

1 **Monitoring low O₂ stress in apples – what we can learn from chlorophyll**
2 **fluorescence and respiratory characteristics**

3 **Authors**

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11 **Highlights**

- 12 • DCA-RQ and DCA-CF differ in their sensitivity to low O₂ stress in apple
- 13 • Chlorophyll fluorescence in apples suffering low O₂ stress reflects the internal
14 state of the fruit
- 15 • DCA-RQ and DCA-CF response differs between ‘Gala’ and ‘Braeburn’
- 16 • Elevated initial CO₂ results in depressed DCA-RQ and DCA-CF responses

17 **Abstract**

18 Dynamic controlled atmosphere (DCA) is becoming a storage method of choice for
19 apple producers seeking to store long-term. There is limited prior work comparing DCA
20 monitoring chlorophyll fluorescence yield (DCA-CF) and respiratory characteristics
21 (DCA-RQ), particularly for the popular UK-grown apple cultivars 'Gala' and 'Braeburn'.
22 However, these techniques can give an insight into the physiological response of apples
23 to low O₂ storage, which could help to design more efficient and sustainable storage
24 regimes. In this study the chlorophyll fluorescence (CF) and respiratory quotient (RQ)
25 responses of 'Gala' and 'Braeburn' are compared in typical and exaggerated DCA
26 conditions. Results show that not only do the two techniques differ in their detection of
27 low O₂ stress but the two cultivars studied also differ noticeably in their behaviour. CF
28 increase was confirmed to be a reaction to a stress signal from inside the fruit.
29 Respiration rate curves were found to reflect the internal structure of the fruit, with
30 'Gala's more homogeneous tissues and greater gas permeability resulting in a strongly
31 bimodal O₂ consumption rate where the change in rate hypothetically corresponds with
32 the majority of the fruit tissue switching to anaerobic respiration at the same time. CF
33 and RQ showed noticeable differences between cultivars, agreeing with previous
34 observations that 'Braeburn' is less tolerant of low O₂ and elevated CO₂ than 'Gala' (CF
35 Lower O₂ Limit (LOL) for Braeburn of approximately 0.75 kPa O₂ relative to 0.5 kPa O₂
36 for Gala). Additionally, the CF and RQ responses indicated different LOLs for 'Gala', at
37 approximately 0.5 kPa O₂ for CF and 0.25 kPa O₂ for RQ. Elevated CO₂ depressed both
38 the CF and RQ response, with a strong depressive effect on the rate of CO₂ evolution
39 but little effect on the rate of O₂ consumption.

40 **Keywords**

41 Apple (*Malus domestica*), controlled atmosphere storage, chlorophyll fluorescence,
42 respiratory quotient

43 **1 Introduction**

44 Classical CA relies on cultivar-specific storage trials to determine a safe atmosphere for
45 storage, at which the development of storage disorders is minimised. As research has
46 continued into the use of CA, the recommended concentrations of O₂ in particular have
47 fallen. Ultra-Low O₂ (ULO, O₂ ≈ 0.7 %) and Initial Low O₂ Stress (ILOS, O₂ of 0.25-
48 0.5 %) regimes have become commonplace (Wang and Dilley, 1999; Zanella, 2003).

49 The most recent development is that of DCA, which exploits various characteristics of
50 the fruit measurable within the storage room to identify the Lower O₂ Limit (LOL) at
51 which metabolism becomes anaerobic. The aim is to maintain an O₂ concentration just
52 above the LOL to minimise respiration and extend storage life without the risk of low O₂
53 damage (Wright et al., 2015). Two important methods of DCA are measures of
54 chlorophyll fluorescence yield (CF) of dark-adapted fruit, which increases when the
55 produce experiences low O₂ stress, and monitoring the respiratory quotient (RQ),
56 defined as the ratio of CO₂ evolution to O₂ consumption, in real time. An increase in RQ
57 at low O₂ concentrations indicates a switch from predominantly aerobic to
58 predominantly anaerobic respiration and can therefore be used to pinpoint the LOL.

59 In chloroplasts, light energy absorbed by the antenna molecules of photosystem II is
60 used to power photosynthesis, with a small proportion of the energy dissipated as
61 fluorescence and heat. Fluorescence can thus be used to probe the response of the

62 photosynthetic material to changes in the surrounding environmental conditions
63 (Strasser et al., 2000). Duysens and Sweers (1963) formulated the idea that
64 fluorescence yield is indicative of the oxidation state of the plastoquinone (PQ) pool in
65 the thylakoid membrane (Schansker et al., 2011). PQ would ordinarily quench the CF by
66 transferring the electrons onwards within the Z-scheme, but reduced PQ cannot perform
67 this function; a sharp spike in CF (above the minimum F_0 level) is seen after brief
68 excitation, followed by a drop as the PQ is gradually re-oxidised (Wright et al., 2011).
69 The redox state of the PQ pool is linked to the cellular NADH/NAD⁺ ratio which
70 increases under anoxic conditions (van Dongen et al. 2003). Therefore, an anaerobic
71 environment, even in darkness, leads to an over reduction of the PQ pool, inhibiting
72 electron transfer from photosystem II and increasing CF (Harris and Heber 1993;
73 Bennoun 2002; Tóth et al. 2007). The HarvestWatch™ DCA system, a pulse-frequency
74 modulated fluorimeter, measures the CF parameter F_α (claimed to be indistinguishable
75 from F_0), and therefore is claimed to detect PQ reduction associated with low O₂ stress
76 (Prange et al., 2003; Wright et al., 2011).

77 In anaerobic respiration, by definition, no O₂ is consumed and therefore the RQ will tend
78 to infinity. Thus, an increase in RQ can indicate when plants or plant organs are
79 experiencing physiological stress. In DCA a threshold RQ is often chosen based on the
80 respiratory characteristics of the produce, and breaching this triggers the addition of
81 more O₂ to the storage atmosphere. RQ is known to be affected by tissue type and age,
82 temperature and atmospheric composition, with high CO₂ sometimes depressing
83 respiration (Fidler and North, 1967). Apples are one of the produce types which exhibit
84 this sensitivity to CO₂ (Knee, 1973) and in apple, chicory and tomato (at 10 °C) the RQ

85 was found to be consistently lower than unity (Hertog et al., 1998). Yearsley et al.
86 (1996) defined the RQ breakpoint as the partial pressure of O₂ at which the steady-state
87 RQ begins to rise as O₂ concentration decreases. This has also become known as the
88 anaerobic compensation point. In apples the LOL based on this breakpoint has been
89 found to be at least partly related to the cuticular permeability of the apple (Gran and
90 Beaudry, 1993). This supports the commercial observation that tolerance to anaerobic
91 conditions varies by cultivar, although this difference has also been attributed to the
92 overall permeance of CO₂ through apple tissues. Elevated CO₂ has also been found to
93 lower the RQ, with this effect more pronounced at lower temperature, thought to be due
94 to temperature-related effects on gas diffusion into the fruit whereby cuticular gas
95 diffusion would be greater at the higher temperature but diffusion through lenticels
96 would not significantly change (Yearsley et al., 1997).

97 Some comparisons have been made between CF and RQ as DCA methods. Identical
98 anaerobic compensation points were observed across the techniques for several
99 cultivars ('Braeburn', 'Idared', 'Maigold', 'Elstar' and 'Golden Delicious'); this was
100 noticeably higher for 'Braeburn' than for the other cultivars (Gasser et al., 2010). When
101 RQ was compared to CF in 'Gala' stored for eight months, a lower temperature
102 disproportionately affected RQ, increasing low O₂ damage in the fruit (Weber et al.,
103 2015). This study recommended RQ (with a threshold of 2) as the DCA technique of
104 choice at 1 °C, but CF as the preferred technique at 0.5 °C. Although not specifically
105 stated, this is presumably because the CF inflection point occurred at a higher O₂
106 concentration than the RQ threshold. In a direct comparison of the two technologies

107 used here, a strong positive correlation ($R=0.77$) was found between CF and RQ
108 responses for both 'Gala' and 'Braeburn' (Rees et al., 2021).

109 Commercially, apples of cultivars 'Gala' and 'Braeburn' require quite different storage
110 atmospheres in order to maximise the quality of the produce coming out of store; 'Gala'
111 fruit are less sensitive to low O_2 than 'Braeburn', and can also be stored at high CO_2 .
112 Under commercial storage conditions, a response in terms of CF increase or RQ
113 increase is observed at higher O_2 concentrations for 'Braeburn' than for 'Gala'. The
114 respiratory response of a bulky plant organ such as an apple to low O_2 will be a
115 cumulative one; reflecting a range of different tissue types, and different densities,
116 across the fruit (Ho et al., 2010). By comparison, the CF response is a localised
117 snapshot of the redox state of the chloroplasts in the periderm and their response to
118 metabolic signals from within the fruit. In practical application DCA-CF and DCA-RQ
119 give very similar results, but the difference between cumulative and localised responses
120 is expected to give differing results under some circumstances.

121 In this context, the study aimed to confirm that the CF response of apple fruit to low O_2
122 stress reflects the response of all the tissues of the fruit, rather than just that of the
123 surface tissues. This was done by exposing whole fruit, cored fruit and fruit peel for
124 'Gala' and 'Braeburn' to a range of low O_2 concentrations. A further objective was to
125 compare CF and respiratory characteristics of 'Gala' and 'Braeburn' over a range of O_2
126 concentrations as well as over a range of different CO_2 concentrations in order to
127 determine whether the two technologies are monitoring the same fruit responses, and
128 lastly, to examine how repeated exposure of fruit to low O_2 concentrations, as would be
129 standard in commercial application of DCA technology, affects the response.

130 **2 Materials and Methods**

131 **2.1 Plant materials, chemicals and equipment**

132 Trials were carried out in temperature-controlled stores at the facilities of the Produce
133 Quality Centre of the Natural Resources Institute, University of Greenwich located in
134 Kent, UK in 2018/19 and 2021/22. Apples of both cultivars used in this study, 'Gala'
135 and 'Braeburn', were harvested from commercial orchards in Kent (UK). Fruit were
136 sampled 1-5 per tree from across an orchard and randomised before storage in nets
137 containing 20 apples. These were refrigerated in air at 1-2 °C within temperature-
138 controlled stores before the trials were set up.

139 The LabPods (430 L Capacity) and LabPod Minis (41 L capacity) used were provided
140 by Storage Control Systems (SCS) Ltd, UK (Rees et al., 2021). These are placed within
141 temperature-controlled rooms, and can be used to control the storage atmosphere, or
142 run with the control switched off so that changes in O₂ and CO₂ can be used to calculate
143 rates of fruit respiration. HarvestWatch™ CF sensors (Isolcell, Italy) were obtained from
144 UKCA Ltd.

145 **2.2 Comparison of CF response of whole fruit, cored fruit and peel**

146 Sequential assessment of the CF response to decreasing O₂ concentration of whole
147 fruit, cored fruit and peel was carried out in February/March 2022 for 'Gala' and
148 'Braeburn' apples harvested in September and October 2021 respectively. Five LabPod
149 Minis each containing approximately 6 kg of fruit were used for each cultivar. Opaque
150 black pod covers were employed to exclude light, and the temperature was maintained

151 at 2 °C, with a starting atmosphere of 1.2 kPa O₂ and 0.4 kPa CO₂. For three LabPod
152 Minis, the fruit was allowed to respire naturally to reduce the O₂ concentration to 0 kPa.
153 The other two were maintained with the starting atmosphere through all stages of the
154 trial. The HarvestWatch™ sensors were suspended above the fruit within each LabPod
155 Mini to record CF from the upper visible surface of all fruit and were set to a 30 min
156 monitoring interval.

157 Once each LabPod Mini of whole fruit had reached 0 kPa O₂ the LabPod Mini was
158 opened and a 3 cm diameter core was removed vertically from the centre of each apple
159 (#18 borer). The fruit were returned to the LabPod Minis and the experiment was run
160 again under the same conditions. Following the cored fruit reaching 0 kPa O₂ the
161 LabPod Mini was again opened. Sufficient fruit were peeled to cover an A4 size plastic
162 tray with peel, skin uppermost. The peeled fruit were returned to the crate and the tray
163 was placed on top so that the HarvestWatch™ sensors recorded CF from the upper
164 surface of the apple skin only. The peeled and/or cored fruit was left within the LabPod
165 Mini to reduce the O₂ naturally through respiration, and the experiment was run for a
166 third time under the same conditions as before. The same fruit/pod/sensor combinations
167 were used throughout to control for possible differences in the HarvestWatch sensor
168 calibrations.

169 **2.3 Evaluation and comparison of CF, respiration rates and RQ response of ‘Gala’**
170 **and ‘Braeburn’ apples to low O₂ and the effect of CO₂**

171 In the 2018-19 season of experiments, ‘Gala’ and ‘Braeburn’ apples were harvested in
172 September and October 2018 respectively and stored under refrigeration in air within
173 the temperature-controlled stores at the Produce Quality Centre.

174 Five LabPod Minis (41 L capacity) each filled with approximately 6 kg of fruit were used
175 for each cultivar, each with a HarvestWatch™ sensor positioned above the fruit. Starting
176 atmospheres were 0.8 kPa O₂ with 0.4 kPa, 2.5 kPa, 5 kPa, 7.5 kPa or 9 kPa CO₂ for
177 ‘Gala’ and 1 kPa O₂ with 0.4 kPa, 1 kPa, 1.5 kPa, 2 kPa and 3 kPa CO₂ for ‘Braeburn’;
178 both cultivars were held at 2 °C. ‘Gala’ experiments started on 10th October 2018 and
179 ‘Braeburn’ started on 12th November 2018 (two replicates each, with different fruit). The
180 ‘Braeburn’ experiment included a repeated drop to 0 kPa O₂ and return to starting
181 atmosphere, with 24 h acclimation each time, to study the repeatability of the CF and
182 RQ response.

183 **2.4 Calculation of respiration rates and statistical analysis**

184 The system recorded the gas concentration and atmospheric pressure every 15 min.
185 Atmospheric pressure was used to adjust the gas readings to the value corresponding
186 to 101.3 kPa using the following equation.

187 Compensated gas value = $G_{\text{meas}} \times 101.3 / P_{\text{meas}}$ (1) Where G_{meas} is the measured gas
188 reading and P_{meas} is measured atmospheric pressure in kPa at the time of the reading.
189 Gas concentrations used in this paper are the adjusted values. To calculate respiration

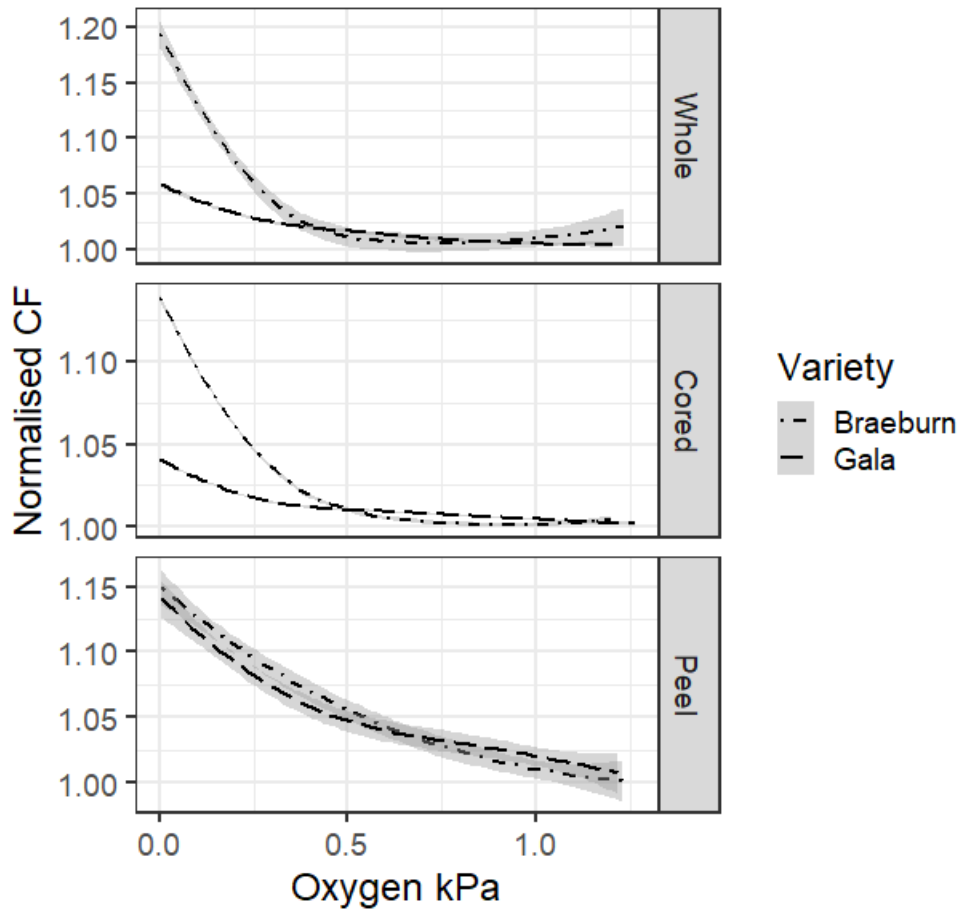
190 rates, the Excel LINEST function was employed to determine the rate of change (slope)
191 of O₂ and CO₂ concentrations over a rolling two-hour period. To calculate the RQ the
192 rate of CO₂ evolution was divided by the rate of O₂ consumption. This ratio was found to
193 change during the cold store defrost cycle, probably due to slight changes in the store
194 temperature, therefore LOESS (localised regression smoothing) was used to fit a curve
195 to plot the change of RQ with O₂ concentration. In all graphs, confidence intervals were
196 calculated as an integral part of the R ggplot/loess function (R version 3.6.1 “Action of
197 the Toes”) and are shown as grey shaded areas. While the LabPod software is capable
198 of calculating RQ, in order to maintain accuracy and repeatability the decision was
199 taken to calculate RQ manually from the raw data.

200 Correct calculation of respiration rates depends on LabPod Minis being well sealed.
201 Where leaks occur they can be detected by a rise in O₂ or decrease in CO₂. Wherever
202 this was observed the data was discarded. This was observed in less than 5% of cases.

203 **3 Results**

204 **3.1 Comparison of CF response of whole fruit, cored fruit and peel**

205 In the case of whole fruit, cored fruit and apple peel for both ‘Gala’ and ‘Braeburn’, it
206 was hypothesised that a difference would be seen between the CF responses if it was
207 reacting to a stress signal from within the fruit, whereas if CF response was solely
208 reflecting the stress state of the chloroplasts in the periderm there would be no
209 difference between response for whole fruit and for peel.



210

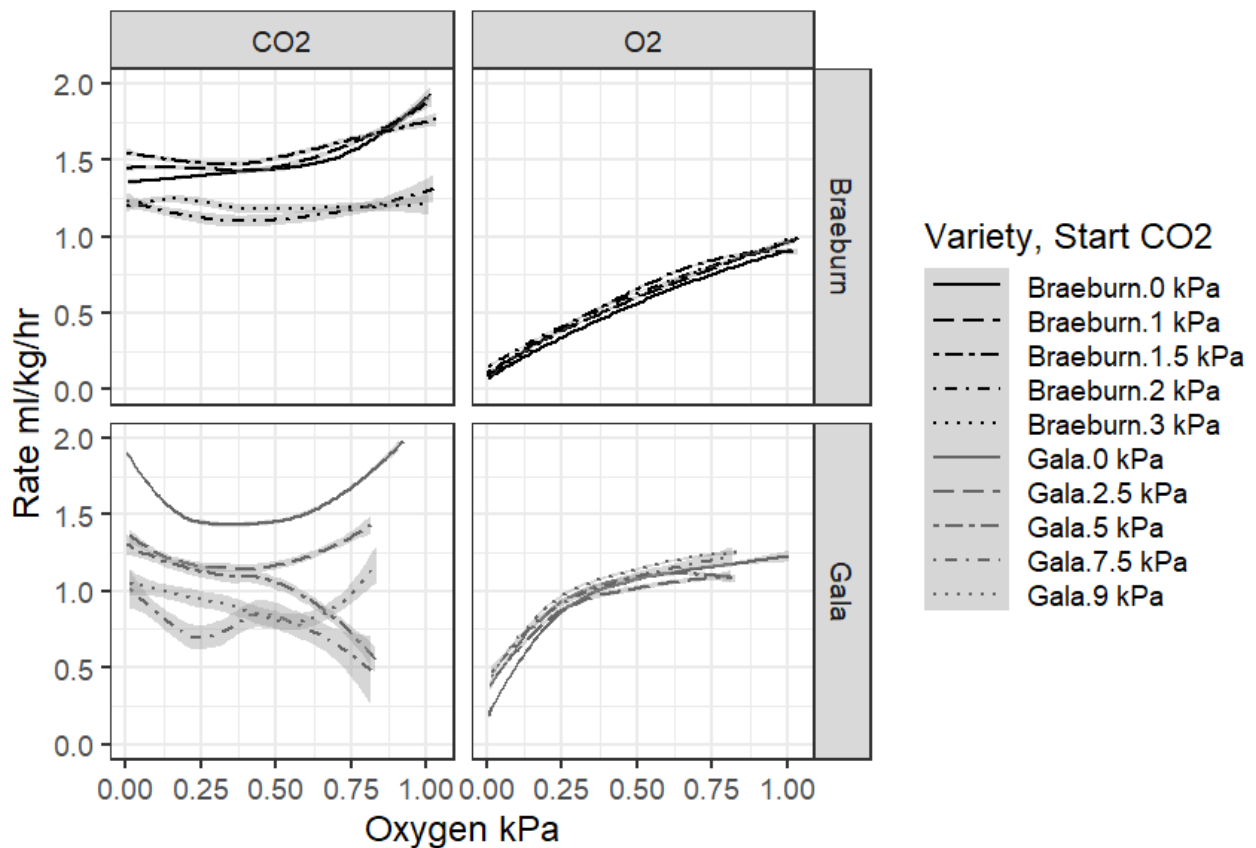
211 Figure 1 Season 2021-22 normalised CF response for 'Gala' and 'Braeburn' whole
 212 apples, cored apples and peel as a function of O₂ concentration. Each data set is the
 213 mean of data from 3 replicate samples, with LOESS confidence intervals. All data sets
 214 were obtained in February-March 2022 using single HarvestWatch sensors positioned
 215 at the top of a LabPod mini containing 6 kg fruit, in a temperature-controlled room set at
 216 2-2.5 °C. 'Braeburn' and 'Gala' fruit were harvested in September and October 2021
 217 respectively and stored under refrigeration in air prior to the measurement. CF response
 218 was normalised to the reading at initial O₂ concentration (1.2 kPa)

219 Under identical atmospheric composition and temperature, whole fruit from 'Braeburn'
220 and 'Gala' displayed clear differences in CF response as a function of O₂ concentration
221 (Figure 1), in agreement with previous observations by the authors (Rees et al. 2021).
222 This difference was retained when the fruit were cored. In both cases, CF from
223 'Braeburn' showed an increase at a higher O₂ concentration than for 'Gala'. The
224 difference between cultivars was not apparent for peel samples indicating that the
225 presence of the apple flesh and core was contributing to the CF response and causing
226 the differences seen between 'Gala' and 'Braeburn' for whole fruit. Controls kept at 1.2
227 kPa O₂ and 0.4 kPa CO₂ throughout the trial had constant CF yield for whole fruit, cored
228 fruit and peel for both cultivars, indicating that the response of CF yield to decreasing O₂
229 was not substantially affected by tissue wounding. Normalised values of chlorophyll
230 fluorescence yield were used as measured CF yield is dependent on the relative
231 positioning of the sensor and fruit; the onset of low O₂ stress is indicated by the
232 increase in CF yield rather than absolute value. It was observed when comparing the
233 shapes of the lines that when stored under the same storage conditions 'Braeburn'
234 displays symptoms of low O₂ stress at higher O₂ concentrations than 'Gala'.

235 **3.2 Evaluation and comparison of CF, respiration rates and RQ response of 'Gala'** 236 **and 'Braeburn' apples to low O₂ and the effect of CO₂**

237 In 2018-19, the effect of elevated initial CO₂ concentrations was studied to mimic the
238 standard UK industry practice of storing 'Gala' in 1 kPa O₂ and 5 kPa CO₂. 'Gala' had a
239 higher O₂ consumption rate than 'Braeburn' throughout the O₂ range considered (0.05
240 kPa to 1.0 kPa, 2.0-2.5 °C) (Figure 2). 'Braeburn' O₂ consumption rate was more

241 sensitive than that of 'Gala' to a decrease in O₂ concentrations. For both cultivars O₂
 242 consumption rate was largely unaffected by differences in the initial CO₂ concentration.
 243 However, for both cultivars the CO₂ evolution rate was strongly affected by initial CO₂
 244 concentration, with higher initial CO₂ concentrations resulting in lower CO₂ evolution
 245 rates.

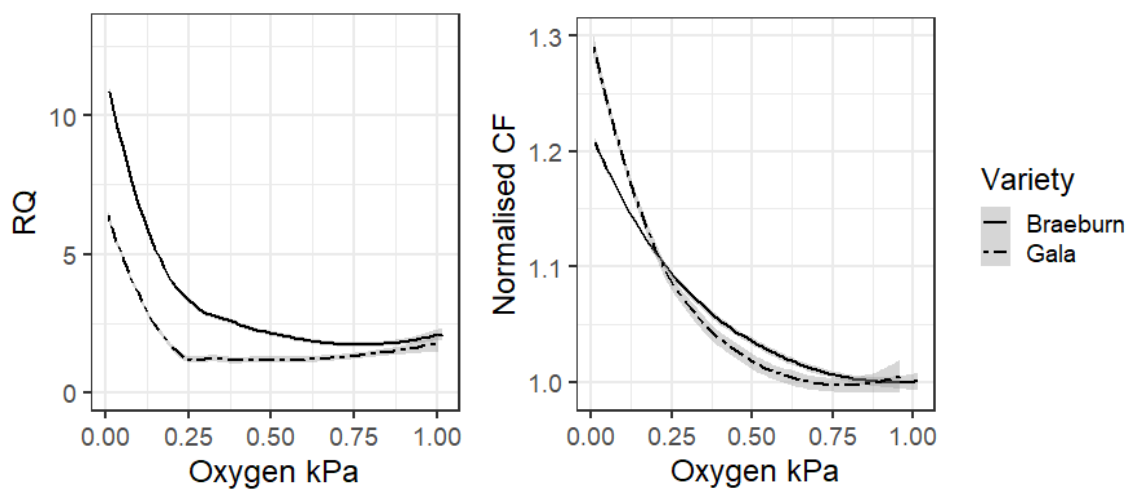


246

247 Figure 2 2018-19 O₂ consumption and CO₂ evolution rates (ml/kg/h) for 'Gala' and
 248 'Braeburn' apples across a range of initial CO₂ concentrations (in kPa, see legend) at
 249 low O₂ concentration and low temperature (2-2.5 °C). Each data set was obtained for a
 250 single LabPod Mini containing approximately 6 kg fruit, between one and four weeks

251 after harvest in 2018. The fruit were placed initially at 1 kPa O₂ and the O₂ concentration
252 decreased through respiration. LOESS smoothing was applied

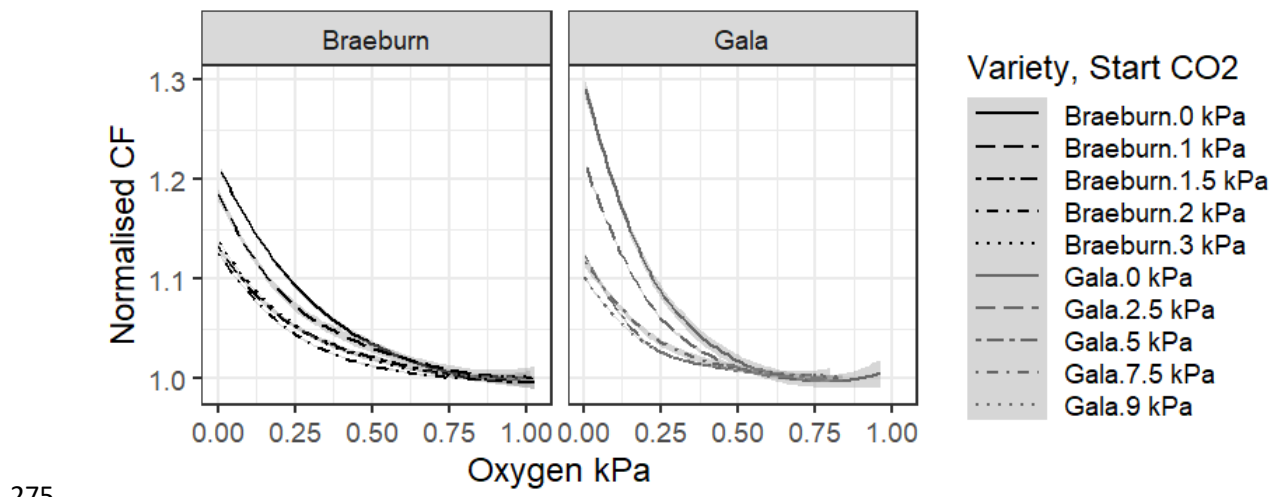
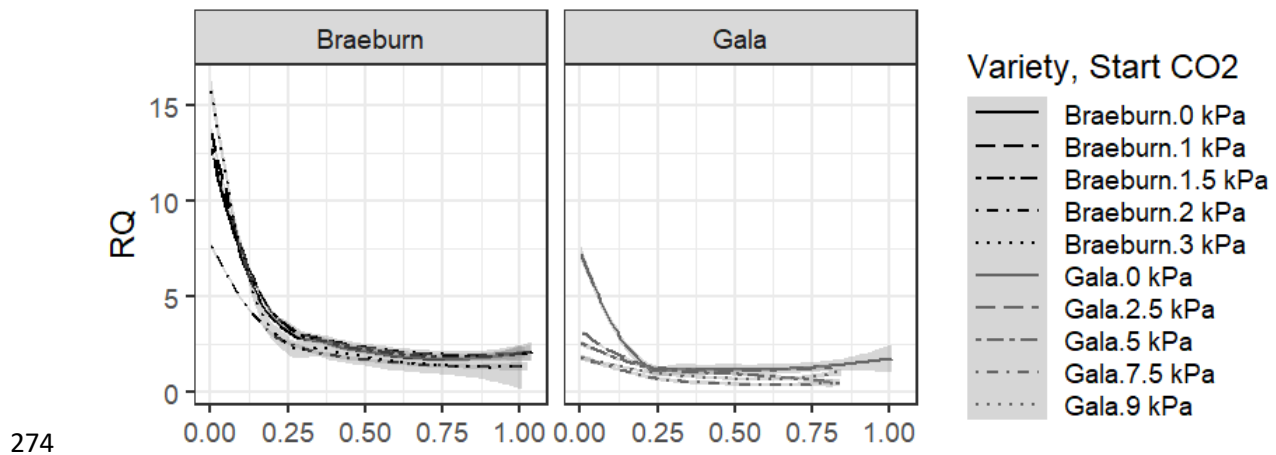
253 The shape of the O₂ consumption curve was very different for the two cultivars; with a
254 bimodal shape for 'Gala' that was not observed for 'Braeburn'. The RQ response to
255 decreasing O₂ concentration showed a difference between 'Gala' and 'Braeburn' (Figure
256 3), with the 'Braeburn' response increasing at a higher O₂ concentration than that for
257 'Gala'. The CF responses were less different but still showed 'Braeburn' starting to
258 increase at a higher concentration of O₂ than 'Gala'. 'Braeburn' RQs were noticeably
259 high relative to 'Gala'.



260

261 Figure 3 2018-19 RQ (left) and normalised CF (right) for 'Gala' and 'Braeburn' fruit at
262 low O₂ concentration, 0.4 kPa CO₂ and low temperature (2-2.5 °C). Each data set was
263 obtained using a single chamber containing approximately 6 kg fruit, between one and
264 four weeks after harvest in 2018. LOESS smoothing was applied. CF response was
265 normalised to the reading at initial O₂ concentration (1.2 kPa)

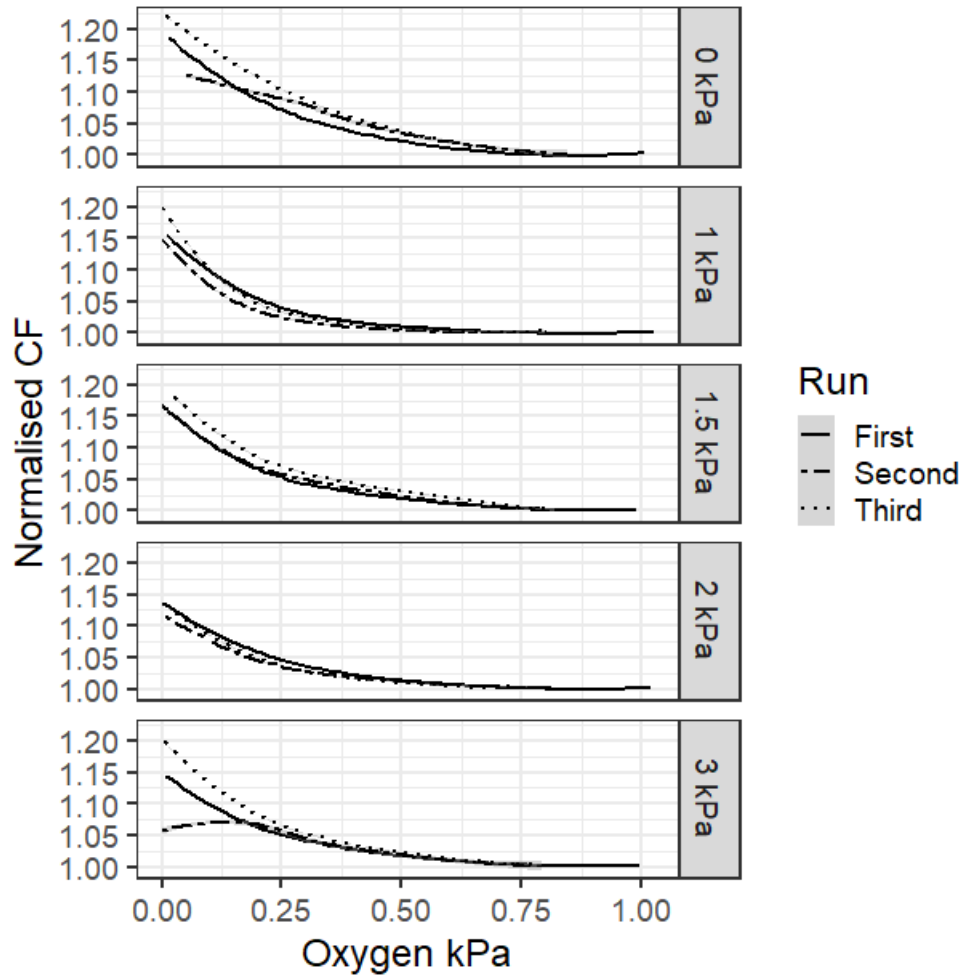
266 For 'Gala' a noticeable depression of the RQ response was seen with increasing initial
 267 CO₂ concentrations, although under all conditions the RQ still demonstrated an
 268 increased response at approximately the same O₂ concentration (Figure 4). A
 269 corresponding depression of the CF response was also seen. 'Braeburn' also showed a
 270 depression of the CF and RQ responses as a result of increased initial CO₂. Lower
 271 concentrations of CO₂ were studied for 'Braeburn' than for 'Gala' to be consistent with
 272 commercial CA conditions. Comparing the 0 and 2.5 kPa CO₂ lines for 'Gala' with the 0
 273 and 2.0 kPa CO₂ lines for 'Braeburn' the depression appeared to be comparable.



276 Figure 4 2018-19 RQ and normalised CF for 'Gala' and 'Braeburn' apples showing the
277 effect of different initial CO₂ concentrations at low O₂ concentration and low temperature
278 (2-2.5 °C). Each data set was obtained using a single chamber containing 6 kg apples,
279 between one and four weeks after harvest in 2018. LOESS smoothing was applied. CF
280 response was normalised to the reading at initial O₂ concentration (1.2 kPa)

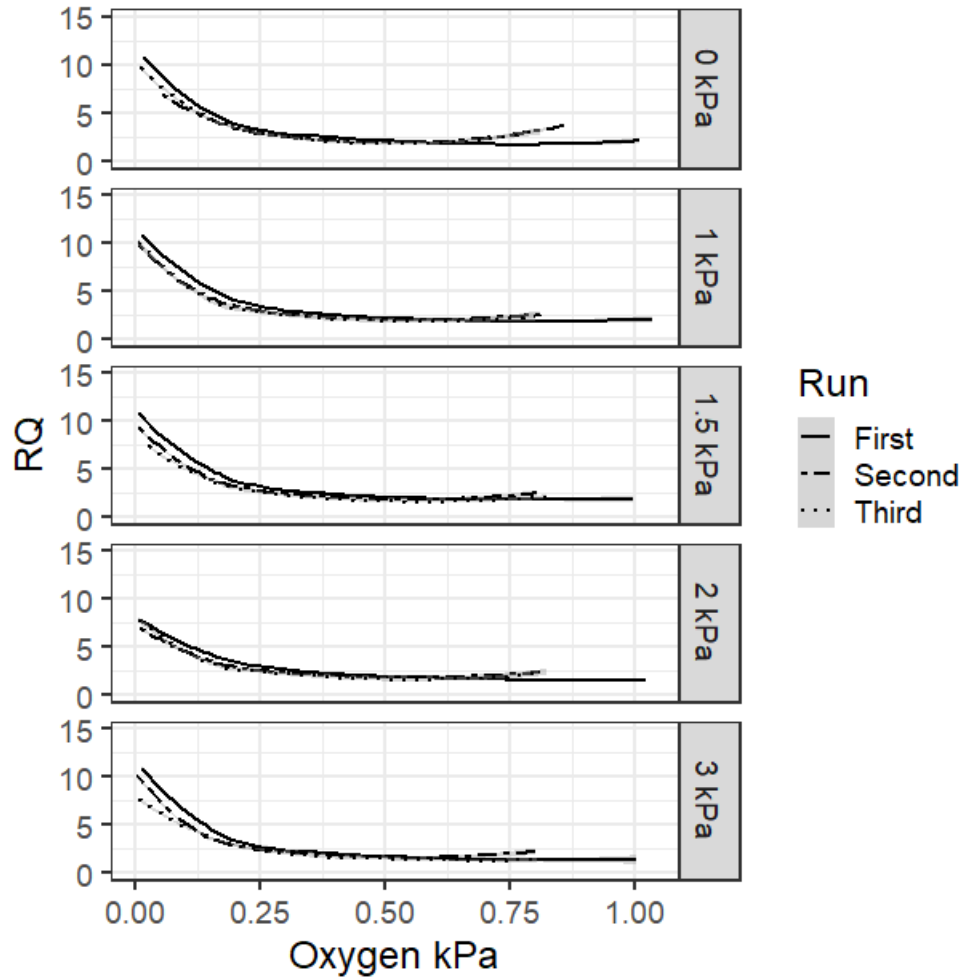
281 **3.3 The effect of repeatedly measuring CF and RQ on the same fruit**

282 The 2018-19 'Braeburn' experiment included repeated runs allowing respiration to take
283 the O₂ concentration to 0 kPa O₂ before the atmosphere was adjusted back to the
284 starting conditions for 24 h (three drops in total) to study the repeatability of the CF and
285 RQ responses under five different initial CO₂ concentrations, conducted within one
286 month of harvest. Only 'Braeburn' was studied due to its greater stress response. In
287 most cases repeated drops to 0 kPa O₂ resulted in increased CF from the first to the
288 third run (Figure 5). By contrast the RQ drops slightly from the first to the third runs
289 (Figure 6).



290

291 Figure 5 2018-19 normalised CF for 'Braeburn' apples across repeated runs showing
 292 the effect of different initial CO₂ concentrations with low O₂ concentration and low
 293 temperature (2-2.5 °C). Normalisation was applied relative to the initial CF yield at 1 kPa
 294 oxygen. Each data set was obtained using a single HarvestWatch sensor positioned at
 295 the top of a LabPod Mini chamber containing approximately 6 kg fruit, within one month
 296 of harvest in 2018. LOESS smoothing was applied



297

298 Figure 6 2018-19 RQ for 'Braeburn' apples across repeated runs showing the effects of
 299 different initial CO₂ concentrations (with CO₂ leak correction applied) with low O₂
 300 concentration and low temperature (2-2.5 °C). Each data set was obtained using a
 301 single chamber containing 6 kg of apples, within one month of harvest in 2018. LOESS
 302 smoothing was applied

303 **4 Discussion**

304 It is important to remember that in all cases the results presented in this study were
 305 obtained at low O₂ concentrations (<1.2 kPa O₂) and low temperature (2-2.5 °C), and

306 therefore the conclusions can only be firmly applied under the stated experimental
307 conditions. In addition, apart from the first reported experiment (comparing whole, cored
308 and peeled apples), the apples were studied between one and four weeks of harvest.
309 The response of the fruit to low O₂ tends to decline during the storage season (Rees et
310 al., 2021). The fruit were not adapted to CA storage.

311 A pronounced difference was observed between 'Gala' and 'Braeburn' in terms of both
312 RQ and CF responses to low O₂ with Braeburn more sensitive to low O₂. This accords
313 with industry experience, but it is believed that this was the first time the two cultivars
314 have been compared under identical conditions. Under low O₂ CA conditions (<1.2 kPa)
315 and when measured within one month of harvest, the differences between respiration
316 rates for 'Gala' and 'Braeburn' were pronounced and consistent, with 'Gala' consuming
317 O₂ faster than 'Braeburn' (Figure 2). This could be due to either differences in the
318 respiratory substrate or a difference in fruit cellular structure. It has been observed that
319 Braeburn has a denser, less homogeneous cellular structure than Gala, with more
320 restricted gas diffusion (Ho et al., 2010). Under CA conditions the range of O₂
321 concentrations to which the underlying cells are exposed would therefore be more
322 variable and at lower concentrations for Braeburn than for Gala; a higher proportion of
323 cells would be in an anaerobic state. The respiratory characteristics of an apple will be a
324 summation of the characteristics of all the different tissues throughout the fruit.
325 Therefore, it is suggested that the different shapes of the O₂ consumption curves for
326 'Gala' compared to 'Braeburn' are a consequence of the differences in fruit structure
327 between the cultivars. Since gas diffusion through 'Gala' tissues is faster than through
328 'Braeburn' (Hertog et al., 1998, Ho et al., 2010), the bimodal nature of the 'Gala' curve

329 indicated a homogeneous situation where the whole fruit started to experience low O₂
330 stress at approximately the same time, while the more gradual curve of 'Braeburn'
331 would be the result of a range of internal O₂ and CO₂ concentrations. In addition slower
332 gas diffusion through the tissues of Braeburn will result in an underestimation of
333 respiration rates measured using external sensors (Bessemans et al., 2020). At O₂
334 concentrations <1.2 kPa 'Braeburn' had a consistently higher RQ than 'Gala' when
335 measured within one month of harvest (Figure 3). This is consistent with core tissues
336 being anaerobic and could be exacerbated due to the gas concentration in internal fruit
337 tissues and the surrounding atmosphere not being in a steady state, causing a build-up
338 of CO₂ inside the fruit.

339 The pronounced difference between 'Gala' and 'Braeburn' CF responses persisted in
340 fruit with the core removed, but largely disappeared in fruit peel with only a small
341 quantity of cortex attached (Figure 1), which strongly suggests that for intact fruit the
342 fruit cortex at some distance from the surface was involved in signalling low O₂ stress to
343 the chloroplasts in the periderm. The results support the assumption that there is redox
344 signalling between cells (Wright et al., 2011), thus providing a mechanism for
345 chloroplasts at the apple surface to respond to the anaerobic environment in the deeper
346 apple tissues. Wright et al (2015) cite a study on wheat roots which indicated that under
347 anoxic conditions the intercellular communication via plasmodesmata was strengthened
348 as a defence against anoxia (Cleland et al., 1994). In the presence of light CF can also
349 be affected by non-photochemical mechanisms of quenching. The increase in CF
350 observed at low O₂ is theorised to be related to reduction of the PQ pool, but it is also
351 known that carotenoids, including those involved in the xanthophyll cycle, are involved

352 in both fluorescence quenching and scavenging reactive oxygen species (ROS) (Das
353 and Roychoudhury, 2014; Latowski et al., 2011). As these compounds are known to be
354 found in both apple peel and flesh (Delgado-Pelayo et al., 2014) it is possible that
355 differences in concentrations of carotenoids and/or xanthophylls could contribute to the
356 differences seen in CF response and other comparisons between 'Gala' and 'Braeburn'.

357 CF and RQ both responded to low O₂ stress, but the pattern of response is not the
358 same (Figure 3): in 'Gala', CF is more sensitive than RQ while little difference was seen
359 for 'Braeburn'. The observations suggest that in 'Braeburn' there is little difference
360 between the points at which these two responses begin, while in 'Gala' the CF response
361 occurs earlier (i.e. at a higher O₂ concentration) than the respiration response. Both RQ
362 and CF provide a signal that responds to low O₂ directly at the fruit surface and also
363 indirectly for the deeper apple tissues. In each case the signal used for the DCA
364 technologies is a composite signal for a range of tissue depths and types. For RQ the
365 observed response depends on the diffusion of O₂ and CO₂ through the apple tissues.
366 For CF the observed response depends on the intercellular redox signalling. Any
367 differences between the observed RQ and CF response will therefore depend on these
368 two mechanisms (gas diffusion and intercellular redox signalling).

369 Research into the effects of wounding of photosynthetic tissues on chlorophyll
370 fluorescence indicate that wounded tissue produces increased fluorescence yield (Bown
371 et al., 2002). However, in the work reported here the wounding was not to the
372 photosynthetic tissues but to the underlying cortex tissue. For both cultivars, elevated
373 CO₂ concentrations in the storage atmosphere did not noticeably affect O₂ consumption

374 rate but significantly depressed CO₂ evolution rate (Figure 2). CO₂ also depressed the
375 magnitude of the response for both CF and RQ while not affecting the location of the
376 LOL inflection point. In commercial practice, both the HarvestWatch and SafePod DCA
377 systems monitor for an increase in CF or RQ with or without specifying a threshold level
378 to be exceeded, therefore the depression in response is not necessarily commercially
379 significant. High external CO₂ concentrations will reduce the diffusion of CO₂ out of the
380 fruit. This would certainly affect the externally measured CO₂ evolution rate and thus the
381 RQ. However in addition high CO₂ is known to depress respiration rate directly and
382 since the mitochondrial electron transport chain is a significant producer of ROS in
383 response to stress (for example low O₂ stress as in this case) it would be expected that
384 depressed activity of the stressed electron transport chain would result in increased
385 ROS production (therefore increase in redox signalling); CO₂ must also be affecting the
386 physiological response which causes the increase in CF yield.

387 Repeated measurements of both RQ and CF with 24 h between measurements resulted
388 in different behaviours for the two parameters. RQ drops slightly from the first to the
389 third measurement run while CF tends to increase. The D1 and to some extent the D2
390 proteins in PSII are known to be susceptible to damage by ROS, among others (Foyer
391 and Noctor, 2000) and therefore the increased CF (decreased quenching) could be
392 explained as a result of either irreversible or very slowly reversible damage to these
393 proteins impairing the function of the photosynthetic electron transport chain. Decreased
394 RQ may be due to the fruit becoming acclimatised (Boersig et al., 1988) to a lower O₂
395 environment during the previous one or two measurements, so anaerobic respiration
396 starts at a lower O₂ concentration each time.

397 **5 Conclusions**

398 The use of CF and RQ to probe the physiological state of 'Gala' and 'Braeburn' apples
399 in conditions similar to commercial CA environments was explored. By comparing the
400 CF response in whole and cored fruit and fruit peel for 'Gala' and 'Braeburn' it was
401 confirmed that the CF increase observed at low O₂ is a response to a stress signal from
402 inside the fruit. CF and RQ showed noticeable differences between cultivars under
403 identical conditions supporting previous observations that 'Braeburn' is less tolerant of
404 low O₂ and elevated CO₂ than 'Gala'.

405 Additionally, the CF and RQ responses indicated different LOLs for 'Gala', with the CF
406 response occurring at a higher O₂ concentration than the RQ response. Elevated CO₂
407 depressed both the CF and RQ response, with a strong depressive effect on the rate of
408 CO₂ evolution but little effect on the rate of O₂ consumption. Given that 'Gala' can be
409 quite difficult to monitor in DCA storage using RQ due to its tolerance of very low O₂
410 concentrations and therefore indistinct RQ response, CF could be more appropriate as
411 a storage monitoring technique for 'Gala' and other cultivars which show similar
412 behaviour, while RQ and CF are equally valid techniques for 'Braeburn'.

413 RQ and CF responses change in repeated runs, with RQ tending to decrease while CF
414 tends to rise. However, in both cases the effect is small and would not interfere with the
415 use of the technology for DCA. During long term CA storage, RQ response has also
416 been found to decrease, which was attributed to fruit acclimatising to low O₂. However,
417 the repeat measurement effect is smaller, and associated with an increase in respiration

418 rate which is not consistent with acclimation. The increase in CF is attributed to
419 accumulated degradation of the photosynthetic apparatus.

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