

MA'AFALA BREADFRUIT MATURITY INDICATORS AND THE
INFLUENCE OF HARVEST MATURITY AND 1-METHYLCYCLOPROPENE
ON ITS POSTHARVEST QUALITY

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ABSTRACT

Fresh breadfruit (*Artocarpus altilis* (Parkins) Fosberg) is difficult to market commercially due to its rapid postharvest ripening and consumers' preference for mature, unripe fruit. Maturity indexes are used in other fruit to identify the harvest point for longest storage duration, but maturity is poorly defined in breadfruit and its relationship to storage quality is not clear. We examined 23 traits in 73 Ma'afala breadfruit harvested at 13, 15, 17, and 19 weeks of development to identify traits that indicate maturity in breadfruit. Maturity was defined as full size with steady internal quality, and breadfruit reached this point at 15 weeks of development. Skin color and intersegment space color were the most accurate indicators of maturity, classifying mature breadfruit with 90% accuracy. The respiration rate, hand-feel, and color of 50 breadfruit were observed during storage to determine the effect of the harvest period on storage quality. Later harvested breadfruit discolored more rapidly (5 vs 10.4 days) but the harvest period did not affect softening rate or magnitude and timing of the respiratory peak.

The ethylene inhibitor 1-methylcyclopropene (1-MCP) is used in other climacteric fruit to delay postharvest ripening, but its effect on breadfruit has not been reported. A portion of breadfruit from each harvest period was treated with 1 ppm active ingredient 1-MCP for 20 hours and observed in storage as described above. Treatment with 1-MCP delayed the onset of the climacteric peak by an average of 6 days (65% delay), delayed softening by an average of 7 days (63% delay), and reduced variation in these traits. Treatment with 1-MCP did not delay discoloration.

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LIST OF ABBREVIATIONS

1-MCP: 1-methylcyclopropene

GDD₂₀: Growing degree days, base 20 °C

HP: Harvest Period

HSD: Tukey's honest significant difference test

LSD: Fisher least significant difference test

RMST: Restricted mean survival time

TA: Titratable acidity

TSS: Total soluble solids

1. INTRODUCTION

Breadfruit (*Artocarpus altilis* (Parkins) Fosberg) are consumed at various stages of maturity, but most often as mature, unripe fruit (Ragone, 1997; Roberts-Nkrumah, 2007; Worrell et al., 1998). Breadfruit ripens and softens rapidly after harvest becoming unsuitable for handling, transport, and sale. Breadfruit marketers face the challenge of transporting mature breadfruit to the consumer before the breadfruit ripens and softens. Harvesting fruit at a specific maturity and the postharvest application of ethylene inhibitors are two postharvest practices used in other fruit crops to delay fruit ripening after harvest, and this study considers these practices in breadfruit postharvest handling.

1.1. Breadfruit Description

Breadfruit is a syncarp with fused perianths forming fused and mostly seedless fruitlets (Jarrett, 1977; Reeve, 1974). The breadfruit surface is covered in polygon units, each unit corresponding to a perianth or fruitlet, which are fused to make up the fleshy, edible portion of the fruit (Jarrett, 1977; Reeve, 1974). Breadfruit size generation follows a single sigmoidal growth curve while weight generation follows a double-sigmoidal growth curve, with an initial growth phase, an intermediate lag phase, and a final phase of rapid accumulation of mass (Worrell et al., 1998).

Ripening in breadfruit follows patterns typical of a climacteric fruit; fruit show a dramatic respiratory rise, a marked increase in ethylene production, and continue to ripen after detachment from the tree (Biale & Barcus, 1970; Thompson et al., 1974; Williams & Golden, 2002). Breadfruit's climacteric peak occurs more rapidly and with greater magnitude compared to other fruit, which is indicative of its rapid ripening after harvest (Biale & Young, 1981). The breadfruit climacteric coincides with the complete softening of the fruit (Worrell et al., 1998). As the breadfruit softens, it becomes very susceptible to compression damage and difficult to handle. Breadfruit softening signals the end of its postharvest life.

1.2. Relationship Between Maturity and Postharvest Quality

A fruit's maturity at harvest is an important determinant of its final texture, flavor, nutritional quality, and postharvest life (Toivonen & Beveridge, 2005). Breadfruit maturity, however, is poorly defined, and its effect on postharvest fitness has not been reported.

1.2.1. Defining Fruit Maturity

Fruit maturity can be defined in a few different ways, depending on perspective. Physiological maturity is based on a fruit's biology, and is defined as the point when a fruit has achieved maximum size and is sufficiently developed to complete the next stage of development even if it is detached from the plant (Wills et al., 2007). Some definitions of physiological maturity do not stipulate that it has reached maximum size, focusing only on the fruit's ability to continue development or ripening after detachment from the plant (Kader, 2002b; Kyriacou & Roupael, 2018; Reid, 2002; Watada et al., 1984).

Horticultural maturity, also referred to as commercial maturity, refers to the point when a fruit is sufficiently developed for its intended use, regardless of whether it is physiologically mature (Kader, 2002b; Kyriacou & Roupael, 2018; Reid, 2002; Watada et al., 1984; Wills et al., 2007). For example, cucumbers are horticulturally mature when their seeds are still soft and physiologically immature. Harvest maturity is sometimes differentiated from horticultural maturity; harvest maturity explicitly considers the dynamics of the marketing chain to define a point when harvest results in the best balance of postharvest performance and eating quality (Kyriacou & Roupael, 2018; Wills et al., 2007).

1.2.2. Relationship of Maturity and Ripening

Ideal harvest maturity is influenced by a fruit's ripening characteristics. Ripening refers to a host of biochemical and physiological changes that generally occur after a fruit reaches full size and results in the characteristic aroma, texture, appearance, and taste associated with the ripe fruit (Watada et al., 1984). Based on their ripening characteristics, fleshy fruit can be separated into two broad categories: non-climacteric and climacteric (Wills et al., 2007). The term *climacteric* comes from *climax* and is used to describe the rise in respiration rate associated with ripening in climacteric fruit (Biale, 1964). The distinction between climacteric and non-climacteric fruit is defined by two factors: presence of the respiratory peak and the fruit's response to ethylene (Paul et al., 2012). Ethylene is a natural plant growth regulator that mediates various stages of plant and fruit development including fruit ripening and senescence (Abeles et al., 1992). Climacteric fruit respond to exogenous ethylene with increased ethylene production (Biale, 1964; Paul et al., 2012; Yamane et al., 2007). Non-climacteric fruit show little change in respiration rate during ripening and do not produce more ethylene in response to exogenous ethylene (Biale, 1964; Paul et al., 2012; Yamane et al., 2007). As the climacteric progresses, it is typically accompanied by other changes in the fruit including softening of the flesh; changes in skin pigmentation; the conversion of starches to soluble sugars; and other changes in the composition of the fruit's carbohydrates, lipids, organic acids, and proteins (Kader, 2002a). Climacteric fruit typically continue to ripen after harvest whereas non-climacteric fruit do not ripen after harvest (Kader, 2002b; Saltveit, 1999; Watada et al., 1984; Wills et al., 2007). Defining harvest maturity for climacteric fruit must account for ripening that will occur after harvest.

1.2.3. Maturity Indexes

Maturity indexes are used to identify the point in a fruit's development when harvesting results in the best combination of eating quality and postharvest life (Kader, 2002b). Maturity indexes correlate objective and subjective quality features of a developing fruit with the fruit's postharvest quality, allowing farmers to harvest fruit at the stage when the fruit has the best chance of arriving to the consumer in optimal condition (Reid, 2002). Maturity indexes use measurements of a fruit's physical and chemical properties to differentiate fruits at different levels of maturity (Table 1). Common measurements used in maturity indexes include skin color, flesh color, specific gravity, size, texture, starch content, dry matter content, soluble solids concentration, titratable acidity, firmness, respiration rate, ethylene production rate, and various electronic sensing measurements (Abbott, 1999; Reid, 2002; Walsh & Anderson, 2020). Since prioritizing eating quality often comes at the expense of a shorter postharvest life and vice versa, maturity indexes must compromise between postharvest life and eating quality based on the needs of the market and what is practical (Kader, 2002b). Appropriately defining and measuring maturity contributes to improved postharvest quality along the marketing chain (Reid, 2002).

Table 1: Examples of physical and chemical properties used as maturity indexes for select fruit.

Fruit	Maturity Index	Source
Apple, Honeycrisp	<ul style="list-style-type: none">• Minimum total soluble solids concentration of 13%.• Starch level resulting in 20-80% flesh staining with iodine.	Call et al., 2015
Papaya	<ul style="list-style-type: none">• Change in skin color from dark green to light green with yellow breaking.• Minimum total soluble solids concentration of 11.5%	Arpaia & Kader, 1997
Avocado	<ul style="list-style-type: none">• Minimum dry matter concentration of 19-25%, depending on the cultivar (eg. 20.8% for Hass).	Kader, 1999
Cucumber	<ul style="list-style-type: none">• Jelly-like material beginning to form in the seed cavity with seeds still soft and immature.• Fruit approaching full size.• Firmness and glossiness specific to variety.	Kader, 1997

A maturity index for the climacteric breadfruit should identify the stage of development when harvesting will result in acceptable eating quality and the longest possible postharvest life. Suggested maturity indexes for breadfruit are limited in scope, do not consider cultivar differences, and are not correlated to postharvest quality. A maturity index for the Caribbean white-flesh breadfruit cultivar identifies maturity by starch accumulation, flattening of the surfaces of fruitlet segments, loss of the central spike (remnant of the exerted perianth style) on the fruitlet surface, and dried latex on the surface of the breadfruit (Worrell et al., 1998). In the Caribbean maturity index, starch content and surface segment flattening are measured objectively, while the loss of central spike and presence of latex are judged subjectively. The index indicates maturity in the Caribbean breadfruit variety 15-19 weeks after flowering (Worrell et al., 1998). A later study also in the Caribbean found a similar pattern for starch development, but identified a shorter maturity period occurring 16-18 weeks after flowering (Nacitas Latchoumia et al., 2014). Both studies found a high variance in starch content (Nacitas Latchoumia et al., 2014; Worrell et al., 1998). Variation in starch content was partially attributed to precipitation, with higher precipitation resulting in lower starch content (Nacitas Latchoumia et al., 2014).

Various descriptions of breadfruit maturity are used in informal and household production to distinguish between maturity stages. These descriptions identify maturity based on latex flow, size, color, surface features, firmness, aroma, pulp discoloration upon cutting, ease of removal from the tree, the degree to which the flesh is separated from the core, and the color of the flesh directly under the skin (Table 2; Graham & DeBravo, 1981; Ragone & Wiseman, 2007). These subjective descriptions of morphological changes associated with maturity are useful, but they lack the specificity of a commercial maturity index.

Table 2: Descriptions of breadfruit maturity.

Maturity Description	Value	Source
flattening of surface segments	~ 7mm segment diameter	Thompson et al., 1974; Worrell et al., 1998
starch accumulation	~50% AIS content (w/w) 13% starch content (w/w)	Worrell et al., 1998; Nacitas Latchoumia et al., 2014
loss of central spike (remnant of exerted perianth style)	subjective	Worrell et al., 1998
dried latex on surface of breadfruit from natural, non-induced flow	subjective	Worrell et al., 1998; Ragone & Wiseman, 2007; Thompson et al., 1974
separation of surface segments (fused perianths)	subjective	Ragone & Wiseman, 2007; Thompson et al., 1974
peduncle tightly held to core and tree	subjective	Ragone & Wiseman, 2007
firm flesh	subjective	Ragone & Wiseman, 2007
little latex from peduncle when cut	subjective	Graham & DeBravo, 1981
yellowing skin	subjective	Graham & DeBravo, 1981
dark green skin color with browning	subjective	Thompson et al., 1974
little discoloration of flesh when cut	subjective	Graham & DeBravo, 1981

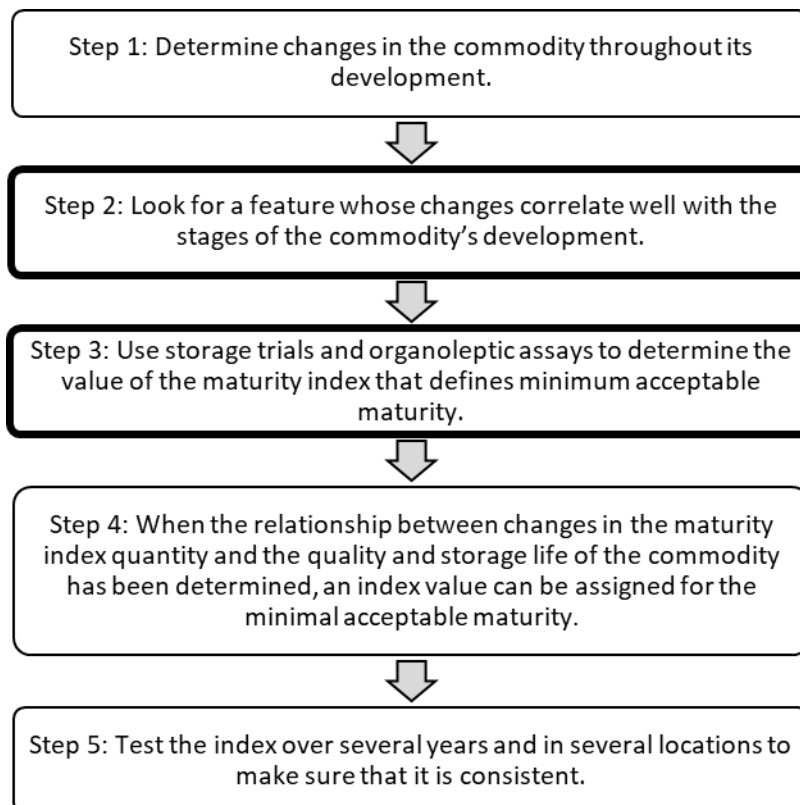


Figure 1: Framework for creating a maturity index from Reid (2002). Bolded steps are the focus of this study.

Compared to the Caribbean, the Pacific has a greater diversity of breadfruit cultivars including cultivars from domesticated breadfruit (*A. altilis*) crossed with its progenitor (*A. mariannensis*; Lincoln et al., 2018; Zerega et al., 2005). The diversity of form between breadfruit cultivars is likely indicative of similar diversity in maturity indicators between cultivars. For example, compared to *A. altilis* varieties, the hybrid cultivars show more subtle changes in texture and color with the onset of maturity (Ragone & Wiseman, 2007).

This study made progress in establishing a formal maturity index for Ma'afala breadfruit using the conceptual framework of a five-step strategy for developing a maturity index (Figure 1; Reid, 2002). The first step of the strategy is to characterize how the fruit changes during its development. This study characterized fruit development in the final stages of growth, building on previous study of breadfruit development in the Caribbean (Nacitas Latchoumia et al., 2014; Worrell et al., 1998). The next step in the framework is to identify specific features that change in correlation with the onset of maturity. Worrell et al. (1998) identified that changes in surface features and starch content can be correlated with maturity (1998), and this study considered those features and others as a maturity index for Ma'afala breadfruit. After identifying an indexing feature, the next step is to identify how changes in the indexing feature influence postharvest quality. After characterizing the postharvest changes, an index value can be assigned for the point of ideal maturity. Finally, the index value should be tested over several locations during several years to ensure it is a reliable indicator of fruit maturity (Reid, 2002). This study focuses on identifying traits that change in correlation with the final stages of breadfruit development and determining how changes in harvest maturity affect postharvest storage quality.

1.3. Practices and Technologies for Breadfruit Storage

In addition to maturity indexes, a host of other pre- and post-harvest practices may be useful in extending breadfruit's shelf-life, some of which have already been studied. Cold storage at 17 °C delays ripening in Ma'afala breadfruit by about 10 days, with chilling injury occurring at lower temperatures (Molimau-Samasoni et al., 2020). Modified atmosphere packaging and wax coatings have been shown to delay ripening but result in off-odors and flesh discoloration (Thompson et al., 1974; Worrell et al., 2002). The effect of ethylene response inhibitors on breadfruit has not been reported.

The ethylene response inhibitor 1-methylcyclopropene (1-MCP) competitively inhibits ethylene perception by binding to ethylene receptors (Watkins, 2015). 1-MCP limits the fruit's perception of ethylene and slows down ethylene-facilitated ripening processes (Sisler & Blakenship, 1994; Watkins, 2015). The effects of 1-MCP are fruit-specific and are influenced by many factors including cultivar, dosing, treatment temperature, fruit maturity, stage of fruit ripening, and interactions with fruit disorders (J. Zhang et al., 2020). While responses to 1-MCP are variable, the most common responses include reduced autocatalytic ethylene production; reduced respiration rates; and delayed softening, loss of greenness, and break-down of organic acids (Watkins, 2006, 2015). Since its discovery and patent in 1992, 1-MCP has been studied in a wide variety of crops and adopted in certain commercial operations to maintain fruit and flower quality during storage (J. Zhang et al., 2020). 1-MCP is an attractive treatment option because it is safe, residue-free, active at low concentrations, and easily applied (Watkins, 2015; J. Zhang et al., 2020). Research has considered the effect of 1-MCP on at least 82 different fruits and vegetables, but the effect of 1-MCP on breadfruit has not been reported (Watkins, 2015).

Since the effect of 1-MCP is dependent on a variety of factors, 1-MCP's effect on breadfruit quality maintenance may be limited to very specific conditions. 1-MCP is typically less effective at delaying ripening as fruit progresses towards ripening; which is likely related to more-mature fruit having already initiated the ripening process and associated ethylene production (Cocci et al., 2014; Mir et al., 2001; Sabir & Agar, 2011; Wang & Sugar, 2015; Z. Zhang et al., 2011). However, this relationship is not always straightforward; for example, optimal response to 1-MCP in tomatoes has been identified at different stages of maturity in seemingly contradictory studies (Guillén et al., 2006; Sabir & Agar, 2011). The lack of predictability may be related to still-undiscovered mechanisms of action by 1-MCP and underscores the limitations of generalizations about 1-MCP's effect on fruit ripening (J. Zhang et al., 2020).

Early, preliminary work on the effect of 1-MCP in breadfruit suggested incomplete penetration of 1-MCP may limit its effectiveness at delaying ripening. In the preliminary study, treatment with 1-MCP delayed softening in only the outer portion of the breadfruit. The inner flesh softened while the outer flesh maintained its firmness (Paull and Sawada, unpublished data).

There is some support in the literature suggesting 1-MCP may improve postharvest quality of breadfruit. For example, 1-MCP has been shown to improve quality maintenance in pre-cut Jackfruit, a fruit related to breadfruit (Vargas-Torres et al., 2017). In soursop, a tropical fruit with similar form to breadfruit, 1-MCP was found to prolong refrigerated storage by reducing chilling injury (Espinosa et al., 2013).

This study examines the effect of 1-MCP on the postharvest quality of Ma'afala breadfruit harvested at various stages of maturity. 1-MCP was chosen for this study based on the success of 1-MCP in similar fruit and preliminary results suggesting it delays breadfruit ripening.

The study considers breadfruit maturity, the effect of maturity on postharvest life, the effect of 1-MCP on postharvest life, and the combined effect of harvest maturity and 1-MCP treatment on postharvest life.

2. MATERIALS AND METHODS

2.1. Sampling

A block of 75 Ma'afala breadfruit trees was selected within a commercial breadfruit orchard in Mililani, Oahu (21.43, -158.02; elevation 160 m). The 8-year-old, clonally-propagated trees were spaced 15 feet (4.5 m) apart in rows 30 feet (9.1 m) apart, and were pruned every year or every other year to a height of approximately 9 ft (2.7 m). The most recent pruning was approximately 3 months prior to the flowering period for fruit included in this study. The selected block did not include orchard border row trees. During the season's first flowering period (May–June), trees were randomly selected from within the block. Initially 74 female inflorescences with equatorial diameter less than 35 mm were tagged and numbered (Figure 2). An additional 74 inflorescences were selected 12 days later, and 89 more after an additional 12 days. Each set of tagged inflorescences served as a replication. The tagging date was assigned as the flowering date and used as the starting point for measuring development.

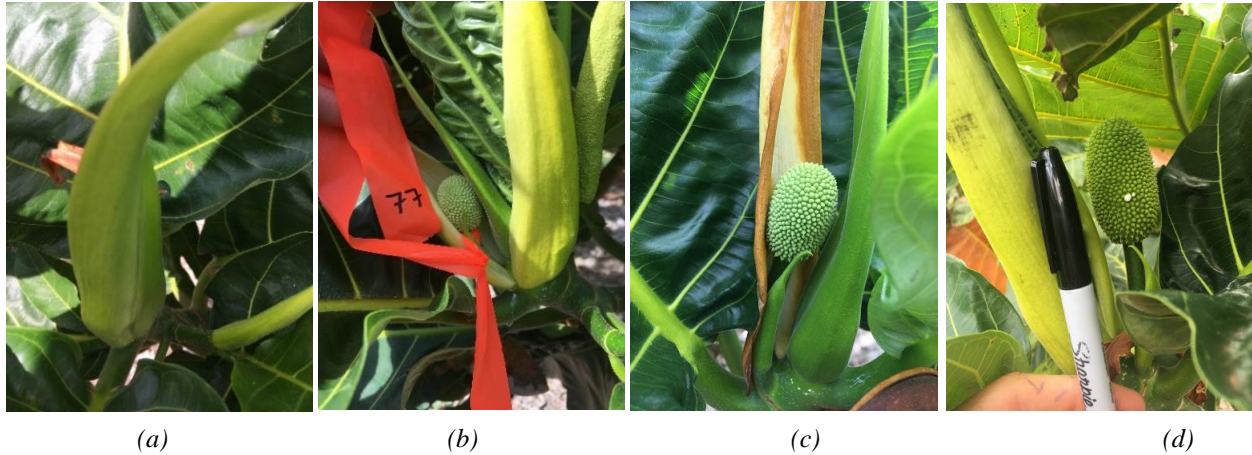


Figure 2: Female inflorescences at time of tagging. Inflorescences with diameter less than 35 mm were tagged, and the tagging date was assigned as the flowering date in order to count the number of weeks from flowering until harvest. (a) The bump near the base of the sheath was a young female inflorescence. This inflorescence was not tagged because it had not emerged from the sheath. Only inflorescences that were visible were tagged. (b) Female inflorescence that was partially emerged from the sheath. The sheath was still white-green and the fruit was white-green. This was the earliest tagging point. The equatorial diameter in the pictured fruit was 21mm. (c) Female inflorescence emerged from the sheath. The sheath was changing color from green to brown, and the fruit was white-green colored. The equatorial diameter was 25mm. (d) Female inflorescence with the sheath no longer present. The inflorescence had darkened from white-green to bright green. The equatorial diameter was 33mm. This specimen was near the oldest point in the tagging range.

Measuring development based on time from flowering aligned with previous studies of breadfruit development (Nacitas Latchoumia et al., 2014; Worrell et al., 1998). Time from flowering, however, was an imperfect proxy for measuring development because it did not account for variation in rate of development due to environmental factors such as light, temperature, and water availability (Ali et al., 2021; Krug, 1997).

Thirteen weeks after tagging, a minimum of six tagged fruit were randomly selected and harvested every two weeks until no tagged fruit remained. All fruit were harvested before 9:00 AM, using pruning shears to cut the peduncle. After harvest, the peduncles were trimmed to be flush with the breadfruit, and the breadfruit were placed peduncle-side down in a newspaper-lined crate to allow latex to drain. Within two hours of harvest, the breadfruit were transported to the lab. In the lab, the fruit were rinsed with cool water, gently washed, and allowed to air dry.

2.2. Experimentation

At the time of harvest, the peduncle color, peduncle latex flow, fraction of skin covered in dried latex, and presence of ants or ant debris at the neck of the peduncle was recorded. In the lab measurements were taken of the harvested fruit's volume, weight, polar diameter, equatorial diameter, surface polygon size, intersegment color, and hand-feel. Skin color was measured by point colorimetry, fractional green space, mean color, and visual judgement related to standard color cards. A portion of the harvested fruit were used for destructive analysis measuring firmness, induced latex flow, total soluble solids (TSS), titratable acidity (TA), starch iodine staining, dry matter, and starch content.

For each harvest period, growing degree days, base 20 °C (GDD₂₀), were calculated as the sum of the daily difference between the base temperature and the average of the high and low temperatures. The base temperature for growing degree-day measurement is typically the estimated minimum temperature needed for the plant to grow vigorously (Clark & Larson, 2020). In breadfruit, chilling sensitivity has been observed at average temperatures as high as 17.3°C (Cao et al., 2006). A base temperature of 20°C was selected as a convenient temperature slightly higher than 17°C.

For each harvest, two-thirds of the fruit were used for storage testing. Breadfruit to be stored were first dipped in fludioxonil suspension (0.58g ai/L, Syngenta Scholar SC) to limit surface mold. After taking the harvest observations, half of the breadfruit were treated with 1 ppm active ingredient 1-MCP for 20 hours at 25°C (77 °F) in a 126-liter chamber, and the other half were held in a similar chamber without 1-MCP. After 20 hours in the enclosed chambers, the breadfruit were transferred to the laboratory benchtop and held at 22 °C (72 °F). Every second day fruit were evaluated for weight, hand-feel, skin color by point colorimetry, respiration rate, and ethylene production; and a photo was taken to calculate green skin fraction and mean skin color. The storage test ended once a fruit was completely soft and misshapen or when a fruit had abundant mold on its surface.

2.2.1. Physical Properties

Fresh weight was measured on a digital scale (Denver Instrument Company, AC-8K). Volume was measured by water displacement. Polar diameter and equatorial diameter were measured at their widest point using calipers (accuracy 1 mm, Haglof, Sweden).

2.2.2. Skin and Peduncle Color

Peduncle color was judged on a three-point scale: all green, mostly green, not green. Skin green fraction was measured using the Canopeo smart phone application that measures the percent green pixels in an image (González-Esquivá et al., 2017; Patrignani & Ochsner, 2015). Skin color was measured with a point colorimeter (Nix ProColor, Nix Sensor Ltd. Hamilton, Ontario, Canada), averaging the CIELAB values from five points around the equator, and by photo analysis using ImageJ software to extract the mean CIELAB values from a photo of the breadfruit (Schneider et al., 2012; Strock, 2021). The photo for fractional green and mean color analyses was taken in the Canopeo app using an iPhone SE (Apple Inc., Cupertino, California) with the breadfruit centered in the frame and the edges of the breadfruit touching the lateral margins of the frame. The green fraction percent was the portion of the photo that is green, including the background. The background was digitally removed from the photo prior to calculating mean color using ImageJ. Skin color was visually judged in comparison with the Royal Horticultural Society Colour Chart (London, England).

2.2.3. Surface Characteristics

Surface polygon segments were measured using electronic calipers (accuracy 0.1 mm), and the widest-point diameter of three polygons on the side opposite of the peduncle were averaged. Intersegment color, which is related to scabbing, was judged on a five-point scale: completely brown, mostly brown, brown and green, mostly green, or completely green (Ragone & Wiseman, 2007). The fraction of skin covered in dried latex prior to harvest was visually approximated as a percent. The presence of ants or ant debris around the peduncle was judged on a five-point scale with 1 being no ants and/or ant debris and 5 being abundant ants and/or ant debris.

2.2.4. Latex Flow

Abundance of latex flow from the cut peduncle at harvest was approximated on a 5-point scale with 1 being no flow and 5 being abundant flow. Abundance of induced latex flow from the skin was measured in destructively analyzed fruit 2 to 5 hours after harvest and after the latex had been allowed to drain from the peduncle. Latex was induced by cutting a conical hole of 5mm diameter into the surface of the breadfruit, and flow was judged on a 3-point scale with 3 being abundant flow and 1 being no flow.

2.2.5. Firmness

The firmness of the breadfruit was judged by hand-feel on a 5-point scale: firm, firm with slight compressibility, less firm with visible compressibility, soft and deforms with pressure, and does not hold shape. In destructively analyzed fruit, a digital force gauge (Zada ZPS-DPU-110; IMADA, Inc., Northbrook, Illinois) was used to measure the force to insert a 15 mm-diameter rod 3 mm into the fruit.

2.2.6. Internal Traits

Dry matter was measured by drying a 75-gram portion of thinly-sliced fresh breadfruit at 65 °C until the sample's weight stabilized, about 48 hours.

Total soluble solids (TSS) and titratable acidity (TA) were measured from fresh breadfruit samples. Breadfruit was sliced longitudinally, and a 75g portion was diluted to 10% by weight with distilled water and blended with a commercial food blender (Model 908, Hamilton Beach Brands, Inc., Virginia) for two minutes. TSS was quantified in a filtered portion using a digital refractometer (Model 300037, Sper Scientific, Scottsdale, Arizona). An unfiltered portion of the sample was titrated in a mini-titrator (Model HI 84432; Hanna Instruments Smithfield, Rhode Island) with units set to percent malic acid. Percent malic acid was converted to milliequivalent H⁺ per 100g dry matter. An attempt was made to use a hand-held infrared brix meter (PAL-Hikari, Atago, Tokyo, Japan) to corroborate the TSS reading, but in a preliminary trial, the meter was found to be unable to measure TSS in breadfruit.

The starch-iodine staining pattern was photographed after dipping a partial latitudinal cross-section of the breadfruit in iodine solution (8.8% potassium iodide + 2.2% iodine w/w; Blankenship et al., 2018; Blanpied & Silsby, 1992).

Starch content was measured in dried breadfruit samples using an enzyme-digestion total starch assay kit (K-TSTA, DMSO format, AOAC Official Method 996.11; Megazyme, Bray, Ireland). Dried breadfruit samples were ground in a coffee grinder then passed through a 0.5 mm screen and washed twice with ethanol (80% v/v) to remove D-glucose and maltodextrins. Washed samples were mixed with dimethylsulfoxide and placed in a boiling water bath for 5 minutes. Samples were incubated with thermostable α -amylase (diluted 30x in MOPS buffer plus calcium chloride) for six minutes in boiling water. Amyloglucosidase in sodium acetate and calcium chloride buffer was added as per the kit instructions, and samples were incubated at 50 °C for 30 minutes. The solution was diluted with sodium acetate and calcium chloride buffer and a portion was incubated with GOPOD reagent (glucose oxidase/peroxidase) with p-hydroxybenzoic acid and 4-aminoantipyrine at 50 °C for 20 minutes. Absorbance was measured at 510 nm. Starch content was calculated by comparing absorbance to glucose standards.

2.2.7. Storage Traits

Storage traits were measured every second day during the storage period. To measure respiration and ethylene production rates, breadfruit were enclosed in sealed jars of 2.5 or 3.5 L volume. After 30 minutes, an air sample from the jar was collected through a rubber septum using a needle and syringe, and carbon dioxide in the sample was measured using a non-dispersive infrared gas analyzer (NDIR, LI-820 CO₂ Analyzer; Li-Cor, Inc. Lincoln, Nebraska). After an additional 90-120 minutes, a second air sample was collected and ethylene was measured using a gas chromatograph (Model GC-8A; Shimadzu Scientific Instruments, Columbia, Maryland). The day with the highest respiration rate was considered the climacteric peak.

Weight, hand-feel, fractional green space, point color, and mean color were measured during storage as described for harvest day measurements.

The number of days until discoloration was calculated for each of the three methods for measuring color. The discoloration endpoint when measuring skin green fraction was the first day with less than 25% green fraction. For mean color and point color, the discoloration endpoint was the first day with a positive a^* value (Table 3).

2.3. Statistical Analysis

Statistics were performed using R 4.1.2 (R Core Team, 2021) with the R packages *agricolae* (DeMendiburu, 2021), *survival* (Therneau, 2021; Therneau & Grambsch, 2000), *survRM2* (Uno et al., 2020), *Hmisc* (Harell Jr., 2021), *rpart* (Therneau & Atkinson, 2019), *rpart.plot* (Milborrow, 2021), and *caret* (Kuhn, 2021). Data was manipulated and visualized using the R packages *tidyverse* (Wickham et al., 2019), *survminer* (Kassambara et al., 2021), *corrplot* (Wei & Simko, 2021), and *colorspace* (Zeileis et al., 2020).











2.3.1. Harvest Index

Spearman rank correlation analysis was used to identify associations between harvest period, harvest features, and storage traits. Spearman coefficients (ρ) were calculated for correlations with p-values greater than 0.05. Spearman rank correlation was used because it allowed comparisons between categorical and continuous variables.

2.3.2. Classification Analysis

Traits that were shown to have a correlation with harvest period and were considered suitable for a field maturity index were trialed as maturity predictor variables using recursive partitioning. The recursive partitioning used one or two predictor variables to group the breadfruit into mature and immature groups. Sorting accuracy was calculated for each predictor variable or combination of variables, and the recursive partitioning was visualized as a tree and plot.

Table 3: Representative example of storage data showing various storage endpoints for a single breadfruit.

day	1	3	5	7	9
photo					
mass (kg)	0.8316	0.8003	0.7709	0.7356	0.7095
respiration (mg CO ₂ /kg/hr)	162	120	92	110	227
ethylene (nl C ₂ H ₄ /kg/hr)	--	--	--	--	--
Handfeel	1	1	2	2	3 ⁽¹⁾
GreenFraction	50.0 %	50.1 %	44.9 %	44.0 %	37.1 %
Mean color (<i>a</i> *)	-11.125	-10.027	-9.217	-10.016	-8.513
Point color (<i>a</i> *)	-7.284	-6.202	-6.248	-6.29	-5.4
day	11	13	15	17	19
photo					
mass (kg)	0.6777	0.6483	0.6237	0.5994	0.5764
respiration (mg CO ₂ /kg/hr)	363 ⁽²⁾	333	170	194	186
ethylene (nl C ₂ H ₄ /kg/hr)	373 ⁽³⁾	--	--	--	--
Handfeel	3	4	5 ⁽⁶⁾	5	5
GreenFraction	18.5 % ⁽⁴⁾	7.7 %	3.0 %	1.9 %	3.2 %
Mean color (<i>a</i> *)	-1.721	3.982 ⁽⁵⁾	8.284	9.033	9.356
Point color (<i>a</i> *)	-2.074	6.016 ⁽⁵⁾	8.304	10.946	11.704

¹ Compressibility endpoint: day with first softness rating of 3 (less firm with visible compressibility).

² Climacteric peak: day with highest CO₂ respiration rate.

³ Ethylene peak: only day with perceived ethylene production.

⁴ Green fraction discoloration endpoint: first day with green fraction less than 25%.

⁵ Point color and mean color discoloration endpoint: first day with *a** value greater than 0.

⁶ Completely soft endpoint: first day with softness rating of 5 (completely soft, does not hold shape).

2.3.3. Storage Duration

Breadfruit storage duration was determined as the number of days to reach a storage endpoint. Multiple storage endpoints were considered including onset of compressibility, complete softening, reaching the climacteric peak, and discoloration measured by skin green fraction, point colorimetry, and mean color. For each endpoint type, the Kaplan-Meier survival curve was plotted, and the log-rank test determined statistical difference between survival curves (Therneau, 2021; Therneau & Grambsch, 2000). The restricted mean survival time (RMST), a representation of the area under the Kaplan-Meier survival curve, was used to quantify differences between survival curves (Uno et al., 2020).

3. RESULTS

Measurements were taken on 73 fruit (Table 4). Of the total fruit quantity, 28 fruit were destructively analyzed at harvest and 50 fruit were observed during storage with 23 of the 50 receiving 1-MCP treatment. Three fruit were already soft and ripening at the time of harvest and were excluded from the correlation analysis and general linear model analyses because the goal of these analyses were to understand indicators for a mature but not ripe breadfruit. Nine fruit had been planned for each collection; however due to extensive fruit drop (25-60% of fruit were aborted before week 13), only six fruit were collected each harvest after the first two harvests.

Table 4: Harvest summary showing quantity by harvest period and repetition.

Repetition	Harvest Period (weeks)				Total
	13	15	17	19	
1	9	6	6	1	22
2	9	6	6	4	25
3	6	6	6	8	26
Total	24	18	18	13	73

3.1. Traits Correlated to Harvest Period

The results of Spearman's rank correlation analysis comparing breadfruit features to harvest period showed that breadfruit harvested in later harvest periods tended to have less latex flow from the peduncle at harvest (Spearman correlation coefficient, $\rho = -0.78$), less skin green fraction ($\rho = -0.78$), browner intersegment spaces ($\rho = 0.76$), less latex flow when induced by skin puncture ($\rho = -0.71$), browner peduncle ($\rho = 0.65$), greater equatorial diameter ($\rho = 0.58$), a larger portion of skin covered in latex ($\rho = 0.52$), a more spherical shape shown by polar:equator diameter ratio ($\rho = 0.49$), larger surface polygons ($\rho = 0.47$), greater volume ($\rho = 0.44$), increased starch content ($\rho = 0.44$), and greater weight ($\rho = 0.42$). There was strong correlation between harvest period and color. Considering mean color, fruit in later harvest periods tended to be darker (L^* , $\rho = -0.74$), less green (a^* , $\rho = -0.76$), and less yellow (b^* , $\rho = -0.64$; Table 5). There was similar correlation between color and harvest period when color was measured using point colorimetry. See Appendix A for correlograms (Figure 14, Figure 15).

Table 5: Summary of harvest trait correlations with harvest period.

Trait	type	ρ	p	distribution	Separation of Mean by Harvest Period	
					HSD	LSD
Peduncle Latex Flow	categorical	-0.78	0	two	a, a, b, b *	a, a, b, b *
Skin Green Fraction	continuous	-0.78	0	skewed	a, a, b, c *	a, a, b, c *
Intersegment Space Color	categorical	0.76	0	skewed	a, b, b, b *	a, b, b, b *
color: mean a^*	continuous	0.76	0	normal	a, a, b, c	a, b, c, d
color: mean L^*	continuous	-0.74	0	normal	a, b, bc, c	a, b, c, c
color: point a^*	continuous	0.73	0	normal	a, b, c, c	a, b, c, d
Induced Latex Flow	categorical	-0.71	0.0005	skewed	a, ab, b, b *	a, b, b, b *
Peduncle Color	categorical	0.65	0	skewed	a, b, b, b *	a, b, b, b *
color: mean b^*	continuous	-0.64	0	normal	a, b, bc, c	a, b, bc, c
color: point b^*	continuous	-0.64	0	near normal	a, ab, c, bc *	a, b, c, c *
Equator Diameter	continuous	0.58	0	normal	a, b, bc, c	a, b, b, c
color: point L^*	continuous	-0.55	0	normal	a, a, b, b	a, a, b, b
Skin Latex Fraction	continuous	0.52	0	skewed	a, a, b, ab *	a, a, b, a *
Polar:Equator Diameter	continuous	0.49	0	near normal	a, ab, bc, c *	a, a, b, b *
Polygon Diameter	continuous	0.47	0	normal	a, b, b, b	a, b, b, b
Volume	continuous	0.44	0.0002	normal	a, ab, b, b	a, b, b, b
Starch Content	continuous	0.44	0.03	normal	a, a, a, a	a, ab, ab, b
Weight	continuous	0.42	0.0003	normal	a, ab, ab, b	a, b, b, b

Density, polar diameter, firmness, water fraction, TSS, TA, and TSS:TA ratio are not included in the table because they were not significantly correlated to the harvest period ($p > 0.05$). Rho (ρ) is the Spearman correlation coefficient, and the p-value gives the probability that the correlation is due to chance. Mean separation by harvest period was considered using Tukey's honestly significant difference test (HSD) and Fisher's least significant difference test (LSD). HSD and LSD results are shown in order of harvest period: 13-, 15-, 17-, 19-week harvest periods, respectively. Harvest periods with different letters have significantly different group means. Results marked with an asterisk () failed to meet the assumption of normal distribution required by the HSD and LSD tests.*

3.1.1. Latex Traits

Three traits associated with latex flow were correlated to harvest period: peduncle latex flow, induced latex flow, and skin latex fraction. The quantity of active latex flow tended to decrease in later harvest periods while the presence of dried latex increased at later harvest periods (Figure 4). Heavy latex flow from the peduncle was observed in all fruit from the 13-week harvest period and most fruit from the 15-week harvest period but was rarely observed in later harvest stages. Induced latex flow from skin puncture was only observed in fruit harvested at 13 weeks (Figure 3). In early harvest periods, there was little or no dried latex on the breadfruit surface, while in late harvest periods, there was a wide range of latex coverage observed, from no coverage to extensive coverage. In all harvest periods, some breadfruit had no latex stain.

3.1.2. Surface Traits

Intersegment space color was correlated to harvest period and its changes were easily observed with the naked eye (Figure 3). Breadfruit at later harvest stages always had mostly brown or completely brown intersegment spaces. Early harvest stages had intersegment space colors ranging from all green to all brown (Figure 4).

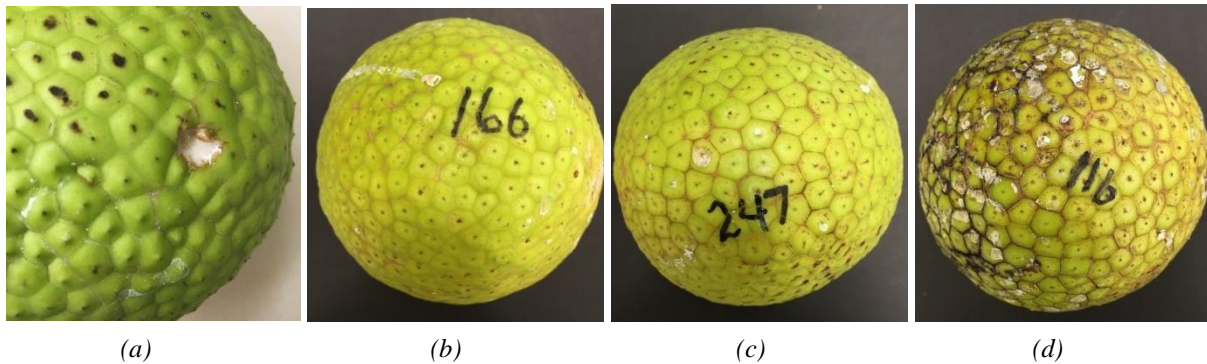


Figure 3: Examples of induced latex flow and intersegment space color. (a) Latex flow from skin puncture in a fruit harvested 13 weeks after flowering. (b) Breadfruit with all green intersegment spaces, (c) mostly brown intersegment spaces, and (d) all brown intersegment spaces.

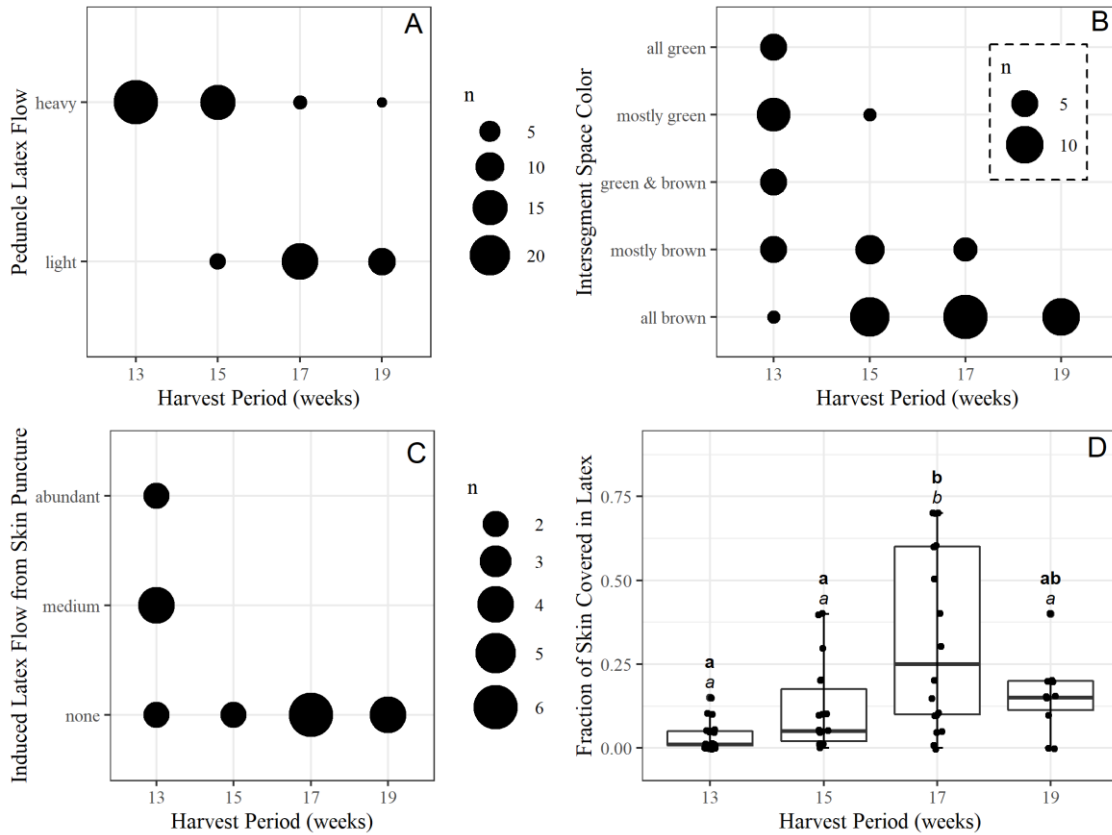


Figure 4: Variation of correlated latex traits and surface traits by harvest period. (A) Peduncle latex flow showed a trend of decreased flow in later harvest periods. (B) Intersegment space color showed high variation in early harvest periods and was mostly brown in later harvest periods. (C) Latex flow from skin puncture was only present in some breadfruit in early harvest periods. (D) Dried latex on the skin was rare in early harvest periods and became more common in later harvest periods; however, even in later harvest periods many fruit had no dried latex on the skin.

3.1.3. Color Traits

Color traits tended to be highly correlated to harvest period with absolute values of Spearman correlation coefficients ranging from 0.52 to 0.78. Peduncle color, skin green fraction, and skin color were all correlated with harvest period.

Skin green fraction correlated strongly to harvest period with later harvest periods showing a smaller green fraction. There was little difference in green fraction between 13 and 15 weeks, but significant drop in green fraction between 15 and 17 weeks and between 17 and 19 weeks.

The peduncles were always completely green in fruit harvested at 13 weeks. At mid to late harvest periods, brown areas were observed on the peduncle, but the color remained mostly green. In a few fruit at the later harvest periods, the color on the peduncle changed to yellow or brown, especially near the fruit. "Not green" peduncles had yellowing and browning near the fruit (Figure 5). While there seems to be some correlation between peduncle and harvest period, this observation and scale were imprecise and poorly defined.

Mean color was significantly different in each harvest period. Luminance as measured by the L^* value decreased in each harvest period, but not significantly after 17 weeks. Color on a green to red spectrum as measured by the a^* value became less green (less negative) in consecutive harvest periods. In the 17- and 19-week harvest periods, a few breadfruit had a positive mean a^* value indicating more red coloration than green. Color on a yellow to blue spectrum as measured by the b^* value was less yellow (less positive) in later harvest periods (Figure 6, Figure 7).

Mean point color had a smaller correlation with harvest period compared to mean color, but showed a similar trend: decreasing luminance (L^*), decreasing green (less negative a^*), and decreasing yellow (less positive b^*) in later harvest periods.

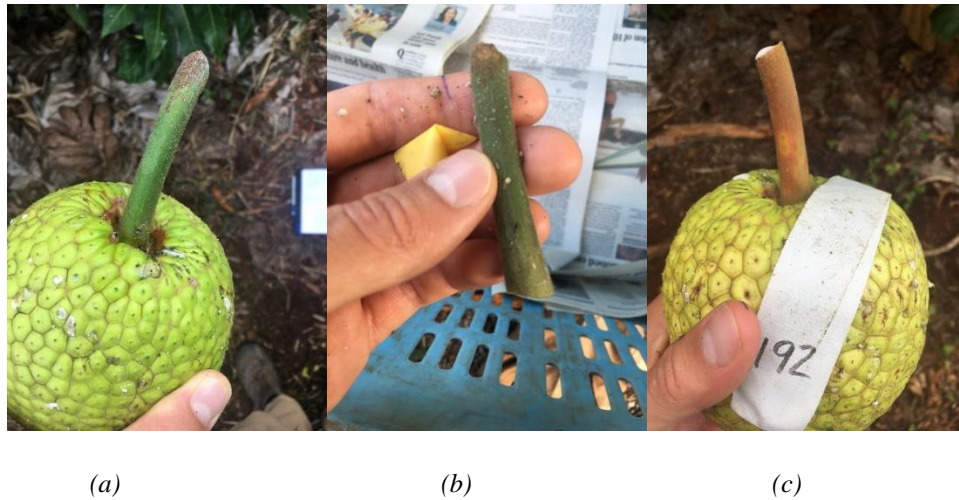


Figure 5: Examples of the ranking scale for peduncle color. Breadfruit exhibited different coloration on the peduncle at the time of harvest; however, the scale used to report peduncle color was imprecise. Examples of color scale: (a) "brown areas", (c) "not green" due to yellowing near the base; (c) "not green."

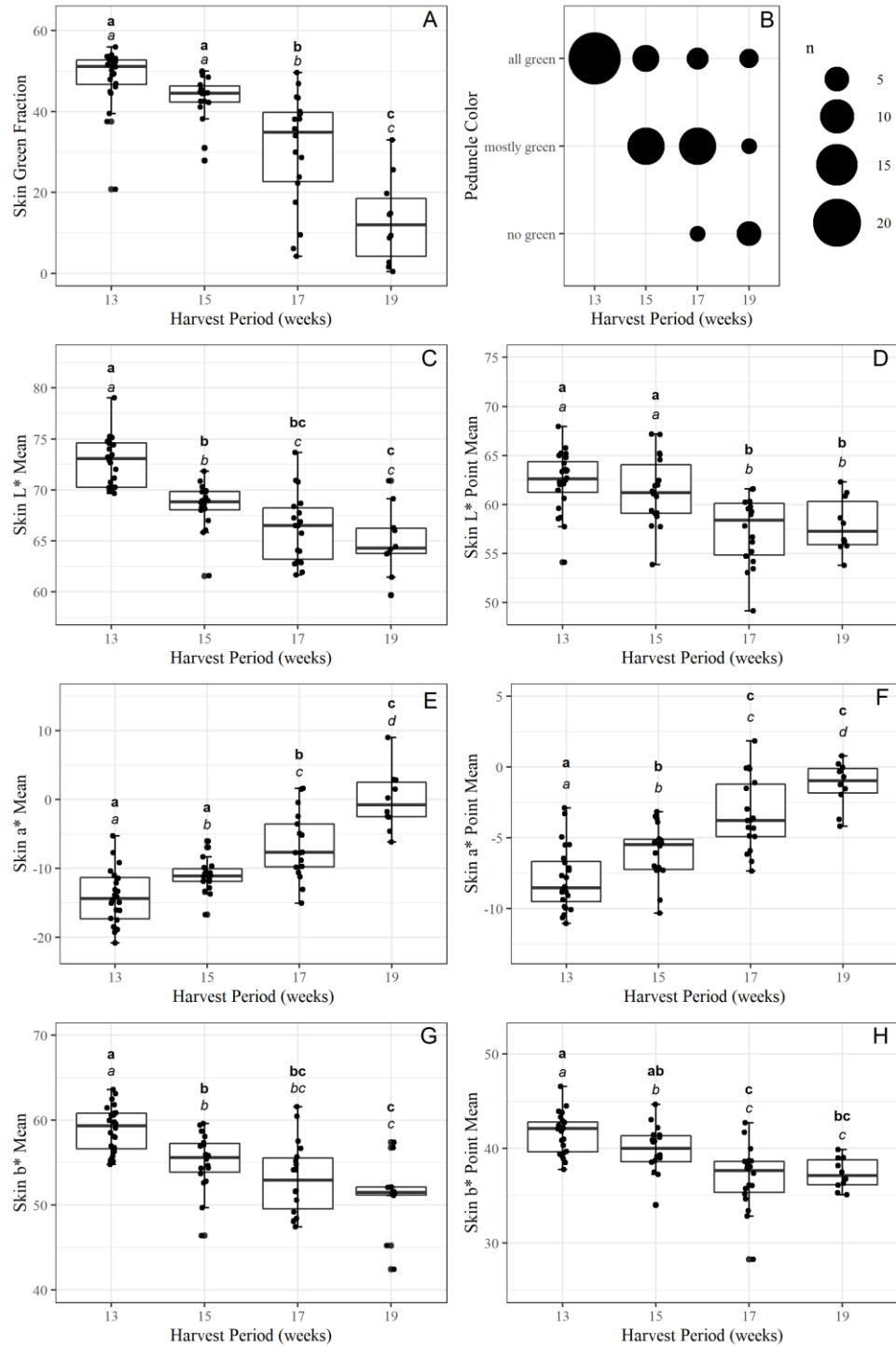


Figure 6: Variation of skin color and peduncle color in Ma'afala breadfruit harvested in the final stages of development. Skin color was measured as green fraction (A), by point colorimetry (D,F,H), and by mean color (C,E,G). Point color and mean color were expressed using the CIELAB color space. The peduncle color was judged visually on a three point scale (B). Most skin color measurements show significant differences in skin color between breadfruit harvested at different harvest periods. Fruit tended to become darker and less green in later harvest periods as shown by decreasing green fraction (A), decreasing luminance (C,D), increasing a^* value (E,F), and decreasing b^* value (G,H).

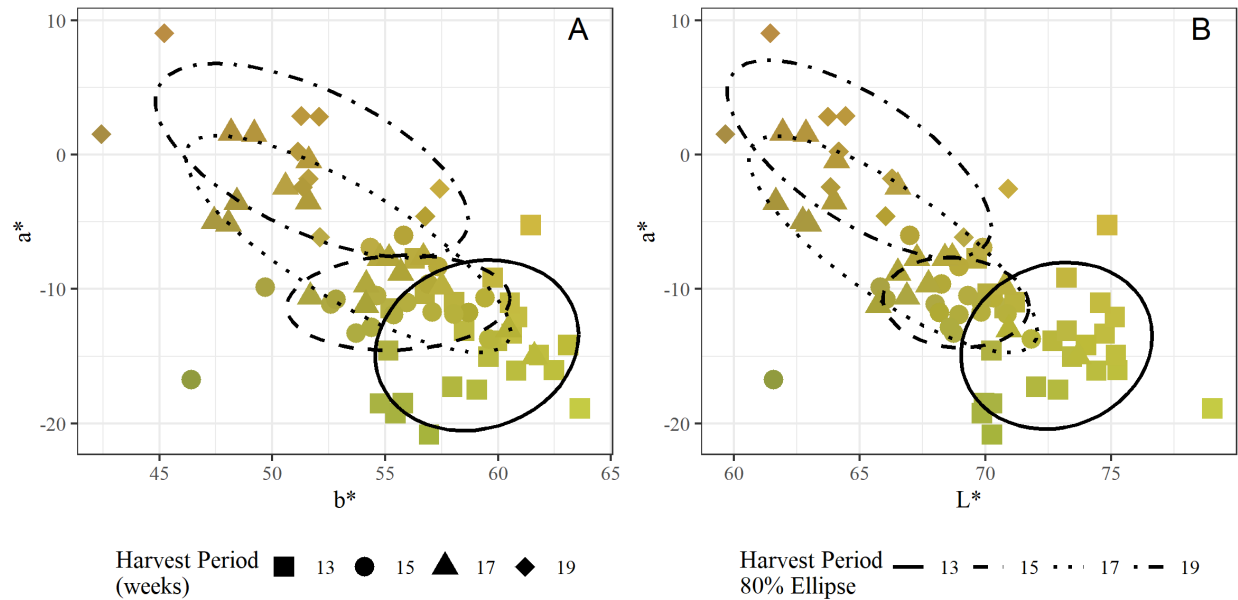


Figure 7: Separation of harvest period groups by mean color. Color is represented by a^* and b^* values (A) and a^* and L^* values (B). Mean color value was extracted from a digital photo of the breadfruit using ImageJ. The marker color correlates to the mean color of the breadfruit it represents. The 80% ellipses show that fruit harvested at the 19 weeks tended to have a color distinct from fruit harvested at either 13 or 15 weeks, and fruit harvested at 13 weeks had mostly distinct coloration from fruit harvested at 15 or 17 weeks. Fruit harvested at 17 weeks had similar coloration to fruit at 15 and 19 weeks.

3.1.4. Physical Traits

Traits related to shape and size tended to be less strongly correlated to harvest period compared to color traits. Spearman correlation coefficients for traits related to size ranged from 42% to 58%. The equatorial diameter tended to increase in relation to the polar diameter in later harvest periods so that the ratio approached 1:1 in later harvest periods. In early harvest periods, the breadfruit were more likely to be longitudinally oblong and in later harvest periods the breadfruit were more likely to be globular. The average volume and average weight increased each harvest period but did not increase significantly after 15 weeks. The average diameter of surface polygons increased between 13 weeks and 15 weeks but did not increase significantly after 15 weeks (Figure 8).

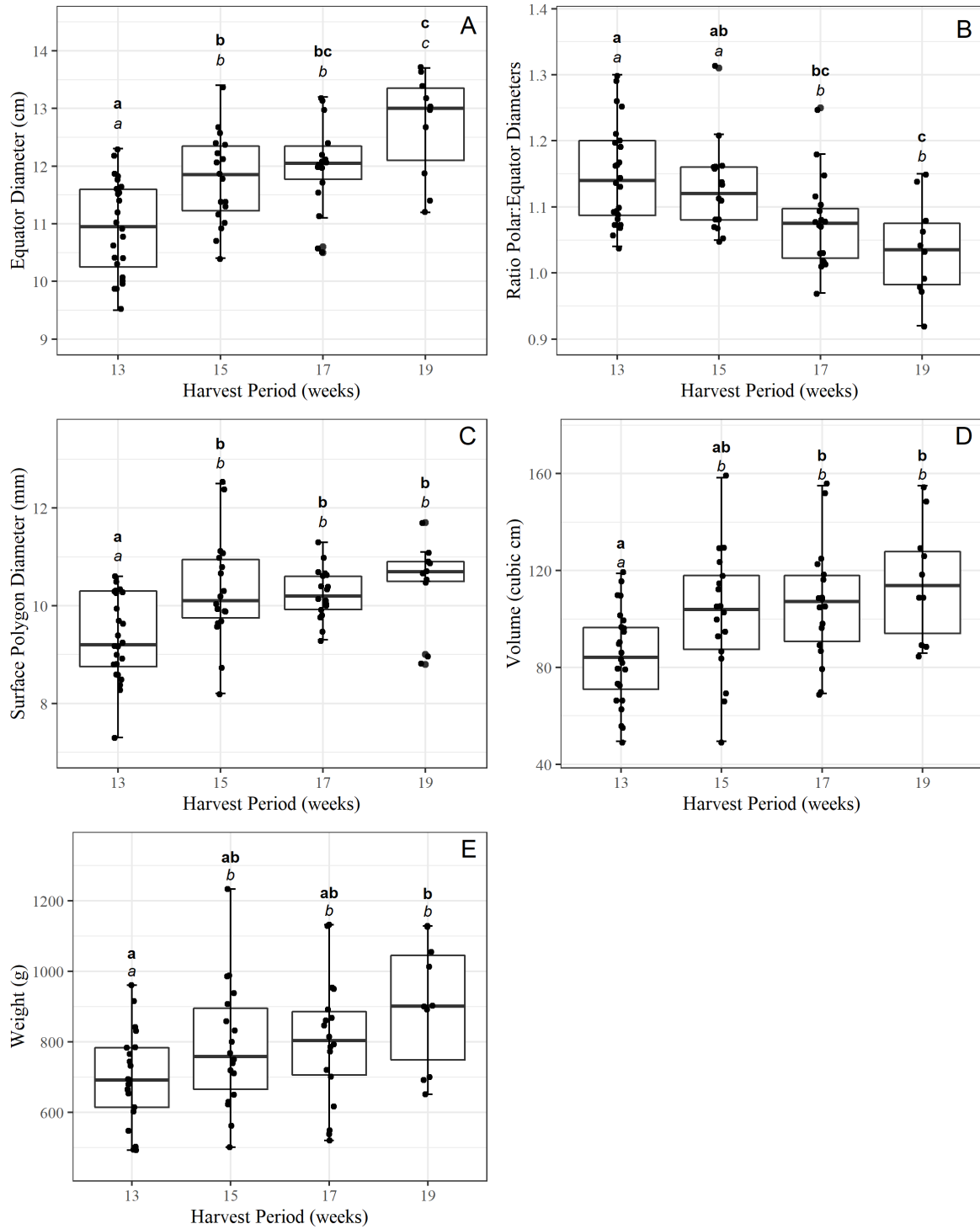


Figure 8: Variation of physical traits of Ma'afala breadfruit harvested during distinct harvest periods in the final stages of development. The equatorial diameter (A) continued to increase through the 19-week harvest period, but the surface polygon size (C), volume (D), and weight (E) plateaued after 15 weeks. The ratio of equator:polar diameter (B) decreased towards one in successive harvest periods, demonstrating that the equatorial diameter continued to increase after 15 weeks while the polar diameter remained mostly constant.

3.1.5. Internal Traits

Most traits related to internal properties were not related to harvest period. Starch had a low but significant correlation with harvest period ($p=0.44$). Average starch content was near 60% (dry weight) for fruit harvested at 13 weeks and increased for each harvest period but not significantly after 15 weeks (Fisher's LSD; Figure 9). Fruit that began to ripen before harvest had dramatically lower starch content, near 15% starch (dry weight) and were not included in the correlation analysis.

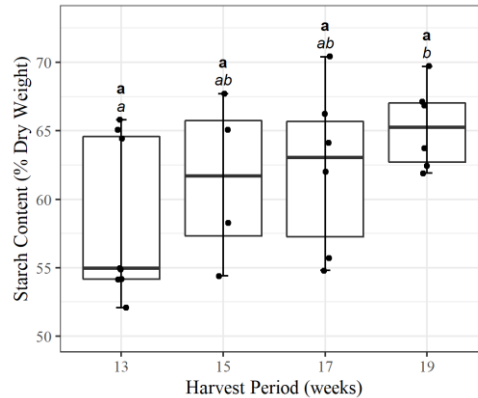


Figure 9: Variation of the starch content in Ma'afala breadfruit harvested during distinct harvest periods in the final stages of development. Starch content was the only internal trait correlated to harvest period and showed only a slight increase between 13 and 15 weeks of development.

3.2. Uncorrelated and Other Traits

Density, polar diameter, firmness measured by penetrometer, water fraction, TSS, TA, TSS:TA ratio, presence of ants or ant debris, and starch iodine staining did not correlate to harvest period (Figure 10). Among these traits, some showed high variation, even if the variation was not correlated with harvest period. For example, starch iodine stain patterns varied from complete to sparse staining, but there was no significant correlation between starch staining pattern and harvest period (Figure 11).

Color according to the Royal Horticultural Society Colour Chart was most frequently in a yellow-green group (145A, 151A, 151B, 151C, 151D, 154C) and occasionally in a grayed-yellow group (160A) or yellow group (8C). One of the fruits that had already commenced softening while on the tree was in a greyed-orange group (165A).

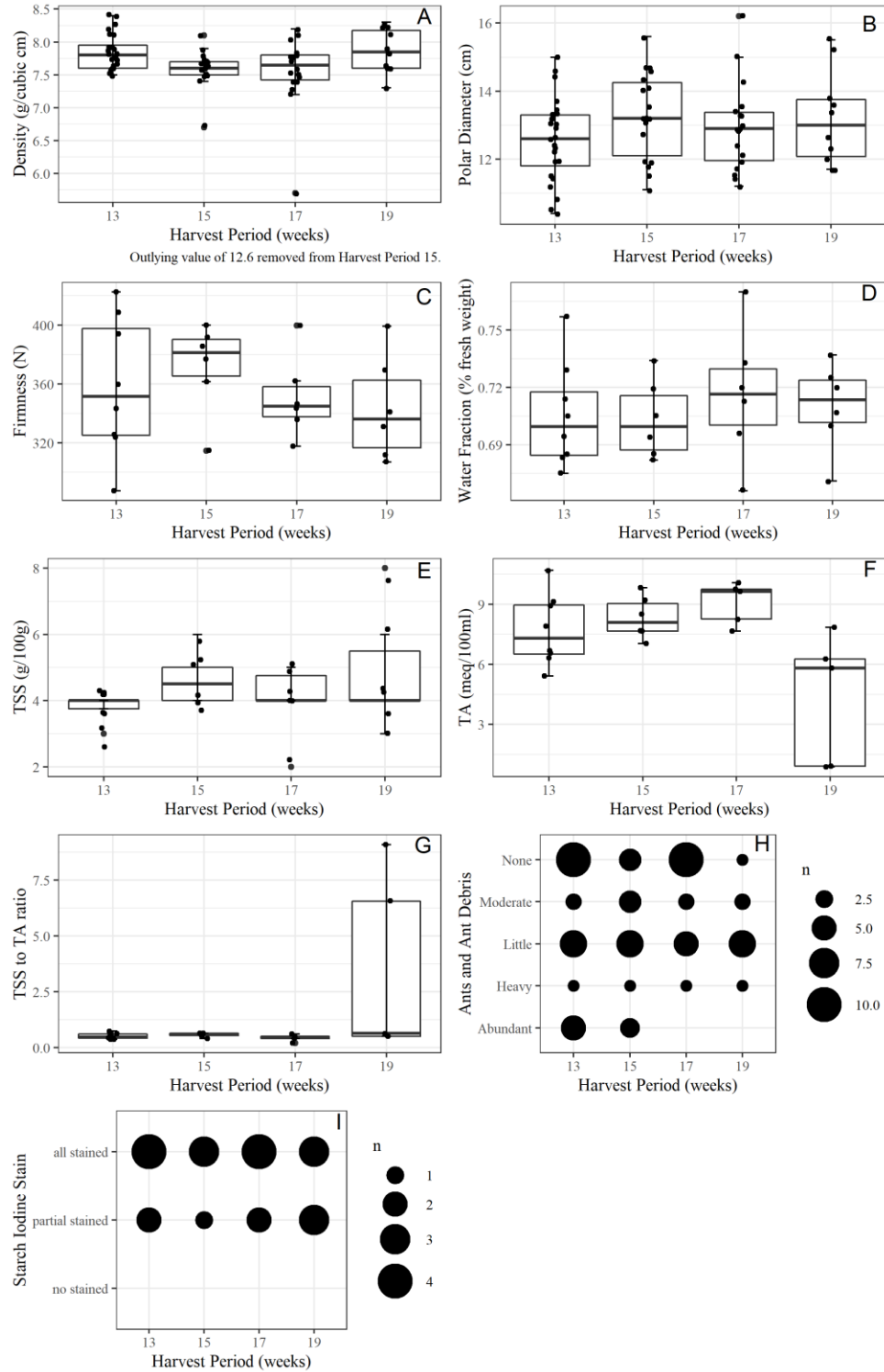


Figure 10: Relationships between Ma'afala breadfruit harvest period and quality traits that were not correlated to harvest period. Most of the uncorrelated traits were related to breadfruit's internal quality such as density (A), water fraction (D), soluble solids concentration (E), titratable acidity (F), ratio of soluble solids to titratable acidity (G), and starch content as demonstrated by starch content staining (I). The physical traits of polar diameter (B) and firmness (C) were also not correlated to harvest period. There was high variation in the presence of ant and ant debris around the peduncle of the breadfruit, but the variation was not correlated to harvest period (H).

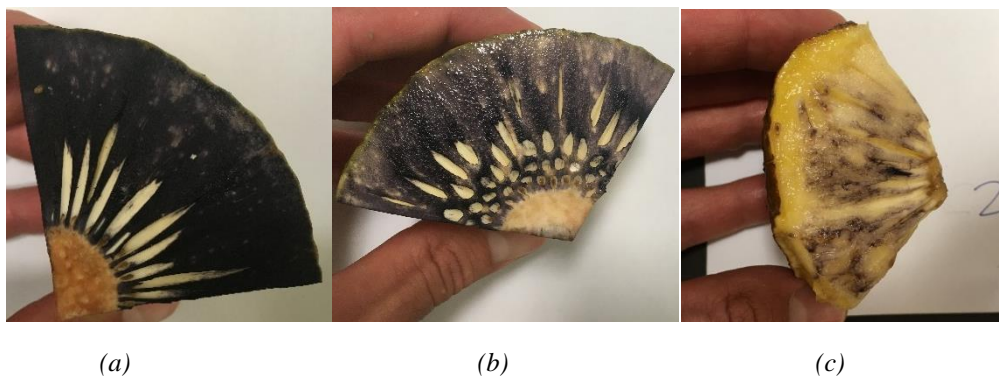


Figure 11: Variation in starch-iodine staining of partial cross-sections of Ma'afala breadfruit. Some breadfruit stained all black (a), while others had partial staining (b), or limited staining (c). Starch iodine staining indicates starch content. Breadfruit that showed little staining (c) had less starch and showed other signs of ripening such as softening and increased soluble solids content. About 60% of breadfruit stained all black (a), 35% of breadfruit had partial staining (b), and the remaining minority had little or no staining (c).

3.3. Effect of Harvest Period on Breadfruit Storage

Discoloration rate varied in correlation with harvest period, but the climacteric and softening rate did not (Figure 12). There was no difference in the timing of the climacteric, magnitude of the climacteric, or softening rate based on harvest period, regardless of treatment with 1-MCP (Figure 12). Fruit from later harvest periods discolored more rapidly than early harvest periods.

3.3.1. Discoloration Variation by Harvest Period

When discoloration was determined by green fraction, the median survival times for fruit harvested at 13, 15, and 17 weeks were 11, 7, and 6 days, respectively (Figure 12). The restricted mean survival times (RMST) for fruit harvested at 13, 15, and 17 weeks was 10.4, 7.5, and 5.0 with standard error of 0.7, 0.5, and 0.8, respectively.

When discoloration was determined by the mean a^* value of the breadfruit photo, the median survival times for fruit harvested at 13, 15, and 17 weeks were 13, 9, and 7 days, respectively (Figure 12). The RMST for fruit harvested at 13, 15, and 17 weeks was 11.8, 9.0, and 6.7 days, respectively with standard error of 0.7 for each.

When discoloration was determined by the mean point a^* value of the breadfruit, the median survival times for fruit harvested at 13, 15, and 17 weeks were 11, 11, and 8 days, respectively (Figure 12). The RMST for fruit harvested at 13, 15, and 17 weeks was 11.6, 10.5, and 7.8 days with standard error of 0.7, 1.0, and 0.9, respectively.

Ethylene was rarely perceived in the storage trials; only 19 of the 45 fruit in storage had perceivable ethylene production at any point during storage. Of these fruit, there was an approximately even distribution by harvest period (6, 6, and 7 for breadfruit harvested at 13, 15, and 17 weeks, respectively).

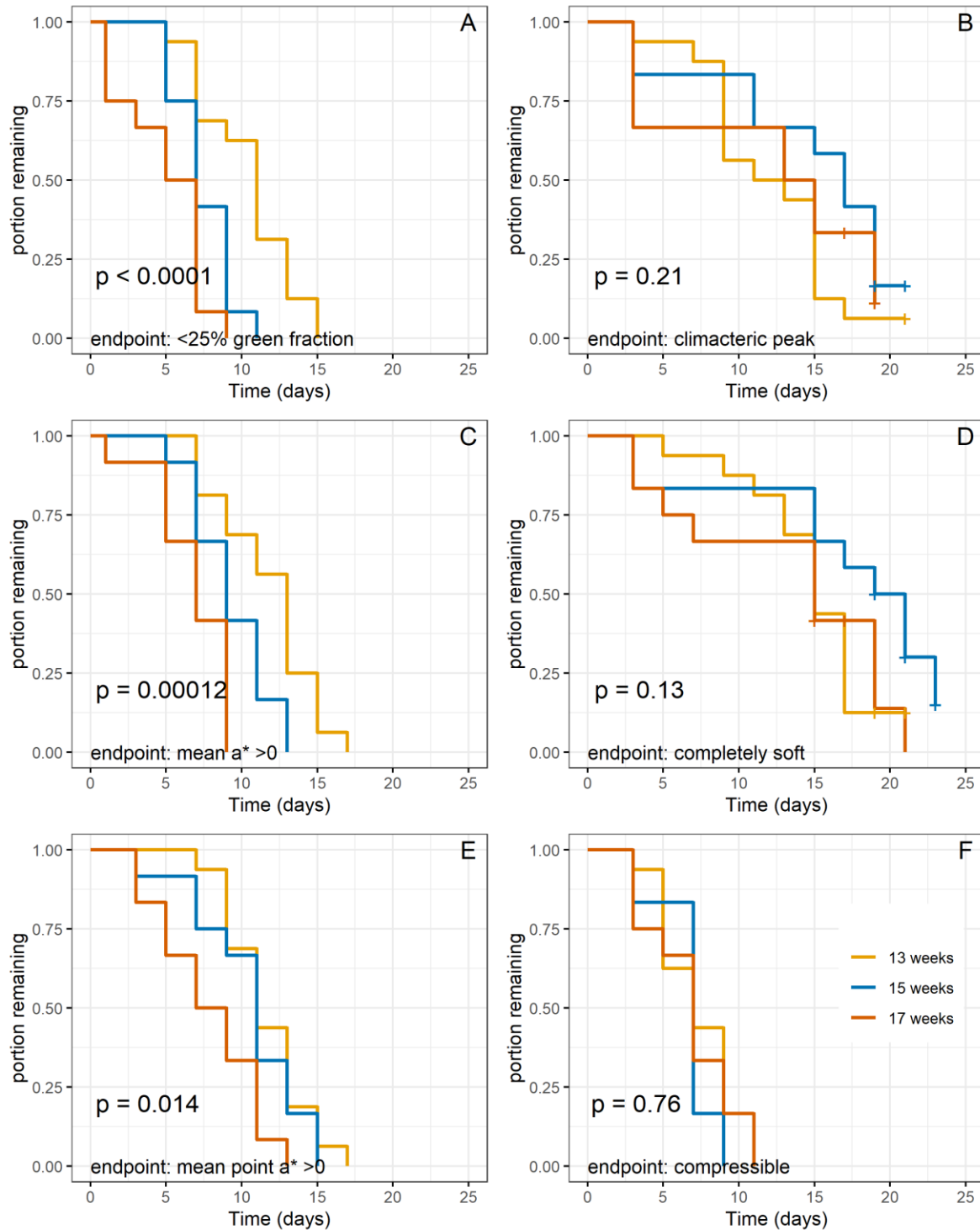


Figure 12: Relationship between harvest period and the duration of Ma'afala breadfruit storage, considering various storage endpoints. Time is the number of days after harvest. P-value is the log-rank test coefficient where $p < 0.05$ is considered significant difference in storage duration between treatments. Storage duration varied significantly when the endpoint was based on skin discoloration (A,C,E) but not when the endpoint was based on the climacteric peak or firmness (B,D,F). Skin discoloration was determined as the point when the green fraction dropped below 25% (A), the mean color a^* value rose above zero (C), and the point color a^* value rose above zero (E).

3.4. Effect of 1-MCP on Breadfruit Storage

Treatment with 1-MCP delayed softening and the climacteric and reduced variation in time until the climacteric and softening. However, 1-MCP did not delay discoloration (Figure 13).

3.4.1. 1-MCP Effect on Climacteric Peak

Breadfruit treated with 1-MCP took longer to reach their climacteric peak compared to breadfruit not treated with 1-MCP (Figure 13). Median survival (50% fruit remaining) for treated and untreated fruit was 16 and 9 days, respectively. Survival range was 9 to 19 days (10 days) and 3 to 17 days (14 days) for treated and untreated, respectively. RMST for treated and untreated fruit were 15.9 and 9.6 days, respectively.

There was no difference in the magnitude of the climacteric peak based on 1-MCP treatment.

3.4.2. Softening

Treatment with 1-MCP delayed softening in breadfruit (Figure 13). Median survival based on complete softness was 17 and 14 days for treated and untreated, respectively. Survival range was 13 to 23 days (10 days) and 3 to 21 days (18 days) for treated and untreated, respectively. RMSTs for treated and untreated fruit were 18.4 and 11.3 days, respectively.

Some of the fruit treated with 1-MCP (6 of 22) showed an irregular softening pattern. In these fruit, the flesh immediately below the surface remained firm while the outer surface softened. In most fruit, the softening of the outer surface coincided with softening of the entire fruit.

3.4.3. Compressibility

Treatment with 1-MCP delayed breadfruit becoming compressible (Figure 13). Median survival based on compressibility was the same for both treatments (7 days), but the overall survival curves for the two treatments were significantly different (log rank p-value <0.01). Variation was similar for both treatments having a range of 5 to 11 days (6 days) and 3 to 9 days (6 days) for treated and untreated fruit, respectively. RMSTs for treated and untreated fruit were 8.6 and 6.9 days, respectively.

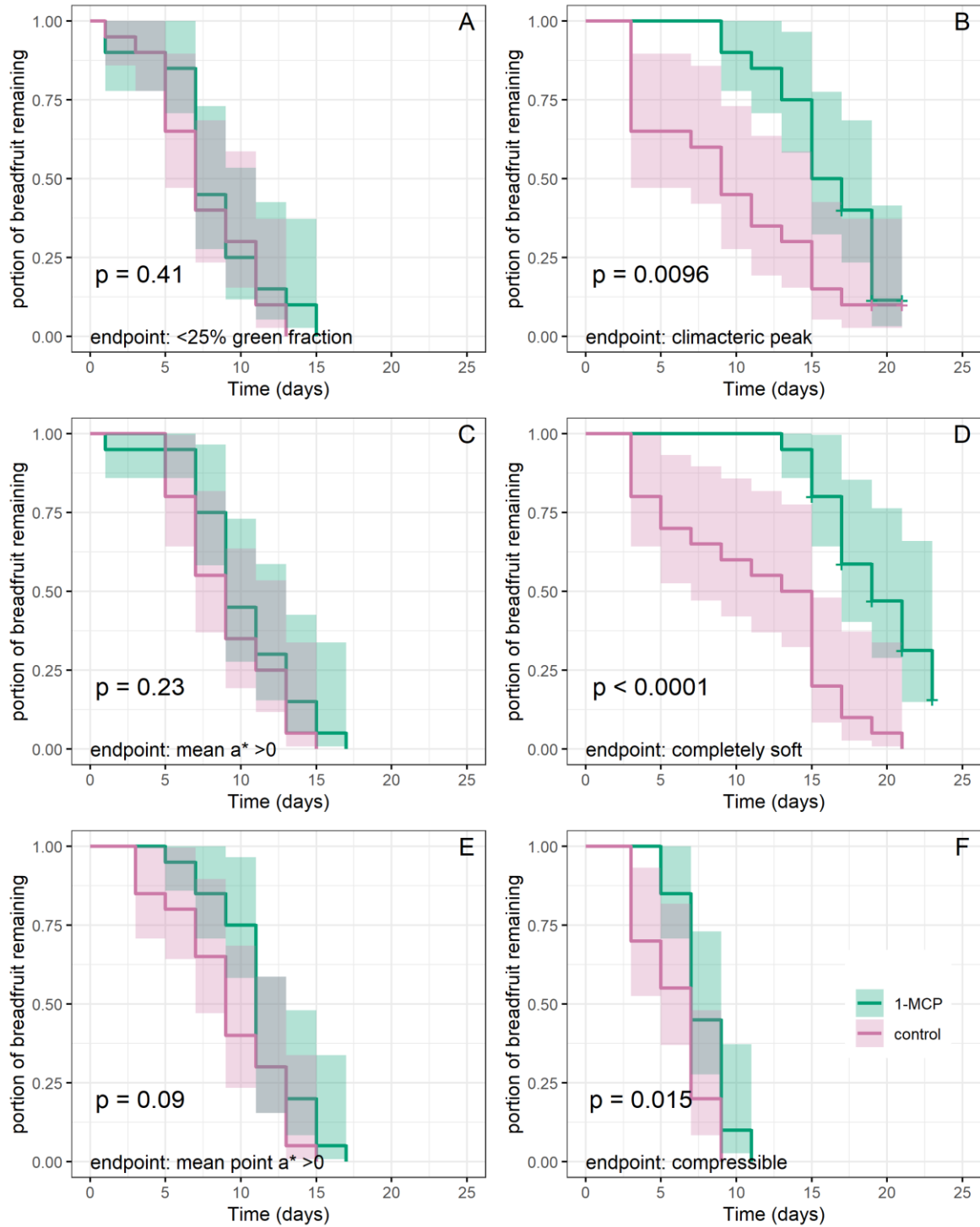


Figure 13: Relationship between 1-MCP treatment and the duration of Ma'afala breadfruit storage, considering various storage endpoints. Breadfruit in the 1-MCP treatment group were treated with 1 ppm active ingredient 1-MCP for 20 hours on the harvest day. Time is the number of days after harvest. P-value is the log-rank test coefficient where $p < 0.05$ is considered significant difference in storage duration between treatments. Treatment with 1-MCP affected the timing of the climacteric peak and softening (B,D,F) but did not affect the time until discoloration (A,C,E). Skin discoloration was determined as the point when the green fraction dropped below 25% (A), the mean color a^* value rose above zero (C), and the point color a^* value rose above zero (E).

3.4.4. Ethylene

Ethylene was rarely perceived in the storage trials, being perceived in only 19 of the 45 fruit observed during storage. Of these fruit, there was an approximately even distribution between 1-MCP treatment groups (9 treated fruit and 10 control fruit). When ethylene was perceived, it was always on the day of the respiration peak or prior. Ethylene was never perceived after the day of the respiration peak. In fruit treated with 1-MCP, the ethylene peak tended to precede the climacteric peak, whereas in fruit not treated with 1-MCP, the ethylene peak and respiration peak tended to coincide. The difference in lag time between ethylene peak and respiration peak was significantly different between treated and untreated groups (omitting one specimen with indiscernible CO₂ peak, ANOVA $p = 0.003$, 4 days mean separation by HSD).

4. DISCUSSION

4.1. Climacteric Patterns

The peak respiration rate of 500 mg CO₂/kg/hr was similar to the peak rate in the Caribbean white-flesh breadfruit, but no relationship between maturity and respiration peak was observed as reported in the Caribbean white-flesh breadfruit (Worrell et al., 1998). A maximum respiration rate of 2025 mg CO₂/kg/hr was observed in one fruit, 5.7 standard deviations away from the average. This outlier may indicate that breadfruit briefly reach a climacteric peak much higher than the average peak observed during the study.

Ethylene rate varied between 166 and 1965 nl/kg/hr, which is similar to the previously reported rates (Worrell et al., 1998). Most of the breadfruit did not have perceivable ethylene production. When ethylene was perceived, it tended to only be perceivable for a single observation point. Since the study only measured ethylene every other day, in fruit that had no perceived ethylene peak, the ethylene peak may have occurred between measurements and thus not been observed.

4.2. Maturity Index

Breadfruit at 15 weeks of development had reached a steady internal state and size. After 15 weeks, there was no change in internal traits or most physical traits suggesting that the fruit was fully developed by 15 weeks. Thus, 15 weeks can be considered the maturity point. Fifteen weeks was also reported as the maturity point for the Caribbean white-flesh breadfruit cultivar (Nacitas Latchoumia et al., 2014; Worrell et al., 1998).

Identifying a maturity index for breadfruit implies identifying an easily observable trait that changes in correlation with the onset of maturity (Reid, 2002). Peduncle color, skin color, and intersegment space color meet this requirement since they can be nondestructively observed and change in correlation with the onset of 15 weeks of maturity. Peduncle color, however, was poorly defined in this study and its variation was not precisely observed. Skin color and intersegment space color showed clear variation that was precisely measured, so these traits were subjected to further analysis as indicators for a maturity index.

Recursive partitioning was used to simulate sorting breadfruit using a maturity index based on skin color and intersegment space color. Traits related to skin color and intersegment space color were trialed as predictor variables in recursive partitioning models, and the most accurate models were identified (Table 6, Table 7). The recursive partitioning models sorted breadfruit into two harvest period groups,

13-weeks or 15-weeks and beyond. Skin green fraction, skin mean CIELAB values, and skin mean point CIELAB values partitioned fruit into the two harvest periods with 75 to 86% accuracy. Skin green fraction, mean L^* value, and mean point a^* value all classified fruit with an accuracy of 85% or higher. Combining two color indicators increased the classification accuracy, for example classification based on L^* and b^* mean skin color resulted in 94% accuracy. Intersegment space color alone partitioned the fruit with 90% accuracy, and intersegment space color paired with a second color trait increased classification accuracy up to 96%.

Polygon diameter and starch content had been previously identified as a maturity index for the Caribbean white-flesh cultivar but were not ideal indicators for Ma'afala breadfruit (Nacitas Latchoumia et al., 2014; Worrell et al., 1998). Diameter was a weak indicator due to large variation only partially attributed to maturity. Starch content had large variation and its central tendency only slightly changed between harvest periods.

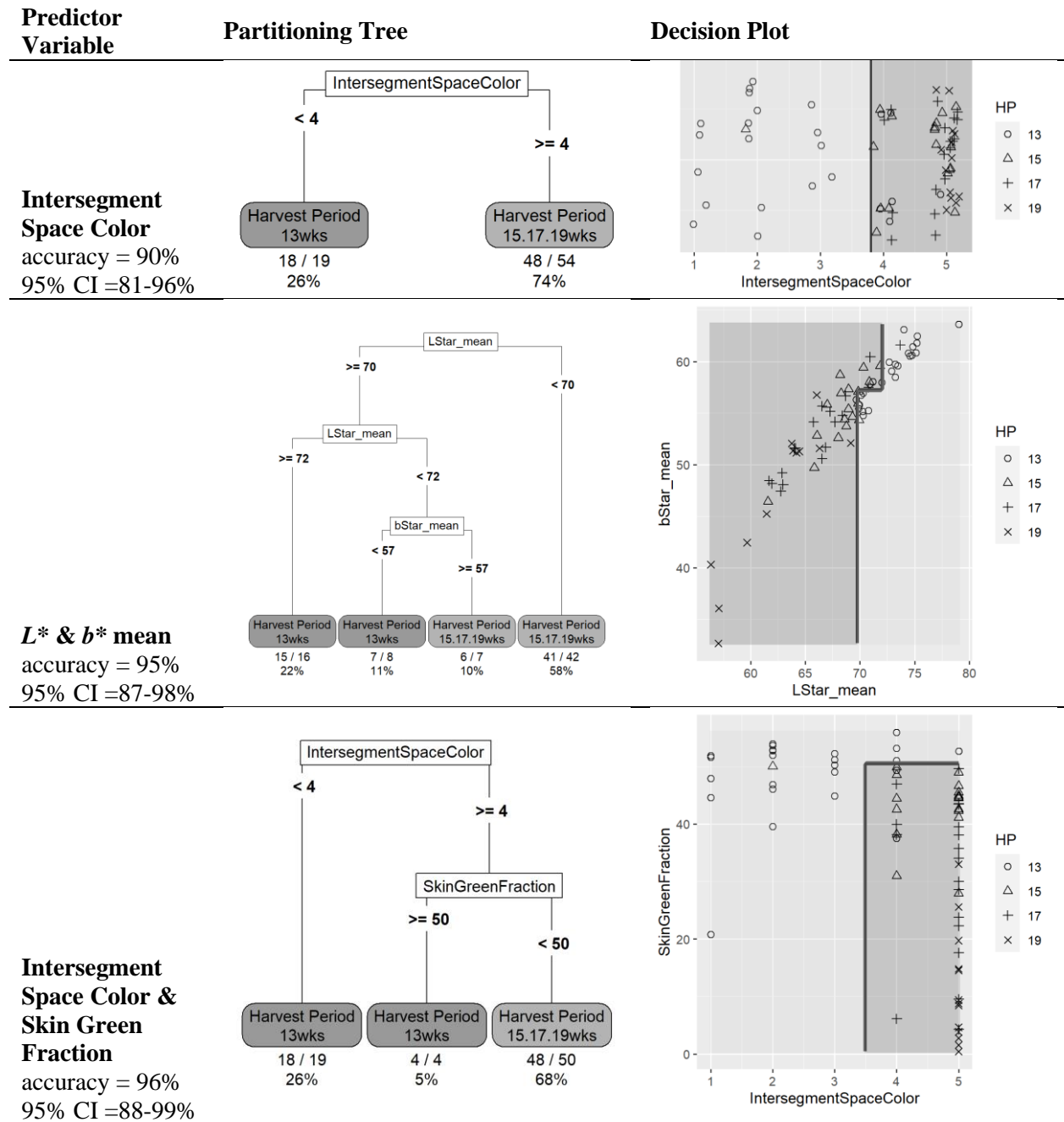
Internal traits of the breadfruit were not useful in differentiating between harvest periods. Of the internal traits measured, only starch was correlated to harvest period and only a slightly increased between 13 and 15 weeks. The TSS:TA ratio was significantly different at 19 weeks due to a few harvested fruit which seemed to have started ripening prior to harvest; these fruit showed a dramatic rise in TSS and decrease in TA, typical of ripening fruit. Some of the fruit in which TSS:TA ratio indicated ripening were not yet soft, suggesting that internal chemical changes associated with ripening preceded physical softening.

Table 6: Accuracy of classification into 13-week harvest period based on select predictor traits.

Trait(s) Used as Predictor Variable(s)	Classification Accuracy (%)	95% CI Lower (%)	95% CI Upper (%)
<i>L*</i> mean	88	78	94
<i>a*</i> mean	84	73	91
<i>b*</i> mean	75	64	85
<i>L*</i> point	79	68	88
<i>a*</i> point	85	75	92
<i>b*</i> point	77	65	85
Intersegment Space Color	90	81	96
Skin Green Fraction	86	76	93
Skin Latex Fraction	74	62	84
<i>L*</i> & <i>a*</i> mean	93	85	98
<i>L*</i> & <i>b*</i> mean	95	87	98
<i>a*</i> & <i>b*</i> mean	90	81	96
<i>L*</i> & <i>a*</i> point mean	90	81	96
<i>L*</i> & <i>b*</i> point mean	82	71	90
<i>a*</i> & <i>b*</i> point mean	85	75	92
Intersegment Space Color & <i>L*</i> mean	92	83	97
Intersegment Space Color & <i>a*</i> mean	95	87	98
Intersegment Space Color & <i>b*</i> mean	92	83	97
Intersegment Space Color & Skin Green Fraction	96	88	99
Skin Green Fraction & <i>L*</i> mean	95	87	97
Skin Green Fraction & <i>a*</i> mean	86	76	93
Skin Green Fraction & <i>b*</i> mean	90	81	96

Bolded entries have classification accuracy of 90% or greater.

Table 7: Repeated partitioning figures for select predictor traits.



Notes:

- HP = Harvest Period, referring to the number of weeks after flowering when the breadfruit were harvested.
- Accuracy percentage refers to the percent of breadfruit predicted to be in the same harvest period as their actual harvest period.
- The numbers at the base of the partitioning trees indicate the number of correctly sorted items out of total items sorted into that category and the percent of the items sorted into that category as a portion of the whole. For example, 18/19 indicates that 18 of the 19 fruit predicted to be in the harvest group were actually in that group.
- A grade of 4 for intersegment space color indicates that more than half of the intersegment spaces are brown.
- Shading in the decision plot indicates the 15-, 17-, or 19-week harvest period.

4.3. Storage

4.3.1. *Harvest Period Effect on Storage*

The study did not identify a correlation between harvest period and most aspects of postharvest quality, contrasting the typical trend for harvest maturity to be an important determinant of postharvest quality (Toivonen & Beveridge, 2005). This conclusion was limited, however, because harvest period was not a perfect proxy for maturity and only a few aspects of postharvest quality were measured.

Discoloration rate was the only aspect of postharvest storage quality affected by harvest period. The amount of time until discoloration decreased in later harvest periods. This trend was complicated by the starting color of the breadfruit; breadfruit harvested at later harvest periods tended to be less green at the time of harvest. Discoloration was thought to be associated with water loss from the epidermis and polyphenol oxidase being brought into contact with phenols resulting in oxidation to polyphenols (Maharaj & Sankat, 1990; Worrell & Carrington, 1997). So, more rapid discoloration at later harvest periods may be the combined effect of being less green at the time of harvest and being more physiologically prone to discoloration, perhaps due to the epidermis being more prone to damage and water loss.

4.3.2. *Effect of 1-MCP on Storage*

Treatment with 1-MCP delayed softening and delayed the climacteric among treated fruit. Since softening and the climacteric indicate ripening, 1-MCP can be considered suitable for delaying ripening in breadfruit. However, treatment with 1-MCP did not delay discoloration which may limit 1-MCP's suitability as a commercial technology for improving breadfruit's postharvest quality.

Discoloration did not show similar patterns compared to ripening events like softening and discoloration and tended to precede ripening, especially in fruit treated with 1-MCP. This supports previous findings that breadfruit tends to discolor prior to ripening and that discoloration is thought to be associated with water loss instead of an ethylene-mediated ripening response (Maharaj & Sankat, 1990; Worrell & Carrington, 1997). While some skin discoloration may be acceptable to the consumer, failure of 1-MCP to retard discoloration undermines its usefulness as a postharvest quality maintenance technology for breadfruit (Molimau-Samasoni et al., 2020).

An irregular softening pattern was observed in some breadfruit treated with 1-MCP and was an additional weakness in the case for 1-MCP as a postharvest quality maintenance technology for breadfruit. The partial softening of the fruit underscores that 1-MCP may alter normal ripening in undesirable ways. While 1-MCP brought about desirable effects of delayed ripening and reduced variation in the timing of ripening, its effect on eating quality, texture, and other commercially-important traits were not considered in this study.

4.3.3. *Combined Effect of 1-MCP and Harvest Period on Storage*

Timing of the climacteric did not change based on the harvest period, regardless of treatment with 1-MCP. However, a rudimentary comparison of the timing of the climacteric based on other proxies for maturity suggested there may be a variable response to 1-MCP based on maturity. Using intersegment space color as a proxy for maturity and comparing it with the timing of the climacteric shows that fruit with more brown intersegment spaces had more delay from 1-MCP compared to fruit with mostly green intersegment spaces ($p=0.02$ by RMST analysis). However, this conclusion was only weakly supported

due to unequal treatment groups and very small number of fruit with green intersegment spaces. Fruit with half or more green intersegment spaces (n=4) had an RMST of 12.5 days while fruit with mostly or completely brown intersegment spaces (n=16) had an RMST of 16.9 days. If fruit with more brown intersegment spaces are assumed to be mature fruit, this finding suggests that mature fruit have a greater response to 1-MCP compared to immature fruit and challenges the previous conclusion that maturity does not affect ripening in fruit treated with 1-MCP.

5. CONCLUSION

5.1. Maturity Index

Color and intersegment space color were useful for identifying maturity in Ma'afala breadfruit. Color changes were, however, subtle. While home breadfruit growers may be able to distinguish by eye the color of a mature breadfruit, a commercial breadfruit operation may benefit from a color sensing technology for measuring the color associated with a mature breadfruit. The point colorimeter and the Canopeo app were a few low cost, field appropriate color sensing technologies that were suitable to measure skin color for a maturity index. Intersegment space was a simple and precise indicator of maturity and can be used as a maturity index without any color sensing technologies.

Since the color of skin and intersegment spaces varies in other breadfruit varieties, the proposed color-based maturity index was specific to Ma'afala breadfruit.

5.2. Storage

Fruit harvested in later harvest periods discolored almost twice as fast as fruit harvested in the earliest harvest period but did not show significant changes in the time until their climacteric peak or softening. Treatment with 1-MCP delayed the climacteric peak and softening regardless of harvest period, but did not affect the discoloration rate of the fruit. Discoloration seems to be a separate process from ethylene-mediated ripening because it was not affected by treatment with 1-MCP and did not align with changes in the climacteric peak. Softening, however, seemed to be an ethylene mediated process since its changes aligned with changes in the climacteric peak and softening was delayed by treatment with 1-MCP.

Picking breadfruit at an early harvest maturity may be beneficial in delaying discoloration, and 1-MCP can be useful for delaying softening.

Correlation of harvest traits exluding destructively analyzed traits (n=70)

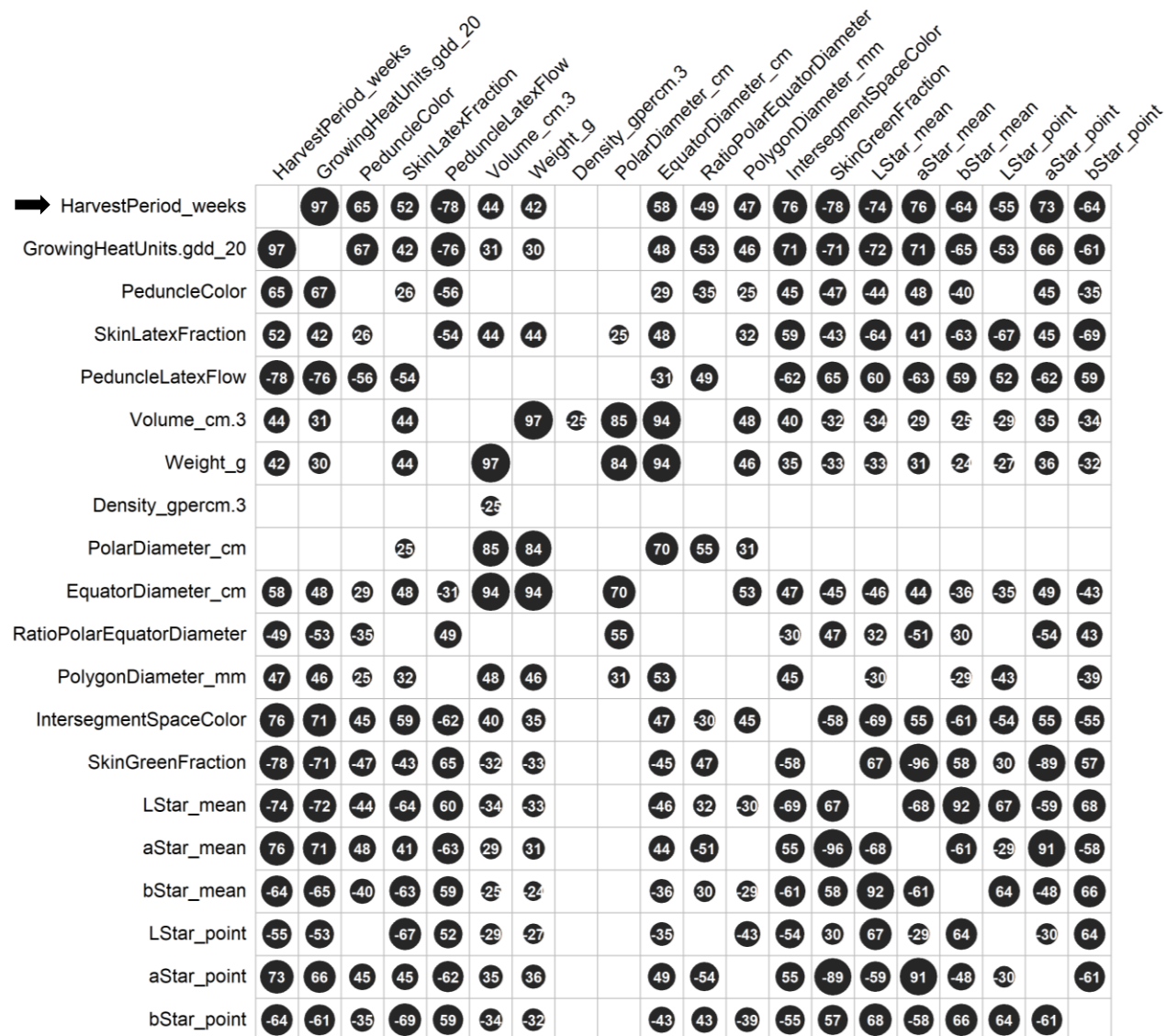
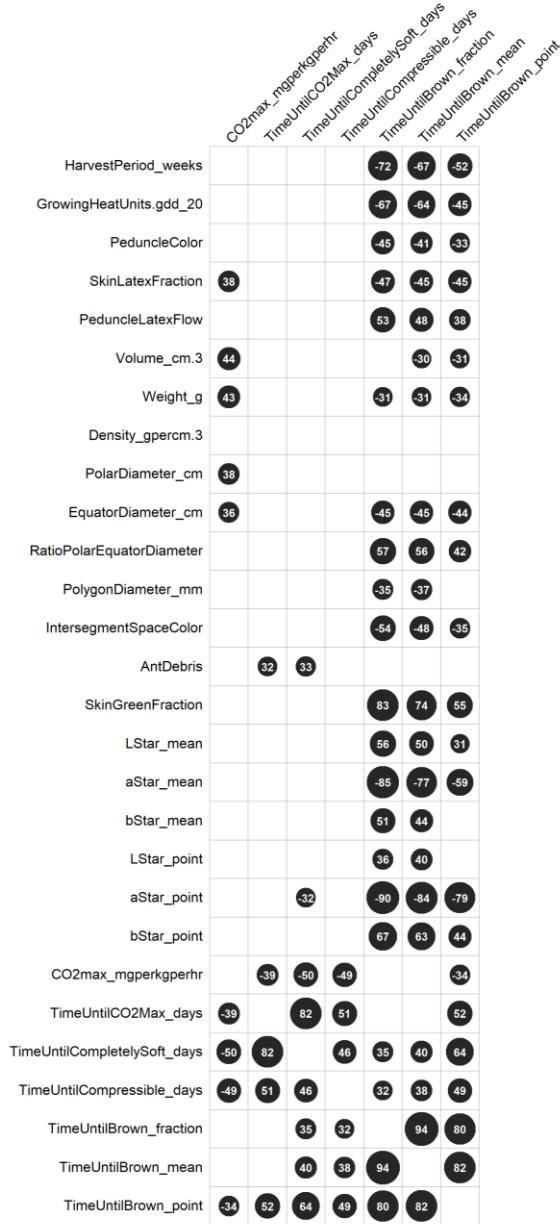


Figure 14: Correlogram of spearman rank correlation coefficients (ρ) for harvest and storage traits of Ma'afala breadfruit harvested at distinct harvest periods in the final stages of development. Coefficients are given for correlations with p -value > 0.05 .

Correlation of storage traits with harvest and storage traits (n=44)



A

Correlation of destructively analyzed harvest traits with other harvest traits (n=26)

B

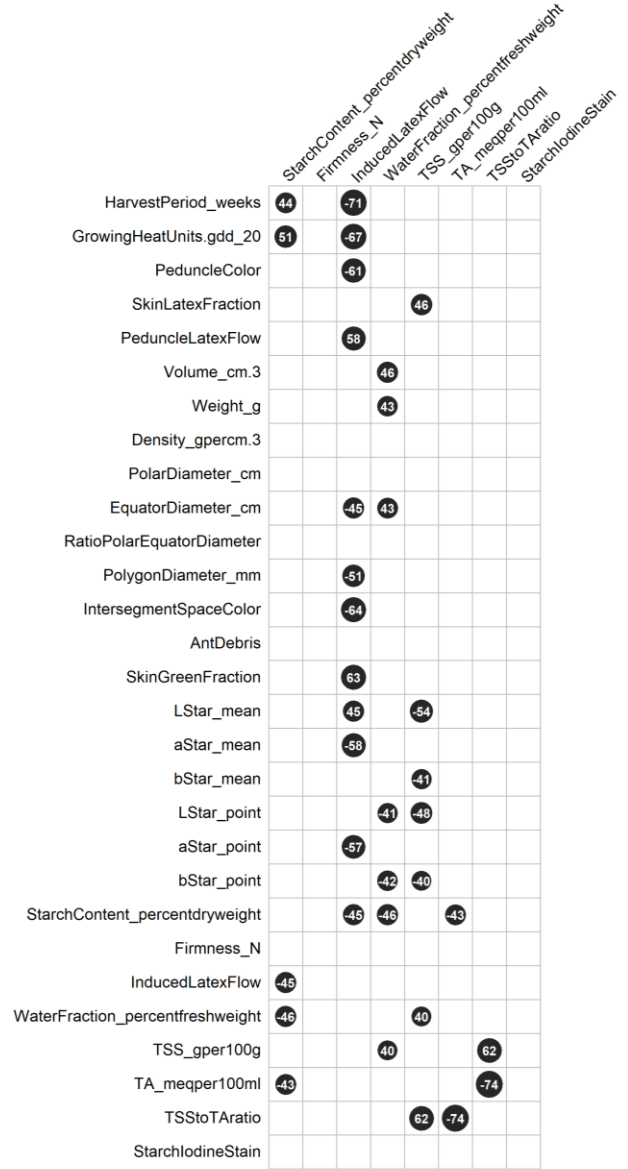


Figure 15: Correlograms of spearman rank correlation coefficients (ρ) showing correlation between harvest traits and storage traits (A) and destructively analyzed harvest traits (B) of Ma'afala breadfruit harvested at distinct harvest periods in the final stages of development. Coefficients are given for correlations with p -value > 0.05 .

APPENDIX B: SUMMARY GRAPHS OF RESPIRATION AND ETHYLENE PRODUCTION

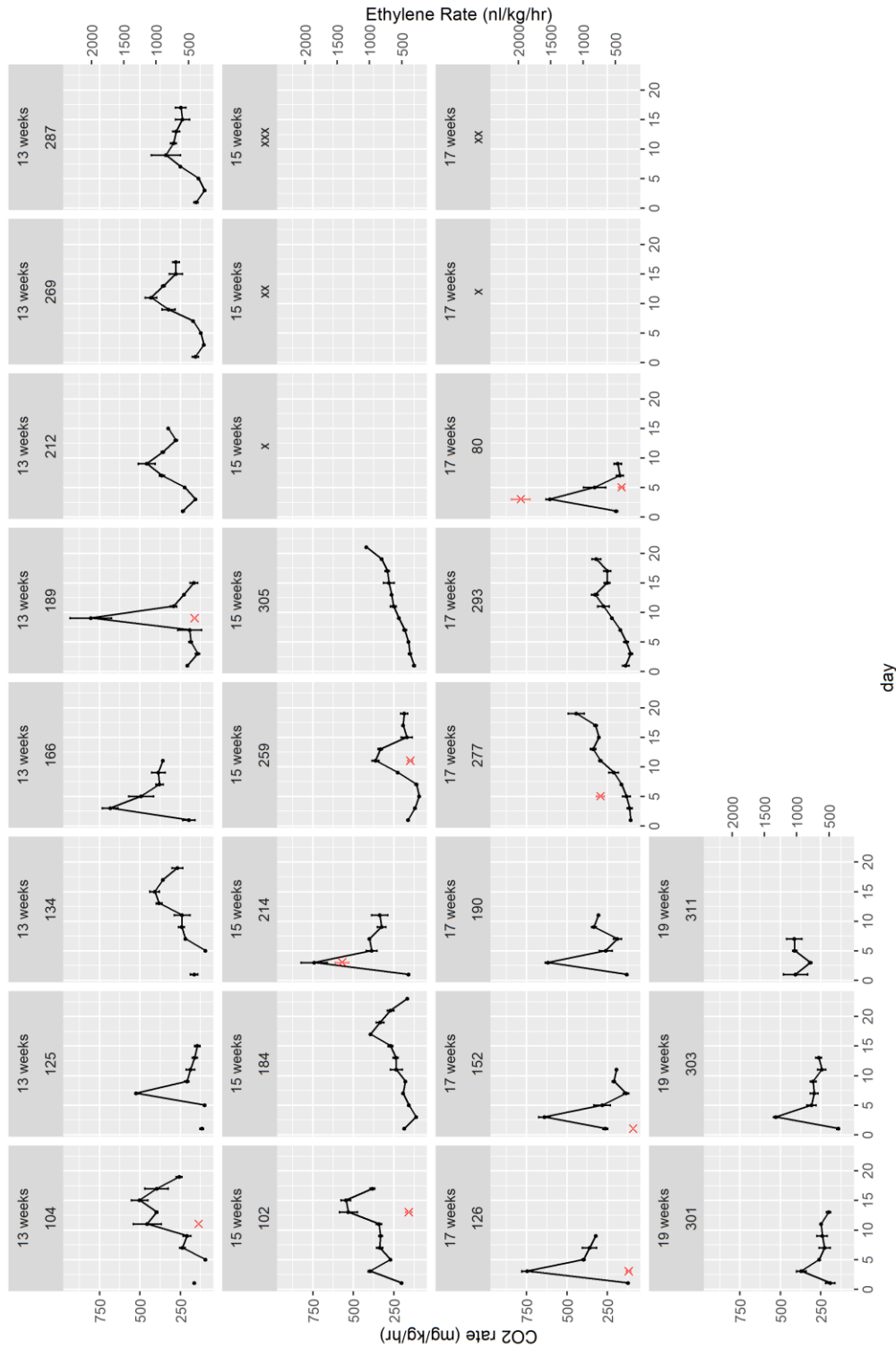


Figure 16: Respiration and ethylene production patterns in stored Ma'afala breadfruit harvested in four different harvest periods. Harvest period is indicated by weeks after flowering. Respiration rate is represented in black and ethylene production is represented in red. Some breadfruit had no ethylene production perceived. Ethylene rates less than 400 nl/kg/hr were not reliably sensed by the experimental procedure.

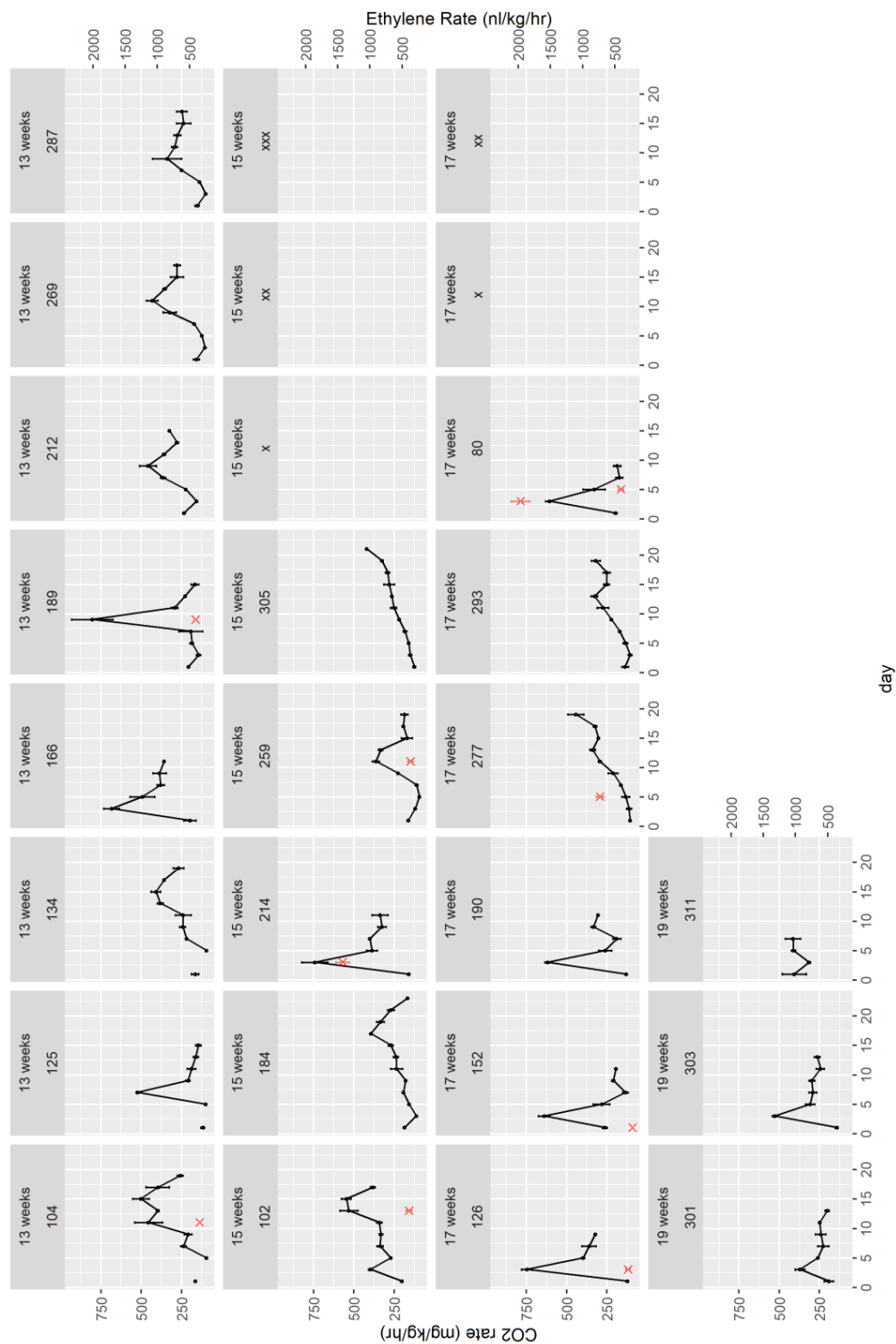


Figure 17: Respiration and ethylene production patterns in stored Ma'afala breadfruit harvested in four different harvest periods and treated with 1 ppm active ingredient 1-MCP for 20 hours on their harvest day. Harvest period is indicated by weeks after flowering. Respiration rate is represented in black and ethylene production is represented in red. Some breadfruit had no ethylene production perceived. Ethylene rates less than 400 nl/kg/hr were not reliably sensed by the experimental procedure.

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