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Respiratory patterns of European pear (*Pyrus communis* L. 'Conference') throughout pre- and post-harvest fruit development

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Abstract

Information on the developmental stage of pear pre-harvest and in shelf-life is crucial to determine the optimum timing of harvest, post-harvest treatment, and time of consumption ensuring high eating quality. In the present study, CO_2 emission and fruit quality of European pear (*Pyrus communis* L.) 'Conference' were analysed pre- and post-harvest with emphasis on shelf life for three years. Additionally, cytochrome and cyanide-resistant O_2 consumption were analysed in the last year of experiments. The respiration rate of pear showed typical climacteric rise of CO_2 emission in two years only, despite daily measurements. However, in each year the fruit quality in shelf life was closely linked to harvest date suggesting climacteric fruit response. Thus, the developmental stage of 'Conference' pear should be analysed by additional methods. Particularly, the cytochrome and cyanide-resistant O_2 consumption showed an encouraging potential to obtain data on characteristic respiratory patterns.

Keywords: Food science, Agriculture, Food analysis, Plant biology

1. Introduction

Both, storability and fruit quality at consumption depend on the maturity stage at time of harvest (Kader, 2002). Kidd and West (1924) showed a relationship between respiratory behaviour and ripening process of pome fruit. The increase of respiration rate of climacteric fruit such as pear (Biale, 1964) and rise of ethylene production (Burg and Burg, 1961) is related to biochemical processes during ripening (Rhodes, 1970). Temperature and atmosphere affect the pathway(s) of respiration as shown for many commodities (Fonseca et al., 2002) including pear (Pedreschi et al., 2008). Apart from cytochrome cyanide-sensitive respiration, a cyanide-resistant pathway was shown for fruits (Lips and Biale, 1966). The percentage of cyanide-resistant path may potentially vary, e.g., due to the physiological stage of fruit development (Lips and Biale, 1966), percentage of CAM (Herppich and Peckmann, 2000) and lack of oxygen (Bahr and Bonner, 1973; Saquet et al., 2000).

Established methods for analysing the developmental stage of pip fruits, including pear, capture gas exchange analysis, but also quality measurements. Gas exchange of fruit has been analysed employing gas chromatography and CO_2 sensors for measuring the fruit respiration rate, while the analysis of fruit quality is usually addressing the fruit flesh firmness [N cm^{-2}] and refractive index of squeezed fruit juice, which is related to the sweetness of fruit and expressed as soluble solids content [%]. Although progress of fruit flesh firmness and refractive index is crucial for consumers acceptance (Kader, 1999), both parameters are not indicative to characterise pre-harvest developmental stage of pear in respect to harvest date and post-harvest treatment (Gamrasni et al., 2015). It was shown in earlier studies for apple, that the NDVI is related to chlorophyll content (Zude, 2003; Seifert et al., 2015), which decreases during fruit development, but wasn't proved to produce reliable results in pear so far.

The respiration rate, on the other hand, is an accepted indicator for the developmental stage of pear pre-harvest as well as post-harvest (Mathooko, 1996). Determining the optimum harvest date at the pre-climacteric minimum of respiration rate, by monitoring pre-harvest respiration rate, showed potential to prolong storage life of 'Spadona' pear in controlled atmosphere (Gamrasni et al., 2015). Post-harvest ripening behaviour is also closely related to gas exchange of fruit (Saltveit, 2016). The higher the respiration rate of fruit, the shorter appears the storage life (Brash et al., 1995). Enhanced storability has been found for 'Conference' pear showing reduced respiration rate compared to 'Abbé Fétel' pear developing enhanced respiration rate (Zerbini and Grassi, 2010).

Harvest quality of pear can be further maintained in storage by post-harvest treatment with 1-methylcyclopropene (1-MCP) (Lurie, 2007). 1-MCP competes for ethylene receptors and thereby inhibits ethylene-based reactions (Eccher Zerbini

et al., 2005; Watkins, 2008). Therefore, 1-MCP decreases softening and respiration rate (Ekman et al., 2004). However, after treatment, pear sometime stay too firm and show a lack of ripening progress (Vanoli et al., 2016) leading to consumer rejection. The developmental stage of fruit at time of treatment is assumed to be most important for the response of pear on 1-MCP determining the eating quality (Calvo and Sozzi, 2004; Baritelle et al., 2001). Consequently, the optimal timing of 1-MCP treatment can reduce losses caused by premature decrease of fruit flesh firmness (Watkins, 2008). Also, here the respiratory patterns may appear informative.

The objective of the present study was to characterise the developmental stage of 'Conference' pear pre- and post-harvest by means of fruit respiration considering CO_2 gas exchange. Data will be shown on the influence of the timing of harvest and post-harvest conditions on respiratory patterns of pear in shelf life, considering cytochrome but also cyanide-resistant respiration, as well as progress of fruit quality in shelf-life.

2. Materials and methods

2.1. Experimental plan for pre-harvest and harvest analyses

'Conference' pear (*Pyrus communis* L.) were grown in commercial orchards located in the Werderan region (Germany), a major fruit production area. All pear trees used in this study were drip irrigated and trained as slender spindle. The day after full bloom (dafb) was determined based on the phenological identification key of pome fruit (Meier, 2001). Sound pear were analysed pre-harvest and post-harvest in three consecutive years. In 2015, 2016, and 2017, CO_2 emission and quality analyses were measured during the pre-harvest fruit developmental period by means of single fruit analysis (2015: n = 150 measuring twice a week; 2016: n = 400 measuring every day around the expected climacteric peak; 2017: n = 150 measuring twice a week). Harvests took place beginning and end of commercial harvest window. Post-harvest treatment and shelf-life analyses were carried out on fruit of all harvest dates.

2.2. 1-MCP treatment

In the three years, 1-MCP treatment with 312 μg kg^{-1} 1-MCP was carried out one day after harvest at 1 °C ± 0.1 K (Hygroflex5 + HC2-S, Rotronic, Germany) in an airtight container (196 L) for 24 h. The 1-MCP was applied as gas by addition of Smartfresh™ (0.14 % 1-MCP complexed with α-cyclodextrin; Agrofresh, USA) to purified water tempered at 20 °C into a wide neck bottle and placed into airtight container. Subsequently to the developing of the gas, the airtight container was sealed.

2.3. Storage

Following 1-MCP treatment, defect-free control and 1-MCP 'Conference' pear were placed into trays (EPS 106) at 1 °C $\pm$ 0.1 K (Hygroflex5 + HC2-S, Rotronic, Germany) in climate chambers dedicated either to storage in controlled atmosphere (CA: 2015, 8 weeks, 3 % CO_2, 1.5 % O_2; 2016, early harvest 8 weeks, late harvest 6 weeks, 1 % CO_2, 2.5 % O_2), in air (2015: 8 weeks; 2016: early harvest 8 weeks, late harvest 6 weeks; 2017: 8 weeks) or in air followed by storage in controlled atmosphere (2015: 13 weeks; 2016: 12 weeks). Static CA conditions were established within two days. Storage conditions were monitored and maintained throughout (Checkmate 3, PBI-Dansensor, Denmark). Repetitions were carried out in 2015 for CA: 3 trays in 4 boxes (12 trays with 20 fruits per tray), results in n = 240 fruits, air: 3 trays in 4 boxes (12 trays with 10 fruits per tray), results in n = 120 fruits, air + CA: 3 trays in 4 boxes (12 trays with 20 fruits per tray), results in n = 240 fruits. In 2016 for all treatments 2 trays in 12 boxes (24 trays with 10 fruits per tray), results in n = 240 fruits. In the third year, the number of measurements in postharvest was reduced allowing the conductance of the analysis of O_2 consumption. In 2017 for all treatments 2 trays in 2 boxes (4 trays with 18 fruits per tray), results in n = 72 fruits.

After removal from storage pear were placed in a climate-controlled laboratory for shelf-life (20 °C; 2015: 14 d; 2016: 10 d; 2017: 5 d) analysis, while adapting the days after storage to the fruit material due to the appearance of decay being different in each year.

2.4. CO$_2$ exchange

2.4.1. Respiration rate

Pre-harvest as well as post-harvest CO_2 emission was measured in closed system by optical infrared sensor (FYA600CO2, Ahlborn, Germany) at 20 °C ($\pm$0.6 °C). Measuring time was dependent on CO_2 evolution, until a steady rise was recorded. This process took between 30 and 180 minutes. Respiration rate was calculated (Eqs. 1 and 2) considering CO_2 emission and fruit mass.

$$R \left[mg \ kg^{-1} h^{-1} \right] = \frac{\Delta CO_2}{\left(\frac{Fresh \ mass \ [g]}{1000} \right) * \left(\frac{\Delta t}{60} \right)} \tag{1}$$

$$\Delta CO_2 \ [mg] = \frac{\left(CO_{2(end)} - CO_{2(start)} \right) \left[\frac{\mu mol}{mol} \right] * (V_C - V_P) \ [L] * 273 \ [K] * \rho_{act} \ [hPa] * 44.3 \left[\frac{g \ CO_2}{mol} \right]}{22.41 \left[\frac{mol}{L} \right] * T_{act} \ [K] * 1013 \ [hPa] * 1000} \tag{2}$$

R = fruit respiration rate [mg kg^{-1} h^{-1}]

Δt = t2 − t1 [min]

V_c = volume cuvette [L]

V_p = volume product [L]

ρ_{act} = actual atmospheric pressure [hPa]

T_{act} = actual temperature [K]

2.4.2. Q_T

In post-harvest, the temperature dependence of CO_2 emission was measured after 2, 4, 6, and 8 weeks of storage and 5 days post-storage shelf-life by optical infrared sensors (GMP222, Vaisala, Finland) using single fruits placed in separated respiration cuvettes (n = 10) at 1 °C ± 0.1 K, 12 °C ± 0.1 K, and 20 °C ± 0.1 K (Hygroflex5 + HC2-S, Rotronic, Germany) located in climate-controlled chambers. The fruits were allowed to reach the temperature for 4 hours before measurement. The temperature coefficient (Q_T) was calculated (eq. 3) considering the impact of temperature (T_i) on respiration rate (R_i) (Zude-Sasse et al., 2000; Fonseca et al., 2002) for the specific range of temperature.

$$Q_T = \left(\frac{R_2}{R_1}\right)^{\left(\frac{10}{T_2 - T_1}\right)} \tag{3}$$

2.5. Ethylene production

The ethylene production was monitored gas chromatographically (GC-17A, Shimadzu, Japan) using 0.5 mL gas samples of the headspace of intact single pears placed in airtight glass jars (n = 10) for 8 h. The helium carrier was set at a flow rate of 21 mL min^{-1} and injector temperature at 80 °C.

2.6. Cytochrome and cyanide-resistant respiration

The measurement of O_2 consumption pre- as well as post-harvest was added in 2017 employing optical oxygen microsensor (PM-PSt7, PreSens, Germany). Fruit flesh samples were taken by cork-borer with a diameter of 4 mm. Length of samples was adjusted at 7 mm using a scalpel. Sensors were placed in the cylindric fruit flesh samples. Two samples were measured concurrently in a light-tight covered conical flask in 25 mL of 2-Amino-2(hydroxymethyl)propane-1,3-diol (TRIS; 10 mM) aerated and, therefore, saturated with oxygen at 23 °C placed on a magnetic stirrer. Measurements were repeated (n = 4). The O_2 concentration in fruit and solution was measured for 10 minutes in TRIS to enable leaching effects and reaching a steady state of O_2 consumption, followed by another 10 minutes measuring in aerated

TRIS. Subsequently, 0.016 g white crystalline potassium cyanide (KCN) was solved in aerated TRIS and added. O_2 concentration was monitored during entire measurement with a measuring interval of 10 s. Data were filtered using moving average over a window size of 60 seconds.

2.7. Fruit quality

Fruit flesh firmness [N cm^{-2}] of each fruit was measured according to Magness-Taylor on a peeled area in the equatorial region with Texture Analyzer (TA-XT Plus, Stable Micro Systems, UK) applying a 11.13 mm diameter, convex plunger with a velocity of 4 mm s^{-1} recording the force value at 8 mm depth of penetration, and correcting it with the plunger size. Soluble solids content (SSC) [%Brix] was measured temperature corrected with a digital refractometer (DR 301-95, Kruess Optronic, Germany) using fresh squeezed juice from two longitudinal slices of each individual pear based on guidance of OECD. The normalised difference vegetation index (NDVI) was calculated from remittance spectra measured with a hand-held spectrophotometer (PA-1101, CP, Germany) on each fruit (NDVI = $[I_{780}\text{-}I_{660}]/[I_{780} + I_{660}]$) (Zude, 2003). The NDVI ranging between 0 and 1, with 1 being the saturation at high chlorophyll content.

2.8. Statistical analyses

Statistical analyses were performed using statistical software R (R core Team, 2018) by addition of packages for Analyses of Variance and *t*-test analysis (agricolae: Mendiburu, 2017; multcompView: Graves et al., 2015) and figures (plyr: Wickham, 2011; ggplot2: Wickham, 2009).

3. Results

3.1. Characterisation of fruit developmental stage by gas exchange analyses

In 2015 (Fig. 1), the typical climacteric respiratory patterns of 'Conference' pear was monitored during pre-harvest as well as at removal from storage and in post-storage shelf-life conditions. Eight fruits were removed from the analysis due to mechanical damage. During pre-harvest, respiration rate of 'Conference' pear decreased throughout fruit development from 94 (48.3 $\pm$ 7.2 mg CO_2 kg^{-1} h^{-1}) till 112 (20.1 $\pm$ 4.4 mg CO_2 kg^{-1} h^{-1}) days after full bloom (dafb) followed by a respiratory peak 126 dafb (39.9 $\pm$ 6.8 mgCO_2 kg^{-1} h^{-1}) (Fig. 1). Based on the respiratory and ethylene progress, early harvest was determined 114 days after full bloom before ethylene and respiration rate increased. Thus, late harvest took place 126 days after full bloom subsequently after increase of CO_2 and ethylene.
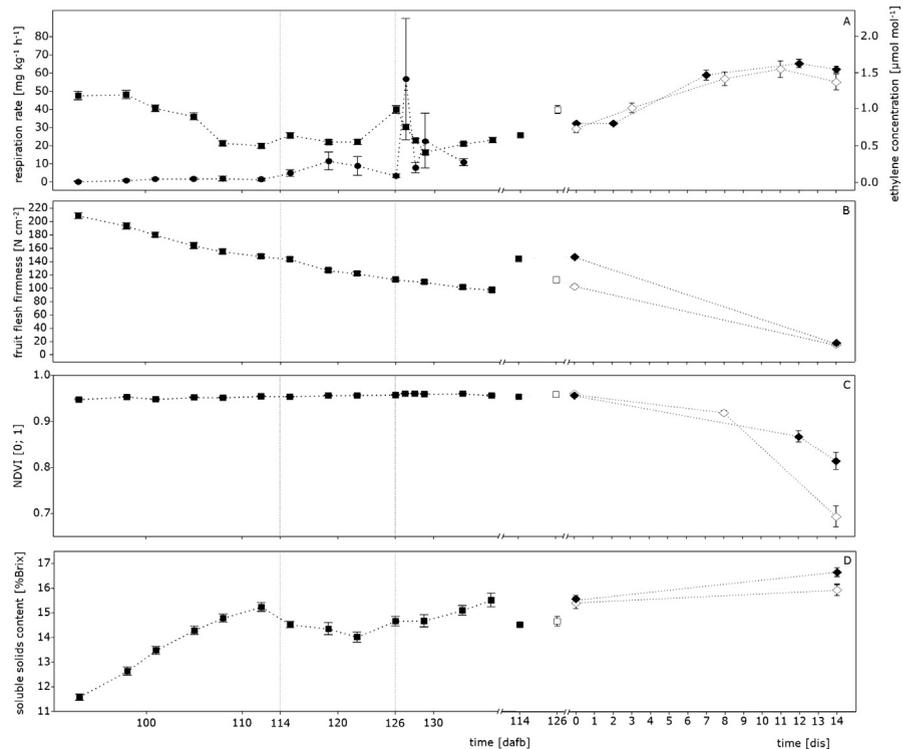
Fig. 1. Typical development of 'Conference' pear, here in 2015, in pre-harvest [days after full bloom, dafb] and in shelf life [days in shelf-life, dis] capturing A) fruit respiration rate [mg kg^{-1} h^{-1}] measured by means of CO_2 emission rate (square) and ethylene concentration [µmol mol^{-1}] (circle); B) fruit flesh firmness [N cm^{-2}]; C) normalised difference vegetation index (NDVI) [0; 1]; and D) soluble solids content [%Brix] in pre-harvest (square). In post-harvest, early (closed symbol, 114 dafb) and late (open symbol, 126 dafb) harvest date were considered. At removal from cold storage (diamond) in air followed by controlled atmosphere and shelf-life (20 °C; dis) for 14 days. Dotted vertical lines indicate harvest dates. Error bars refer to standard error.

In 2016, the typical decrease of respiration rate was measured 101 dafb with 33.9 ± 4.8 mg CO_2 kg^{-1} h^{-1} at the beginning of pre-harvest fruit development (Fig. 2). However, despite daily sampling, no distinct increase of CO_2 emission appeared. Instead many smaller peaks occurred around harvest date. In contrast, ethylene production increased at expected time of climacterical rise. Ethylene values ranged from 0.006 ± 0.004 µg kg^{-1} h^{-1} 101 dafb till the ethylene burst recorded 142 dafb (0.45 ± 1.34 µg kg^{-1} h^{-1}) till highest measured value 156 dafb (5.49 ± 7.62 µg kg^{-1} h^{-1}). Thus, early (131 dafb) and late (148 dafb) harvest date in 2016 was determined by progress of ethylene production only. However, the fluctuation of ethylene values was huge, providing information on on and off ethylene burst, but no distinction between the two stages or further potential to monitor fruit in shelf life.

In 2017, 2 fruits were removed from the analysis due to mechanical damage. The respiration rate of 'Conference' pear decreased from 68 dafb (224.67 ± 36.60 mg CO_2 kg^{-1} h^{-1}) till 138 dafb (17.00 ± 2.00 mg CO_2 kg^{-1} h^{-1}) followed by an
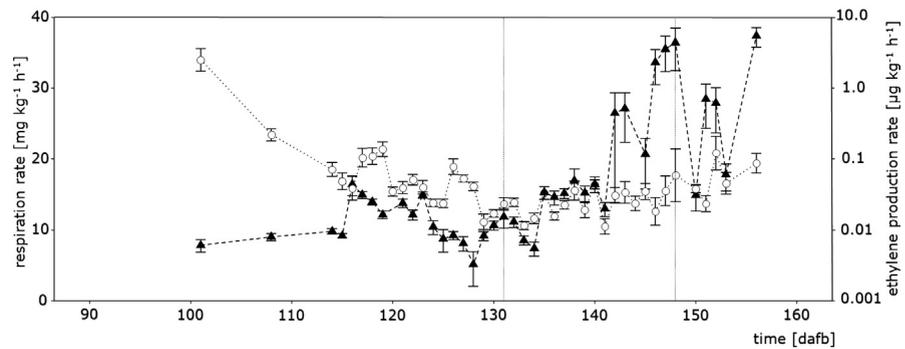
Fig. 2. Respiration rate [mg kg^{-1} h^{-1}] (○) of 'Conference' pear throughout pre-harvest fruit development in 2016 [days after full bloom, dafb], measured by means of CO_2 emission in a closed system on intact fruit and ethylene production rate [μg kg^{-1} h^{-1}] (▲). Dotted vertical lines indicate harvest dates. Error bars refer to standard error.

increase to 26.17 ± 4.49 mg CO_2 kg^{-1} h^{-1} (143 dafb) and a subsequent drop (Fig. 3). Invasively measured O_2 consumption of pear tissue proceeded analogously to patterns of CO_2 emission measured on the entire fruit. The climacteric peak was highlighted by means of O_2 drastically enhanced consumption rate, appearing 142 days after full bloom. Cyanide resistant O_2 consumption was measured from 64 dafb till 158 dafb and an effect of the addition of cyanide on the O_2 consumption was found in early stages of fruit development, when also CO_2 production was high. The cyanide resistant O_2 consumption decreased till 129 dafb. From this point of time, O_2 consumption after addition of cyanide showed no change (Fig. 3).

In post-storage shelf-life, respiration rate of 'Conference' pear proceeded with similar patterns regardless of harvest date (Fig. 1). Respiration rate increased after removal from storage with a decrease at the end of shelf-life. Due to heavily reduced fruit flesh firmness, shelf-life was abbreviated in 2016 and 2017. However, the
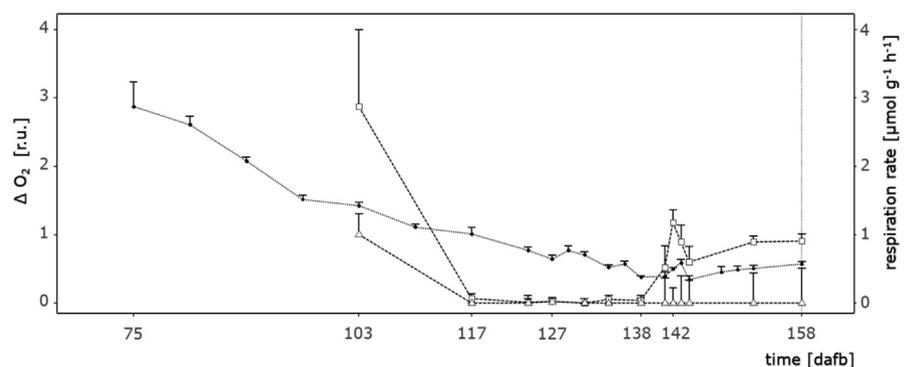

Fig. 3. Respiration rate of 'Conference' pear [mg kg^{-1} h^{-1}] (●) measured by means of CO_2 emission of intact fruit in a closed system, Δ O_2 with difference given in relative units [r.u.] of pear tissue in TRIS (□), and exposed to KCN (△) throughout pre-harvest fruit development in 2017. Error bars refer to standard error considering positive bars for enhanced readability.

pattern of initially enhanced and subsequently decreased respiration rate was found
in all years (Fig. 4). The extent of this effect appeared according to the storage con-
ditions. Pear stored in air showed enhanced respiration rate already at removal from
storage with high variability (Fig. 4), while pear stored in controlled atmosphere ap-
peared more homogeneous and shelf life patterns were even more clearly measurable
(Fig. 5).

Pear treated with 1-MCP showed constant low respiration rate in shelf-life for both,
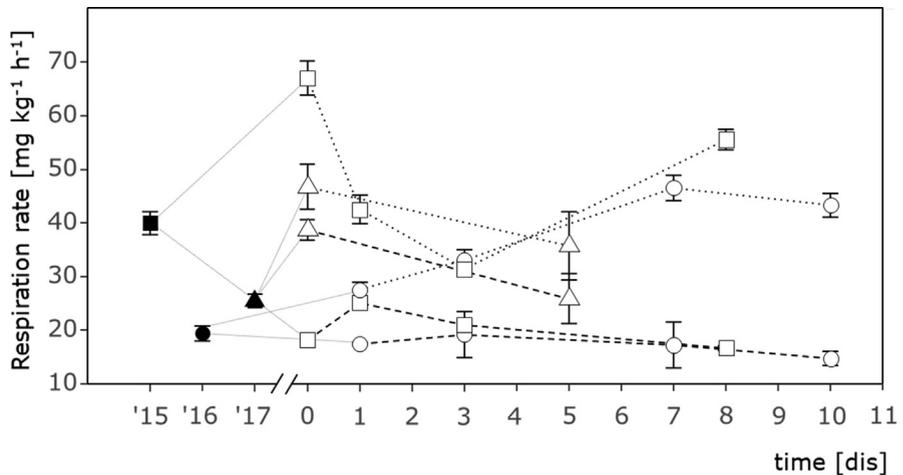pear stored in air (Fig. 4) and in controlled atmosphere (Fig. 5). In contrast to control

Fig. 4. Seasonal differences in respiration rate [mg kg^{-1} h^{-1}] of 'Conference' pear harvested late in 2015
(□), 2016 (○), and 2017 (△) considering control (· · ·) and 1-methylcyclopropene (– –) treated fruit stored
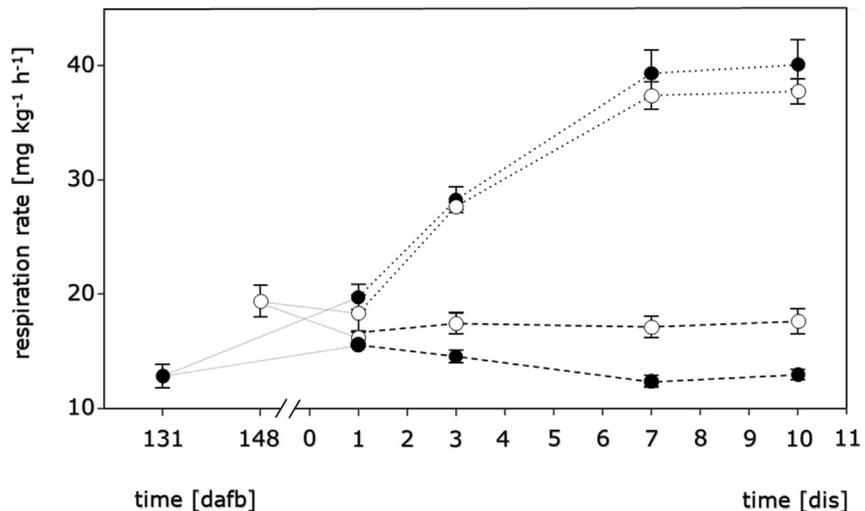in air (1 °C) followed by shelf-life (20 °C).

Fig. 5. Progress of respiration rate in 2016 of control (· · ·) and 1-methylcyclopropene (– –) treated
'Conference' pear stored in controlled atmosphere with 1 % CO$_2$, 2.5 % O$_2$ followed by shelf-life for
8 days (20 °C) considering pears harvested early (●) and late (○).

pear, no influence of storage conditions was found on the respiration rate of 1-MCP pear at the end of shelf-life in all years (Fig. 4). While respiration rate of control pear stored in CA showed similar values in shelf-life, respiration rate of late harvested pear treated with 1-MCP was slightly enhanced compared to early harvested pear ($p < 0.01$).

Comparing respiration rate measured by means of CO_2 emission and O_2 consumption point to similar patterns also post-harvest. Considering pear stored for 2, 4, 6, and 8 weeks in air at 1 °C and subsequently for 5 days in shelf life, a decrease in the respiratory activity was found from week 2 to week 6. After 8 weeks of storage, an increase of O_2 consumption was measured in TRIS as well as after addition of cyanide. After 8 weeks of cold storage fruits appeared over-mature already after removal from storage. In parallel to the increasing respiration rate at the end of potential storage time, the cyanide resistant respiratory pathway appeared enhanced from 0.9 % to 23.1 % of entire respiratory activity (Fig. 6).

As mentioned, respiration rate of control pear was enhanced compared to respiration rate of pear treated with 1-MCP, when measured at the same fruit temperature. However, the influence of temperature, measured as temperature coefficient (Q_T) was equally found in control and 1-MCP treated pear (Table 1). Results revealed 4 times enhanced Q_T values in control as well as 1-MCP treated pear stored 2 weeks compared to fruit stored for an extended period of time. The Q_T values of pear appeared non-linear over the relevant temperature range (Fig. 7) and, therefore, Q_T was calculated for two temperature ranges (Table 1).

3.2. Quality progress pre- and post-harvest

Typical progress of fruit flesh firmness, pre- as well as post-harvest, was found in all years, shown for 2015 for overview (Fig. 1). Pre-harvest, fruit flesh firmness
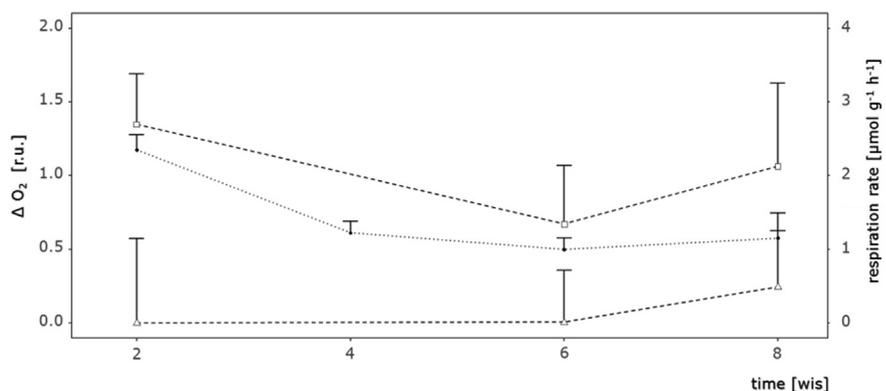
Fig. 6. Respiration rate (●) measured by means of CO_2 emission on intact fruit in a closed system at 20 °C [mg kg^{-1} h^{-1}] of 'Conference' pear. The O_2 consumption in relative units [r.u.] of fruit tissue in TRIS (□) and exposed to KCN (△) after 2, 4, 6, and 8 weeks in storage (wis) in air (1 °C) followed by 5 days in shelf-life (20 °C). Error bars refer to standard error with positive bars presented to enhance readability.

Table 1. Temperature coefficient, Q_T (mean $\pm$ standard deviation) of control and 1-MCP treated 'Conference' pear derived from respiration rate measured at 1, 12, and 20 °C after 2, 4, 6, and 8 weeks of cold storage at 1 °C and 5 days shelf-life at 20 °C. Different letters indicate significant differences of the mean.

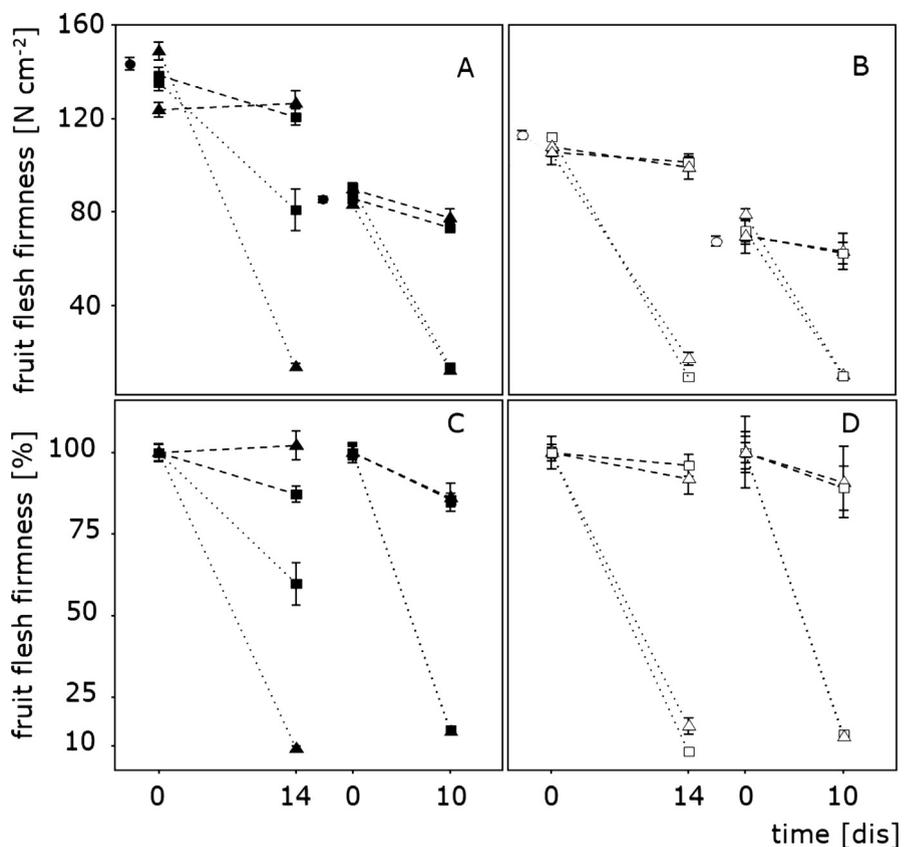|	Weeks in storage			
	2	**4**	**6**	**8**
control				
Q_{1-20}	8.12 $\pm$ 0.93 a	2.59 $\pm$ 0.21 c	2.05 $\pm$ 0.23 c	1.94 $\pm$ 0.12 c
Q_{1-12}	8.13 $\pm$ 1.21 a	2.39 $\pm$ 0.48 c	2.04 $\pm$ 0.36 c	1.79 $\pm$ 0.31 c
Q_{12-20}	8.22 $\pm$ 1.47 a	3.07 $\pm$ 0.88 c	2.09 $\pm$ 0.24 c	2.26 $\pm$ 0.61 c
1-MCP				
Q_{1-20}	8.40 $\pm$ 0.69 a	3.03 $\pm$ 0.42 c	2.00 $\pm$ 0.09 c	1.91 $\pm$ 0.18 c
Q_{1-12}	9.24 $\pm$ 1.75 a	2.17 $\pm$ 0.75 c	2.01 $\pm$ 0.24 c	1.73 $\pm$ 0.23 c
Q_{12-20}	7.78 $\pm$ 2.35 a	5.50 $\pm$ 2.42 b	2.02 $\pm$ 0.37 c	2.27 $\pm$ 0.58 c

Fig. 7. Fruit flesh firmness of control [····] and 1-methylcyclopropene treated [– –] 'Conference' pear harvested early (A, C; 2015: 114 dafb; 2016: 131 dafb) and late (B, D; 2015: 126 dafb; 2016: 148 dafb). Pear were cold-stored in air [▲] (1 °C) or controlled atmosphere [■] for 8 (6) weeks followed by 14 (2015)/10 (2016) days in shelf-life (dis; 20 °C). Data are given in absolute (N cm^{-2}; A, B) and normalised (%; C, D) values. Error bars refer to standard error.

decreased throughout fruit development. 'Conference' pear were harvested mature-firm early (2015: 143.5 ± 15.2 N cm^{-2}; 2016: 85.5 ± 7.0 N cm^{-2}) and late (2015: 113.0 ± 10.8 N cm^{-2}; 2016: 67.4 ± 11.7 N cm^{-2}; 2017: 102.5 ± 9.82 N cm^{-2}).

While at removal from storage the fruit flesh firmness of control and 1-MCP treated pear was similar for air and air + CA storage, while fruit flesh firmness was highly influenced by 1-MCP treatment considering the end of shelf-life (Table 2). In shelf-life, fruit flesh firmness of control 'Conference' pear without 1-MCP treatment decreased and revealed buttery and mellow fruit flesh, regardless of harvest date and storage atmosphere (Fig. 7). In contrast, fruit flesh firmness of 1-MCP treated pear remained high with 99.02 ± 18.82 N cm^{-1} or above in 2015 and 62.35 ± 15.04 N cm^{-1} in 2016 throughout shelf-life conditions ($p < 0.01$). Similar patterns of the decrease of fruit flesh firmness were monitored in 2017, but on less fruit.

At the tree, NDVI of 'Conference' pear showed steady values throughout fruit development due to high chlorophyll content and resulting NDVI reaching saturation (Fig. 1). However, in shelf life NDVI changed. In 2015, the NDVI of early harvested control pear decreased by 14.7%, while NDVI of pear harvested late decreased by 27.6%. The NDVI of pear treated with 1-MCP remained 0.955 ± 0.005 or above. Similar patterns were found in all years.

Throughout pre-harvest quality development at the tree, refractometrically measured soluble solids content (SSC) followed double sigmoid progress (Fig. 1). In years 2015 and 2016, at time of harvest SSC differed between 13.97 ± 0.92 %Brix and 14.77 ± 0.83 %Brix (Fig. 8). The SSC value at time of early and late harvest was similar. For control as well as 1-MCP treated pear stored in air, an early and late harvest date leaded to increased SSC at the end of shelf-life. Similar patterns were found in 2017, but with a reduced sample size. Inconsistently, in 2016, control pear stored in CA, showed decreased SSC at the end of shelf-life.

Table 2. Analysis of variance (F-ratio) of fruit flesh firmness after removal from storage and end of shelf life considering the factors harvest date and 1-methylcyclopropene treatment.

Atmosphere	State	2015		2016	
		Harvest date	Treatment	Harvest date	Treatment
Air	Removal	57.97^{*}	8.47^{*}	21.84^{*}	0.26^{ns}
	Shelf-life	9.61^{*}	617.47^{*}	3.48^{ns}	181.67^{*}
CA	Removal	102.80^{*}	0.40^{ns}	13.87^{*}	0.54^{ns}
	Shelf-life	77.42^{*}	161.32^{*}	8.04^{*}	444.80^{*}
Air + CA	Removal	244.38^{*}	5.21^{ns}	3.13^{ns}	1.49^{ns}
	Shelf-life	65.92^{*}	1322.08^{*}	8.69^{*}	339.06^{*}

ns = no significant influence, $^{*}p < 0.01$. Storage conditions: 8 weeks in air (1 °C), 8 weeks in controlled atmosphere (CA), and 8 weeks storage in air followed by 5 weeks in CA (air + CA) and at the end of 14 (2015) or 10 (2016) days in shelf-life (20 °C).
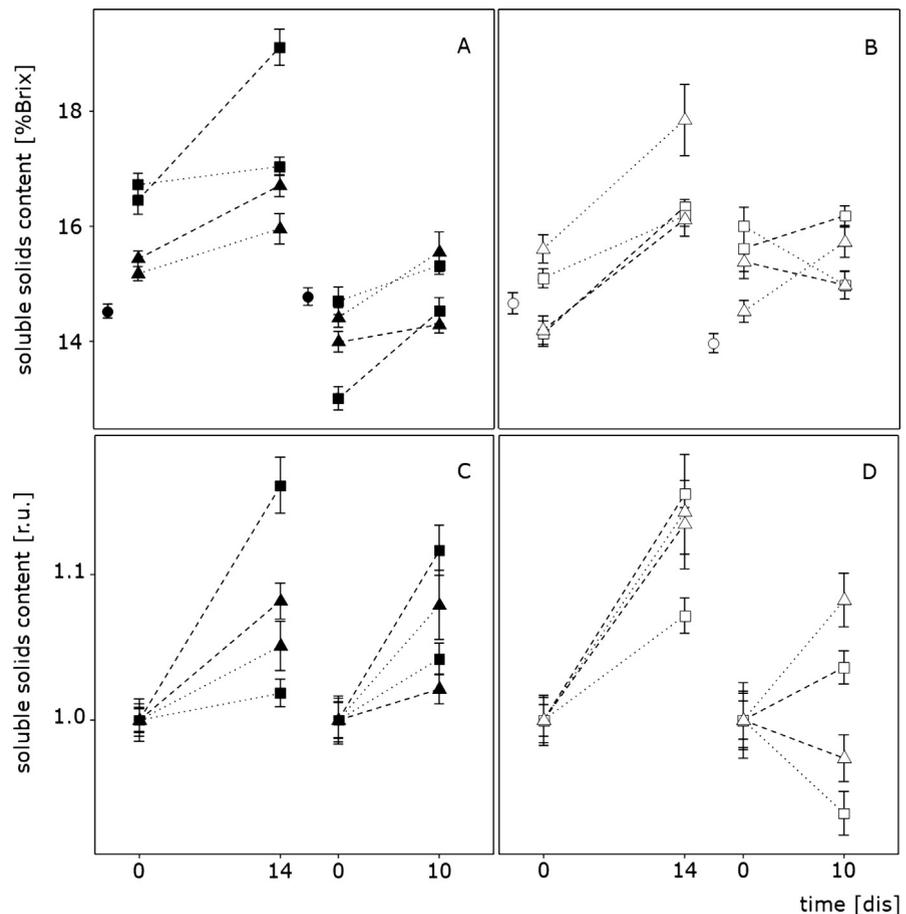
Fig. 8. Soluble solids content in absolute (%Brix; A, B) and normalised (r.u.; C, D) values of control [····] and 1-MCP treated [– –] 'Conference' pear harvested early (A, C; 2015: 114 dafb; 2016: 131 dafb) and late (B, D; 2015: 126; 2016: 148 dafb). Pear were cold stored in air [▲] or CA [■] at 1 °C for 8 (6) weeks followed by 14 (2015)/10 (2016) days in shelf-life (dis; 20 °C). Error bars refer to standard error.

4. Discussion

4.1. Characterisation of fruit developmental stage by respiratory behaviour

In a typical year such as in 2015, the pre-harvest onset of the climacteric of 'Conference' pear can be monitored by a distinct increase of respiration rate. This change in respiration rate provides patterns, which appear independently from absolute values. However, despite daily sampling the appearance of the sharp onset of climacteric rise could not be monitored reliably in every year of this study. These findings coincide with the respiratory behaviour of 'Moltke' pears, monitored over a period of six years, showing differing onset of the climacteric (Kvåle, 1977). The many smaller peaks, indifferent from the climacteric peak, appear in all years and may be explained by patchy photosynthetically active areas with chloroplasts or varying skin and flesh resistance considering the gas exchange and transport.

Pre-harvest ethylene burst (Figs. 1 and 2) indicated climacteric rise. However, owing to the high standard deviation of ethylene measurements (Saquet and Almeida, 2017), another parameter is necessary to analyse the climacteric stage of fruit development, particularly post-harvest. The additional distinction between cytochrome and cyanide resistant O_2 consumption complemented the gas exchange patterns of 'Conference' pear, characterising the developmental stage in more detail (Fig. 3). The highlighted appearance of climacteric rise of cytochrome respiration may be due to the removal of varying parallel processes affecting the gas exchange of the fruit. Furthermore, results of O_2 consumption in fruit tissue after exposure to cyanide, revealed a cyanide resistant respiratory pathway of 'Conference' pear, pre- as well as post-harvest (Figs. 3 and 6) as shown for climacteric apple (Duque and Arrabaça, 1999), banana (Theologies and Laties, 1978), cherimoya (Solomos and Laties, 1976), and avocado fruits (Theologies and Laties, 1978). An increase of cyanide-resistant respiration rate simultaneously to the increase of ethylene emission was shown for apple (Duque and Arrabaça, 1999) and tomato (Xu et al., 2012). Thus, for pear cyanide resistant-respiration was expected at time of ethylene burst. However, despite showing cyanide-resistant pathway at the beginning of fruit development and during later ripening and decay progress in shelf life after storage, no cyanide-resistant respiration was measured at time of pre-harvest ethylene burst. This is in contrast to previous studies, characterising the long-term response of fruit after exposure to CN^- (Theologies and Laties, 1978). Considering the results, the response time of cyanide-resistant respiration at least varies throughout fruit development. Particularly regarding time depending cyanide-resistant response and influence of tissue gas resistance of pear further research is required.

In control pear, pre-harvest patterns of respiration rate continue post-harvest. In years with high respiration rate at time of harvest, respiration rate of control 'Conference' pear is also high at removal from storage (Fig. 4). At the end of shelf-life, storage time is more important than storage condition, which requires rapid marketing after long-term storage. Respiration rate of control pear increased during shelf-life and decreased in the end (Figs. 1 and 4). Consistently, respiration rate of control 'Bartlett' pears increased in shelf-life during first 4 days and decreased afterwards (Trinchero et al., 2004). Also, 'Anna' apples showed decrease of respiration rate after 7 days in shelf-life conditions (Lurie, 2007). At the end of shelf-life pear showed high variability of respiration rate.

Regardless of year, harvest date, storage condition, and storage time, the respiration rate of 1-MCP treated 'Conference' pear remained low throughout quality progress in shelf-life, providing a benefit for keeping the quality in enhanced shelf life. The impact of temperature on reaction rate of 1-MCP treated pear, expressed as temperature coefficient Q_T, was unchanged compared to the Q_T of control fruit (Table 1). The non-linearly enhanced Q10 values at temperature range 12−20 °C compared to 1−12 °C quantifies the influence of temperature on respiration rate of 'Conference'

pear as shown for apple and guava (Zude-Sasse et al., 2000; Bron et al., 2005). Even more relevant for shelf life quality, the present results also show a major influence of storage time on Q_T. The shorter storage time, the higher divergence of reaction rate was found, usually ranging from 2 to 3 when increasing temperature by 10 K (Reyes et al., 2008). In the present study, after short term storage, Q_T values of control and 1-MCP treated 'Conference' pear ranged from 7.40 to 9.25. Results reveal increased respiration rate of intact pear after 2 and 4 weeks of storage, than reported for pear earlier (pear: 2.50, Cameron et al., 1995; Watkins, 2016) After 6 and 8 weeks of storage, respiration rate of control and 1-MCP treated pear differed. However, temperature-dependent respiratory behaviour after storage of 'Conference' pear pointed to no influence of 1-MCP treatment.

4.2. Post-harvest quality progress of pear depends on respiratory behaviour at time of harvest

The progress of varietal eating quality of 'Conference' pear after harvest differed depending on harvest date. Fruit flesh firmness is a commercially used indicator to predict the optimal harvest date of pear (Wang and Sugar, 2015). However, while pre-harvest characterisation of developmental stage by respiratory patterns highlighted differences, analyses of quality parameter did not allow the determination of harvest date by diagnostic changes. In this study, fruit flesh firmness at time of harvest differed between the years by 57.97 N cm^{-1} harvested early and 45.59 N cm^{-1} harvested late (Fig. 7). Since fruit flesh firmness is highly influenced by the production system and growth factors (Sams, 1999), an additional parameter to characterise the optimal harvest date appears reasonable. The results of this study revealed an influence of harvest date, and therefore of respiratory behaviour at time of harvest, on eating quality progress of 'Conference' pear in storage and shelf-life (Table 2). Control pear of both, early and late harvest date, revealed the full range of eating quality beginning with crispy texture at removal from storage and buttery flesh at the end of shelf-life (Fig. 7). However, decrease of fruit flesh firmness of pear treated with 1-MCP was greatly inhibited in agreement with previous studies showing an evergreen effect of 'Conference' pear (Chiriboga et al., 2011) and 'Bartlett' pear (Trinchero et al., 2004).

The SSC progress in shelf life was depending on respiratory behaviour at time of harvest and affected by storage atmosphere and treatment in shelf-life as well. Pre-harvest measurement of SSC only, did not indicate the change of developmental stage. Since pear harvested late and stored in air revealed higher SSC than pear harvested early and stored in CA, the determination of harvest date by means of the respiration rate - with regard to storage atmosphere - could improve SSC progress and eating quality in storage and shelf-life. For both, pear harvested early and late, 1-MCP treatment resulted in similar SSC values at the end of shelf-life,

regardless of storage conditions. These findings are consistent with previous studies of 'Bartlett' pear harvested at optimal maturity stage for long term storage (Trinchero et al., 2004).

The non-destructively measured NDVI showed steady values throughout fruit development at the tree (Fig. 1). However, the change of NDVI in shelf-life conditions was affected by harvest date. In previous studies it was reported for 'Conference' pear, that a late harvest date leads to more pronounced decrease of chlorophyll content in shelf-life (Błaszczyk, 2012). This was confirmed in the present study, also considering storage conditions.

5. Conclusions

The results point out the link between fruit quality and respiration rate. Data show the respiratory patterns in 'Conference' pear when characterising the fruit developmental stage pre- as well as post-harvest aimed at determining the date of harvest and post-harvest treatment. The method for measuring cytochrome and, particularly, cyanide-resistant respiration rate in fruit still needs more studies due to variable responses.

Declarations

Author contribution statement

Nicole Brandes: Performed the experiments; Analyzed and interpreted the data; Contributed reagents, materials, analysis tools or data; Wrote the paper.

Manuela Zude-Sasse: Conceived and designed the experiments; Analyzed and interpreted the data; Contributed reagents, materials, analysis tools or data; Wrote the paper.

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The authors declare no conflict of interest.

Additional information

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