FURTHER OBSERVATIONS ON THE EFFECTS THAT ARTIFICIAL CO_2 ENRICHMENT HAS ON 'HASS' AVOCADO FRUIT STORAGE POTENTIAL

FJ Kruger, GO Volschenk and E Volschenk

Lowveld Postharvest Services
PO Box 4001, Mbombela 1200, SOUTH AFRICA

E-mail: fjkruger58@gmail.com

ABSTRACT
During 2020, the dry ice-based CO_2 atmosphere-enrichment procedure developed during 2019 was further refined by doing three sets of laboratory-based trials with the Hass cultivar. Each trial consisted of 17 treatments. The results can best be expressed in terms of the delay in ripening achieved with regards to the first fruit in each treatment to reach the ready-to-eat stage. When a balanced atmosphere (BA) was passively built up as from Day 8 (representing a typical export scenario), a 63% improvement was attained in relation to the regular atmosphere (RA) control. This proportion progressively shrunk as the passively built up BA was applied on later dates and by Day 16 no additional storage potential was attained. A 150% improvement over the RA control was achieved when simulating door-to-door BA storage. When replacing the packhouse to harbour leg of the Day 8 BA scenario with an artificial 8% CO_2 insertion, a 10% improvement on the passively built up door-to-door BA simulation was recorded. When lowering the CO_2 concentration to 4%, the reaction was 10% weaker than the door-to-door scenario. However, it was still 38% better than the RA until Day 8 option.

An interesting observation was made when simulating the insertion of CO_2 on Day 1 (during the initial cooling stage in the cold room). A similar set of reactions to the above were expected. However, the results showed that, instead of shifting the normal distribution curve to the right (as was the case with the CO_2 applications performed on Days 3 and 4), the Day 1 applications caused the right leg of the bell curve to move towards the left. More condensed ripening thus occurred. This was an unexpected result and we hypothesise that the higher CO_2 levels caused an increase in the sugar content of slower ripening fruit due to an acceleration of the dark stage of photosynthesis that may still have been running at the time. This observation has potential commercial value, especially in so far as improved ripening of SmartFresh treated consignments is concerned.

Abbreviations used:
RA – regular atmosphere, BA – balanced atmosphere, CA – controlled atmosphere

INTRODUCTION

During the 2019 season, Kruger *et al*. (2020a) found that an 8% CO_2 : 13% O_2 balanced atmosphere (BA) combination has similar ripening inhibition qualities to the traditional 6% CO_2 : 4% O_2 controlled atmosphere (CA) combination. In a preliminary study, we also found that temporary enrichment of the storage atmosphere with CO_2 during the period that the fruit would have been traveling by refrigerated truck from the packhouse to the harbour, may contribute towards suppressing the respiration rate of the fruit (Kruger *et al.*, 2020b).

During the 2020 season we conducted a further set of three trials aimed at confirming and refining the above procedures. A trial was also conducted to determine what effect the addition of CO_2 during the initial cooling phase (on the day of packing) will have on the storage potential of the fruit.

MATERIALS AND METHODS

Carbon dioxide enriching on days 3 and 4

These trials simulated an export situation whereby Days 1 and 2 represented the time spent in the packhouse cold room under RA; Days 3 and 4 represented the time spent on the truck to the harbour (with either 0%, 4% or 8% CO_2 added) and Days 5 to 30 represented the time on the dock and the ship. During this period, the fruits were either immediately sealed into a container (BA) or left unsealed (RA) for 4, 8 or 12 days before going into a sealed container.

Three trials were performed with fruits from different producers during the mid- to late-season. A temperature of 5 °C was maintained throughout the storage period for all treatments. The treatments are summarised below and are listed in Tables 1 - 3.

- RA for 30 days - no CO_2 addition or build-up (Treatment 1)
- RA in the packhouse, truck and at the harbour for 0, 4, 8 or 12 days, followed by BA to Day 30 (Treatments 2 - 5)
- RA in the packhouse, passive BA build up in the truck, return to RA at the harbour for 0, 4, 8 or 12 days and then BA again up to Day 30 (Treatments 6 - 9)
- RA in the packhouse, 4% CO_2 insertion in the truck, return to RA at the harbour for 0, 4, 8 or 12 days and then BA again up to Day 30 (Treatments 10 - 13)
- RA in the packhouse, 8% CO_2 in the truck, return to RA at the harbour for 0, 4, 8 or 12 days and then BA again up to Day 30 (Treatments 14 - 17).

Each treatment contained 30 mid-season 'Hass' fruits that were placed in 25 litre drums that were slightly ventilated to represent a commercial truck.

After the 30-day storage period at 5 °C, the fruits were ripened at 20 °C and the ripening period of each fruit recorded. This was followed by a full quality appraisal of each fruit.

Table 1: Ripening rates recorded during the first export simulation trial performed with the Hass cultivar during the 2020 season

Treatment no	Description	Atmosphere days 1-2	Atmosphere days 3-4	Last 26 days		Ripe fruits per day (%)										
				N days RA	N days BA	1	2	3	4	5	6	7	8	9	10	11
1	RA all the way	RA	RA without CO_2 build up	26	0		10	20	20	13	20	17				
2	Leaky truck + BA	RA	RA without CO_2 build up	0	26				23	17	13	17	17	13		
3	Leaky truck + BA	RA	RA without CO_2 build up	4	22			17	20	10	17	27	10			
4	Leaky truck + BA	RA	RA without CO_2 build up	8	18			23	20	23	10	17	7			
5	Leaky truck + BA	RA	RA without CO_2 build up	12	14		10	17	13	27	17	17				
6	Door to door BA	RA	RA with CO_2 build up	0	26					17	23	13	17	13	17	
7	Airtight truck + BA	RA	RA with CO_2 build up	4	22				17	17	13	20	13	10	10	
8	Airtight truck + BA	RA	RA with CO_2 build up	8	18			13	17	13	23	10	17	7		
9	Airtight truck + BA	RA	RA with CO_2 build up	12	14			17	13	13	17	20	13	7		
10	Truck 4% CO_2+BA	RA	4% CO_2 added at start	0	26					17	13	17	13	20	20	
11	Truck 4% CO_2+BA	RA	4% CO_2 added at start	4	22				20	17	13	13	20	17		
12	Truck 4% CO_2+BA	RA	4% CO_2 added at start	8	18			17	23	23	17	20				
13	Truck 4% CO_2+BA	RA	4% CO_2 added at start	12	14			17	23	13	20	13	13			
14	Truck 8% CO_2+BA	RA	8% CO_2 added at start	0	26						13	13	27	20	13	13
15	Truck 8% CO_2+BA	RA	8% CO_2 added at start	4	22					20	10	23	17	13	17	
16	Truck 8% CO_2+BA	RA	8% CO_2 added at start	8	18				17	13	13	27	17	13		
17	Truck 8% CO_2+BA	RA	8% CO_2 added at start	12	14			17	13	23	23	13	10			

Carbon dioxide enriching on Day 1

This trial consisted of five treatments. These were 0, 2, 4, 6 and 8% CO_2 that were applied to 30 late season 'Hass' fruits in slightly ventilated drums directly after packing. The drums were kept at 5 °C for two days during which the CO_2 concentration was measured on a four-hourly basis. This was followed by ripening at 20 °C.

RESULTS AND DISCUSSION

Carbon dioxide enriching on Days 3 and 4

The results are shown in Tables 1 - 3. It was striking how similar the results of the three trials were. The effect of the BA progressively diminished as the period from packing to CA/BA containerisation increased and by Day 16 no additional ripening retardation effect was attained when compared with the RA control treatment. In addition, in all instances, the application of both 4% and 8% CO_2 during Day 3 made a significant impact on the ripening rates of the fruits.

One way of interpreting the results is to compare the number of days that the first fruit in each treatment took to reach the ready-to-eat stage. The five most important treatments were selected for this purpose and are shown in Table 4 (the results from a trial with similar goals that was performed towards the end of the 2019 season are also included in the table). When the BA was passively built up as from Day 8 (representing a typical export scenario), a 63% improvement was attained in relation to the RA control. This proportion progressively shrunk as the BA was

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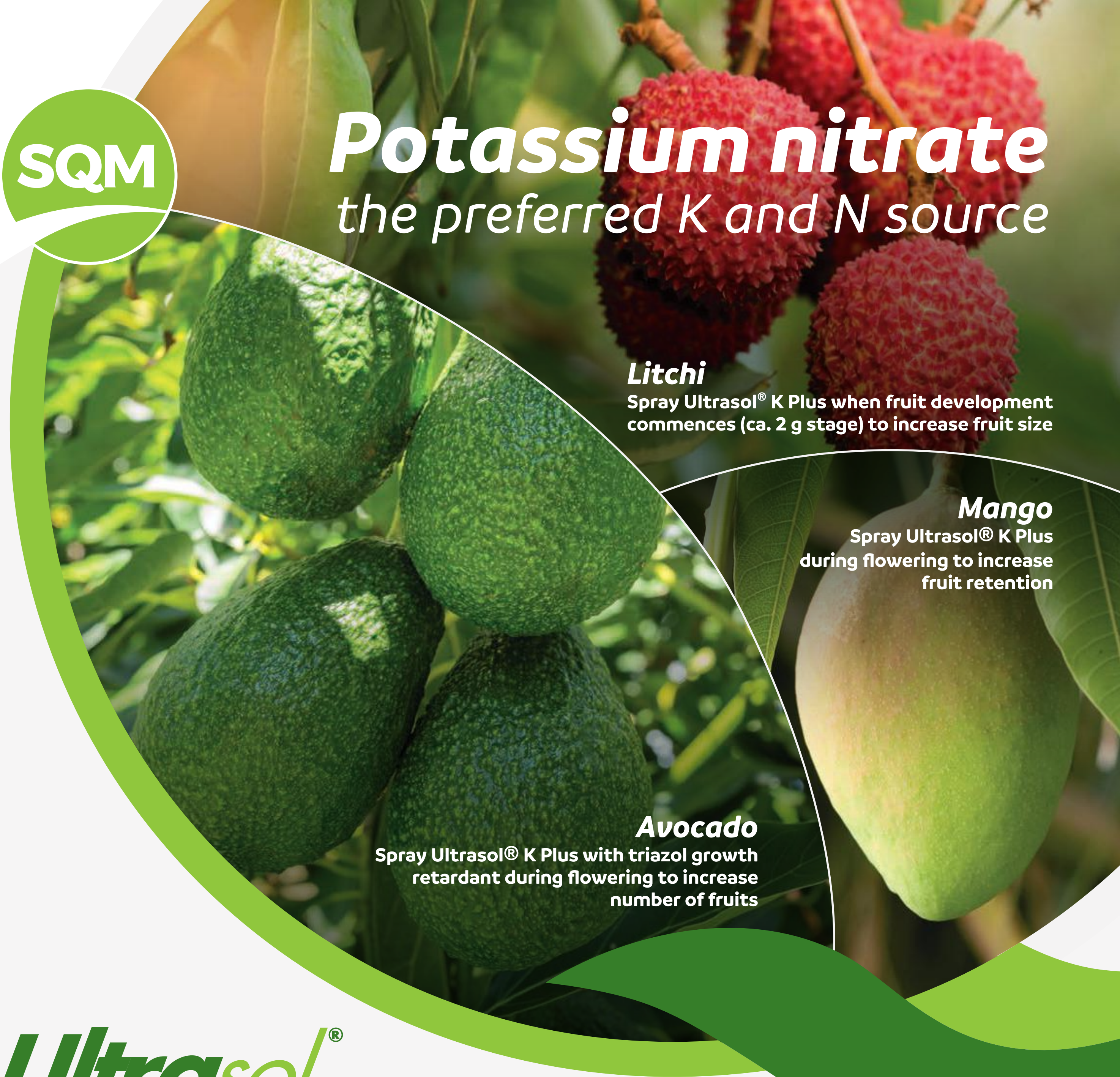
Table 2: Ripening rates recorded during the second export simulation trial performed with the Hass cultivar during the 2020 season

Treatment no	Description	Atmosphere days 1-2	Atmosphere days 3-4	Last 26 days		Ripe fruit per day (%)										
				N days RA	N days BA	1	2	3	4	5	6	7	8	9	10	11
1	RA all the way	RA	RA without CO_2 build up	26	0		7	13	30	20	20	10				
2	Leaky truck + BA	RA	RA without CO_2 build up	0	26				17	27	17	20	20			
3	Leaky truck + BA	RA	RA without CO_2 build up	4	22			26	23		17	23	10			
4	Leaky truck + BA	RA	RA without CO_2 build up	8	18		13	13	7	20	13	20	13			
5	Leaky truck + BA	RA	RA without CO_2 build up	12	14		17	13	20	13	17	13	7			
6	Door to door BA	RA	RA with CO_2 build up	0	26					17	17	13	17	10	17	10
7	Airtight truck + BA	RA	RA with CO_2 build up	4	22				13	20	17	20	10	17	3	
8	Airtight truck + BA	RA	RA with CO_2 build up	8	18			10	23	13	17	13	23			
9	Airtight truck + BA	RA	RA with CO_2 build up	12	14		17	13	20	17	13	20				
10	Truck 4% CO_2+BA	RA	4% CO_2 added at start	0	26					3	13	17	17	23	17	10
11	Truck 4% CO_2+BA	RA	4% CO_2 added at start	4	22					17	23	13	10	20	17	
12	Truck 4% CO_2+BA	RA	4% CO_2 added at start	8	18			7	17	17	23	17	13	7		
13	Truck 4% CO_2+BA	RA	4% CO_2 added at start	12	14		23	20	17	10	10	20				
14	Truck 8% CO_2+BA	RA	8% CO_2 added at start	0	26							10	20	17	17	20
15	Truck 8% CO_2+BA	RA	8% CO_2 added at start	4	22						17	13	23	13	13	17
16	Truck 8% CO_2+BA	RA	8% CO_2 added at start	8	18				7	17	13	20	13	10	20	
17	Truck 8% CO_2+BA	RA	8% CO_2 added at start	12	14		7	17	17	13	7	13	10	17		

Potassium nitrate

the preferred K and N source



Litchi

Spray Ultrasol® K Plus when fruit development commences (ca. 2 g stage) to increase fruit size

Mango

Spray Ultrasol® K Plus during flowering to increase fruit retention

Avocado

Spray Ultrasol® K Plus with triazol growth retardant during flowering to increase number of fruits

Ultrasol® K Plus

Foliar fertilization is an important tool for the sustainable and productive management of crops:

- When soil conditions limit availability of soil applied nutrients;
- In conditions when high loss rates of soil applied nutrients may occur
- When the stage of plant growth, the internal demand and the environment conditions interact to limit a delivery of nutrients to critical plant organs
- When certain foliar applications are tested and proved to result in measurable and positive plant parameter responses.

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Contact details:

Mr. Duncan Napier: (082) 772 2207

E-mail: spn-emea@sqm.com

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Table 3: Ripening rates recorded during the third export simulation trial performed with the Hass cultivar during the 2020 season

Treat-ment no	Description	Atmos-phere days 1-2	Atmosphere days 3-4	Last 26 days		Ripe fruits per day (%)										
				N days RA	N days BA	1	2	3	4	5	6	7	8	9	10	11
1	RA all the way	RA	RA without CO_2 build up	26	0		10	20	23	17	20	10				
2	Leaky truck + BA	RA	RA without CO_2 build up	0	26				7	17	17	10	13	17	20	
3	Leaky truck + BA	RA	RA without CO_2 build up	4	22				20	17	13	17	23	10		
4	Leaky truck + BA	RA	RA without CO_2 build up	8	18			17	23	13	17	13	17			
5	Leaky truck + BA	RA	RA without CO_2 build up	12	14		17	17	23	10	13	13	7			
6	Door to door BA	RA	RA with CO_2 build up	0	26					20	20	13	3	13	13	17
7	Airtight truck + BA	RA	RA with CO_2 build up	4	22				20	13	20	17	10	20		
8	Airtight truck + BA	RA	RA with CO_2 build up	8	18			17	13	17	23	17	13			
9	Airtight truck + BA	RA	RA with CO_2 build up	12	14		13	10	20	17	10	13	17			
10	Truck 4% CO_2+BA	RA	4% CO_2 added at start	0	26						10	23	20	17	13	17
11	Truck 4% CO_2+BA	RA	4% CO_2 added at start	4	22					20	17	10	20	13	17	3
12	Truck 4% CO_2+BA	RA	4% CO_2 added at start	8	18				10	17	13	17	13	20	10	
13	Truck 4% CO_2+BA	RA	4% CO_2 added at start	12	14		10	17	20	17	13	10	13			
14	Truck 8% CO_2+BA	RA	8% CO_2 added at start	0	26							20	20	17	23	20
15	Truck 8% CO_2+BA	RA	8% CO_2 added at start	4	22						20	17	13	20	10	20
16	Truck 8% CO_2+BA	RA	8% CO_2 added at start	8	18				7	20	17	13	17	17	10	
17	Truck 8% CO_2+BA	RA	8% CO_2 added at start	12	14		10	17	17	17	13	20	7			

Table 4: Day number on which the first fruit of five treatments in four trials performed during the 2019 and 2020 seasons reached the ready-to-eat stage

Treatment no in Tables 1-3	Treatment represents	Day number on which the first fruit ripened				Mean day no that first fruit ripened*
		Trial 1 of 2019	Trial 1 of 2020	Trial 2 of 2020	Trial 3 of 2020	
1	RA all the way	2	2	2	2	2 a
3	Truck to Cape Town then BA on day 8	3	3	3	4	3.25 b
6	BA door to door	5	5	5	5	5 cd
11	Truck with 4% CO_2 then BA in Cape Town on day 8	4	4	5	5	4.5 c
15	Truck with 8% CO_2 then BA in Cape Town on day 8	5	5	6	6	5.5 d

Student's t-test; $p<0.05$

applied on later dates and by Day 16 no additional storage potential was attained. A 150% improvement over the RA control was achieved when simulating door-to-door BA storage. When replacing the packhouse to harbour leg of the Day 8 BA scenario with an artificial 8% CO_2 insertion, a 10% improvement on the passively built up door-to-door BA simulation was recorded. When lowering the CO_2 concentration to 4%, the reaction was 10% weaker than the door-to-door scenario. However, it was still 38% better than the above Day 8 CA/BA option.

The enhanced respiration-suppressing effect attained with the artificial CO_2 insertions is an interesting observation and may possibly be associated with non-competitive inhibition related reactions (Hertog *et al*., 1998; Maarten *et al*., 2003; Cruz *et al.*, 2019).

Carbon dioxide enriching on Day 1

An interesting observation was made when simulating the insertion of CO_2 during the initial cooling phase in the cold room. A similar set of reactions to the above was expected. Instead, the results revealed that the right leg of the normal distribution curve shifted to the left (Fig. 1) (only the 8% CO_2 application is shown in the figure to prevent cluttering). More condensed ripening thus occurred.

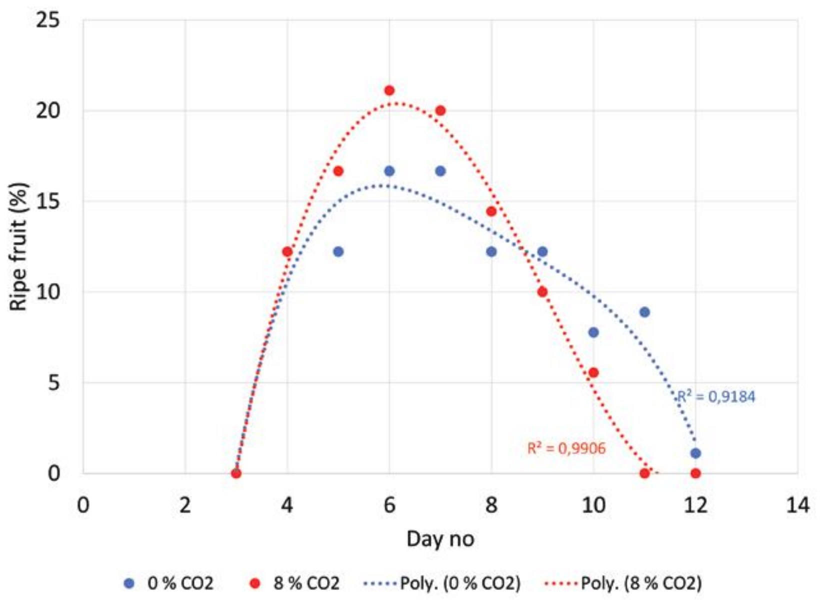

Figure 1: Percentages of fruit that ripened per day after administering 8% CO_2 during the initial cooling phase on the day of harvest.

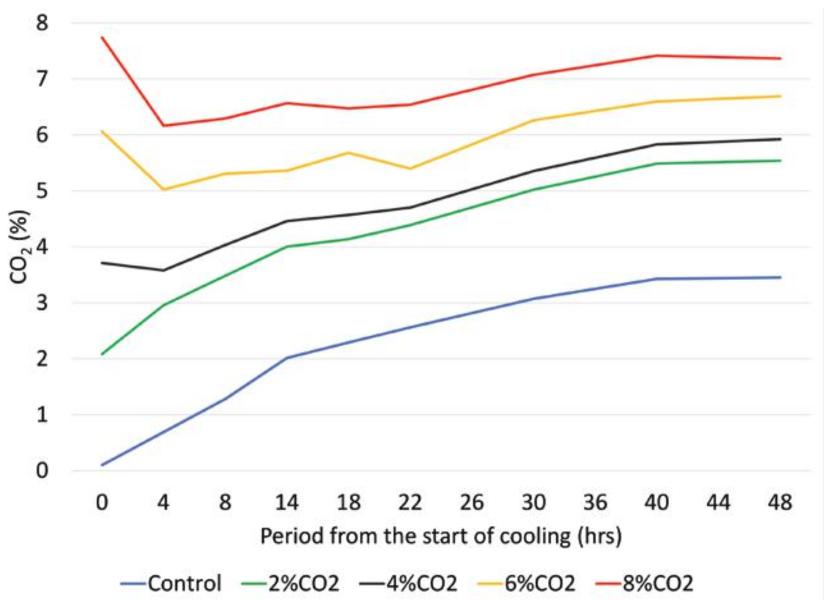

Figure 2: Carbon dioxide concentrations recorded during five artificial CO_2 applications performed during the initial cooling phase directly after packing.

This was an unexpected result and we hypothesise that the higher CO_2 levels caused an increase in the sugar content of slower ripening fruit due to improved dark stage photosynthesis. This conjecture was supported by the CO_2 readings in the drums that were taken during the application period. These indicated that, directly after application, more CO_2 was absorbed from higher dosage treatments than from lower dosage treatments (Fig. 2).

COMMERCIALISATION OF THE PROCEDURES

Carbon dioxide enriching on Days 3 and 4

Door-to-door CA/BA is used for specific purposes by the South African avocado export industry. The largest percentage of door-to-door consignments occurs during the late season to reduce the incidence of soft landings in the Hass, Fuerte and Pinkerton cultivars. However, a packhouse may not have the necessary stock available to make up count-dependent door-to-door consignments for specific clients. The insertion of dry-ice-derived CO_2 into trucks will enable the exporter to assemble consignments in the harbour, using stock from different runs and/or packhouses.

Another example where door-to-door CA/BA is critical, concerns the Maluma Hass cultivar. This variety has a fast metabolic rate with a high prevalence of fruit with dead seeds that prematurely ripen during the export process. It is therefore necessary that all consignments be placed under CA/BA within four days after harvest (Kruger *et al*. 2019). During the 2021 season, we will start applying the present dry ice based CO_2 enriching technique to 'Maluma Hass' fruits in cases where door-to-door CA/BA transportation is not possible.

Carbon dioxide enriching on Day 1

The above observations may have commercial potential in so far as SmartFresh treatments are concerned. We have previously observed that slower ripening fruit in SmartFresh treated consignments have lower sugar contents (Kruger *et al*., 2018) and postulated that these fruit need to first convert starch to sugars in order to generate the necessary energy for the ripening process. We aim to increase the energy levels of the laggard fruit by applying CO_2 in combination with SmartFresh in an attempt to improve the sugar levels of the slower ripening fruit.

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