

I_{AD} Values in Relation to Physiological Storage Disorders of ‘Honeycrisp’ Apples

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Abstract. The ‘Honeycrisp’ apple is highly prone to developing physiological disorders such as soft scald, bitter pit, and core browning during storage, but disorder incidences are affected by fruit maturity stage. To investigate relationships between fruit maturity and physiological disorders, a difference of absorbance meter was used to separate fruit into index of absorbance difference (I_{AD}) value categories with the objective of reducing fruit-to-fruit variability within a population of fruit harvested at any one time. Fruit from two harvest dates, 10 days apart, were separated by I_{AD} values and stored at 0.5 or 3 °C. The internal ethylene concentration (IEC) of fruit during storage and the incidence of physiological disorders after 18 weeks were assessed. The results indicate that earlier harvest, colder storage temperature, and lower I_{AD} values (less green fruit) were associated with lower IECs during storage. The incidences of soft scald, bitter pit, and core browning, as well as superficial scald, were affected by I_{AD} values within each harvest date and storage temperature, and more precisely than an evaluation of bulked fruit samples. This study highlights the potential of categorizing fruit by I_{AD} values as a tool to minimize variation of fruit within a population and therefore increase sensitivity of investigations into factors affecting storage performance.

Harvest of fruit at the optimal time for its intended use is a critical part of many apple industries, especially those based on medium- to long-term storage (Little and Holmes 2000; Watkins 2017). Fruit maturity and quality are typically assessed by measuring the following at harvest: internal ethylene concentration (IEC), starch pattern index (SPI), flesh firmness, soluble solids concentration (SSC), and titratable acidity (TA). Background color has been used for bicolor apples such as ‘Gala’ (Brookfield et al. 1993); however, the pressure on the industry to use high-red-color sports has resulted in fruit with very-high-red coverage. As a result, the use of background color cards

is difficult. The development of the difference of absorbance (DA) meter has overcome the limitations of background color charts. The DA meter produces an index of absorbance difference between 670 and 720 nm that is known as the I_{AD} value. This value is an indirect measure of chlorophyll concentration in the skin, with higher values indicating greater chlorophyll concentrations. Strong associations between I_{AD} values and chlorophyll have been found (DeLong et al. 2014, 2020; Toivonen and Hampson 2014), although not always (Moran et al. 2020). The most common use of the DA meter has been to add an additional harvest index for determining fruit maturity along with the IEC and SPI, as well as the quality indices, flesh firmness, TA, and SSC (DeLong et al. 2014, 2020; Doerflinger et al. 2016; Moran et al. 2020; Mostofi and DeEll 2024; Nyasordzi et al. 2013; Serra et al. 2016). All of these cited studies have used bulked apple samples harvested over time.

Less well investigated is the separation of fruit into I_{AD} value categories as a method to reduce variability of samples compared with a typical harvest protocol based on bulked samples. Such separation can be used to investigate relationships between skin color and other physiological factors with greater

precision. To date, research has focused on ethylene production, flesh firmness, and the SPI (Cocetta et al. 2017; Moran et al. 2020; Nyasordzi et al. 2013; Sjöstrand et al. 2024; Williamson et al. 2018), and ethylene responses to fruit exposed to low storage temperatures (Cai et al. 2023). However, the relationships between I_{AD} value categories and the susceptibility of fruit to physiological disorders has been limited to superficial scald (Farneti et al. 2015) and soft scald (Al Shoffe et al. 2018; Moran et al. 2020).

In our study, we used the DA meter to separate ‘Honeycrisp’ fruit into different I_{AD} value categories at two harvest dates. Our primary objective was to investigate differences in the susceptibility of fruit to physiological disorders based on these categories that are not revealed when using bulked samples. We chose the cultivar Honeycrisp because the fruit has well-known responses to different storage temperatures; it is susceptible to chilling injuries (CIs) such as soft scald, superficial scald, and core browning at temperatures close to 0 °C, and bitter pit at warmer temperatures such as 3 °C (Al Shoffe et al. 2020; Moran et al. 2020). To maximize the susceptibility of fruit to low-temperature-related disorders, fruit were not conditioned before storage (10 °C for 7 d) (Watkins et al. 2004), which is a standard commercial practice to reduce the risk of soft scald development of ‘Honeycrisp’ apples.

Materials and Methods

‘Honeycrisp’ apple fruit (*Malus × domestica* Borkh) were obtained from trees planted in 2000, grafted on M.9 rootstock at the Cornell Orchard (Ithaca, NY, USA) on 3 Sep [harvest 1 (H1)] and 13 Sep [harvest 2 (H2)] 2014. The dates represent the commercial harvest window for the cultivar at this location. All fruit from six trees were harvested and bulked to obtain the maximum number of fruit per I_{AD} value category, and they were then separated based on I_{AD} values using a DA meter (TR Turoni Srl, Forlì, Italy). The I_{AD} value of each fruit was measured on the blushed and nonblushed side, and the average was taken to sort into categories of 0 to 0.20, 0.21 to 0.40, 0.41 to 0.60, 0.61 to 0.80, 0.81 to 1.00, 1.01 to 1.20, 1.21 to 1.40, and 1.41 to 1.60. The categories with sufficient fruit numbers to allow measurement of the IEC at harvest, sampling during storage, and evaluation of disorder incidence were chosen for further experimentation.

The IEC of five fruit per category was measured by taking gas samples from each apple using a 1-mL syringe. Gas samples were injected into a Hewlett-Packard 5890 series II gas chromatograph (Hewlett-Packard, Wilmington, DE, USA) equipped with a flame ionization detector and an alumina column (1.5 m × 3 mm). Gas analyses were conducted isothermally at 160 °C. Nitrogen carrier, air, and H₂ flows were 45, 400, and 45 mL·min⁻¹, respectively. The injector and detector were kept at 230 and 245 °C, respectively.

The remaining fruit were stored in air at 0.5 or 3 °C. The IEC of five fruit per category and the temperature was measured as described

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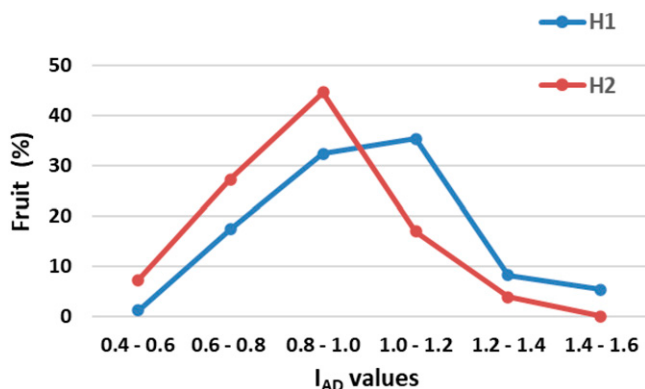


Fig. 1. The distribution of fruit in each index of absorbance difference (I_{AD}) value category at first (H1) and second (H2) harvest. The total number of fruit harvested at H1 and H2 was 484 and 620, respectively.

Table 1. The average and range of internal ethylene concentrations of 'Honeycrisp' fruit in each index of absorbance difference value category at harvests 1 and 2.

I_{AD} value	Harvest 1 IEC ($\mu\text{L}\cdot\text{L}^{-1}$)		Harvest 2 IEC ($\mu\text{L}\cdot\text{L}^{-1}$)	
	Avg	Range	Avg	Range
0.41–0.60	0.566	0.079–1.668	0.575	0.293–0.942
0.61–0.80	0.357	0.089–0.854	0.767	0.179–2.196
0.81–1.00	0.097	0.029–0.144	2.395	0.157–5.301
1.01–1.20	0.155	0.032–0.432	0.082	0.051–0.106
1.21–1.40	0.095	0.025–0.282	0.061	0.043–0.112
1.41–1.60	0.033	0.033–0.113	—	—

I_{AD} = index of absorbance difference; IEC = internal ethylene concentration.

earlier at 1, 2, 3, 4, 6, 8, 10, 14, and 18 weeks while still cold. At week 18, all remaining fruit were cut equatorially at least five times from the calyx end to the stem end of the fruit. External and internal disorders were calculated based on the percentage of the total fruit assessed.

Statistical analysis. Tukey's honestly significant different (HSD) test, Student's t test, and the least significant difference (LSD) were used to compare means at the 5% confidence level. Means and standard errors were used to present data in figures. Principal component

analysis (PCA) was used to visualize the effects of harvest date, I_{AD} category, and storage temperature on fruit quality and incidences of disorders after storage. The eigenvectors, a special set of vectors associated with a linear system of equations, were used to show the correlation between PCx and the variables. The eigenvalues, which indicate the amount of variance captured by each principal component, were analyzed. Higher eigenvalues correspond to components that capture greater variance in the data. All statistics were carried out with JMP[®] Pro 15 (SAS Institute Inc., Cary, NC, USA). Percentage data were arcsine transformed for analysis and presented as back-transformed means.

The distribution of the I_{AD} data for the IEC and physiological disorders varied based on category, harvest time, storage temperature, and storage duration. Different factors affected the variance. Only the I_{AD} data for fruit percentage in the two harvests followed a normal distribution.

Results and Discussion

The distribution of I_{AD} values at the two harvest dates showed a shift toward lower values (less chlorophyll concentrations) at H2 compared with H1 (Fig. 1).

The IECs of fruit across all categories with at least five fruit were measured (Table 1). The IECs at H1 were low, averaging $0.220 \mu\text{L}\cdot\text{L}^{-1}$. There was a single fruit in the 0.41- to 0.60-unit range in which the IEC exceeded $1 \mu\text{L}\cdot\text{L}^{-1}$ (Table 1). At H2, the average IEC was $1.14 \mu\text{L}\cdot\text{L}^{-1}$, with several fruit with ethylene concentrations exceeding $1 \mu\text{L}\cdot\text{L}^{-1}$.

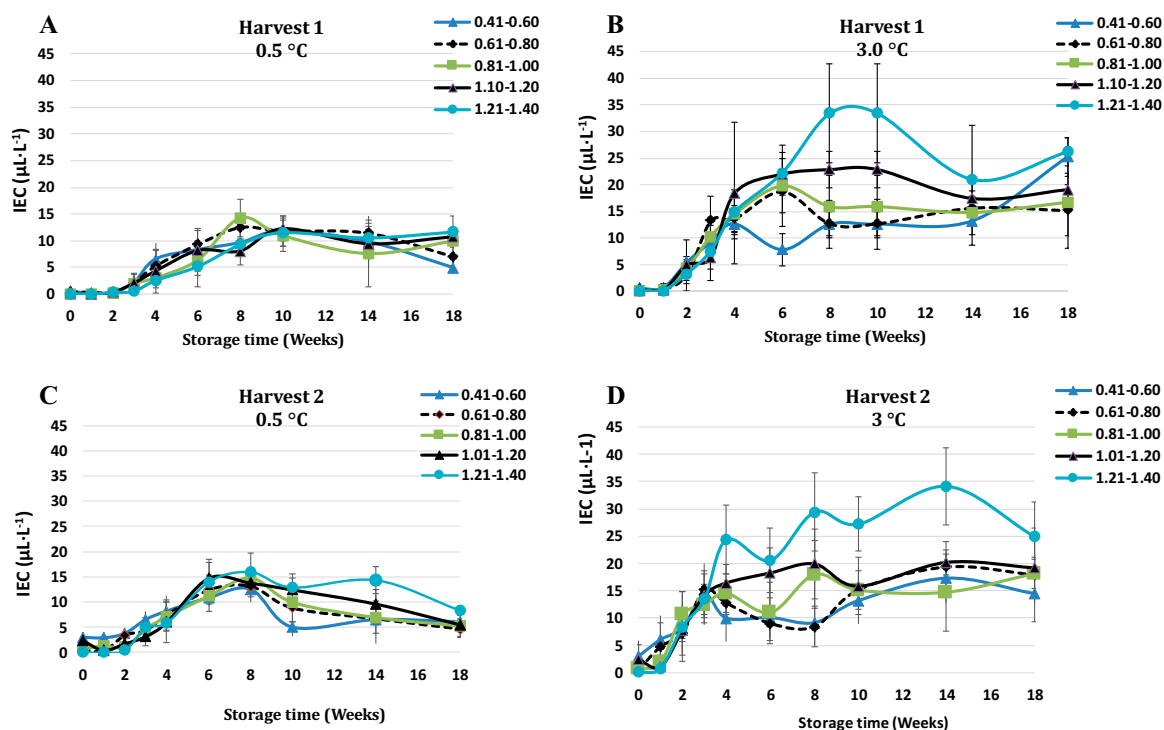


Fig. 2. The internal ethylene concentration (IEC) of 'Honeycrisp' apples harvested 10 d apart, separated into index of absorbance difference value categories and stored at 0.5 or 3 °C for up to 18 weeks. Each mean is the average of five individual apples $\pm$ the standard error.

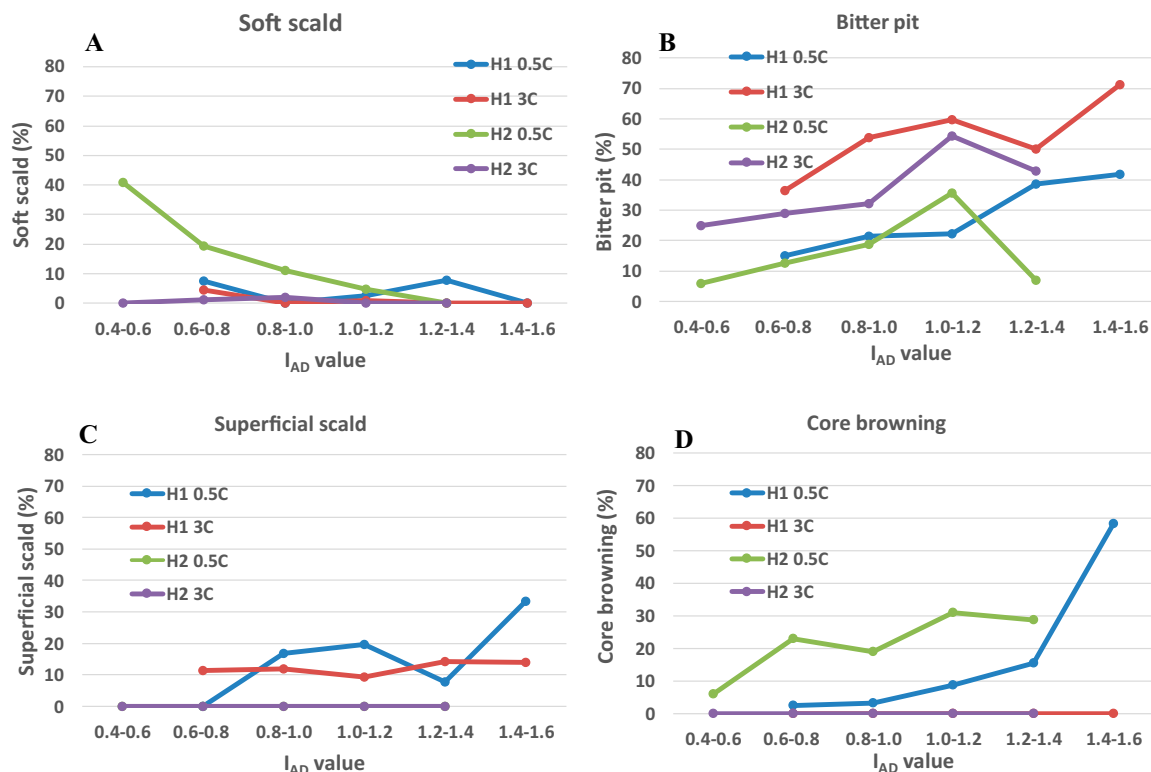


Fig. 3. The incidences of soft scald (A), bitter pit (B), superficial scald (C), and core browning (D) of 'Honeycrisp' apples harvested 10 d apart [harvest 1 (H1) and harvest 2 (H2)], separated into index of absorbance difference (I_{AD}) value categories and stored at 0.5 or 3°C for up to 18 weeks.

The categories with sufficient fruit numbers for sampling over time ranged from 0.61 to 1.60 units at H1 and 0.41 to 1.40 units at H2. During storage, the IECs of the fruit kept at 0.5°C remained low until week 3, with maximum concentrations reached at ~6 to 8 weeks, before leveling off or declining (Fig. 2A and B). Increases in the IEC were earlier in fruit from H2 than H1. There was little differentiation in IECs among I_{AD} value categories at 0.5°C. At 3°C, however, the increase in the IECs of the fruit was faster and at greater concentrations than at 0.5°C (Fig. 2A and B). Also, the differences among I_{AD} value categories at 3°C were more pronounced, with generally greater IECs in the 1.21-to-1.40 category than in the others.

Greater IECs in the least mature fruit (higher I_{AD} values) were not expected. However, this effect has also been found in 'Gala' fruit in which harvest maturity interacted with the effect of cold temperature on stimulation of ethylene production (Cai et al. 2023). The relationships between ethylene production of fruit with I_{AD} values warrant further investigation.

Separation of fruit into I_{AD} value categories revealed effects of fruit maturity and storage temperature with greater insight (Fig. 3). Soft scald is a major disorder of 'Honeycrisp' apples stored at 0.5°C. The incidence of the disorder is decreased by increasing the storage temperature with or without conditioning of the fruit at 10°C for 7 d (Al Shoffe et al. 2020; Moran et al. 2020; Watkins et al. 2004). Soft scald also

tends to be associated with later harvests (Watkins et al. 2004; Ehsani-Moghaddam and DeEll 2013). In our experiment, soft scald incidence was high in fruit only for H2 stored at 0.5°C and was clearly associated with lower I_{AD} values (Fig. 3). Soggy breakdown, a low-temperature disorder that is affected by the same factors as soft scald, was detected only in fruit in the 0.41-to-0.6 category, being 29% and 6% at H1 and H2, respectively.

In contrast, bitter pit is a major disorder of 'Honeycrisp' apples at a warmer storage temperature such as 3°C and it is further exacerbated by conditioning (Al Shoffe et al. 2020). However, in fruit from susceptible orchard blocks, the disorder will occur at all storage temperatures. Consistent with these observations, bitter pit incidence was high at each harvest date and at both storage temperatures (Table 2), with incidences at 3°C being approximately twice that at 0.5°C and tending to be associated with less mature fruit at each harvest date (Fig. 3). The susceptibility of fruit to bitter pit is usually higher in less mature fruit and at warmer storage temperatures, but is often associated with low Ca concentrations and ratios of Ca with other minerals (Ferguson and Watkins 1989). However, bitter pit incidence decreases as fruit maturity increases, as shown in 'Cox's Orange Pippen' (Perring 1986) and in 'Honeycrisp' (Prange et al. 2011) apples. Interestingly, this decrease in bitter pit incidence occurs while Ca concentrations are also decreasing as a result of increasing fruit size, indicating that

factors other than maturity are involved in the susceptibility of fruit to the disorder (Perring 1984; Watkins 2025).

Superficial scald is rarely reported in 'Honeycrisp' apples (Al Shoffe et al. 2023). However, it was found in H1 fruit stored at either 0.5°C or 3°C, and incidence was not associated consistently with I_{AD} values (Fig. 3). Although the disorder is thought to be a CI, less mature fruit stored at low temperatures tend to be the most susceptible (Lurie and Watkins 2012). We do not have an explanation for its occurrence in the fruit used in our experiment.

Core browning occurred only in fruit stored at 0.5°C. At H1, the incidence was strongly affected by I_{AD} value, with less mature fruit having an increasingly higher disorder incidence. At H2, the relationship with I_{AD} values was less pronounced (Fig. 3). In 'Honeycrisp', the disorder is associated with storage temperatures close to 0.5°C (Al Shoffe et al. 2021), and in general is

Table 2. The incidences of soft scald, bitter pit, superficial scald, and core browning in 'Honeycrisp' apples harvested 10 d apart and stored at 0.5 or 3°C for 18 weeks plus 7 d at 20°C.

Physiological disorder (%)	0.5 °C		3 °C	
	H1	H2	H1	H2
Soft scald	0.3	15	1	1
Bitter pit	23	18	50	34
Superficial scald	15	0.4	11	0
Core browning	9	20	0	0

H1 = harvest 1; H2 = harvest 2.

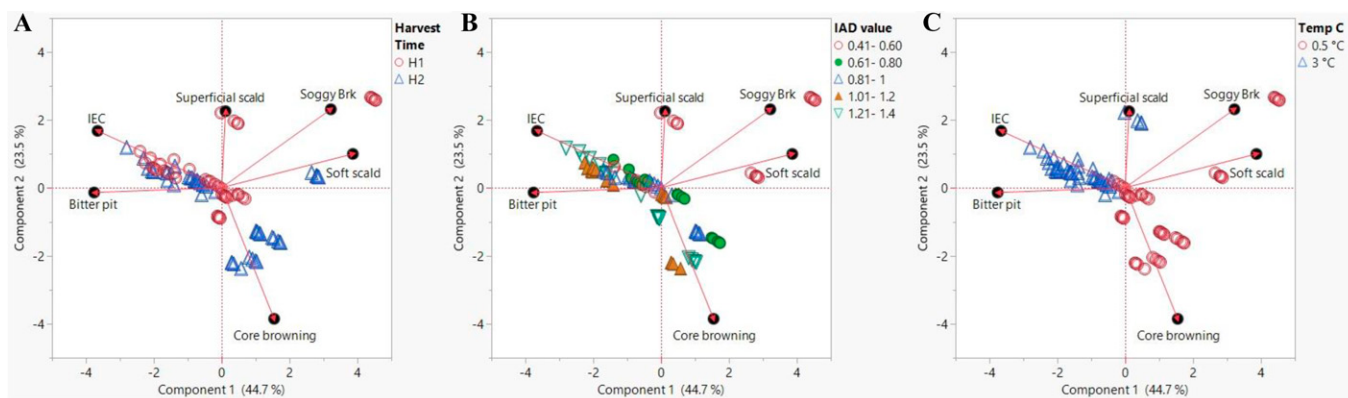


Fig. 4. Principal component analysis of the postharvest physiological disorders and harvest (A), index of absorbance difference (I_{AD}) values (B), and storage temperature (C). Eigenvalues for principal components 1 and 2 were 2.3 and 1.3, respectively, and were significant at $P < 0.0001$. H1 = harvest 1; H2 = harvest 2; IEC = internal ethylene concentration.

regarded as a low-temperature disorder of apple fruit (Watkins and Mattheis 2019).

To visualize the data and explain the variation in the data, PCA, which reduces the dimensionality of the data while preserving as much variance as possible, was undertaken. The analyses revealed that PC1 accounts for 45% of the variation, whereas PC2 explains 24% (Fig. 4). The eigenvectors revealed that the IECs, bitter pit, soft scald, and soggy breakdown were correlated with PC1 at -0.5 , -0.5 , 0.5 , and 0.4 , respectively. However, core browning and superficial scald were correlated with PC2 with $r = -0.7$ and 0.4 , respectively. Eigenvalues for PC1 and PC2 were 2.6 and 1.4, respectively, and were significant at $P < 0.0001$. Our analyses reinforce the results shown in Fig. 3. Harvest date did not influence the IEC, soft scald, soggy breakdown, or core browning. However, H1 fruit had greater bitter pit and superficial scald compared with those at H2 (Fig. 4A). The IEC, bitter pit, and core browning were greater in fruit with higher I_{AD} values, whereas soft scald development increased at lower I_{AD} values. Superficial scald and soggy breakdown, however, showed inconsistent patterns in relation to the I_{AD} values (Fig. 4B). When fruit were categorized by storage temperature, soft scald, soggy breakdown, and core browning were higher at 0.5°C , whereas bitter pit, superficial scald, and the IEC were greater at 3°C (Fig. 4C).

In most studies, the evaluation of I_{AD} values as an additional measure of fruit maturity is based on bulked fruit samples (DeLong et al. 2014, 2020; Doerflinger et al. 2016; Moran et al. 2020; Mostofi and DeEll 2024; Nyasordzi et al. 2013; Serra et al. 2016). To compare the results based on fruit categorization by I_{AD} values, we also determined disorder incidences in the population if bulked samples were used to investigate maturity and storage effects. For each harvest date and storage temperature, the percentage of each disorder in each category was recalculated based on the proportion of fruit in each one (Table 2).

The results of our study confirm the usefulness of fruit categorization by I_{AD} values to allow a more granular understanding of the effects of fruit maturity on the susceptibility

of fruit to physiological disorders. Similar approaches to date have been taken for superficial scald (Farneti et al. 2015) and soft scald (Al Shoffe et al. 2018; Moran et al. 2020). These approaches could improve the precision of studies of biochemistry and the development of different physiological disorders. Studies of this type can be confounded by a range of maturities of fruit in a typical bulked sample of fruit, which results in difficulties distinguishing susceptible and unsuitable fruit.

Conclusion

In conclusion, this study highlights the potential of categorizing fruit by I_{AD} values as a tool to minimize variation of fruit within a population, and therefore increases the sensitivity of investigations into factors affecting storage performance. Both I_{AD} values and storage temperatures affect the IEC and incidences of physiological disorders of 'Honeycrisp' apples. This could be especially useful when studying changes at the biochemical level when minimizing the variation of fruit could reduce confounding factors associated with averaged fruit maturity.

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