate dehydration such as coatings applied after harvest or management of storage humidity could be explored to maintain yield and fruit quality (Fisk et al. 2008).

Fruit weights were consistent across harvests within year

After three weeks of storage, fruit weights were relatively consistent across harvests with slightly higher fruit weights in 2019 and 2020 harvests for *A. arguta* (Figure 5). Relatively consistent fruit weights for each accession across harvests indicate that harvest timing was likely appropriate for achieving full sized fruit within a year.

Soluble solids content

Soluble solids content differed among and within Actinidia species

Actinidia species varied for SSC which provides future targets for breeding efforts in kiwiberry. Higher SSC is often associated with sweeter tasting fruit and greater consumer acceptance; therefore, *A. arguta* accessions in this study might be more appealing to consumers than *A. kolomikta* accessions. In this study, a threshold of 12 °Brix was chosen as a minimum threshold for kiwiberry quality based on the results of previous studies focused on kiwifruit and kiwifruit pulp. Rossiter et al. (2000) found that sensory sourness ratings were higher when SSC values were less than 11 °Brix.

Additionally, consumer liking has been reported to be positively correlated with SSC (Marsh et al. 2003; McMath et al. 1991). Mean SSC values for all *A. arguta* accessions were greater than the threshold of 12 °Brix with accession E093 having the lowest SSC. Averaged species harvests at postharvest week three indicate that SSC for *A. arguta* was above the minimum threshold indicating harvest timings may be appropriate for consumer acceptable fruit quality (Figure 5). *A. kolomikta* accessions, except D013, have consistent mean SSC above minimal thresholds in 2020 and 2021 indicating that harvest timings are appropriate for SSC (Figure 6b). SSC generally increased in storage for accessions of both *Actinidia* species which is expected as previous studies have reported that SSC of kiwiberry increases after storage primarily due to the conversion of starches to sugars (Fisk et al. 2006a; Han et al. 2019; Lin et al. 2022; Oh et al. 2017; Park et al. 2022).

Titratable acidity

Average TA decreased in storage across accessions

Previous studies have reported decreases in TA after storage for *Actinidia* species (Fisk et al. 2006a; Han et al. 2019; Park et al. 2022). In this study, decreases in TA after storage were observed for both *A. arguta* and *A. kolomikta* (Figure 3). The range of TA for *A. arguta* fell within previously reported values, but variation was observed between accessions where E075 had lower average TA than the other *A. arguta* accessions. Initial average TA for *A. kolomikta* accessions were higher but then reached similar amounts as *A. arguta* in storage. However, D013 and D031 had higher TA than other *A. kolomikta* accessions. Variation in TA in both *Actinidia* species could provide future breeding directions as the amount of TA impacts perceptions of sweetness and flavor in fruits.

The ratio between soluble solids content and titratable acidity

The sugar (SSC) and acid (TA) ratio (SAR) has been used as a harvest maturity metric for consumer preferences in fruits like grape (Jayasena and Cameron 2008; Ough and Alley 1970) and pomegranate (Chater et al. 2018). In grape, previous studies have reported that the ratio between SSC and TA should be between 30 and 40 for consumer consumption (Jayasena and Cameron 2008; Ough and Alley 1970). The desired ratio depends on the type of fruit and consumer preferences, but higher ratio fruit might be considered sweeter and preferred, while lower ratio fruit are tarter (Chater et al. 2018). The ratio should be considered along with SSC and TA, and all measurements should be associated with consumer taste panels. SAR has been previously reported in kiwifruit with ranges for puree between 7.36 and 11 (Castaldo et al. 1992) and for fresh fruit between 19.37 and 23.24 after storage (Choi et al. 2019). Overall, *A. kolomikta* had lower SAR values than *A. arguta*. *A. arguta* accessions might be more favorable for marketing to a wide range of consumers. However, niche consumer segments might be interested in lower SSC fruit (Harker et al. 2009). A consumer group for low SSC might provide an avenue for marketing current *A. kolomikta* cultivars which display lower SARs possibly indicating lower perception of sweetness.

Tactile firmness (TF) and mechanical firmness (MDT)

TF and MDT displayed an inverse relationship indicating that MDT values are correlated with human perception estimates of firmness in kiwiberry. Overall, fruits were successfully harvested at firm stages appropriate for handling and shipping while allowing fruit to ripen after storage (Figure 3). Following cold storage and ripening at room temperature, fruits of both *Actinidia* species softened rapidly after two weeks in

storage and reached a firmness plateau for the remaining weeks in storage (Figure 3). *A. kolomikta* accessions started with firmer fruit based on MDT values, but more quickly reached a TF appropriate for eating (Figure 3). The rapid ripening of *A. kolomikta* might reduce the storage life for *A. kolomikta* fruit, which frequently rotted by the end of storage treatment. *A. arguta* fruits typically remained firm after six weeks of storage indicating that *A. arguta* fruits could be stored longer than *A. kolomikta* fruit.

A. arguta retained firmness in storage longer than A. kolomikta

When examining individual accessions, *A. arguta* accessions reached the TF threshold, a rating of three indicating eating firmness, between three and four weeks of storage (Figure 4). The *A. arguta* accession E093 displayed low TF throughout storage in 2021 reaching adequate TF at six weeks postharvest. Slow ripening of E093 fruits could indicate that fruit were harvested too early, though other fruit quality traits for E093 are within previous years' ranges for appropriate harvest. *A. kolomikta* accessions reach the TF threshold after one or two weeks of storage and continued ripening throughout storage becoming too soft after four or five weeks of storage (Figure 4). The decrease in TF ratings in 2020 and 2021 indicated that harvest timing impacted kiwiberry fruit firmness throughout storage.

Firmness values indicate appropriate harvests

High MDT values of *A. arguta* accessions could indicate that fruit were harvested too early. *A. arguta* accession E093 had higher MDT at harvest one in 2021 than harvest two, but all values were higher than previous years indicating that E093 was harvested

too early. Both harvests for E093 were below the acceptable TF threshold at postharvest week three indicating that harvests could have occurred later to allow fruit to develop further on the vine. TF ratings for E065 and E075 were both at the TF threshold, indicating appropriate eating firmness (Figure 6). However, in 2021, E065 had higher MDT values, which indicated that E065 fruits could have been harvested slightly later to improve fruit quality trait levels.

TF values for *A. kolomikta* accessions in 2020 and 2021 were consistent across harvest around the TF threshold rating indicating appropriate harvests (Figure 6). The MDT values were consistent across harvests for *A. kolomikta*. However, both S40 and S45 were higher for MDT values than other *A. kolomikta* accessions throughout the observed years. Increased firmness through postharvest week three might indicate that accessions S40 and S35 retain firmness longer than other *A. kolomikta* accessions. Overall, TF and MDT values demonstrated that appropriate harvest timing was achieved for most accessions in this study.

Conclusion

A harvest recommendation balancing fruit quality and storage can be provided using fruit quality trait averages. Fruits of *A. kolomikta* accessions harvest windows range for periods between August and September, while *A. arguta* accessions harvest windows are early to mid-September in Minnesota (Figure 7). By planting a combination of *A. kolomikta* and/or *A. arguta* accessions, Minnesota growers might be able to extend their kiwiberry production season.

Tables

Table 1. Overview of fruit collection methods over three years.

Species	Accessions repeated across years	Year	Number of accessions harvested	Number of harvests	Fruit per harvest	Fruit per postharvest	Total fruit harvested
A. arguta	3	2019	6	4	40	5	720
		2020	6	4	40	5	720
		2021	6	2	130	20	1170
A. kolomikta	8	2019	45	4	40	5	5400
		2020	40	4	40	5	4800
		2021	15	2	130	20	2925

Table 2. Mean fruit quality traits by year for harvest, postharvest week 3 (PH3), and postharvest week 6 (PH6). Fruit quality traits were soluble solids content (SSC), titratable acidity (TA), sugar-acid ratio (SAR), tactile firmness rating (TF), instrumental firmness (MDT), and fruit weight.

Accessions	Year	Weight (g)			TF (rating)			MDT (N)			TA (g/100ml)			SSC (°Brix)			SAR		
		H	PH3	PH6	H	PH3	PH6	H	PH3	PH6	H	PH3	PH6	H	PH3	PH6	H	PH3	PH6
D013	2019	2.89	2.82	2.38	2.10	4.40	4.80	20.99	1.76	1.22	1.86	1.58	1.01	8.14	15.55	15.30	4.34	10.75	15.11
	2020	3.20	2.49	1.96	1.50	3.54	3.37	19.66	1.79	1.91	2.18	1.92	1.67	15.90	15.80	13.65	7.30	8.32	8.10
	2021	2.10	2.11	1.91	1.28	3.50	4.05	34.50	1.68	1.62	2.30	1.94	1.99	10.95	15.33	18.43	4.78	7.95	9.26
D031	2019	3.39	3.29	2.48	2.84	4.70	5.00	5.44	1.03	0.91	1.84	1.59	1.36	11.64	15.32	17.25	6.34	10.31	12.66
	2020	2.92	2.25	2.13	2.60	4.05	3.39	9.09	1.52	1.30	1.85	1.77	1.68	14.75	13.88	14.70	7.86	7.79	8.63
	2021	2.07	1.91	1.95	1.08	3.38	3.68	38.34	2.03	1.90	1.61	1.93	2.07	9.51	16.80	17.96	6.26	8.73	8.75
S35	2019	4.46	4.05	4.21	2.70	4.26	4.78	8.74	1.31	0.97	1.26	1.44	1.10	10.26	9.30	8.10	8.89	6.47	7.38
	2020	5.11	4.15	2.72	2.05	3.69	NA	11.83	1.33	NA	1.73	1.23	NA	11.55	14.40	NA	6.83	12.11	NA
	2021	2.76	2.47	2.45	1.19	2.99	4.15	27.45	1.57	1.19	1.80	1.65	1.52	7.95	12.26	12.09	4.43	7.47	7.97
S37	2019	2.34	2.51	2.05	2.15	3.58	4.89	15.10	1.86	0.93	1.55	1.23	NA	6.55	9.45	NA	4.25	8.71	NA
	2020	2.36	2.15	2.18	2.13	2.88	3.85	20.72	2.15	1.09	1.73	1.48	1.24	11.93	13.45	10.50	6.92	9.11	8.47
	2021	1.75	1.63	1.54	2.05	3.38	4.05	11.55	1.48	1.26	1.76	1.41	1.42	15.79	14.03	14.18	8.99	9.95	10.00
S40	2019	2.65	2.79	2.27	2.65	3.26	5.00	8.31	2.26	NA	1.56	1.57	NA	10.70	12.49	NA	6.85	8.00	NA
	2020	1.87	1.96	1.83	1.20	2.38	3.29	31.80	3.66	1.49	1.74	1.66	NA	8.85	10.80	NA	4.84	6.49	NA
	2021	1.56	1.69	1.65	1.94	2.98	3.86	18.70	2.99	1.56	1.44	1.87	1.45	14.25	16.61	16.61	10.54	8.94	11.62
S45	2019	3.62	3.55	3.28	2.55	3.15	4.63	6.85	2.22	1.13	1.58	1.60	1.28	10.10	11.10	10.35	6.47	6.98	8.11
	2020	2.82	2.74	2.32	1.87	2.43	3.25	8.90	2.56	1.69	1.71	1.46	1.31	12.00	12.20	9.90	6.98	8.36	7.53
	2021	2.23	2.44	1.93	1.95	2.85	3.26	14.58	3.22	2.01	1.64	1.54	1.65	10.79	13.61	15.49	6.56	8.86	9.43
S47	2019	3.63	3.48	3.09	2.35	4.42	4.78	17.73	1.29	1.01	1.55	1.35	1.19	7.55	10.20	8.55	4.85	7.58	7.20
	2020	2.82	2.51	2.16	1.00	3.44	2.60	26.88	1.58	2.16	1.54	1.24	1.04	10.28	11.66	10.65	6.67	9.49	10.22
	2021	1.31	1.34	1.24	1.40	3.70	4.26	22.38	1.67	1.54	1.81	1.35	1.37	11.49	12.86	13.01	6.51	9.58	9.51

Accessions	Year	Weight (g)			TF (rating)			MDT (N)			TA (g/100ml)			SSC (°Brix)			SAR		
		H	PH3	PH6	H	PH3	PH6	H	PH3	PH6	H	PH3	PH6	H	PH3	PH6	H	PH3	PH6
E065	2019	13.29	12.66	11.38	1.65	3.05	4.00	10.99	2.18	1.55	2.12	1.54	1.36	20.35	14.33	19.85	9.20	9.20	14.63
	2020	10.32	9.70	9.39	2.15	2.93	3.85	3.88	2.46	1.61	1.56	1.68	1.71	19.35	18.05	16.85	12.53	10.87	10.32
	2021	5.85	5.03	5.60	1.63	2.80	2.71	11.08	4.47	2.99	1.17	1.61	1.45	10.61	21.94	15.58	9.07	13.57	10.75
E075	2019	15.85	16.96	16.08	3.00	3.78	4.00	2.96	1.76	1.20	1.05	0.93	0.88	15.69	13.15	18.45	14.86	14.09	21.06
	2020	11.07	11.37	10.48	2.20	2.70	3.57	2.92	2.62	1.91	1.00	1.05	0.98	12.53	15.04	15.00	12.43	14.42	15.32
	2021	7.76	8.09	7.84	1.33	2.85	3.00	12.93	2.89	2.31	1.04	0.99	1.10	11.91	15.03	19.31	11.52	15.14	17.50
E093	2019	13.55	13.68	13.09	2.47	2.95	4.83	8.26	2.57	1.62	1.92	1.43	1.40	15.40	10.88	12.35	7.89	7.99	8.78
	2020	14.05	12.98	12.66	2.63	3.35	4.17	3.17	2.58	1.66	1.42	1.62	1.24	15.70	16.75	13.50	11.67	10.38	10.88
	2021	12.96	12.35	12.30	1.33	2.14	2.50	15.45	5.12	2.67	1.36	1.22	1.29	12.04	11.64	12.96	8.88	9.74	10.12
C025	2019	3.28	2.87	1.95	1.90	4.79	NA	16.17	1.01	NA	1.69	1.45	NA	9.23	11.18	NA	5.52	7.71	NA
	2020	2.61	1.91	1.46	2.27	4.00	4.31	5.05	1.25	1.17	1.71	1.25	NA	13.00	12.98	13.50	7.54	10.99	NA
	2021	2.02	2.42	2.44	1.59	4.00	4.83	17.32	1.12	0.91	2.07	1.61	1.57	10.26	12.34	13.20	5.05	7.66	8.37

Figures

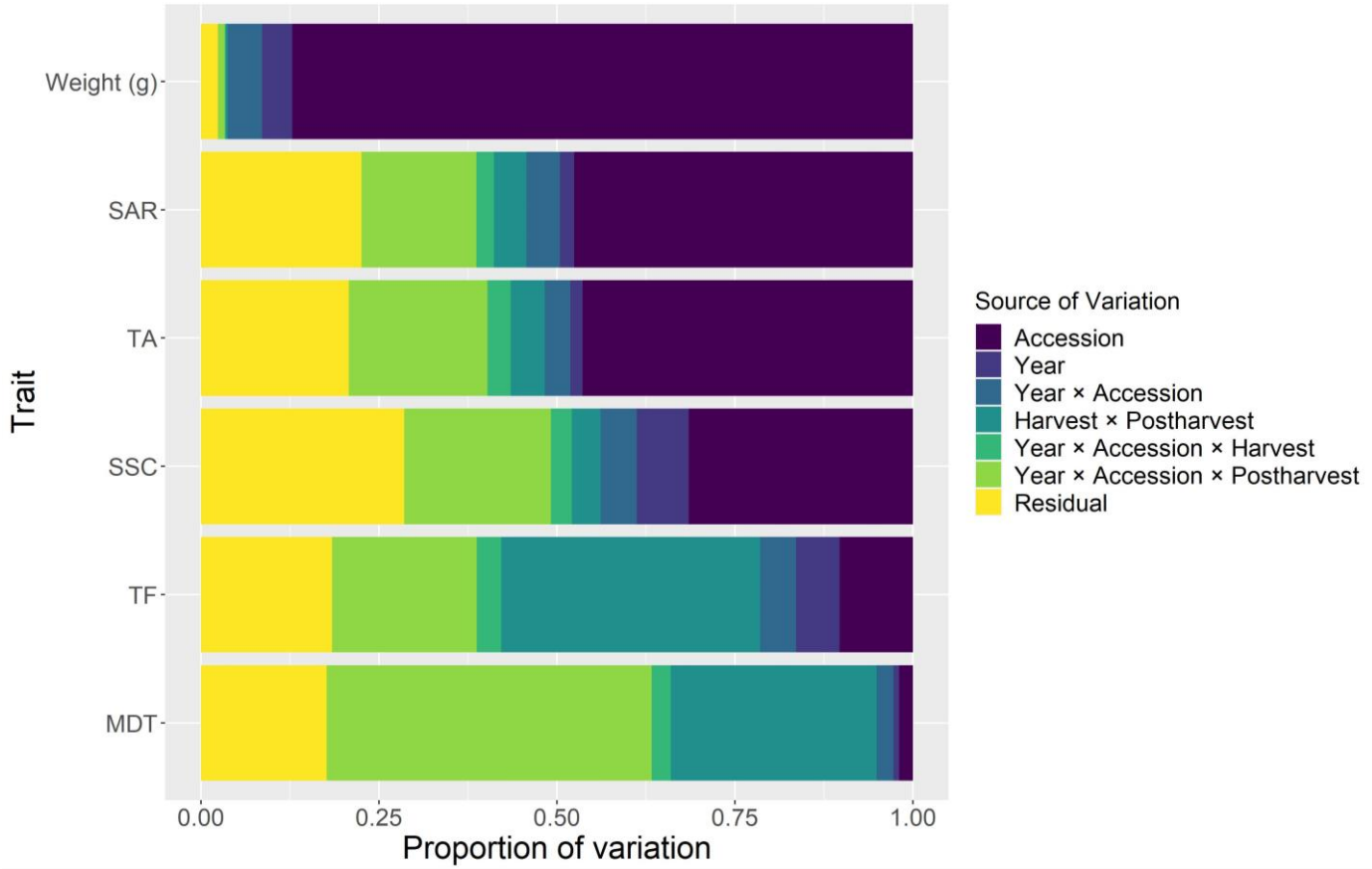

Figure 1. Proportions of variation for all harvests and postharvest timepoints associated with accession, year, year × accession, harvest week × postharvest week, year × accession × harvest week, year × accession × postharvest week, and residual error for fruit weight, sugar-acid ratio (SAR), titratable acidity (TA), soluble solids content (SSC), tactile firmness (TF), and instrumental firmness (MDT) sample means.

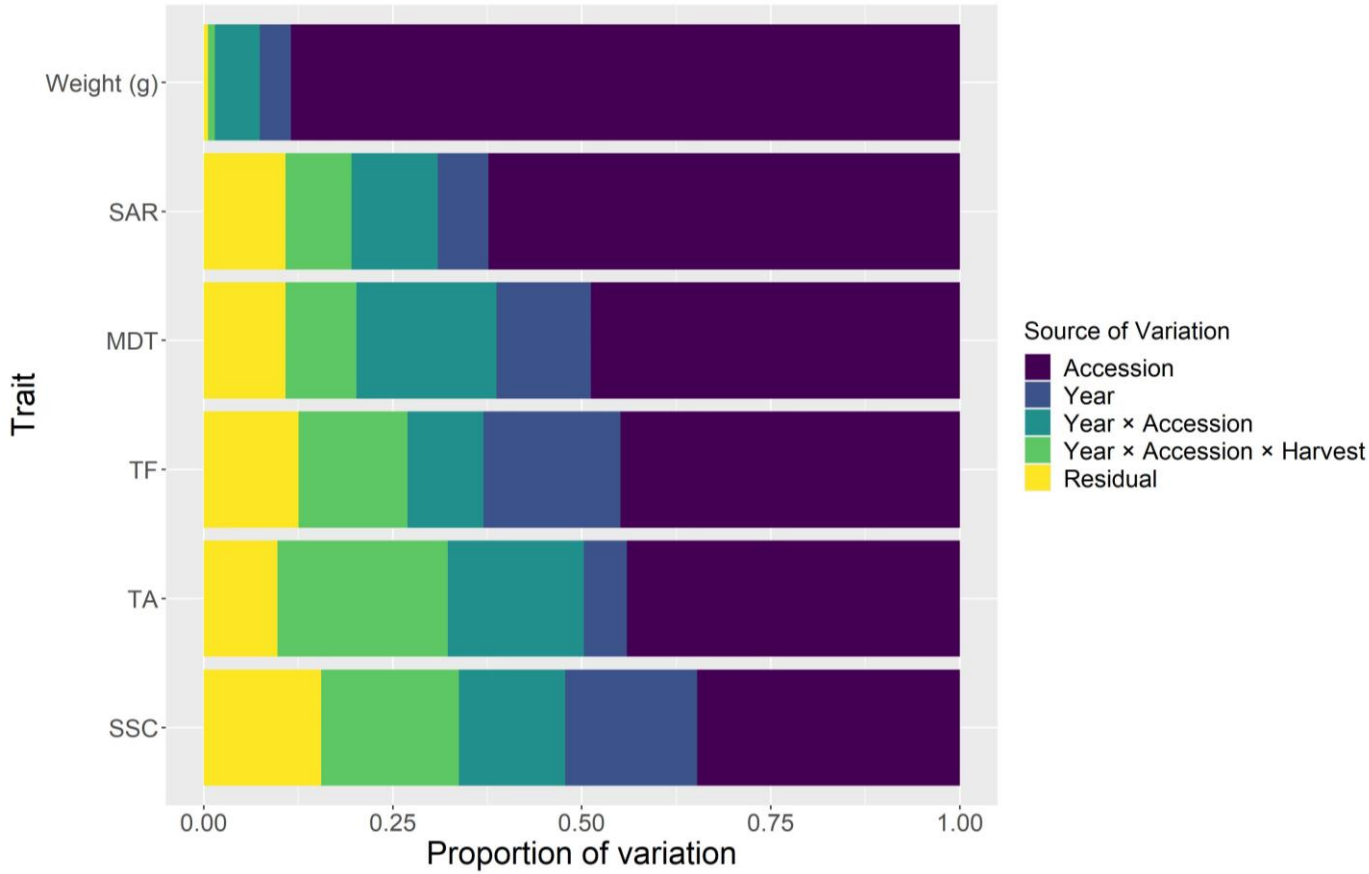

Figure 2. Proportions of variation for postharvest week 3 samples associated with accession, year, year × accession, year × accession × harvest week, and residual error. Fruit quality traits were soluble solids content (SSC), titratable acidity (TA), sugar-acid ratio (SAR), tactile firmness rating (TF), instrumental firmness (MDT), and fruit weight.

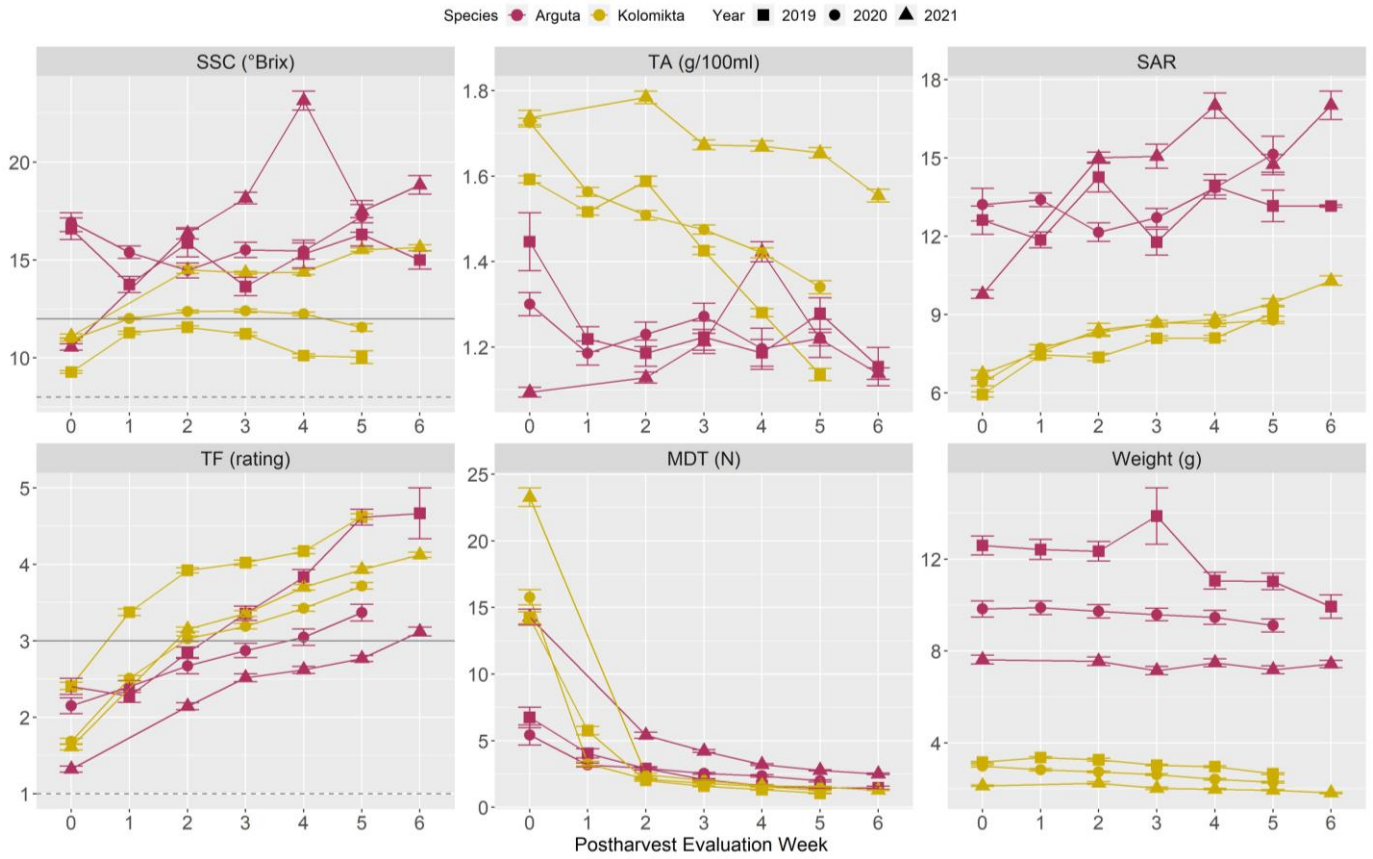

Figure 3. Fruit quality trait means with standard error are displayed for *A. arguta* and *A. kolomikta* at six postharvest evaluations within three consecutive years. Fruit quality traits were soluble solids content (SSC), titratable acidity (TA), sugar-acid ratio (SAR), tactile firmness rating (TF), instrumental firmness (MDT), and fruit weight. Solid horizontal lines indicate minimum consumer acceptability for traits. Dashed horizontal lines indicate trait threshold required to initiate harvest. Traits without threshold lines have limited information available to estimate thresholds.

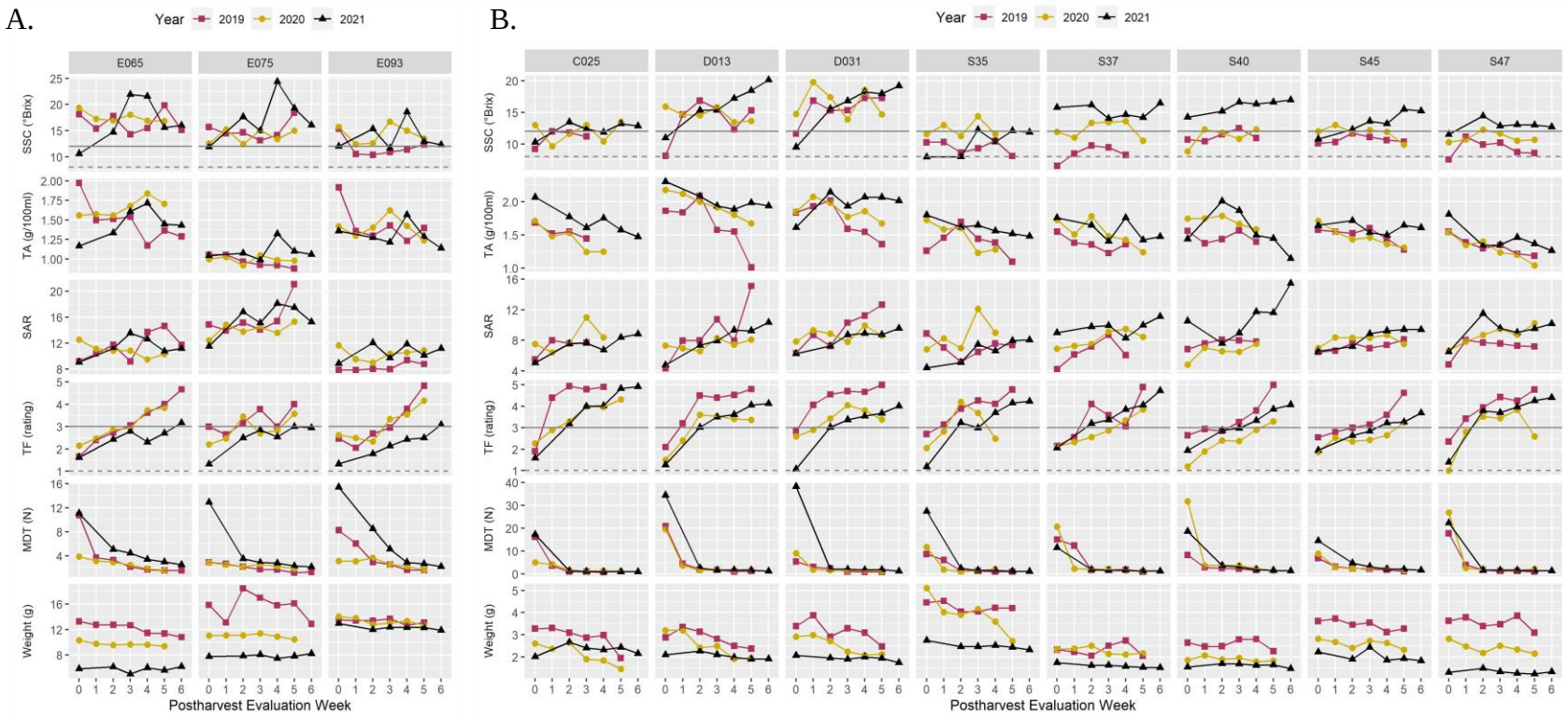

Figure 4. Yearly fruit quality trait means from initial harvest across six weeks of postharvest evaluations for (A) *A. arguta* and (B) *A. kolomikta* accessions are shown. Fruit quality traits were soluble solids content (SSC), titratable acidity (TA), sugar-acid ratio (SAR), tactile firmness rating (TF), instrumental firmness (MDT), and fruit weight. Solid horizontal lines indicate minimum consumer acceptability for traits. Dashed horizontal lines indicate trait threshold required to initiate harvest. Traits without threshold lines have limited information available to estimate thresholds.

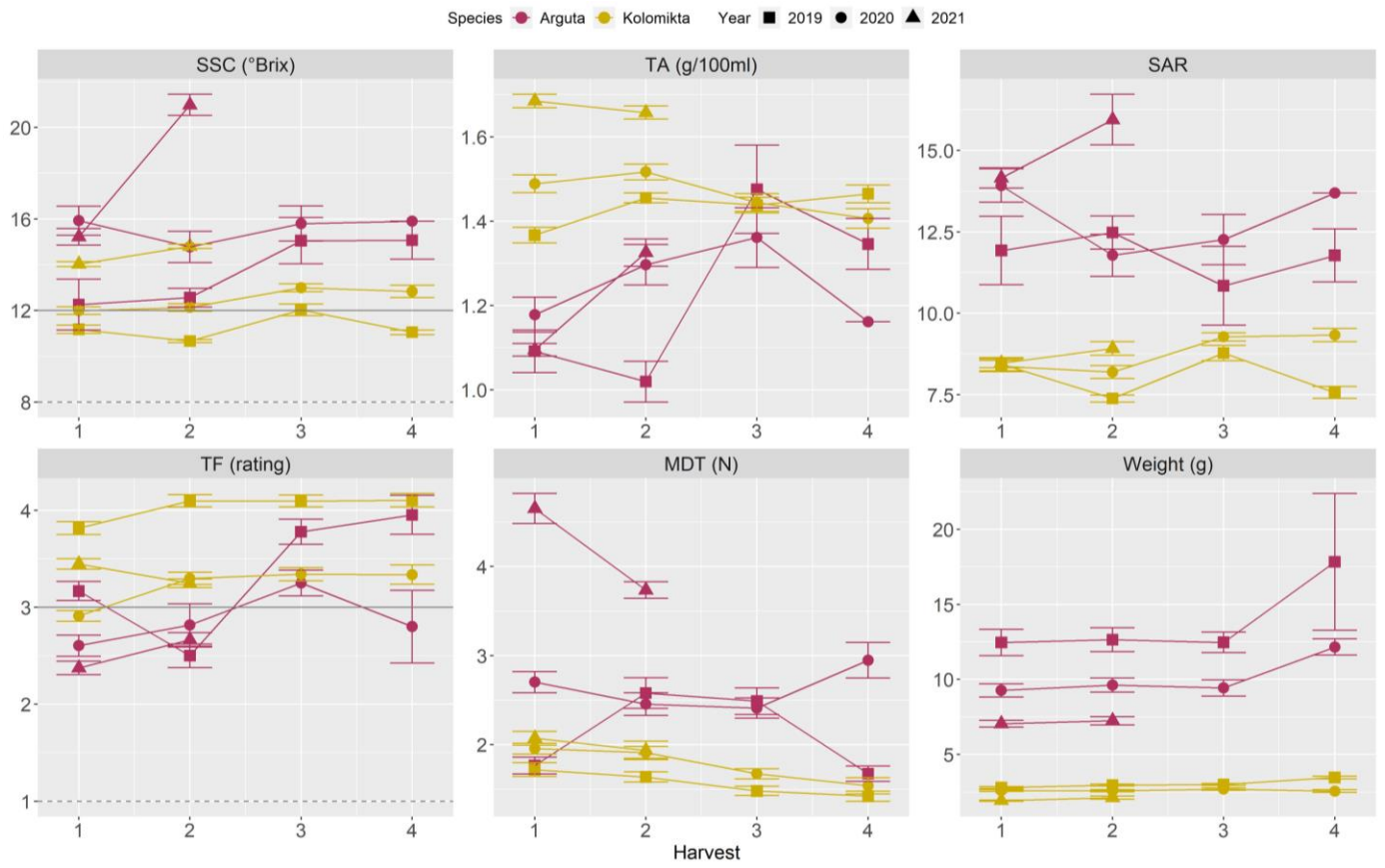

Figure 5. Fruit quality trait means from postharvest evaluation week three are displayed for *A. arguta* and *A. kolomikta* across harvests within three consecutive years. Fruit quality traits were soluble solids content (SSC), titratable acidity (TA), sugar-acid ratio (SAR), tactile firmness rating (TF), instrumental firmness (MDT), and fruit weight. Solid horizontal lines indicate minimum consumer acceptability for traits. Dashed horizontal lines indicate trait threshold required to initiate harvest. Traits without threshold lines have limited information available to estimate thresholds.

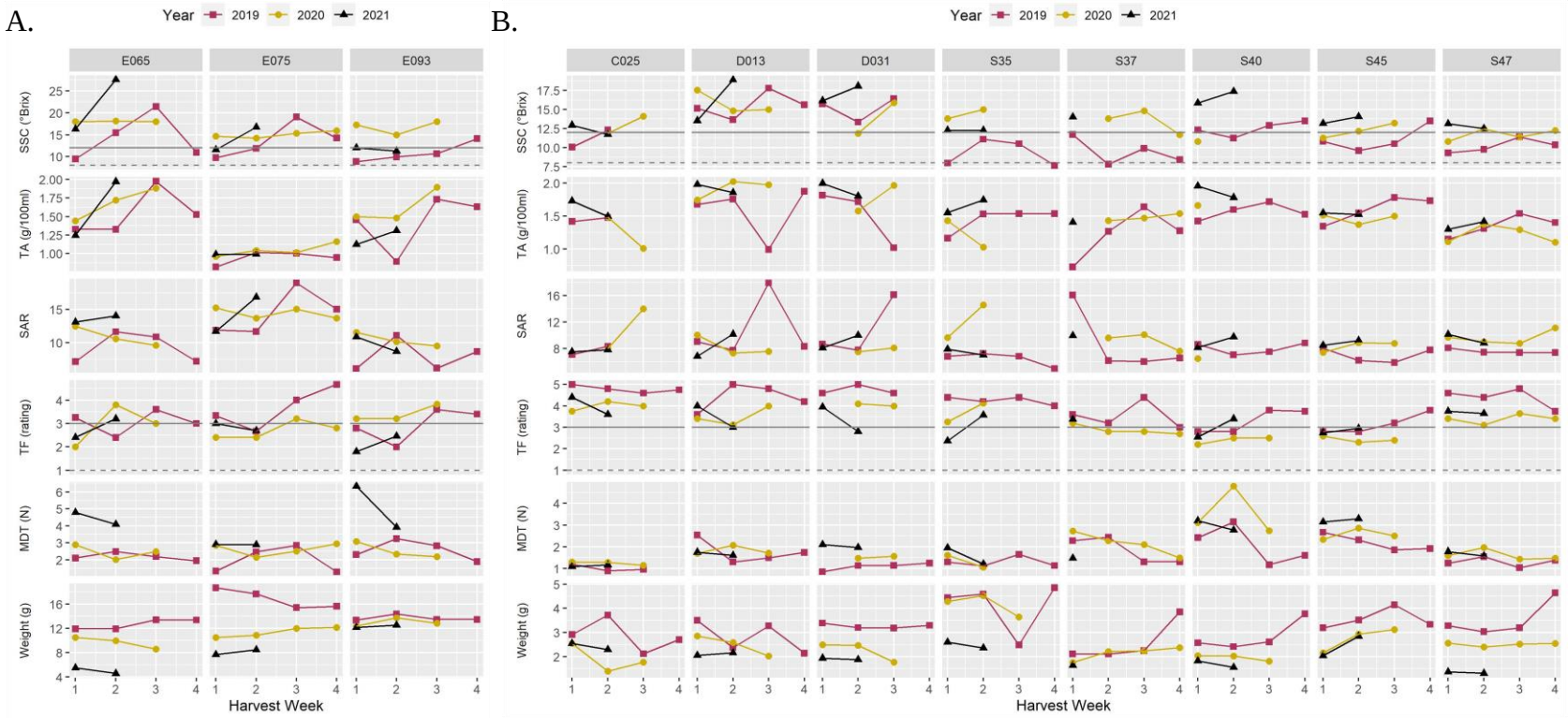

Figure 6. Postharvest week three evaluation means for (A) *A. arguta* and (B) *A. kolomikta* accessions are shown for each harvest within each year. Fruit quality traits were soluble solids content (SSC), titratable acidity (TA), sugar-acid ratio (SAR), tactile firmness rating (TF), instrumental firmness (MDT), and fruit weight. Solid horizontal lines indicate minimum consumer acceptability for traits. Dashed horizontal lines indicate trait threshold required to initiate harvest. Traits without threshold lines have limited information available to estimate thresholds.

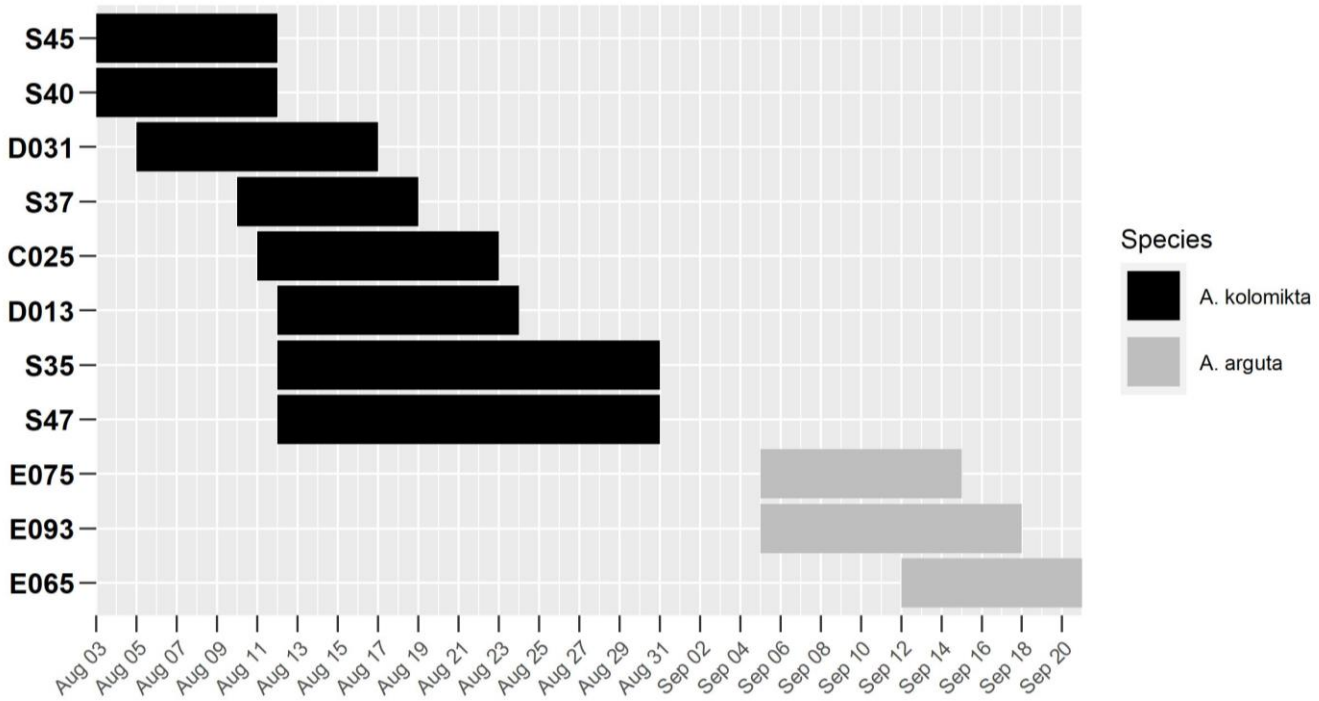

Figure 7. Analysis of 2019 to 2021 fruit quality evaluations identified harvest windows for eleven accessions. Harvest windows were determined by examining multiple harvests for three years and identifying harvests within each year for each accession that balance fruit quality with shelf life.

Chapter 4: Evaluation of spotted-wing drosophila infestation of kiwiberry and impacts on fruit quality

Introduction

The term kiwiberry (*A. arguta* and *A. kolomikta)* is used to refer to several species within the genus *Actinidia* that have smooth, green skinned berries (Cossio et al. 2015). With limited plantings in the United States, determining fruit susceptibility to pests is important for new growers. Field observations have shown that kiwiberry is susceptible to spotted-wing drosophila (SWD), *Drosophila suzukii* Matsumura, oviposition (Wannemuehler and Tran 2021). SWD is a polyphagous invasive vinegar fly that has emerged as a pervasive pest of soft-skinned fruits in Asia, Europe, and North America (Walsh et al. 2011). Economically important SWD hosts include blueberry (*Vaccinium corymbosum*), raspberry (*Rubus idaeus*), blackberry (*Rubus fruticosus*), strawberry (*Fragaria ×ananassa*), sweet cherry (*Prunus avium*), and peach (*Prunus persica*) (Bellamy et al. 2013). Since 2008, SWD has rapidly spread throughout the United States, leading to significant economic losses, particularly for small fruits such as raspberry and strawberry (Farnsworth et al. 2017; Goodhue et al. 2011). SWD was first detected in the Upper Midwest in 2010, and its presence in Minnesota was confirmed by August 2012, which subsequently resulted in economic losses for regional small fruit producers (Asplen et al. 2015).

The spread of SWD can be attributed to its short generation cycles and unique ability to infest ripening fruit (Walsh et al. 2011). SWD exhibits rapid reproduction rates, with females laying approximately 20 to 400 eggs that mature into adults within 12 to 14 days (Hamby et al. 2016). Additionally, female SWD possess a serrated ovipositor which

allows them to oviposit in intact, ripening fruit (Karageorgi et al. 2017). However, the presence of wounds on fruit facilitates SWD infestation, allowing establishment of populations in resistant crops with fruit qualities unfavorable for oviposition such as thick exocarps in apple (*Malus* × *domestica*) or grape (*Vitis* spp.) (Holle et al. 2017; Steffan et al. 2013).

Fruit quality traits, including pH and soluble solids content (SSC), have been examined in relation to their impacts on SWD host suitability (Bellamy et al. 2013). Physiological barriers, like fuzz on peaches, have been associated with lower infestation rates (Stewart et al. 2014). Skin thickness has been positively associated with reduced infestation levels (Pelton et al. 2017; Steffan et al. 2013). Additionally, studies have shown that fruit susceptibility to oviposition increases as fruit firmness decreases (Gullickson et al. 2023; Ioriatti et al. 2015; Kinjo et al. 2013; Wohner et al. 2020). For example, grape skin with penetration force less than 40 cN resulted in increased chance of eggs being laid (Ioriatti et al. 2015) and artificial diets in laboratory settings above 50 cN resulted in fewer oviposit attempts (Burrack et al. 2013). Another study demonstrated that when calcium silicate was applied to blueberries, fruit firmness increased resulting in fewer oviposit attempts (Lee et al. 2016). However, oviposition attempts still occur despite increased fruit firmness which has been suggested could contribute to increased secondary infections of the fruit (Ebbenga et al. 2021; Ioriatti et al. 2015). This relationship between firmness and oviposition could be exploited for cultural control of SWD on climacteric fruit as fruits can be harvested firm when fewer infestation attempts are likely.

Currently, the most widely accepted control method for SWD throughout the season is the application of insecticides, both in conventional and organic systems (Van Timmeren and Isaacs, 2013). However, the limited number of effective insecticides available for organic applications increases the risk for development of resistance, therefore necessitating improved integrated pest management (IPM) practices. IPM practices include physical barriers such as high tunnels (Rogers et al. 2016), strategic watering to reduce relative humidity (Rendon et al. 2019), pruning to reduce canopy cover (Schöneberg et al. 2020), and development of appropriate harvest schedules (Leach et al. 2018). Kiwiberry fruits are climacteric which means that they are harvested firm for shipping and packaging and will then ripen in storage. This quality is important for producing quality fruit for market as the firm kiwiberry is more resilient to handling damage. The objective of this study was to examine whether the climacteric nature of kiwiberry allows for reduced SWD infestation pressure at harvest (as a function of harvest date or fruit firmness) and the subsequent impacts of infestation on fruit quality of two kiwiberry species (*A. arguta* and *A. kolomikta*) in the Upper Midwest.

Materials and Methods

Spotted-wing drosophila (SWD) colonies

Flies were sourced from a laboratory-reared *D. suzukii* colony established from infested raspberries in 2020 at the University of Minnesota. Flies were maintained following protocols described by Dalton et al. (2011). Growth media was provided in tubes and stopped with a foam plug. Vials were stored in a growth chamber at 25 °C (77 °F) with a L16:D8 photoperiod and 90% relative humidity.

No choice bioassays

2020

Bioassays of three *A. kolomikta* accessions were conducted based on the methodology described by Gullickson et al. (2019). Approximately 100 pollinated flowers with observable fruit set were covered with white organza bags to prevent field infestation for three accessions (C041, C061, and D031). Thirty-five fruits were harvested per accession at two time points: prior to fruit softening with < 6 °Brix (July 27) and with > 7.5 °Brix (August 10). Fruits were examined under a dissecting microscope for evidence of prior infestation and fruit with prior infestation were set aside for observation. For each harvest, thirty fruits per accession were individually placed in 30 mL plastic cups with a moistened 1 cm^2 Kimwipe and covered with an aerated lid. Two male and five female SWD (*Drosophila suzukii* Matsumura (Diptera: Drosophilidae)) were identified via wing spot and ovipositor and added to each plastic cup with a single fruit. Plastic cup incubation units were placed in a growth chamber with a temperature of 25 °C (77 °F), 90% humidity, 16:8 light:dark cycle for 48 hours. After 24 hours, SWD were removed from containers and fruits were examined under a dissecting microscope for presence of egg breathing tubes, oviposition scarring, and eggs on fruit surface. Incubation units were returned to the growth chamber for a week after which developed larva were counted. The units were then returned to the growth chamber for further SWD development, but larvae expired prior to pupation due to fruit degradation releasing excess fluids.

2021

Bioassays were repeated with slight modifications. Approximately 120 developing fruits per accession were covered with organza bags which were harvested at one time point. Accessions from 2020 were repeated with the addition of one *A. kolomikta* (C045) and one *A. arguta* (E065). Thirty fruits were harvested for baseline harvest fruit quality and thirty additional fruits were harvested and stored at 3 °C for four weeks. Thirty fruits were used in the bioassay and twenty fruits were used as controls in the growth chamber. Incubation unit construction was repeated from 2020. For the bioassay, applied SWD numbers were altered from the previous year with longer time in the growth chamber. Three female and one male SWD were applied to each incubation unit based on (Elsensohn et al. 2021).

After infestation, fruits were examined with a dissecting microscope for signs of oviposition attempts. Additionally, a nondestructive tactile fruit firmness (Chapter 3) rating was recorded. Fruits were returned to incubation units and placed in cold storage at 2 °C (36 °F) with no other produce to limit exogenous ethylene. After four weeks of storage, fruits were evaluated for fruit quality including: disease presence (score), tactile ripeness rating (score), weight (g), length (mm), diameter of rounded side (mm), diameter of flat side (mm), mechanical firmness (N), titratable acidity (g/100mL), pH, and soluble solids content (°Brix).

2022

Accessions examined in 2021 were also examined in 2022. Two hundred fruits per accession were covered with organza bags to prevent field infestation. For each accession: fifty fruits were infested in the bioassay; thirty fruits were used as controls in the growth chamber; thirty fruits were processed for baseline harvest fruit quality; and an

additional thirty fruits were stored immediately after harvest as a storage control. Bioassay methods and materials were the same as in prior years. Storage and fruit quality evaluation methods were repeated from 2021.

Statistical analyses

The normality of the data was examined for the no-choice bioassays using R v 4.0.5 (R Core Team, 2020). The infestation count data were then examined using both negative binomial and zero-inflated Poisson models, followed by a Dunnett's post hoc test for pairwise comparison using *MASS* (Venables and Ripley 2002) and *dunn.test* (Dinno 2017) packages. Fruit quality data was normally distributed and assessed using ANOVAs with the following linear model: $Y_{\text{fruit quality trait}} = \beta_{\text{accession}} + \beta_{\text{treatment}} + \beta_{\text{year}} + \beta_{\text{accession} \times \text{treatment}} + \beta_{\text{accession} \times \text{treatment} \times \text{year}}$. Tukey post hoc tests were used to determine differences between treatments within each accession.

Results

Pilot study showed no difference in infestation between harvest times

The 2020 pilot study indicated that the mean number of eggs laid per accession did not significantly differ between harvest times ($p = 0.089$). Mean number of eggs laid for D031 was significantly different from other accessions ($p < 0.01$) with fewer eggs laid. All eggs laid for D031 were on fruit surface with no observed eggs under the fruit skin. Approximately 50% of eggs for C041 and C061 developed into larvae over two weeks (Table 1).

Minimal differences in total oviposition attempts between accessions

When examining fruits after 48 h in no-choice bioassays, egg breathing tubes, oviposition wound, and eggs on fruit surface across years, significant differences between accessions were observed. For egg breathing tube count, three accessions (C031, C041, C045) had significantly ($p < 0.05$) more egg breathing tubes when compared to D031. No significant difference in egg breathing tube count was found between E065 and D031 across years. Significantly fewer oviposition wounds were observed for C031, C041, C045 and D031 when compared to E065 across the years (Table 2). In 2022, E065 had a mean oviposition wound count approximately three times more than other accessions. There were no differences between accessions for the number of eggs laid on fruit surfaces between years (Table 2).

Across years, the mean number of total oviposition attempts (i.e., egg breathing tubes, oviposition wounds, and eggs on fruit surfaces) did not significantly differ among kiwiberry accessions (Figure 1). However, D031had significantly fewer oviposition attempts than other accessions in 2022 with a mean total of 0.54 (Table 2). E065 had the highest mean oviposition attempts value of 7.64 in 2022 with a mean of 4.87 oviposition attempts across years. The remaining accessions fell between D031 and E065 with yearly mean oviposition attempts between 3.51 and 4.56. Remaining accessions did not statistically differ for total oviposition attempts in either 2021 or 2022 when examined using Dunnett's test. Accessions vary between years for mean total oviposition attempts, but were not significantly different from each other.

Firmness impacted infestation event counts

Mean initial tactile firmness (TF) ratings were approximately 1.0 in both years (Table 2). C031 and C045 mean initial TF were slightly higher than other accession in 2021 with each at a rating of approximately 1.73. After 48 h in the growth chamber, TF ratings increased (i.e., softer fruit) for all accessions, but D031 remained firm in both years with mean ratings between 1 and 1.08 after 48 h. In 2021, C031, C041, and C045 had the highest TF ratings after 48 h at 1.62, 1.45, and 1.75, respectively. In 2022, C031 and C041 had the highest TF ratings after 48 h at 1.48 and 1.45, respectively. E065 had low TF after 48 h ratings (1.06) in 2021 but median ratings in 2022 (1.26) (Table 2). Overall, D031 and E065 berries retained lower tactile firmness ratings after 48 h than other accessions.

As berry TF after 48 h ratings in the growth chamber increased (softened), the number of observed total eggs laid (i.e., egg breathing tube count and eggs laid on fruit surfaces) increased for all accessions (Figure 2). A Spearman's rank correlation indicated a significant ($p < 0.001$) positive correlation (rho = 0.342) between TF ratings and total eggs laid. The number of oviposition wounds on fruit was also found to have a significant ($p < 0.001$) positive correlation (rho = 0.213) with softening ratings (Figure 3).

Fruit quality did not significantly differ between treatments

Greater significant differences in fruit quality (i.e., TF, MDT, SSC TA) were observed between years and between accessions than fruit quality differences between storage treatments (Table 3). Differences in fruit quality between 2021 and 2022 included: mean mechanical firmness was greater for all accessions except E065; mean SSC was lower for all accessions; and mean TA was higher than 2021 for all accessions.

Within accessions, differences were observed between storage treatments for mechanical firmness values but not in a consistent pattern indicating storage control was improved compared to infested fruit. Mean SSC was significantly different between treatments for only accessions C041 and C045 (Table 3). Both accessions had higher SSC for storage control than for infested fruit. TA did significant differ between storage treatments for all accessions except C031. For three of the accessions (C041, D031, E065), storage control had higher TA than infested and growth chamber control. Growth chamber control TA was not significantly different from infested fruit. Accession C045 had the inverse trend with infested fruit having higher TA, but was not significantly different from the storage control. Significant difference trends for fruit quality between treatments were not consistent across accessions.

Discussion

Fruit firmness was inversely correlated to oviposition, where relatively firmer fruit resulted in no to low oviposition (Figure 2). This was expected as similar inverse relationships between firmness and infestation have been noted in blueberry and grape (Gullickson et al. 2023; Ioriatti et al. 2015; Kinjo et al. 2013; Wohner et al. 2020). In 2020, fruit were harvested at different time points to determine if this would impact SWD infestation and the results did not show significantly different infestation level. The similarity in infestation amounts between harvest dates in 2020 was likely because firm fruits were harvested at both time points; which 2021 and 2022 data indicates firmness is inversely correlated with infestation. The firm fruit of D031 in all years had the least infestation and though the total number of infestation events across years was

significantly different between D031 and other accessions, this result was impacted by exceptionally firm fruits in 2022. In both years D031 had slower fruit softening in the growth chamber as initial TF and TF-after-48 h indicate that the D031 fruits did not soften while in the growth chamber. D031 may have slower initial softening than the other cultivars as D031 fruit firmness after storage was within expected ranges (see Chapter 3). However, this does support that firmness of fruit at harvest is associated with the amount of SWD infestation.

The present study also found that although firm fruit were less likely to have as many infestation events occur, there were still oviposition wounds and eggs laid on fruit surfaces. The oviposition wounds could promote secondary infections, though no difference in development of storage fungal growth was observed between treatments as all treatments had similar percentages of fruit with fungal growth (data not shown). Eggs laid on the fruit surface could develop into larvae as the fruit softens and result in fruit infestation. There are no reports of survivorship of eggs laid on fruit surfaces or a rationale why they are deposited this way. Eggs could be laid on fruit surfaces because fruit firmness prevents oviposition, but the fly detects other cues of fruit ripening resulting in attempted oviposition. High fly fecundity could also be responsible for this response. Future work could investigate if fruit firmness is a main component resulting in eggs deposited on fruit surfaces.

Even though infested fruits received punctures with or without eggs deposited, no differences among treatments were observed for the fruit quality traits evaluated. Though statistical differences in fruit quality were observed among treatments, these differences indicated no clear negative trends impacting kiwiberry fruit quality for early infestation

when fruits had SWD eggs oviposited. For instance, some accessions had higher SSC and lower TA for storage controls indicating that the fruit in storage was riper than the infested fruits and growth chamber controls. However, greater fruit quality trait variation was observed among years and individual fruits on a vine than observed among the treatments presented. For example, though each accession underwent harvest evaluation examining multiple fruits for SSC greater than 7-8 °Brix and darkening of seed coats before experiment harvest, fruits in 2022 would be considered underripe based on postharvest SSC and TA despite field sampling of SSC at time of harvest indicating appropriate harvest time. Sampling with an even greater number of fruits could resolve a portion of the variation observed between treatments, but the data does not indicate that the infested fruits were of lower quality than the control fruits.

One main reason for this may be that following infestation, fruits were placed in cold storage at 2 °C which can reduce SWD development (Aly et al. 2017; Kim et al. 2018a). Postharvest cold storage at 1.7 °C for 72 h has previously been reported to completely prevent SWD egg survival and reduce larvae development by up to 41% (Aly et al. 2017). Since kiwiberries were placed in cold storage immediately after infestation for four weeks, eggs and larvae were unable to develop and cause greater damage to the fruit. Another impact of cold storage is that sound, whole kiwiberry fruits are known to desiccate during cold storage (see Chapter 3). Water loss from SWD ovipositor punctures likely only exacerbates the natural desiccation of the fruit in cold storage. Finally, variability in fruit quality between years due to environmental conditions certainly impacts fruit quality and results in confounding noise. Overall, the possibility of

infestation events decreasing the quality of firm fruit in cold storage was not supported by the results of the current study.

The climacteric nature of kiwiberry fruits allows for reduced SWD infestation susceptibility at harvest if fruit are picked when still firm. Therefore, growers in the Upper Midwest would be able to reduce SWD infestation when harvesting if they consider the firmness of the fruit along with SSC. Even with relatively high infestation, there were marginal effects on fruit quality for the sampled species (*A. arguta* and *A. kolomikta*) suggesting SWD at the egg stage will not strongly impact fruit quality for growers in the region. More studies will be needed to fully understand the impacts and how to address SWD in kiwiberries.

Conclusion

These results indicate that infestation is less likely to occur for firm kiwiberry fruits, as expected, with minimal impacts on fruit quality. This inverse relationship between infestation and firmness can be leveraged by growers to harvest at early stages to reduce crop loss due to SWD infestation. Further examination using uncovered, field samples should be examined for validation.

Tables

Table 1. Observed counts of total eggs laid and larvae developed on three kiwiberry (*A. kolomikta*) accessions in 2020.

Accession	Total eggs laid (count)	Larva (count)
C041	103	52
C061	95	54
D031	21	0

Table 2. Mean tactile fruit firmness and infestation events for five kiwiberry accessions by year. Tactile fruit firmness ratings were recorded before and after infestation in the growth chamber. Total infestation events include counts of egg breathing tubes, oviposition wounds, and eggs laid on fruit surfaces.

Year	Accession	Tactile firmness^{0}		Tactile firmness^{48hr}		Egg breathing tubes	Oviposition wounds	Eggs laid on fruit surface	Total infestation events
2021						f, h, g, i	e, f, i, j	-	-
	C031	1.73 ± 0.92	a	1.62 ± 0.57	ab	3.00	1.23	0.30	4.57
	C041	1.01 ± 0.12	b	1.45 ± 0.59	b	1.60	1.30	0.57	3.47
	C045	1.73 ± 0.93	a	1.75 ± 0.67	a	3.80	0.33	0.30	4.43
	D031	1.00 ± 0.00	b	1.00 ± 0.00	c	0.00	0.37	0.77	1.13
	E065	1.02 ± 0.29	b	1.06 ± 0.24	c	0.00	1.90	0.20	2.10
2022						j	c, d, f, i, j	-	j
	C031	1.00	a	1.48 ± 0.80	a	1.14	1.36	0.58	3.08
	C041	1.00	a	1.45 ± 0.74	a	2.06	0.80	0.68	3.54
	C045	1.00	a	1.25 ± 0.65	ab	2.18	1.84	0.66	4.68
	D031	1.00	a	1.08 ± 0.35	b	0.00	0.04	0.50	0.54
	E065	1.00	a	1.26 ± 0.44	ab	1.72	5.68	0.24	7.64

a = statistical difference ($p < .05$) between C031 and D031; b = statistical difference ($p < .05$) between C031 and E065
c = statistical difference ($p < .05$) between C041 and D031; d = statistical difference ($p < .05$) between C041 and E065;
e = statistical difference ($p < .05$) between C045 and D031, f = statistical difference ($p < .05$) between C045 and E065;
g = statistical difference ($p < .05$) between D031 and E065

Table 3. Mean fruit quality traits for five kiwiberry accessions in four treatments in 2021 and 2022. Fruit quality traits were tactile firmness rating after four weeks in storage (TF), instrumental firmness (MDT), soluble solids content (SSC), and titratable acidity (TA). Treatments include initial harvest (IH), cold-storage control (SC), growth chamber control (GCC), and infested fruit (INF).

Accession	Treatment	2021				2022				Means across years			
		TF (rating)	MDT (N)	SSC (°Brix)	TA (%)	TF (rating)	MDT (N)	SSC (°Brix)	TA (%)	TF (rating)	MDT (N)	SSC (°Brix)	TA (%)
C031	IH	-	1.73	8.20	1.91	-	32.0	6.23	1.81	-	16.86	7.21	1.86
	SC	4.24	1.07	13.92	1.47	2.93	2.67	6.05	1.83	3.59 a	1.87 a	9.99 a	1.65 a
	GCC	5.00	-	-	-	3.37	2.22	6.28	1.86	4.18 a	2.22 a	6.28 a	1.86 a
	INF	4.93	-	-	-	3.76	1.78	6.38	1.83	4.35 a	1.78 a	6.38 a	1.83 a
C041	IH	-	36.3	8.59	1.56	-	32.7	6.08	1.60	-	34.56	7.33	1.58
	SC	3.25	1.60	17.29	1.58	3.40	2.00	13.08	1.65	3.33 a	1.78 a	15.18 a	1.61 a
	GCC	3.75	0.98	11.48	1.48	3.47	1.65	12.95	1.58	3.61 a	1.33 b	12.21 b	1.53 ab
	INF	3.60	0.93	13.00	1.40	3.44	1.69	12.18	1.53	3.52 a	1.33 b	12.59 b	1.47 b
C045	IH	-	1.33	15.41	1.77	-	27.3	5.28	1.70	-	14.32	10.34	1.73
	SC	3.5	1.02	8.74	1.59	4.63	1.96	7.59	1.70	4.08 a	1.47 b	8.16 a	1.64 b
	GCC	4.7	-	-	-	2.80	3.20	6.20	1.71	2.80 c	3.20 a	6.20 ab	1.71 a
	INF	4.9	-	-	-	3.36	2.36	5.43	1.69	3.36 b	2.36 ab	5.43 b	1.69 ab
D031	IH	-	41.2	7.69	1.88	-	38.8	5.88	2.15	-	39.81	6.78	2.03
	SC	3.15	1.56	13.54	2.15	3.47	1.78	11.70	2.17	3.31 a	1.69 a	12.44 a	2.16 a
	GCC	2.85	1.65	16.13	1.44	2.77	3.83	9.78	1.72	2.80 b	2.94 a	12.32 a	1.60 b
	INF	3.07	1.33	20.60	1.41	2.80	2.71	10.98	1.74	2.90 b	2.18 a	14.59 a	1.61 b
E065	IH	-	15.3	9.49	1.17	-	28.0	6.45	1.71	-	23.00	7.67	1.49
	SC	1.55	3.60	23.89	1.98	3.03	2.31	6.33	1.70	2.44 b	2.85 b	13.35 a	1.71 a
	GCC	1.80	5.29	16.84	1.37	2.93	2.45	6.40	1.69	2.48 ab	3.60 a	10.58 a	1.57 b
	INF	2.37	4.89	18.28	1.36	3.08	2.54	6.53	1.69	2.81 a	3.43 ab	10.93 a	1.49 b

Figures

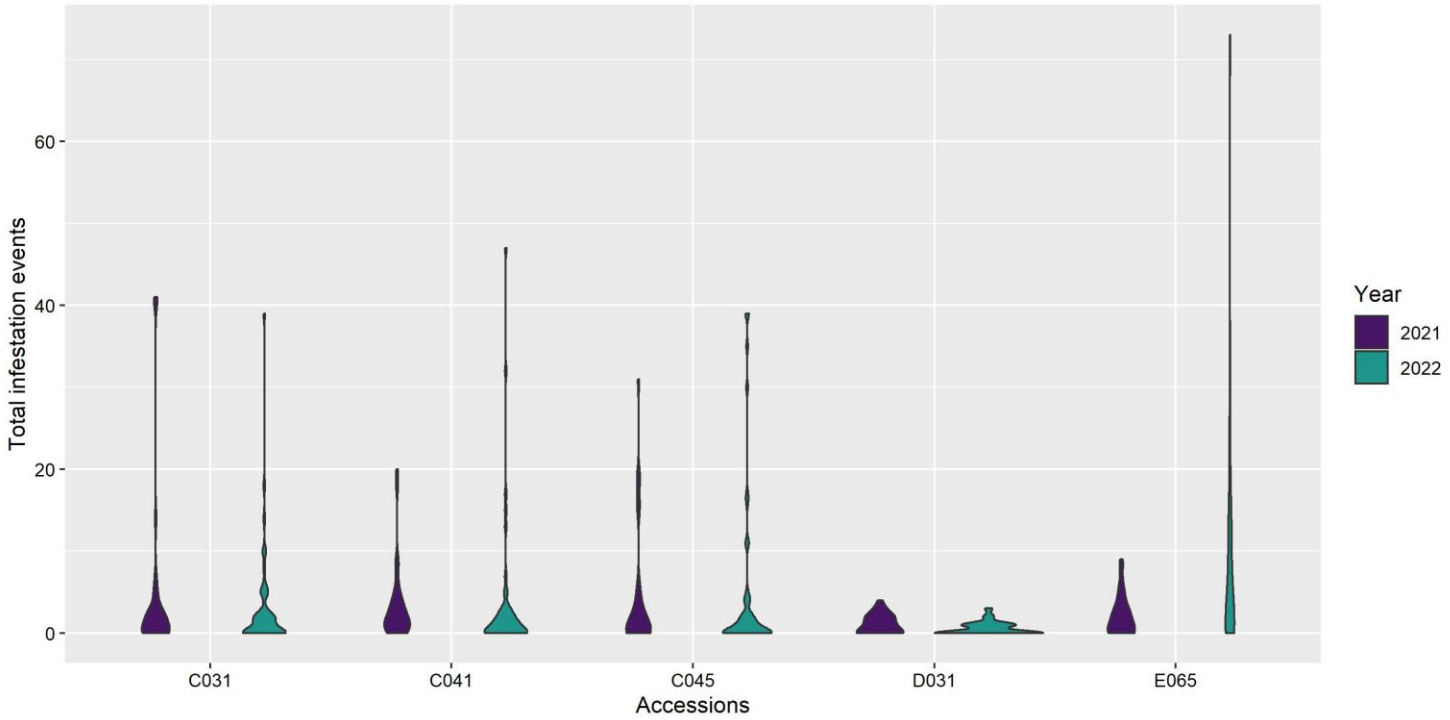

Figure 1. The count distribution of total infestation events (i.e., egg breathing tubes, oviposition wounds without egg breathing tubes, eggs laid on fruit surface) of fruits from five kiwiberry accessions from the University of Minnesota Horticultural Research Center in Victoria, MN in 2021 and 2022.

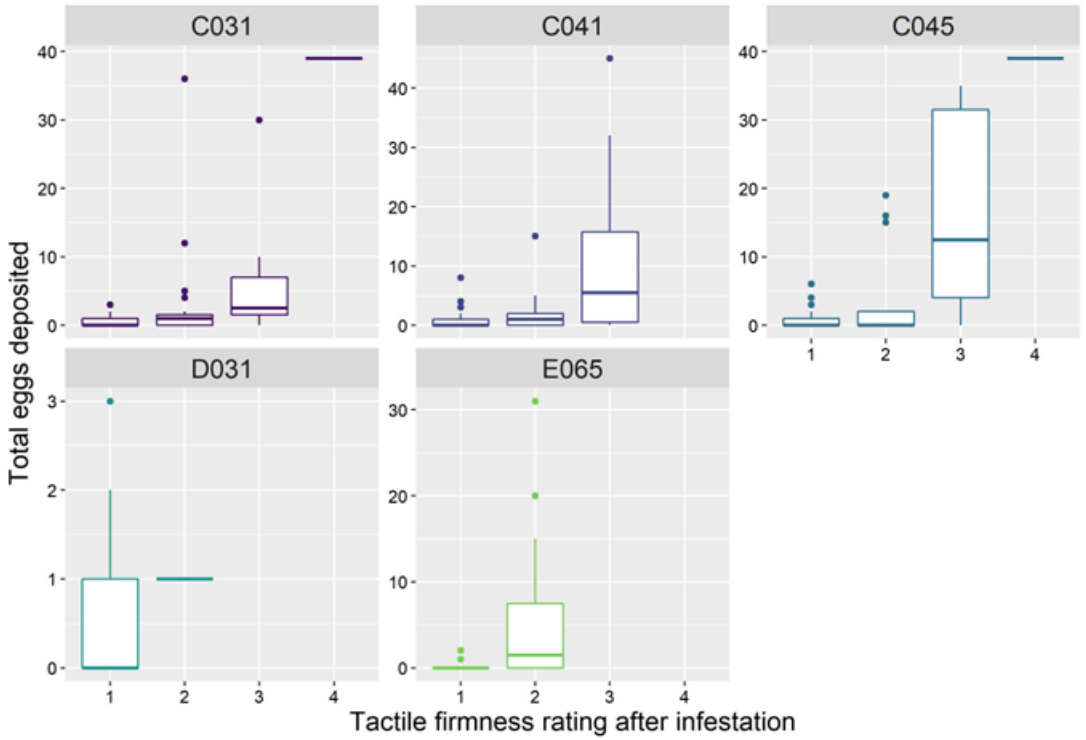

Figure 2. The count distribution of total eggs deposited per fruit (i.e., egg breathing tube count, eggs laid on fruit surface) across tactile firmness ratings after 48 h in the growth chamber. Fruits were harvested from five kiwiberry accessions from the University of Minnesota Horticultural Research Center in Victoria, MN in 2021 and 2022.

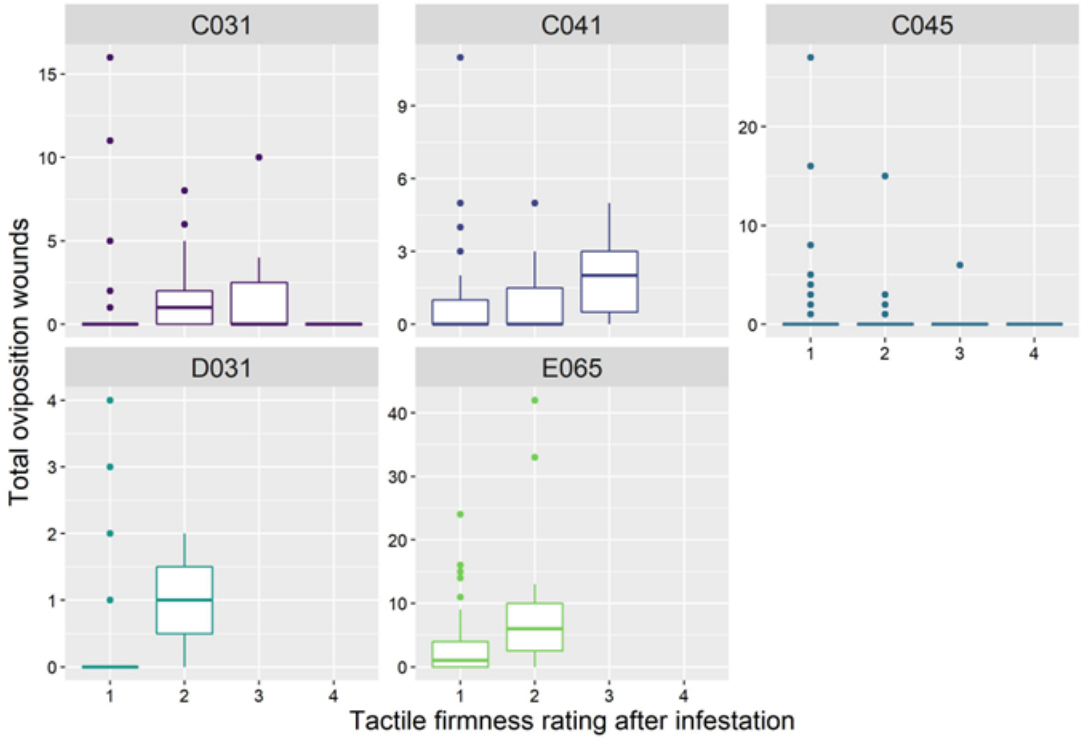

Figure 3. The count distribution of total oviposition wounds (i.e., oviposition wounds without egg breathing tubes) across tactile firmness ratings after 48 h in the growth chamber. Fruits were harvested from five kiwiberry accessions from the University of Minnesota Horticultural Research Center in Victoria, MN in 2021 and 2022.

Conclusion and future breeding

Implications of this work are 1) identification of consumer segments interested in purchasing kiwiberry; 2) improved curation of kiwiberry germplasm for more informed future breeding; 3) recommendations of harvest timing balancing fruit quality and storage; and 4) leveraging the climacteric nature of kiwiberry to reduce SWD infestation. The results of these studies provide a base understanding of variation for fruit quality traits in the UMN kiwiberry germplasm and can be used to inform future breeding efforts. Importance of various fruit quality traits is dependent on specific breeding program objectives, but size, SSC, TA, texture, and taste have been suggested as important breeding targets (Latocha et al. 2021). Additionally, although not targeted in this research, cold-hardiness and spring acclimation are two important traits to consider for northern kiwiberry breeding.

When comparing all fruit quality trait levels across the observed accessions, recommendations can be made for pistillate parents. Of the *A. arguta* accessions, E075 (‘Tatyana’), which had large fruit, the highest SAR, and adequate firmness ratings for multiple weeks in storge, should be considered. *A. kolomikta* accessions that should be considered as parents are D031 (‘Krupnopladnaya’) and S47 (‘Nahodka’), which both had adequately large fruit, SAR, and acceptable fruit firmness. Other accessions could be considered if individual traits are of breeding importance. Larger fruit will likely be a priority for greater harvest efficiency and the variation in both species suggests that there are opportunities for increasing berry size. Possible female vines with the large average fruit size included E075 (‘Tatyana’) and E093 (Chang Bai seedling), *A. arguta* and D031 (‘Krupnopladnaya’) and S35 (‘Pautske’) for *A. kolomikta.* If targeting higher SAR or

other fruit chemistry traits, there are differences to exploit in accessions for SSC and TA. E065 (‘Natasha’) and E075 (‘Tatyana’) could be considered for cold-hardy *A. arguta* with high SARs. Although no significant differences in SAR were observed for *A. kolomikta* in all years, a few accessions (i.e., D013 (‘Sentyobraskaya’), S40 (‘Oluyckos’) might have interest for breeding higher SARs.

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Appendix A

Supplementary Table 1. Summary of Illumina NovaSeq for *Actinidia* samples submitted by the University of Minnesota to the University of Minnesota Genomics Center

Sample	Barcode sequence	PF Clusters	% of the lane	% Perfect barcode	% One mismatch barcode	Yield (Mb)	% PF Clusters	% >= Q30 bases	Mean Quality Score
A011	TGAGTGCC+GAGGCCGT	5,741,736	0.21	93.21	6.79	1,734	100	87.06	34.68
A015	CACTGGTG+TATACCAG	4,545,252	0.16	92.42	7.58	1,373	100	88.74	34.97
A023	TGAGTGCC+CGGTCCGC	4,165,951	0.15	93.22	6.78	1,258	100	89.23	35.05
A024	TGAGTGCC+AGCGATCC	5,380,719	0.19	93.84	6.16	1,625	100	89.22	35.05
A025	CACTGGTG+TCATTACG	4,847,708	0.18	92.6	7.4	1,464	100	88.78	34.97
A031	TGAGTGCC+TCTCGACC	4,411,570	0.16	93.99	6.01	1,332	100	89.48	35.1
A032	AACGGCGC+ACGAACAT	5,528,726	0.2	90.79	9.21	1,670	100	89.2	35.05
A033	AACGGCGC+TTGATACA	5,357,938	0.19	92.94	7.06	1,618	100	89.37	35.08
A035	AACGGCGC+ACCGTCCA	3,873,147	0.14	91.29	8.71	1,170	100	88.85	34.98
A052	AACGGCGC+GGCGGCAA	7,711,658	0.28	93.4	6.6	2,329	100	87.1	34.68
A054	AACGGCGC+GAGGCCGT	6,066,369	0.22	91.26	8.74	1,832	100	87.43	34.75
A061	AACGGCGC+CGGTCCGC	4,752,650	0.17	92.41	7.59	1,435	100	88.83	34.98
A063	AACGGCGC+AGCGATCC	4,462,374	0.16	92.95	7.05	1,348	100	89.14	35.04
A072	AACGGCGC+TCTCGACC	4,529,231	0.16	93.18	6.82	1,368	100	89.15	35.04
A075	TTGGCTGA+ACGAACAT	5,868,710	0.21	91.36	8.64	1,772	100	89.03	35

A081	TTGGCTGA+TTGATACA	5,056,825	0.18	93.84	6.16	1,527	100	88.98	34.98
A082	TTGGCTGA+ACCGTCCA	5,333,661	0.19	92.04	7.96	1,611	100	89.14	35.03
A083	TTGGCTGA+GGCGGCAA	6,571,071	0.24	94.36	5.64	1,984	100	84.6	34.19
A085	TTGGCTGA+GAGGCCGT	6,106,836	0.22	92.98	7.02	1,844	100	85.91	34.45
A091	TTGGCTGA+CGGTCCGC	4,791,789	0.17	92.15	7.85	1,447	100	88.43	34.89
A093	TTGGCTGA+AGCGATCC	5,009,683	0.18	93.73	6.27	1,513	100	88.74	34.95
A095	TTGGCTGA+TCTCGACC	4,671,476	0.17	94.23	5.77	1,411	100	88.9	34.97
A102	GAACGTGG+ACGAACAT	5,301,958	0.19	76.82	23.18	1,601	100	80.62	33.42
A105	GAACGTGG+TTGATACA	4,765,223	0.17	92.04	7.96	1,439	100	89.5	35.1
A112	CACTGGTG+AGACACTA	4,807,802	0.17	88.51	11.49	1,452	100	86.86	34.65
A123	GAACGTGG+ACCGTCCA	5,596,441	0.2	91.19	8.81	1,690	100	88.43	34.9
A131	GAACGTGG+GGCGGCAA	6,322,077	0.23	93.75	6.25	1,909	100	85.57	34.38
A133	GAACGTGG+GAGGCCGT	5,512,627	0.2	80.95	19.05	1,665	100	86.17	34.52
A135	GAACGTGG+CGGTCCGC	4,107,424	0.15	91.37	8.63	1,240	100	88.94	35
A151	GAACGTGG+AGCGATCC	4,994,580	0.18	88.69	11.31	1,508	100	87.71	34.79
A152	GAACGTGG+TCTCGACC	4,590,174	0.17	91.51	8.49	1,386	100	84.01	34.01
B001	CAGACACA+ACGAACAT	4,946,518	0.18	90.96	9.04	1,494	100	88.74	34.94
B022	CACTGGTG+ATGGTAAC	4,700,962	0.17	92.04	7.96	1,420	100	88.83	34.98
B023	CAGACACA+TTGATACA	5,089,780	0.18	93.28	6.72	1,537	100	88.72	34.93
B024	CAGACACA+ACCGTCCA	5,613,576	0.2	93.05	6.95	1,695	100	88.2	34.86
B041	CAGACACA+GGCGGCAA	7,017,284	0.25	93.91	6.09	2,119	100	86.4	34.54
B042	CAGACACA+GAGGCCGT	5,700,249	0.21	92.62	7.38	1,721	100	86.48	34.56
B043	CAGACACA+CGGTCCGC	4,075,056	0.15	92.82	7.18	1,231	100	88.42	34.88
B044	CAGACACA+AGCGATCC	4,054,040	0.15	93.36	6.64	1,224	100	88.65	34.92
B051	CAGACACA+TCTCGACC	4,820,012	0.17	94.09	5.91	1,456	100	88.7	34.93

B052	CATGGTCA+ACGAACAT	5,358,600	0.19	93.57	6.43	1,618	100	88.85	34.99
B053	CATGGTCA+TTGATACA	4,337,658	0.16	94.21	5.79	1,310	100	89.39	35.07
B054	CATGGTCA+ACCGTCCA	5,371,460	0.19	93.64	6.36	1,622	100	89.04	35.03
B071	CATGGTCA+GGCGGCAA	6,254,010	0.23	94.64	5.36	1,889	100	87.52	34.76
B073	CATGGTCA+GAGGCCGT	5,624,212	0.2	93.13	6.87	1,699	100	87.61	34.79
B074	CATGGTCA+CGGTCCGC	3,869,762	0.14	93.32	6.68	1,169	100	89.08	35.02
B103	CATGGTCA+AGCGATCC	4,746,256	0.17	93.85	6.15	1,433	100	89.21	35.05
B105	CATGGTCA+TCTCGACC	4,588,454	0.17	94.33	5.67	1,386	100	89.11	35.03
B114	GGCAGCCA+ACGAACAT	6,565,729	0.24	90.02	9.98	1,983	100	88.69	34.93
B122	GGCAGCCA+TTGATACA	5,622,521	0.2	93.5	6.5	1,698	100	88.92	34.97
B131	GGCAGCCA+ACCGTCCA	6,061,393	0.22	91.17	8.83	1,831	100	88.68	34.93
B133	GGCAGCCA+GGCGGCAA	3,869,755	0.14	88.96	11.04	1,169	100	78.48	32.94
B135	GGCAGCCA+GAGGCCGT	6,463,619	0.23	91.42	8.58	1,952	100	83.85	34.03
B145	GGCAGCCA+CGGTCCGC	4,944,526	0.18	92.97	7.03	1,493	100	88.15	34.83
B151	GGCAGCCA+AGCGATCC	6,482,624	0.23	93.05	6.95	1,958	100	88.8	34.96
B152	GGCAGCCA+TCTCGACC	5,616,435	0.2	93.84	6.16	1,696	100	88.77	34.95
B153	AAGGTCCA+ACGAACAT	5,464,134	0.2	89.67	10.33	1,650	100	88.95	34.98
B154	AAGGTCCA+TTGATACA	5,193,829	0.19	91.27	8.73	1,569	100	89.12	35.01
C001	AAGGTCCA+ACCGTCCA	5,744,252	0.21	91.06	8.94	1,735	100	89.1	35.02
C003	AAGGTCCA+GGCGGCAA	6,873,025	0.25	94.24	5.76	2,076	100	87.41	34.73
C011	AAGGTCCA+GAGGCCGT	5,710,162	0.21	92.01	7.99	1,724	100	87.38	34.73
C013	AAGGTCCA+CGGTCCGC	4,690,714	0.17	91.24	8.76	1,417	100	88.5	34.91
C021	AAGGTCCA+AGCGATCC	4,684,785	0.17	91.57	8.43	1,415	100	89.05	35.01
C023	AAGGTCCA+TCTCGACC	4,689,159	0.17	91.03	8.97	1,416	100	89.09	35.01
C025	TCGTTAGA+ACGAACAT	5,296,670	0.19	92.08	7.92	1,600	100	88.64	34.93

C035	TCGTTAGA+TTGATACA	4,915,706	0.18	93.83	6.17	1,485	100	88.67	34.93
C043	TCGTTAGA+ACCGTCCA	5,292,414	0.19	92.88	7.12	1,598	100	88.82	34.97
C071	TCGTTAGA+GGCGGCAA	6,753,370	0.24	94.63	5.37	2,040	100	85.84	34.44
C081	TCGTTAGA+GAGGCCGT	4,565,174	0.17	91.2	8.8	1,379	100	85.89	34.46
C091	CACTGGTG+ACACCTCT	5,483,552	0.2	91.69	8.31	1,656	100	88.58	34.95
C093	TCGTTAGA+CGGTCCGC	4,217,364	0.15	92.68	7.32	1,274	100	88.4	34.88
C131	TCGTTAGA+AGCGATCC	4,695,783	0.17	93.72	6.28	1,418	100	88.46	34.91
C133	TCGTTAGA+TCTCGACC	4,393,430	0.16	94.04	5.96	1,327	100	88.49	34.9
C135	CACTGGTG+ACGAACAT	4,973,138	0.18	92.19	7.81	1,502	100	89.04	35.03
CACT_100_001	GTGTGAGC+TGGAGGAA	13,092,356	0.47	89.52	10.48	3,954	100	89.46	35.1
CACT_101_001	GTGTGAGC+CGGTCCGC	5,374,281	0.19	92.76	7.24	1,623	100	88.49	34.91
CACT_104_001	AACGGCGC+TCCGCTGG	4,649,380	0.17	92.78	7.22	1,404	100	89.21	35.05
CACT_108_001	TGAGTGCC+TGGAGGAA	9,278,135	0.34	90.3	9.7	2,802	100	89.71	35.14
CACT_109_001	CACTGGTG+CGTGTACC	3,877,288	0.14	92.97	7.03	1,171	100	89.18	35.05
CACT_111_001	TCGTTAGA+CTGTGAGT	4,627,259	0.17	92.04	7.96	1,397	100	88.93	34.99
CACT_114_001	GTGTGAGC+TCCGCTGG	5,749,715	0.21	92.92	7.08	1,736	100	87.11	34.63
CACT_116_001	CATGGTCA+CGTAATAC	3,785,458	0.14	94.42	5.58	1,143	100	88.91	34.97
CACT_120_001	AACGGCGC+GCAAGTGA	5,044,492	0.18	92.7	7.3	1,523	100	87.33	34.72
CACT_121_001	AACGGCGC+TGAGAAGT	4,890,162	0.18	91.96	8.04	1,477	100	89.36	35.08
CACT_122_001	AACGGCGC+TGGAGGAA	9,805,875	0.36	87.1	12.9	2,961	100	89.58	35.12
CACT_123_001	GGCAGCCA+CAGCACAA	6,672,970	0.24	93.45	6.55	2,015	100	87.56	34.7
CACT_124_001	CATGGTCA+TCCGCTGG	4,075,318	0.15	93.78	6.22	1,231	100	89.23	35.05
CACT_126_001	TCGTTAGA+TGAGAAGT	4,750,939	0.17	93.21	6.79	1,435	100	88.77	34.95
CACT_127_001	GTGTGAGC+AGCGATCC	6,411,457	0.23	93.21	6.79	1,936	100	88.25	34.87

CACT_128_001	GTGTGAGC+TCTCGACC	5,453,948	0.2	93.71	6.29	1,647	100	88.92	34.99
CACT_130_001	AAGGTCCA+TGGAGGAA	3,971,002	0.14	87.57	12.43	1,199	100	89.43	35.08
CACT_136_001	GGCAGCCA+TGGAGGAA	4,167,749	0.15	86.31	13.69	1,259	100	89.17	35.02
CACT_138_001	GGCAGCCA+GCAAGTGA	6,102,421	0.22	91.74	8.26	1,843	100	88.92	34.94
CACT_139_001	GGCAGCCA+TGAGAAGT	5,778,667	0.21	91.79	8.21	1,745	100	88.98	34.98
CACT_140_001	TCGTTAGA+CGTGTACC	4,329,537	0.16	93.36	6.64	1,308	100	88.78	34.95
CACT_141_001	CACTGGTG+CTGTGAGT	4,180,904	0.15	92.13	7.87	1,263	100	89.46	35.11
CACT_162_001	TCGTTAGA+CGTAATAC	3,987,947	0.14	94.15	5.85	1,204	100	88.6	34.92
CACT_163_001	CACTGGTG+TGGAGGAA	5,144,570	0.19	88.86	11.14	1,554	100	89.62	35.13
CACT_164_001	AAGGTCCA+CGTGTACC	4,106,804	0.15	90.29	9.71	1,240	100	89.01	34.99
CACT_165_001	TCGTTAGA+CAGCACAA	7,501,208	0.27	94.22	5.78	2,265	100	88.47	34.89
CACT_169_001	TGAGTGCC+CGTGTACC	4,865,032	0.18	93.71	6.29	1,469	100	89.3	35.06
CACT_170_001	GTGTGAGC+CTGTGAGT	5,873,701	0.21	92.64	7.36	1,774	100	88	34.85
CACT_171_001	CAGACACA+GCAAGTGA	3,228,325	0.12	93.83	6.17	975	100	86.8	34.61
CACT_172_001	GGCAGCCA+CGTGTACC	5,260,584	0.19	93.16	6.84	1,589	100	88.55	34.89
CACT_174_001	AAGGTCCA+TGAGAAGT	5,226,396	0.19	91.77	8.23	1,578	100	88.84	34.98
CACT_175_001	AAGGTCCA+CTGTGAGT	4,664,044	0.17	90.01	9.99	1,409	100	89.51	35.1
CACT_176_001	TCGTTAGA+TGGAGGAA	2,725,683	0.1	90.01	9.99	823	100	88.93	35
CACT_178_001	CTCGAGCA+TCCGCTGG	4,153,187	0.15	93.78	6.22	1,254	100	89.12	35.02
CACT_179_001	CATGGTCA+GCAAGTGA	3,805,982	0.14	93.84	6.16	1,149	100	87.97	34.84
CACT_184_001	TGAGTGCC+TGAGAAGT	6,961,059	0.25	93.81	6.19	2,102	100	89.62	35.12
CACT_186_001	CACTGGTG+TGAGAAGT	4,009,826	0.15	93.17	6.83	1,211	100	89.15	35.05
CACT_188_001	TCGTTAGA+TCCGCTGG	4,304,202	0.16	93.49	6.51	1,300	100	88.73	34.94
CACT_229_001	CTCGAGCA+TGGAGGAA	4,737,777	0.17	86.7	13.3	1,431	100	89.49	35.1

CACT_238_001	CTCGAGCA+CAGCACAA	20,227,253	0.73	94.64	5.36	6,109	100	89.01	35
CACT_239_001	CACTGGTG+GCAAGTGA	5,711,584	0.21	91.52	8.48	1,725	100	88.5	34.96
CACT_241_001	GTGTGAGC+ACGAACAT	6,166,925	0.22	92.06	7.94	1,862	100	88.88	34.99
CACT_244_001	CAGACACA+CGTAATAC	2,764,502	0.1	93.99	6.01	835	100	88.65	34.91
CACT_245_001	GGCAGCCA+TCCGCTGG	10,063,528	0.36	93.26	6.74	3,039	100	87.7	34.73
CACT_246_001	GGCAGCCA+CGTAATAC	3,697,360	0.13	94.05	5.95	1,117	100	88.63	34.91
CACT_248_001	CTCGAGCA+GCAAGTGA	4,736,897	0.17	94.13	5.87	1,431	100	87.73	34.8
CACT_249_001	TGAGTGCC+ACGAACAT	5,896,949	0.21	92.68	7.32	1,781	100	89.56	35.11
CACT_250_001	CACTGGTG+CGTAATAC	4,299,938	0.16	93.55	6.45	1,299	100	89.24	35.06
CACT_251_001	TGAGTGCC+GCAAGTGA	3,752,818	0.14	90.65	9.35	1,133	100	84.12	34.08
CACT_252_001	GTGTGAGC+TTGATACA	6,254,245	0.23	93.54	6.46	1,889	100	89.16	35.03
CACT_253_001	CACTGGTG+CAGCACAA	9,987,388	0.36	93.75	6.25	3,016	100	88.94	35.01
CACT_254_001	CACTGGTG+TCCGCTGG	6,210,589	0.22	89.78	10.22	1,876	100	86.76	34.64
CACT_255_001	CTCGAGCA+TGAGAAGT	1,824,589	0.07	92.1	7.9	551	100	89.16	35.03
CACT_256_001	CTCGAGCA+CTGTGAGT	3,748,700	0.14	91.65	8.35	1,132	100	89.11	35.02
CACT_30_001	GAACGTGG+TCCGCTGG	5,447,698	0.2	91.34	8.66	1,645	100	87.81	34.77
CACT_31_001	AAGGTCCA+TCCGCTGG	7,020,697	0.25	90.83	9.17	2,120	100	89.22	35.04
CACT_43_001	GAACGTGG+CTGTGAGT	6,604,242	0.24	90.69	9.31	1,994	100	89.53	35.12
CACT_44_001	GAACGTGG+TGAGAAGT	5,776,904	0.21	91.18	8.82	1,745	100	89.25	35.06
CACT_45_001	GAACGTGG+GCAAGTGA	4,945,673	0.18	85.08	14.92	1,494	100	88.83	35.01
CACT_46_001	GGCAGCCA+CTGTGAGT	4,050,705	0.15	91.08	8.92	1,223	100	89.08	35.01
CACT_47_001	GAACGTGG+CGTGTACC	5,100,072	0.18	90.75	9.25	1,540	100	88.68	34.94
CACT_48_001	CAGACACA+TGAGAAGT	3,839,511	0.14	92.15	7.85	1,160	100	88.96	34.98
CACT_49_001	GAACGTGG+CGTAATAC	4,392,422	0.16	90.76	9.24	1,327	100	88.6	34.94
CACT_50_001	CAGACACA+TCCGCTGG	3,800,081	0.14	93.51	6.49	1,148	100	88.72	34.93

CACT_50_002	CAGACACA+CGTGTACC	3,788,430	0.14	93.22	6.78	1,144	100	88.82	34.95
CACT_50_003	GTGTGAGC+ACCGTCCA	7,045,469	0.26	91.75	8.25	2,128	100	89.07	35.02
CACT_50_004	CATGGTCA+CAGCACAA	6,676,050	0.24	94.44	5.56	2,016	100	89.24	35.05
CACT_50_005	CATGGTCA+TGGAGGAA	6,394,942	0.23	89.19	10.81	1,931	100	89.6	35.12
CACT_51_001	TGAGTGCC+TCCGCTGG	6,389,267	0.23	94.11	5.89	1,930	100	89.36	35.08
CACT_54_001	TTGGCTGA+TCCGCTGG	3,855,504	0.14	94.04	5.96	1,164	100	88.77	34.95
CACT_56_001	AACGGCGC+CGTGTACC	3,661,027	0.13	92.74	7.26	1,106	100	89.16	35.04
CACT_58_001	CATGGTCA+CTGTGAGT	11,844,200	0.43	92.82	7.18	3,577	100	89.67	35.14
CACT_59_001	GTGTGAGC+GGCGGCAA	7,077,630	0.26	93.75	6.25	2,137	100	84.43	34.15
CACT_60_001	GTGTGAGC+CGTAATAC	4,967,975	0.18	93.62	6.38	1,500	100	88.79	34.97
CACT_65_001	GTGTGAGC+CGTGTACC	3,861,485	0.14	88.76	11.24	1,166	100	85.88	34.43
CACT_66_001	TTGGCTGA+TGGAGGAA	6,962,112	0.25	86.36	13.64	2,103	100	89.25	35.04
CACT_67_001	AAGGTCCA+CGTAATAC	5,416,351	0.2	90.92	9.08	1,636	100	89.18	35.02
CACT_69_001	GTGTGAGC+GCAAGTGA	5,755,977	0.21	85.2	14.8	1,738	100	83.82	34.03
CACT_70_001	TTGGCTGA+GCAAGTGA	5,841,732	0.21	94.38	5.62	1,764	100	87.65	34.77
CACT_71_001	GTGTGAGC+TGAGAAGT	4,951,881	0.18	91.07	8.93	1,495	100	88.7	34.97
CACT_72_001	GAACGTGG+CAGCACAA	5,202,675	0.19	92.22	7.78	1,571	100	89.2	35.04
CACT_73_001	TTGGCTGA+TGAGAAGT	3,982,882	0.14	92.45	7.55	1,203	100	89.06	35
CACT_74_001	CATGGTCA+TGAGAAGT	4,206,413	0.15	93.36	6.64	1,270	100	89.46	35.09
CACT_75_001	CAGACACA+CTGTGAGT	5,913,110	0.21	91.21	8.79	1,786	100	89.31	35.06
CACT_76_001	CAGACACA+CAGCACAA	6,213,497	0.23	94.47	5.53	1,876	100	88.31	34.87
CACT_78_001	TGAGTGCC+CGTAATAC	3,486,277	0.13	94.4	5.6	1,053	100	89.44	35.08
CACT_79_001	GAACGTGG+TGGAGGAA	8,712,757	0.32	91.73	8.27	2,631	100	89.58	35.13
CACT_80_001	TTGGCTGA+CGTAATAC	6,550,640	0.24	94.56	5.44	1,978	100	88.86	34.95
CACT_81_001	AACGGCGC+CTGTGAGT	8,181,787	0.3	91.51	8.49	2,471	100	89.56	35.12

CACT_84_001	TTGGCTGA+CAGCACAA	6,932,321	0.25	94.64	5.36	2,094	100	88.93	34.97
CACT_85_001	CATGGTCA+CGTGTACC	4,220,010	0.15	93.66	6.34	1,274	100	89.24	35.05
CACT_86_001	TTGGCTGA+CGTGTACC	3,545,157	0.13	93.43	6.57	1,071	100	88.75	34.94
CACT_87_001	AACGGCGC+CAGCACAA	12,168,841	0.44	93.77	6.23	3,675	100	89.04	35.01
CACT_88_001	AAGGTCCA+GCAAGTGA	1,896,165	0.07	83.78	16.22	573	100	82.68	33.79
CACT_89_001	TGAGTGCC+CTGTGAGT	5,378,335	0.19	93.87	6.13	1,624	100	88.68	34.96
CACT_90_001	CAGACACA+TGGAGGAA	3,781,657	0.14	85.76	14.24	1,142	100	89.15	35.01
CACT_91_001	TTGGCTGA+CTGTGAGT	6,873,648	0.25	91.39	8.61	2,076	100	89.23	35.04
CACT_92_001	AACGGCGC+CGTAATAC	3,125,217	0.11	93.41	6.59	944	100	89.12	35.03
CACT_93_001	TGAGTGCC+CAGCACAA	6,709,283	0.24	94.47	5.53	2,026	100	89.5	35.1
CACT_94_001	AAGGTCCA+CAGCACAA	5,824,433	0.21	92	8	1,759	100	88.9	34.98
CACT_96_001	GTGTGAGC+GAGGCCGT	6,009,675	0.22	84.3	15.7	1,815	100	78.38	32.96
CACT_97_001	TCGTTAGA+GCAAGTGA	4,528,964	0.16	91.41	8.59	1,368	100	86.35	34.54
CACT_98_001	GTGTGAGC+CAGCACAA	7,535,880	0.27	94.23	5.77	2,276	100	89.16	35.03
D003	CACTGGTG+TTGATACA	3,960,545	0.14	93.37	6.63	1,196	100	89.37	35.08
D011	CACTGGTG+ACCGTCCA	5,235,385	0.19	92.54	7.46	1,581	100	88.97	35.03
D023	CACTGGTG+GGCGGCAA	6,473,303	0.23	94	6	1,955	100	87.48	34.76
D055	CACTGGTG+GAGGCCGT	5,059,258	0.18	91.17	8.83	1,528	100	87.98	34.87
D071	CACTGGTG+CGGTCCGC	4,640,274	0.17	92.52	7.48	1,401	100	88.74	34.98
D073	CACTGGTG+AGCGATCC	4,592,320	0.17	92.96	7.04	1,387	100	89.01	35.02
D091	CACTGGTG+TCTCGACC	4,945,858	0.18	93.13	6.87	1,494	100	88.84	34.98
D093	CTCGAGCA+ACGAACAT	3,768,377	0.14	91.4	8.6	1,138	100	89.11	35.02
D095	CTCGAGCA+TTGATACA	11,321,858	0.41	94.02	5.98	3,419	100	89.3	35.05
D105	CTCGAGCA+ACCGTCCA	4,863,774	0.18	93.58	6.42	1,469	100	87.78	34.81
E001	CTCGAGCA+CGGTCCGC	4,145,444	0.15	92.97	7.03	1,252	100	88.48	34.92

E002	CTCGAGCA+AGCGATCC	4,298,930	0.16	93.7	6.3	1,298	100	88.93	34.99
E011	CTCGAGCA+TCTCGACC	4,138,745	0.15	94.37	5.63	1,250	100	88.51	34.93
E013	CTCGAGCA+ACTCATGG	3,967,233	0.14	91.4	8.6	1,198	100	89.12	35.03
E015	CTCGAGCA+TTCTAAGA	3,822,899	0.14	92.22	7.78	1,155	100	89.13	35.02
E021	CTCGAGCA+GGTCAGCG	4,753,425	0.17	93.72	6.28	1,436	100	87.02	34.66
E023	CTCGAGCA+AGGTTGAT	3,836,582	0.14	89.62	10.38	1,159	100	89.11	35.01
E025	CTCGAGCA+AGTGCTAA	3,654,722	0.13	91.58	8.42	1,104	100	88.98	34.99
E031	CTCGAGCA+GGTTCAGA	3,879,245	0.14	94.88	5.12	1,172	100	87.87	34.82
E033	CTCGAGCA+CAATACTT	3,948,615	0.14	93.66	6.34	1,192	100	89.04	35
E035	CTCGAGCA+TCTATGTT	3,942,763	0.14	90.56	9.44	1,191	100	89.56	35.11
E041	CACTGGTG+ACTCATGG	4,561,575	0.17	92.33	7.67	1,378	100	89.05	35.03
E043	CACTGGTG+TTCTAAGA	4,379,919	0.16	92.44	7.56	1,323	100	89.53	35.11
E045	CACTGGTG+GGTCAGCG	4,703,117	0.17	94.45	5.55	1,420	100	86.46	34.57
E051	CACTGGTG+AGGTTGAT	4,476,424	0.16	91.35	8.65	1,352	100	89.31	35.08
E053	CACTGGTG+AGTGCTAA	4,191,187	0.15	92.35	7.65	1,266	100	89.24	35.06
E055	CACTGGTG+GGTTCAGA	4,531,896	0.16	93.13	6.87	1,369	100	88.82	35
E061	CACTGGTG+CAATACTT	4,935,432	0.18	92.7	7.3	1,491	100	88.94	35
E063	CACTGGTG+TCTATGTT	3,873,607	0.14	91.38	8.62	1,170	100	89.46	35.1
E065	TCGTTAGA+ACTCATGG	4,542,038	0.16	92.77	7.23	1,372	100	88.11	34.84
E071	TCGTTAGA+TTCTAAGA	4,085,148	0.15	92.58	7.42	1,234	100	88.71	34.94
E073	TCGTTAGA+GGTCAGCG	4,665,466	0.17	93.82	6.18	1,409	100	87.08	34.67
E075	TCGTTAGA+AGGTTGAT	4,347,730	0.16	90.18	9.82	1,313	100	88.73	34.95
E081	TCGTTAGA+AGTGCTAA	4,059,413	0.15	92.4	7.6	1,226	100	88.65	34.93
E083	TCGTTAGA+GGTTCAGA	4,525,955	0.16	93.91	6.09	1,367	100	88.01	34.84
E085	TCGTTAGA+CAATACTT	4,391,290	0.16	92.92	7.08	1,326	100	88.07	34.81

E091	TCGTTAGA+TCTATGTT	4,405,358	0.16	91.15	8.85	1,330	100	89.06	35.01
E093	AAGGTCCA+ACTCATGG	5,303,252	0.19	89.81	10.19	1,602	100	89.16	35.03
E101	AAGGTCCA+TTCTAAGA	4,397,400	0.16	90.25	9.75	1,328	100	89.23	35.03
E103	AAGGTCCA+GGTCAGCG	6,129,901	0.22	93.58	6.42	1,851	100	87.5	34.75
F001	AAGGTCCA+AGGTTGAT	5,167,624	0.19	89.56	10.44	1,561	100	89.18	35.03
F011	AAGGTCCA+AGTGCTAA	4,793,026	0.17	90.46	9.54	1,447	100	89.15	35.02
F013	AAGGTCCA+GGTTCAGA	4,922,737	0.18	93.17	6.83	1,487	100	88.4	34.91
F015	AAGGTCCA+CAATACTT	4,684,833	0.17	90.05	9.95	1,415	100	88.86	34.95
F02-05	TGAGTGCC+TTGATACA	4,078,898	0.15	94.28	5.72	1,232	100	89.55	35.1
F021	AAGGTCCA+TCTATGTT	4,227,000	0.15	89.18	10.82	1,277	100	89.43	35.08
F023	GGCAGCCA+ACTCATGG	5,115,138	0.19	90.23	9.77	1,545	100	88.91	34.97
F025	GGCAGCCA+TTCTAAGA	3,987,602	0.14	92.08	7.92	1,204	100	88.95	34.97
F033	GGCAGCCA+GGTCAGCG	5,624,275	0.2	93.19	6.81	1,699	100	87.74	34.78
F035	GGCAGCCA+AGGTTGAT	5,408,869	0.2	88.87	11.13	1,633	100	89.02	34.99
F03_05	TGAGTGCC+ACCGTCCA	2,902,029	0.11	92.83	7.17	876	100	89.6	35.12
F045	GGCAGCCA+AGTGCTAA	5,817,297	0.21	90.99	9.01	1,757	100	88.85	34.95
F051	GGCAGCCA+GGTTCAGA	5,781,271	0.21	92.31	7.69	1,746	100	88.73	34.94
F053	GGCAGCCA+CAATACTT	4,592,160	0.17	93.52	6.48	1,387	100	88.71	34.92
F055	GGCAGCCA+TCTATGTT	9,204,214	0.33	90.29	9.71	2,780	100	89.18	35.02
F05_09	TGAGTGCC+GGCGGCAA	4,228,590	0.15	94.76	5.24	1,277	100	85.8	34.42
F061	CATGGTCA+ACTCATGG	4,072,981	0.15	92.88	7.12	1,230	100	87.57	34.73
F063	CATGGTCA+TTCTAAGA	3,640,203	0.13	93.26	6.74	1,099	100	89.32	35.06
F073	CATGGTCA+GGTCAGCG	5,068,421	0.18	94.54	5.46	1,531	100	86.98	34.65
F081	CATGGTCA+AGGTTGAT	5,057,751	0.18	91.21	8.79	1,527	100	89.37	35.08
F091	CATGGTCA+AGTGCTAA	4,371,910	0.16	92.71	7.29	1,320	100	89.32	35.07

F093	CATGGTCA+GGTTCAGA	4,593,273	0.17	94.77	5.23	1,387	100	88.56	34.95
F095	CAGACACA+ACTCATGG	4,748,433	0.17	90.48	9.52	1,434	100	89.05	35.01
F101	CAGACACA+TTCTAAGA	3,762,587	0.14	91.58	8.42	1,136	100	88.75	34.93
F103	CAGACACA+GGTCAGCG	4,591,761	0.17	93.41	6.59	1,387	100	84.45	34.14
F105	CAGACACA+AGGTTGAT	4,276,676	0.15	88.81	11.19	1,292	100	88.92	34.97
F135	CTCGAGCA+CACGAAGC	3,969,582	0.14	93.93	6.07	1,199	100	88.98	34.97
G001	CAGACACA+AGTGCTAA	3,924,652	0.14	90.77	9.23	1,185	100	88.7	34.92
G011	CAGACACA+GGTTCAGA	4,676,363	0.17	94.72	5.28	1,412	100	87.12	34.67
G013	CAGACACA+CAATACTT	4,122,041	0.15	93.53	6.47	1,245	100	88.59	34.9
G015	CAGACACA+TCTATGTT	4,355,670	0.16	90.05	9.95	1,315	100	89.37	35.07
G021	GAACGTGG+ACTCATGG	4,255,132	0.15	90.69	9.31	1,285	100	88.37	34.88
G023	GAACGTGG+TTCTAAGA	3,970,233	0.14	91.95	8.05	1,199	100	89.56	35.11
G025	GAACGTGG+GGTCAGCG	4,304,992	0.16	93.94	6.06	1,300	100	88.22	34.89
G031	GAACGTGG+AGGTTGAT	4,272,340	0.15	92.01	7.99	1,290	100	89.26	35.06
G033	GAACGTGG+AGTGCTAA	4,137,408	0.15	91.53	8.47	1,249	100	89.02	35.02
G035	GAACGTGG+GGTTCAGA	4,240,668	0.15	89.13	10.87	1,281	100	89.1	35.04
G041	GAACGTGG+CAATACTT	4,339,552	0.16	91.07	8.93	1,311	100	89.21	35.04
G043	GAACGTGG+TCTATGTT	4,254,607	0.15	91.12	8.88	1,285	100	89.68	35.14
G045	TTGGCTGA+ACTCATGG	5,107,240	0.18	92.91	7.09	1,542	100	88.72	34.96
G051	TTGGCTGA+TTCTAAGA	4,092,759	0.15	92.07	7.93	1,236	100	89.15	35.02
G053	TTGGCTGA+GGTCAGCG	5,951,515	0.22	93.76	6.24	1,797	100	86.89	34.63
G055	TTGGCTGA+AGGTTGAT	4,848,900	0.18	89.53	10.47	1,464	100	88.93	34.98
G061	TTGGCTGA+AGTGCTAA	4,481,874	0.16	91.46	8.54	1,354	100	89.02	35
G063	TTGGCTGA+GGTTCAGA	4,601,946	0.17	94.89	5.11	1,390	100	88.24	34.88
G065	TTGGCTGA+CAATACTT	5,115,058	0.19	93.76	6.24	1,545	100	88.81	34.95

G071	TTGGCTGA+TCTATGTT	3,831,520	0.14	90.4	9.6	1,157	100	89.48	35.09
G073	AACGGCGC+ACTCATGG	4,522,768	0.16	90.52	9.48	1,366	100	89.16	35.04
G075	AACGGCGC+TTCTAAGA	3,781,984	0.14	91.67	8.33	1,142	100	89.19	35.04
G081	AACGGCGC+GGTCAGCG	4,775,539	0.17	93.75	6.25	1,442	100	85.87	34.44
G083	AACGGCGC+AGGTTGAT	4,293,237	0.16	89.42	10.58	1,297	100	89.31	35.07
G085	AACGGCGC+AGTGCTAA	4,257,153	0.15	91.12	8.88	1,286	100	89.16	35.04
G091	AACGGCGC+GGTTCAGA	4,443,759	0.16	93.55	6.45	1,342	100	88.58	34.95
G093	AACGGCGC+CAATACTT	8,948,984	0.32	93.02	6.98	2,703	100	89.02	35.01
G095	AACGGCGC+TCTATGTT	4,564,353	0.17	90.2	9.8	1,378	100	89.52	35.11
G101	TGAGTGCC+ACTCATGG	4,917,605	0.18	92.71	7.29	1,485	100	89.42	35.09
G103	TGAGTGCC+TTCTAAGA	4,194,073	0.15	94.05	5.95	1,267	100	89.24	35.05
G105	TGAGTGCC+GGTCAGCG	4,868,334	0.18	94.48	5.52	1,470	100	88	34.85
G111	TGAGTGCC+AGGTTGAT	5,387,595	0.2	93.11	6.89	1,627	100	89.31	35.08
G113	TGAGTGCC+AGTGCTAA	4,849,000	0.18	93.45	6.55	1,464	100	89.23	35.05
G121	TGAGTGCC+GGTTCAGA	3,494,974	0.13	94.25	5.75	1,055	100	86.95	34.65
G123	TGAGTGCC+CAATACTT	5,763,164	0.21	93.96	6.04	1,740	100	89.43	35.08
G125	TGAGTGCC+TCTATGTT	4,605,452	0.17	92.09	7.91	1,391	100	89.79	35.16
G131	GTGTGAGC+ACTCATGG	5,409,229	0.2	91.57	8.43	1,634	100	89.38	35.08
G141	GTGTGAGC+TTCTAAGA	4,735,183	0.17	92.79	7.21	1,430	100	89.25	35.05
G143	GTGTGAGC+GGTCAGCG	5,650,682	0.2	94.43	5.57	1,707	100	87.8	34.82
G151	GTGTGAGC+AGGTTGAT	5,485,277	0.2	91.58	8.42	1,657	100	89.45	35.1
G153	GTGTGAGC+AGTGCTAA	5,850,816	0.21	92.96	7.04	1,767	100	89.2	35.05
N01	GTGTGAGC+GGTTCAGA	3,307,936	0.12	92.72	7.28	999	100	88.68	34.98
N02	GTGTGAGC+CAATACTT	6,249,336	0.23	93.54	6.46	1,887	100	89.4	35.08
N03	GTGTGAGC+TCTATGTT	6,621,599	0.24	91.25	8.75	2,000	100	89.76	35.16

N04	GTGTGAGC+TATACCAG	4,794,398	0.17	92.04	7.96	1,448	100	88.94	34.98
N05	GTGTGAGC+TCATTACG	5,227,547	0.19	93.25	6.75	1,579	100	89.22	35.04
N06	GTGTGAGC+AGACACTA	5,365,709	0.19	91.95	8.05	1,620	100	88.43	34.91
N07	GTGTGAGC+ATGGTAAC	5,352,691	0.19	92.7	7.3	1,617	100	89	35.01
N08	GTGTGAGC+ACACCTCT	5,127,418	0.19	92.22	7.78	1,548	100	88.93	34.98
N09	TGAGTGCC+CACGAAGC	5,305,868	0.19	93.76	6.24	1,602	100	89.36	35.06
N10	TGAGTGCC+CTTCAATC	4,175,851	0.15	93.03	6.97	1,261	100	89.67	35.13
N11	TGAGTGCC+GTAATAAT	5,581,161	0.2	94.75	5.25	1,686	100	88.67	34.96
N12	TGAGTGCC+TATACCAG	5,589,375	0.2	92.51	7.49	1,688	100	89.33	35.07
N13	TGAGTGCC+TCATTACG	5,187,386	0.19	93.6	6.4	1,567	100	89.47	35.09
N14	TGAGTGCC+AGACACTA	5,878,760	0.21	94.07	5.93	1,775	100	89.02	35.03
N15	TGAGTGCC+ATGGTAAC	4,771,721	0.17	92.38	7.62	1,441	100	89.65	35.13
N16	TGAGTGCC+ACACCTCT	4,449,838	0.16	92.75	7.25	1,344	100	89.17	35.02
N17	AACGGCGC+CACGAAGC	5,133,690	0.19	93.17	6.83	1,550	100	89.02	35.02
N20	AACGGCGC+CTTCAATC	3,491,892	0.13	91.47	8.53	1,055	100	88.63	34.92
N21	AACGGCGC+GTAATAAT	3,839,312	0.14	92.81	7.19	1,159	100	88.22	34.84
N22	AACGGCGC+TATACCAG	4,521,965	0.16	91.19	8.81	1,366	100	88.58	34.9
N23	AACGGCGC+TCATTACG	5,078,797	0.18	92.98	7.02	1,534	100	88.97	35
N24	AACGGCGC+AGACACTA	5,664,015	0.21	92.46	7.54	1,711	100	88.11	34.86
N25	AACGGCGC+ATGGTAAC	5,295,979	0.19	90.63	9.37	1,599	100	89.13	35.03
N26	AACGGCGC+ACACCTCT	5,501,820	0.2	91.09	8.91	1,662	100	89.05	35.02
N27	TTGGCTGA+CACGAAGC	4,865,614	0.18	94.02	5.98	1,469	100	89.01	35
N28	TTGGCTGA+CTTCAATC	4,114,642	0.15	91.74	8.26	1,243	100	89.11	35.01
N29	TTGGCTGA+GTAATAAT	5,284,872	0.19	94.86	5.14	1,596	100	88.22	34.85
N30	TTGGCTGA+TATACCAG	4,967,335	0.18	91.83	8.17	1,500	100	88.84	34.95

N31	TTGGCTGA+TCATTACG	4,917,568	0.18	93.46	6.54	1,485	100	88.9	34.96
N33	TTGGCTGA+AGACACTA	5,379,873	0.19	94.01	5.99	1,625	100	88.77	34.97
N34	TTGGCTGA+ATGGTAAC	4,395,043	0.16	90.43	9.57	1,327	100	89.04	34.99
N35	TTGGCTGA+ACACCTCT	5,115,572	0.19	92.22	7.78	1,545	100	89	35
N36	GAACGTGG+CACGAAGC	4,819,996	0.17	83.34	16.66	1,456	100	72.28	31.38
N38	GAACGTGG+CTTCAATC	3,962,634	0.14	90.77	9.23	1,197	100	89.14	35.03
N39	GAACGTGG+GTAATAAT	4,612,331	0.17	86.94	13.06	1,393	100	89.16	35.05
N40	GAACGTGG+TATACCAG	4,186,977	0.15	91.07	8.93	1,264	100	89.4	35.07
N41	GAACGTGG+TCATTACG	4,156,878	0.15	91.76	8.24	1,255	100	89.48	35.09
N42	GAACGTGG+AGACACTA	4,873,318	0.18	86.79	13.21	1,472	100	88.73	34.97
N43	GAACGTGG+ATGGTAAC	4,960,621	0.18	90.49	9.51	1,498	100	88.92	35.01
N44	GAACGTGG+ACACCTCT	5,515,118	0.2	90.49	9.51	1,666	100	89.24	35.05
S01	CAGACACA+CACGAAGC	4,090,122	0.15	93.51	6.49	1,235	100	88.29	34.83
S02	CAGACACA+CTTCAATC	3,927,177	0.14	91.8	8.2	1,186	100	88.96	34.98
S03	CAGACACA+GTAATAAT	4,707,503	0.17	94.24	5.76	1,422	100	87.86	34.8
S04	CAGACACA+TATACCAG	4,809,727	0.17	92.59	7.41	1,453	100	88.27	34.86
S05	CAGACACA+TCATTACG	4,340,245	0.16	92.92	7.08	1,311	100	88.84	34.96
S06	CAGACACA+AGACACTA	4,834,380	0.18	93.78	6.22	1,460	100	87.67	34.74
S07	CAGACACA+ATGGTAAC	4,740,785	0.17	89.16	10.84	1,432	100	88.76	34.95
S08	CAGACACA+ACACCTCT	4,895,090	0.18	91.87	8.13	1,478	100	88.85	34.97
S09	CATGGTCA+CACGAAGC	4,497,779	0.16	93.75	6.25	1,358	100	89.38	35.07
S10	CATGGTCA+CTTCAATC	4,165,051	0.15	92.5	7.5	1,258	100	89.41	35.09
S11	CATGGTCA+GTAATAAT	4,130,502	0.15	94.6	5.4	1,247	100	88.53	34.93
S12	CATGGTCA+TATACCAG	4,472,732	0.16	93.17	6.83	1,351	100	89.04	35.01
S13	CATGGTCA+TCATTACG	4,680,640	0.17	93.8	6.2	1,414	100	89.26	35.05

S14	CATGGTCA+AGACACTA	5,538,814	0.2	94.08	5.92	1,673	100	88.88	35.01
S15	CATGGTCA+ATGGTAAC	3,790,075	0.14	92.19	7.81	1,145	100	88.86	34.95
S16	CATGGTCA+ACACCTCT	5,109,476	0.19	92.53	7.47	1,543	100	89.3	35.07
S18	GGCAGCCA+CACGAAGC	6,217,236	0.23	93.64	6.36	1,878	100	88.68	34.93
S19	GGCAGCCA+CTTCAATC	4,809,455	0.17	91.95	8.05	1,452	100	88.82	34.95
S20	GGCAGCCA+GTAATAAT	5,764,871	0.21	93.13	6.87	1,741	100	88.58	34.91
S21	GGCAGCCA+TATACCAG	5,832,357	0.21	91.39	8.61	1,761	100	88.62	34.91
S22	GGCAGCCA+TCATTACG	5,078,748	0.18	93.22	6.78	1,534	100	88.75	34.9
S23	GGCAGCCA+AGACACTA	5,206,196	0.19	91.93	8.07	1,572	100	88.79	34.9
S25	GGCAGCCA+ATGGTAAC	6,182,639	0.22	90.17	9.83	1,867	100	88.95	34.98
S26	GGCAGCCA+ACACCTCT	7,310,784	0.26	91.26	8.74	2,208	100	88.79	34.96
S27	AAGGTCCA+CACGAAGC	3,024,997	0.11	90.5	9.5	914	100	89.05	35
S29	AAGGTCCA+CTTCAATC	3,695,515	0.13	88.93	11.07	1,116	100	88.77	34.93
S30	AAGGTCCA+GTAATAAT	5,331,856	0.19	92.24	7.76	1,610	100	88.42	34.91
S31	AAGGTCCA+TATACCAG	5,543,791	0.2	90.5	9.5	1,674	100	88.99	35
S32	AAGGTCCA+TCATTACG	5,176,716	0.19	89.93	10.07	1,563	100	88.94	34.97
S33	AAGGTCCA+AGACACTA	4,817,759	0.17	89.24	10.76	1,455	100	87.6	34.74
S35	AAGGTCCA+ATGGTAAC	4,897,217	0.18	88.74	11.26	1,479	100	88.97	34.98
S38	AAGGTCCA+ACACCTCT	5,672,578	0.21	89.85	10.15	1,713	100	89.08	35.01
S39	TCGTTAGA+CACGAAGC	8,550,793	0.31	93.67	6.33	2,582	100	88.72	34.94
S40	TCGTTAGA+CTTCAATC	3,839,964	0.14	92.23	7.77	1,160	100	88.43	34.88
S41	TCGTTAGA+GTAATAAT	4,953,686	0.18	93.74	6.26	1,496	100	88.38	34.89
S42	TCGTTAGA+TATACCAG	4,027,191	0.15	91.93	8.07	1,216	100	88.73	34.94
S43	TCGTTAGA+TCATTACG	4,077,726	0.15	93.36	6.64	1,231	100	88.64	34.92
S45	TCGTTAGA+AGACACTA	4,656,112	0.17	93.18	6.82	1,406	100	88.28	34.88

S46	TCGTTAGA+ATGGTAAC	4,517,644	0.16	90.89	9.11	1,364	100	88.4	34.88
S47	TCGTTAGA+ACACCTCT	4,865,984	0.18	92.13	7.87	1,470	100	88.51	34.9
S48	CACTGGTG+CACGAAGC	12,291,204	0.45	93.12	6.88	3,712	100	89.32	35.08
S50	CACTGGTG+CTTCAATC	5,835,411	0.21	92.3	7.7	1,762	100	89.38	35.09
S51	CACTGGTG+GTAATAAT	4,682,277	0.17	92.78	7.22	1,414	100	88.8	34.9

Appendix B

Soluble oxalate analysis

Materials and Methods

For soluble oxalate analysis, samples were lyophilized or freeze dried in conical vials for a minimum of 72 hours. After samples completely dried, the conical vial and dried fruit was weighed to the hundredth of a gram. Three 2 mm tungsten carbide beads were placed in each vial. Then vials were balanced on a block within 0.2 g and a SPEX SamplePrep model 2010 Geno/Grinder® was used to pulverize samples for 5 minutes at 1400 rpm. After ensuring vials were sound, pulverizing was repeated twice more. Tungsten carbide beads were removed and ground tissue was stored in a 1.5 mL Eppendorf tube with silica and stored in -80 °C until soluble oxalate analysis.

Soluble oxalate analysis was conducted using a Sigma-Aldrich Oxalic Acid Colorimetric Assay Kit. Samples were prepared by weighing out 10 mg of lyophilized fruit tissue into a centrifuge filter tube. Then 300 µL assay buffer was added and incubated on ice for 100 minutes followed by spinning at 10,000 rom for 5 minutes. Sample supernatant was then diluted (1:2 for *A. arguta* samples; 1:4 for *A. kolomikta* samples) and organized onto a 96-well flatbottomed plate with two replicates and one spiked well (4 µL oxalate standard) per postharvest sample. Standards of 0-10 µL of 1 mM oxalate standard were included for each plate. Additionally, Master mix (46 µL oxalate development buffer; 2 µL oxalate enzyme mix; 2 µL oxalate probe) was added to each cell and the plate was then incubated for 60 minutes at 37 °C (98.6 °F). After incubation, the plate was run on a plate-reader spectrophotometer (SpectraMax 190,

Molecular Devices) at 450 nm. A standard curve was generated for each plate to calculate mg oxalate per sample.

Results and Discussion

Actinidia arguta accessions ranged from 0.15 to 0.54 mg SOC/100g fresh weight. *Actinidia kolomikta* accessions ranged from 1.38 to 5.91 mg SOC/100g fresh weight. Overall, *A. arguta* accessions had much lower soluble oxalate content than *A. kolomikta.* A lower soluble oxalate content may make *A. arguta* more favorable for consumers who are sensitive to oxalates. However, the variability observed in *A. kolomikta* accessions provides opportunities for breeding future cultivars with lower oxalate content.

Table 1. Mean soluble oxalate content (SOC) by year for all harvests within a year for three years.

Accession	Year	SOC (mg/100g FW)
D013	2019	-
	2020	1.69
	2021	1.38
D031	2019	2.67
	2020	2.28
	2021	2.91
S35	2019	4.22
	2020	2.11
	2021	2.75
S37	2019	-
	2020	3.35
	2021	3.09
S40	2019	-
	2020	2.39
	2021	-
S45	2019	-
	2020	3.61
	2021	4.69
S47	2019	-
	2020	5.91
	2021	4.12
E065	2019	-
	2020	0.34
	2021	0.17
E075	2019	0.21
	2020	0.14
	2021	0.15
E093	2019	-
	2020	0.54
	2021	0.23
C025	2019	-
	2020	-
	2021	-