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Measurement of ethylene production as a method for determining the optimum harvest date of 'Jonagored' apples

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ABSTRACT

The measurement of ethylene concentration in the apple core is considered a good method of determining OHD, but requires high accuracy and expensive equipment. The measurement of ethylene production seems to be a technologically easier method. During research conducted in the years 2003-2008, measurements of ethylene production were evaluated as a method for determining the harvest maturity of 'Jonagored' apples intended for storage. Measurements were carried out every 4-5 days starting a few weeks before the estimated harvest date. Apples were picked on four dates and after five months the loss of mass, firmness, TSS and TA was measured, the incidence of physiological disorders and fungal diseases was assessed and sensory tests were conducted to evaluate storability. Based on this evaluation it was determined which of those dates was OHD. After a period of low ethylene production, the production accelerated rapidly to reach an average level between 10.0 and 12.5 ppm kg^{-1} hr^{-1} on the OHD. Later, ethylene production rose still more sharply and quickly reached several dozens of ppm kg^{-1} hr^{-1}. The accuracy of the new method was compared with other methods used to determine the maturity stage. The results obtained through the measurement of ethylene production showed the least deviation from the six-year average and only the results obtained using the Streif index method, which may be unreliable for some cultivars according to the literature, had a comparable margin of error.

Key words: firmness, fungal diseases, maturity, physiological disorders, Streif index

Abbreviations:

OHD – Optimum harvest date, TA – titratable acidity, TSS – total soluble solids

INTRODUCTION

Poland is one of world's leaders in apple production, and 'Jonagored', one of the 'Jonagold' mutants, has for many years been among the country's most important apple cultivars (Lieberz et al. 2008). This is because 'Jonagored' is highly productive,

easy to cultivate and enjoys great popularity among consumers. However, being a late-autumn cultivar, 'Jonagored' is not well suited to long cold storage (Łysiak 2011). Therefore, to ensure the high quality of 'Jonagored' apples throughout the winter season and longer, proper account must be taken of all factors that influence its storability. Besides such

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factors as the rootstock (Łysiak 1998, Łysiak and Kurlus 2000) or the age of the tree (Kapłan and Baryła 2005), the correct determination of the harvest date is also crucial for the storability of apples (Zude-Sasse et al. 2000).

The storability of autumn and winter apple cultivars intended for long storage crucially depends on the ability to determine the optimum harvest date (OHD) (Łysiak 2011). Fruit harvested when still unripe will shrivel more easily (Łysiak and Kurlus 2000, Nguyena et al. 2008) and will be more vulnerable to physiological disorders and diseases (Valero and Serrano 2010, Łysiak 2013). Over-mature fruit is likely to become soft and mealy (Kader 1997) and has an insipid flavour after a short period of storage (Kays 1991). Only apples picked at the optimum maturity stage are suitable for storage over six months because of better storage potential and organoleptic quality (Streif 1996).

The most popular methods for determining OHD include the starch index (Brookfield et al 1997, Peirs et al. 2002), colour development (Blanpied and Silsby 1992, Łysiak 2012), the Streif index (Streif 1983, De Jager and Roelofs 1996, Skrzyński 1996, Łysiak 2011) or the sum of active temperatures (Łysiak 2012). Their advantage is their simplicity, but they may not always be sufficiently accurate. A method regarded as the most accurate is based on the ethylene concentration because ethylene is considered to be the plant hormone responsible for the ripening process (Valero and Serrano 2010). As a climacteric fruit, apples show a sharp increase in the respiration rate and ethylene production at the start of ripening.

Ethylene production is most commonly determined by measuring ethylene concentration in the apple core (Blanpied 1989). However, to apply this method, samples must be collected very precisely because the gas has to be sucked out from the core using a syringe with a needle. The needle must be fixed to the apple with paste and covered with a serum stopper (Bender and Seibert 2004). Only one measurement is made on each apple so many replications must be carried out in order to obtain a sufficient sample. Another downside of this method is that it only enables the measurement of the gas concentration but not the direct production of ethylene, which could be different and depends on many factors (Dadzie et al. 1996, Larrigaudierea et al. 1997, Valero and Serrano 2010).

Ethylene concentration in the apple core has to be measured immediately after harvest because the harvest itself triggers a stress response in apples which might be misinterpreted (Morgan and Drew 1997). In some experiments this risk was eliminated by measuring ethylene concentration in the core before harvest, when the fruit was still on the tree (Bender and Seibert 2004). This method of measuring gas concentration requires a gas chromatograph, and because this is highly sophisticated and expensive equipment this method will never be popular in apple production.

Between 2003 and 2008, research was conducted to evaluate a method of measurement of ethylene production that would allow the reduction of factors causing misinterpretation and to determine ethylene production in 'Jonagored' apples.

MATERIAL AND METHODS

The research was conducted in the experimental orchard, the cold storage facility and the laboratory of the Department of Pomology of the Poznan University of Life Sciences between 2003 and 2008 (52°31' north latitude and 16°38' east longitude). Fruit was picked from 'Jonagored' apple trees planted in 1995 on M.9 rootstock in single rows (tree spacing 4 × 1.5 m), and the trees had a wide spindle shape. The orchard was protected and maintained in line with the recommendations for commercial orchards.

Sampling for OHD estimation

Fruit was collected every 4-5 days starting a few weeks before the estimated OHD (Tab. 1). The starting time for fruit collection varied depending on the flowering period and weather conditions prevailing during the growing season. The OHD was estimated on the basis of starch index measurements and Streif index calculations. The sample size was 20 apples picked from a minimum of 10 trees, from a height between 140 and 160 cm, from the outer canopy, from the southeastern side of the trees. Apples over or under size or infected by pests or diseases were rejected. The apples had to represent the stage of maturity (in size and colour) of the apples from the samples harvested for cold storage.

Sampling for cold storage

Besides samples intended for OHD estimation, there were four harvests of fruit intended for storage. The harvest was conducted according to the rules applicable to the samples intended for OHD estimation, but the size of a single sample intended for storage was considerably larger and amounted to four boxes with 10 kg in each box (50-60 apples

Table 1. Schedule of experiments

Measurement number	Year and date					
	2003	2004	2005	2006	2007	2008
1	27 Aug	27 Aug	1 Sept	26 Aug	27 Aug	2 Sept
2	1 Sept	2 Sept	5 Sept	31 Aug	1 Sept	6 Sept
3	6 Sept	6 Sept	10 Sept	5 Sept	6 Sept	12 Sept
4	11 Sept	10 Sept	14 Sept	9 Sept	11 Sept	16 Sept
5	<u>15 Sept</u>	15 Sept	2 Sept	<u>14 Sept</u>	<u>15 Sept</u>	22 Sept
6	<u>20 Sept</u>	<u>20 Sept</u>	<u>20 Sept</u>	<u>19 Sept</u>	<u>20 Sept</u>	<u>27 Sept</u>
7	<u>25 Sept</u>	<u>24 Sept</u>	<u>24 Sept</u>	<u>25 Sept</u>	<u>25 Sept</u>	2 Sept
8	<u>30 Sept</u>	<u>29 Sept</u>	<u>29 Sept</u>	<u>30 Oct</u>	<u>1 Oct</u>	<u>7 Sept</u>
9		<u>4 Oct</u>	<u>4 Oct</u>			<u>11 Oct</u>
Date of full bloom	6 May	2 May	3 May	9 May	27 Apr	1 May
Date of end of storage of first harvest	23 Feb	1 Mar	10 Mar	27 Feb	1 Mar	14 Mar
Length of storage in days	160	163	168	164	168	169

*Underlined dates present dates of harvesting samples for storage
**Double underlined dates present OHD based on evaluation after storage

per box). The schedule of all measurements is shown in Table 1.

Evaluation of ethylene production

Samples of fruit were immediately transported to the laboratory. Six to eight apples (depending on their size) were weighed with an accuracy of up to 1 g and sealed in a gastight container at 20°C. This procedure was carried out four times (in total, 24-32 apples were taken for measurement). After two hours, the concentration of ethylene in the container was measured using an ICA 56 ethylene meter (International Controlled Atmosphere Ltd. Instrument Division UK) with an accuracy of up to 0.1 ppm, within a time of 15 s, at a flow of 0.8 l min^{-1} (Response Sample flow, 0.8 l min^{-1}). Based on the result and assuming for the apple a specific weight of 830 kg $\times$ m^{-3} (Kheiralipour et al. 2008), ethylene production per 1 kg of fruit per hour was calculated.

Storage conditions and evaluation of storability

Fruit was stored in a cold storage room at 1-2°C and RH of around 90% for over five months. Within each year, the length of storage was equal irrespective of the harvest date (Tab. 1). The evaluation of storage efficiency and storability carried out after storage was based on judgments (sensory tests and assessment of incidence of diseases and disorders) and on measurements (fruit mass loss, internal quality features, i.e. firmness, TSS, TA).

Each evaluation criterion was scored separately for each date of harvest. The scores were given according to the following rules (Łysiak 2013):

1. Loss of fruit mass was measured in each stored box. Ten fruit were numbered and weighed with an accuracy of 0.1 g before and after storage. Scores were given according to an analysis of variance between the harvest dates. If there were no significant differences, each sample received 1 point. If the analysis showed a significant difference, a sample could receive 1, 2 or 3 points.

2. Incidence of disorders and diseases was scored separately according to the analysis of variance. If the percentage of non-healthy fruit was higher than 10% for, respectively, disorders or diseases, the sample received 1 point independently of the analysis of variance results.

3. Firmness was scored according to the following point scale:
     0 – below 39.3 N
     0.5 – 39.3 – 44.1
     1.0 – 44.2 – 49.0
     2.0 – 49.1 – 53.9
     3.0 – over 53.9 N.

The point scale was developed independently based on research by Konopacka et al. (2003), which examined the relationship between texture attributes and consumer perception and found that the minimum firmness preferences for three examined cultivars were between 39.3 and 49.0 N.

4. TSS and TA were scored separately according to the same rules as mass loss (scores 1-3) and based on the analysis of variance. If TSS for 'Jonagored' was below 11.5% and if TA was below 0.25, all samples received 1 respectively for each criterion, independently of the analysis result.

5. Sensory tests were made by 3-5 professional judges who judged the fruit according to the overall acceptance on the market along the following scale: 0 – no acceptance on market, 1 – poor acceptance, 2 – good, 3 – excellent. The average judgment was rounded to 0.5 points. Sensory attributes were: firmness, crispness, mealiness, juiciness, sweetness, sourness and aroma.

All the measured data were subjected to the analysis of variance. The mean comparisons were performed using the Duncan test to examine differences (p = 0.05) among the harvest dates.

RESULTS AND DISCUSSION

As could be expected (Streif 1983, De Jager and Roelofs 1996, Skrzyński 1996, Łysiak 1998, Łysiak 2013), a harvest date that was too early or too late negatively affected fruit storability due to higher incidence of physiological disorders and, in some years, the occurrence of infections with pathogens causing fungal diseases (Fig. 1). The number of apples affected by fungal diseases varied depending on the harvest year and date. Often, the later the harvest date, the greater the incidence of storage diseases causing fruit decay (Błaszczyk 2006); however, such a pattern was clearly observed only in two years of the research (2003 and 2004). In 2005, no differences were found in the occurrence of infections, regardless of the harvest date, and in 2006 and 2007 those differences were only insignificant. In 2008, the infection incidence declined with the harvest date, which allows the conclusion that the vulnerability of 'Jonagored' apples to bull's eye rot (*Neofabraea* sp.), blue mould (*Penicillium expansum*) and brown rot (*Monilinia* sp.) does not depend solely on the stage of maturity. Such variable results suggest that other factors are also at play, including natural variability in quality parameters at harvest (Johnson and Ridout 2000), weather development (Schreiner et al. 2000), correctness and timeliness of protection measures and mineral nutrition (Łysiak 2013). However, it should be emphasised that during the six-year study period, infection by fungal diseases observed on OHD was never higher than that observed on the remaining harvest dates.

Bitter pit is the most frequent physiological disorder in countries of high apple production (Ferguson and Watkins 1989). In all years of the research, a large part of the fruit losses caused by post-cold storage physiological disorders were attributable to bitter pit. The harvest date is believed to influence the incidence of bitter pit (Ferguson and Watkins 1989). In this research, the incidence of bitter pit increased with the number of days preceding OHD. Superficial scald is another important disease that is correlated with maturity and harvest date (Anet 1972). In four years of the research (2005-2008), the highest incidence of superficial scald was observed in apples picked on the earliest harvest date. In those years, two harvest dates preceded OHD. In 2003 and 2004, when two harvest dates followed OHD, the highest incidence of superficial scald was found in apples picked on the last harvest date. This observation confirmed the correlation between the incidence of superficial scald and the harvest date. Recent studies on superficial scald showed a correlation between the incidence of this disorder and ethylene biosynthesis (Ju and Bramlage 2000) or activity (Fan et al. 1999). Ethylene production induced in apples picked on the last harvest dates of 2003 and 2004 was much higher than that induced in apples picked on all other dates (Tab. 2). This suggests that the incidence of superficial scald is correlated with the amount of ethylene produced during the harvest of apples intended for storage.

In addition to the incidence of disorders and diseases, the chemical and physical properties of the fruit (Fig. 2) should also be taken into account during the final evaluation after storage (Shewfelt 2000). The firmness value below which some consumers consider fruit as unattractive amounts to 44.2 N for apples (Konopacka et al. 2003). The firmness value of fruit collected on some harvest dates in two years covered by the study (2006 and 2007) fell under this threshold after storage, which shows that the storage period of five months was too long. But regardless of this, there were significant differences in the firmness of fruit after storage (except 2003) as measured by the firmness of flesh, and although the differences were small, those measurements were helpful in evaluating the method of determining harvest maturity and storability. The sum of quantity parameters (diseases, disorders and weight loss) and quality, physical and chemical parameters (firmness, TSS

Figure 1. Fruit losses after storage caused by physiological disorders and fungal diseases
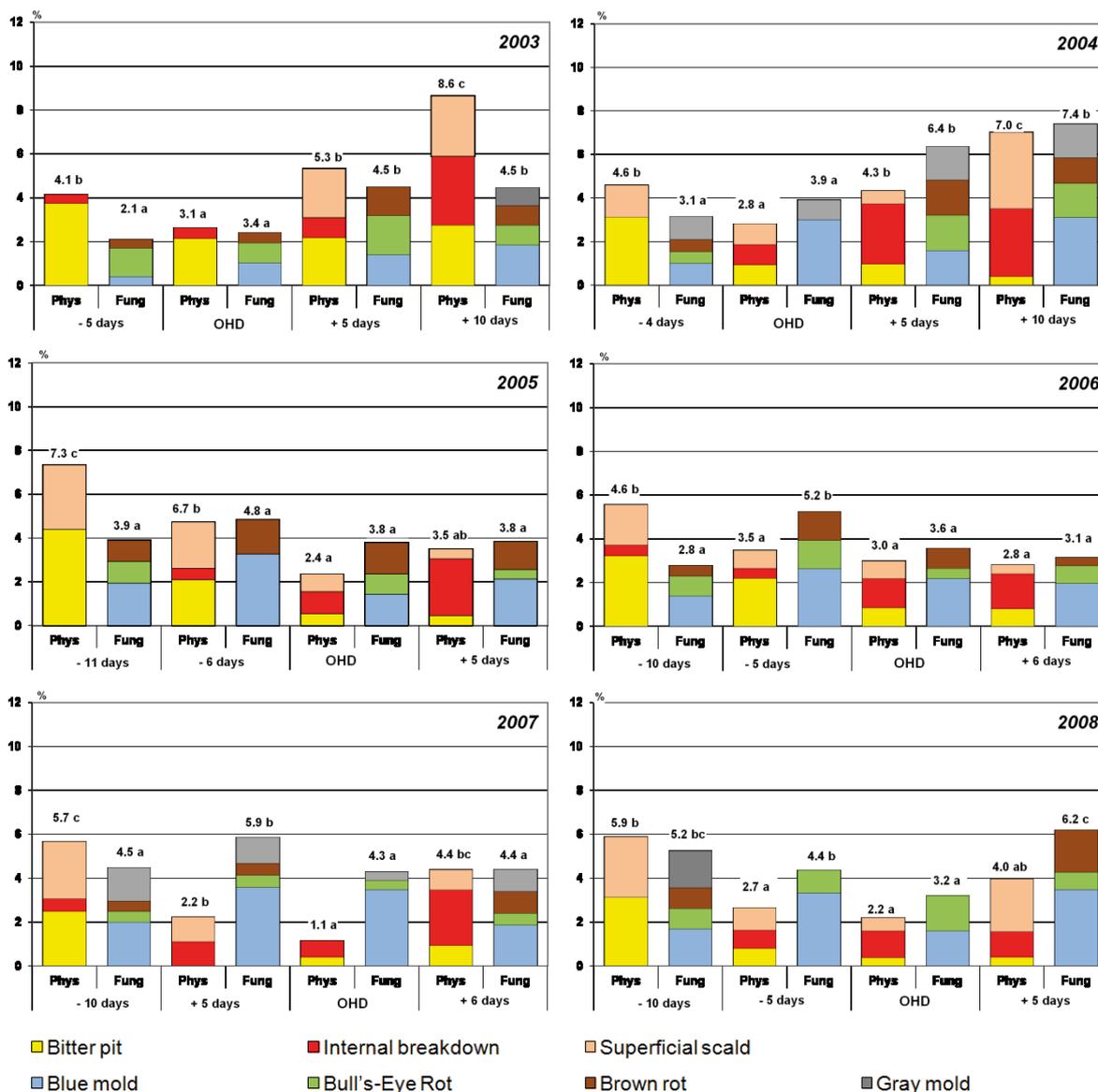
*Values designated with the same letters do not significantly differ in maturity stages, according to Duncan's test (P > 0.05)
**Phys – Incidence of physiological disorders
***Fung – Infection by fungal diseases
****Second row of X axis – numbers of days before or after the optimum harvest date (OHD)

and TA) evaluated after storage allowed us to determine which of the four harvest dates was correct (Fig. 2).

In Poland, the starch index is often used to determine OHD. It is based on the measurement of starch degradation (Brookfield et al. 1997). The Streif index method, which is based on the evaluation of three parameters – starch, firmness and TSS during the harvest period – seems to be more accurate (Łysiak 2011). Although it is not accurate enough for all cultivars (Skrzynski 1996), the results obtained using this method for 'Jonagored' have been highly consistent and therefore the Streif index has been often recommended for the

measurement of OHD of this cultivar (De Jager and Roelofs 1996). The table below shows the values of the Streif index and some other indices (Tab. 2).

Ethylene is produced by fruit throughout the season; however, in climacteric fruits like apple, a sharp increase in the production of ethylene before maturation is observed (Valero and Serrano 2010). The research showed that ethylene production per 1 kilogram of fruit ranged between 3.7 and 5.7 ppm hr^{-1} five days before OHD. During harvest this value was between 10.0 and 12.5 ppm kg^{-1} hr^{-1} and considerably increased (two or threefold) during the five days immediately following OHD. The range of ethylene production values observed in fruit during
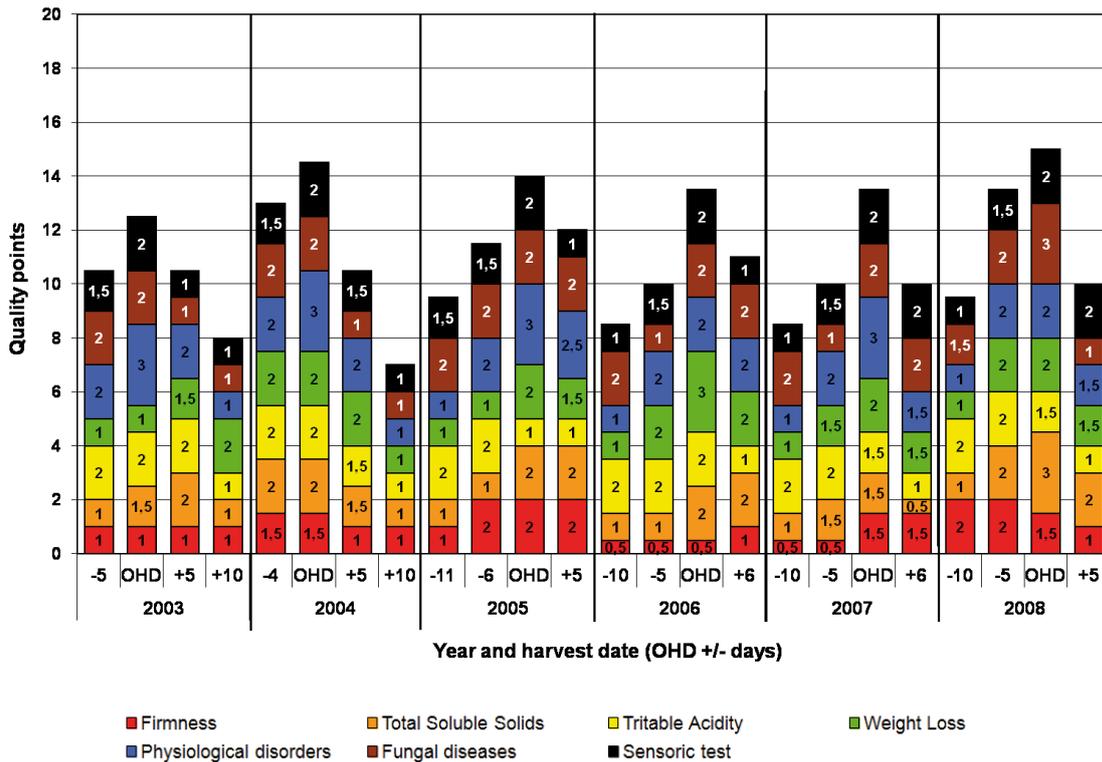
Figure 2. Assessment according to a point scale of the quality aspects of fruit samples harvested on the respective dates and examined after storage in the years 2003-2008

OHD is very narrow and the standard deviation amounts only to 0.85. In view of a considerable difference between the smallest and the largest number of days between full bloom and OHD (two weeks between the number of days in 2003 and in 2005) and the difference in the starch degradation values (2.2 points – which represents a difference in degradation of more than 20%) – both values largely depending on the location and weather conditions prevailing during the entire season (Brookfield et al. 1997) and ethylene production seems to be at the same level. The result is comparable to that obtained using the Streif index, although there have sometimes been reservations about the latter method. The percentage deviation from the six-year average is even ca. 4 points smaller. A clear advantage of the method based on the measurement of ethylene production is that it truly reflects the physiological state of apples, which cannot be said about the Streif index, as the parameters used in this method (TSS, firmness) depend on weather conditions, agricultural treatment, plant nutrition or the physical properties of the fruit. Another advantage of this method is a smaller measurement error. The accuracy of the measurement of ethylene concentration in the apple core strongly depends on correct sampling because results are obtained

for each individual apple measured. Therefore, differences in ethylene concentration between individual apples often reach several hundred per cent. Taking a gas sample from the apple core using a syringe, properly securing the sample content, and later its correct analysis using a gas chromatograph constitute a series of operations during which a mistake can easily occur (Bender and Seibert 2004). In the measurement of ethylene concentration method, an average result could only be calculated based on the results obtained for each of several dozens of apples, which would be very cost and labour intensive. The method based on the measurement of ethylene production allows us to avoid the above difficulties and risks. This method relies on average results obtained from one sample consisting of several apples and from several samples. In this method, it is possible to analyse a large number of fruit within a short time, in a simple way and without the necessity to use sophisticated equipment. The results of the analysis of variance show that each year ethylene production on OHD significantly differed from ethylene production on the measurement dates preceding and following OHD.

However, as in each method used to determine OHD, when using the proposed method one must

Table 2. Ethylene production as compared to harvest indices used for fruit intended for long-term storage

Year	Harvest	Days after bloom	Firmness [N]	Starch index [1-10]	Streif index	Ethylene production [ppm kg^{-1} hr^{-1}]
2003	-5	141	81.4 c*	5.7 a	0.121	3.7 a
	OHD**	**146**	**78.5 b**	**7.4 b**	**0.081**	**11.2 b**
	+5	151	74.5 b	8.1 c	0.069	19.7 c
	+10	156	73.6 a	8.9 d	0.059	50.3 d
2004	-4	153	77.5 b	4.4 a	0.138	5.7 a
	OHD	**157**	**74.5 b**	**6.9 b**	**0.082**	**10.0 b**
	+5	162	71.6 a	8.0 c	0.067	24.0 c
	+10	167	78.6 a	9.2 d	0.055	43.5 d
2005	-11	149	76.5 d	4.6 a	0.139	2.5 a
	-6	154	72.6 c	5.0 ab	0.119	3.7 a
	OHD	**160**	**67.7 b**	**5.9 b**	**0.088**	**10.7 b**
	+5	165	61.8 a	7.3 c	0.071	16.5 c
2006	-10	147	82.4 b	5.4 a	0.127	2.4 a
	-5	152	79.4 b	6.2 b	0.103	5.2 b
	OHD	**157**	**72.6 a**	**7.4 c**	**0.076**	**10.5 c**
	+6	163	69.6 a	8.3 d	0.063	28.8 d
2007	-10	145	76.5 c	4.5 a	0.145	2.1 a
	-5	150	72.6 b	6.4 b	0.096	3.8 a
	OHD	**155**	**71.6 ab**	**7.0 c**	**0.081**	**10.9 b**
	+6	161	68.6 a	8.5 d	0.065	30.1 c
2008	-10	138	82.4 b	4.2 a	0.161	2.6 a
	-5	143	80.4 b	5.7 b	0.115	4.5 b
	OHD	**148**	**80.4 b**	**8.1 c**	**0.082**	**12.5 c**
	+5	153	74.5 a	9.2 d	0.063	25.5 d
OHD Average		153	74.5	7.12	0.081	11.0
SD		5.73	41.2	1.73	3.8	0.85

*Values followed by the same letters do not differ significantly at p = 0.05
**Bold dates present dates of OHD based on evaluation after storage

also remember that increased ethylene production is a function of cultivar, and it is greatly affected within a cultivar by factors such as growing region, orchard within a region, cultivar strain, growing season conditions and nutrition (Watkins et al. 2004).

CONCLUSIONS

Since the measurement of ethylene production is relatively easy and no expensive equipment is needed (ICA 56 ethylene meter), as in the case of the measurement of ethylene concentration in the apple core (gas chromatograph), this method can be recommended for determining OHD for 'Jonagored'. The level of ethylene produced by the fruit of this cultivar on OHD was between 10.0 and 12.5 ppm kg^{-1} hr^{-1}. The application of this method to apples grown in other conditions might yield different results. However, the consistency of the results obtained during six years of research suggests that the differences will not be large. Thus, the measurement of ethylene production is a promising method for determining the OHD of

apples, but it must be examined whether it is also suitable for other cultivars and growing locations and whether other threshold levels of induced ethylene production should be used as indicators of OHD in those cultivars and locations.

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