

1 **Design and construction of a flexible laboratory-scale mixing apparatus for continuous**  
2 **ethylene supplementation of fresh produce**

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7

8 **Abstract**

9 The design and construction of a laboratory-scale apparatus for generating variable  
10 concentrations and flow rates of exogenous ethylene for fresh produce supplementation during  
11 storage trials is described. A stock of compressed ethylene in nitrogen ( $5000 \mu\text{l l}^{-1}$ ) was blended  
12 into a continuous flow stream of air and diluted to the desired concentrations. The ethylene and  
13 air flow rates were controlled with calibrated mass flow control valves. An empirical  
14 mathematical model was derived for real-time variation of both the mixed concentration and  
15 flow rate during continuous flow. Validation of the model was performed using fresh sweet  
16 potato as a case study where a steady continuous ethylene concentration of  $10 \mu\text{l l}^{-1}$  was  
17 achieved for three months. The bespoke system offers easy-to-manage ethylene  
18 supplementation for research.

19 **Keywords:** Ethylene blending, Controlled atmosphere, Mass flow controller, Fresh produce,  
20 Storage

21

22 **Nomenclature**

23	CA	Controlled atmosphere
24	MAP	Modified atmosphere packaging
25	MFC	Mass flow controller
26	MFC-4	Four-channel mass flow control unit (Sable systems)
27	Ve	Ethylene flow control valve
28	Va	Air flow control valve
29	SLPM	Standard litres per minute
30	FS	Calibrated full-scale mass flow rate of ethylene control valve (SLPM)
31	EE	Continuous ethylene-flushed boxes

32	EA	Ethylene, then air-flushed boxes
33	AA	Continuous air-flushed boxes
34	AE	Air, then ethylene-flushed boxes
35	Me	Ethylene gas distribution manifold
36	Me	Air distribution manifold
37	A	Hydrovane air compressor
38	C	Ethylene cylinder
39	OD	Outside diameter of tubing (mm)
40	ID	Inside diameter of tubing (mm)
41	RH	Relative humidity
42	HWD	Hot wire detector
43	FID	Flame ionisation detector
44	CO <sub>2</sub>	Carbon dioxide
45	<i>C1</i>	Stock concentration of ethylene in nitrogen (5000 $\mu\text{l l}^{-1}$ )
46	<i>C2</i>	Target concentration of ethylene in air ( $\mu\text{l l}^{-1}$ )
47	<i>M1</i>	Total mass flow rate of compressed air and ethylene stock ( $\text{ml l}^{-1}$ )
48	<i>M2</i>	Target mass flow rate of ethylene in air ( $\text{ml l}^{-1}$ )
49	<i>E</i>	Any given stock ethylene concentration ( $\mu\text{l l}^{-1}$ )
50	<i>Fe</i>	Any given calibrated full-scale flow through the ethylene valve (SLPM)
51	<i>Fa</i>	Any given calibrated full-scale flow through the air valve (SLPM)
52	x	Percentage of the calibrated full-scale flow through the ethylene valve (%)
53	y	Percentage of the calibrated full-scale flow through the air valve (%)
54	z	Percentage of the calibrated full-scale flow through the control (pure air) valve
55		(%)
56	Q1	Total gas flow rate through the ethylene/air mix tubing ( $\text{ml l}^{-1}$ )
57	Q2	Gas flow rate through the pure air tubing ( $\text{ml l}^{-1}$ )
58	$\chi^2$	Chi-square

59	$R^2$	Correlation coefficient
60	RMSE	Root mean square error
61	df	Degrees of freedom
62	$K_{pred}$	Model-predicted ethylene concentration
63	$K_{expt}$	Experimentally obtained ethylene concentration

64

## 65 **1 Introduction**

66 Postharvest research often requires controlling the atmospheric composition of the storage  
67 environment. Monitoring and regulating the proportion of respiratory gases is critical and finds  
68 practical applications in controlled atmosphere (CA) storage and modified atmosphere  
69 packaging (MAP) techniques. CA storage and MAP are primarily concerned with the balance  
70 between oxygen, carbon dioxide and nitrogen in a headspace (Kader, 2002). However, many  
71 systems are not flexible enough for continuous ethylene to be exogenously applied into the  
72 atmosphere mix.

73 Besides the familiar role ethylene plays in promoting the ripening of climacteric fresh produce,  
74 ethylene supplementation has been shown to inhibit sprouting in many bulbs, tubers and roots  
75 (Cools et al., 2011; Foukaraki et al., 2016b; Amoah et al., 2016). Exogenous ethylene, however,  
76 elicits variable, and sometimes contrasting, responses in plant tissues depending on a matrix of  
77 crop factors and the application regime employed. In particular, the ethylene concentration and  
78 timing of exposure have been shown to have distinctive effects (Cools et al., 2011; Foukaraki  
79 et al., 2014; Amoah et al., 2016). Therefore, it is desirable to optimise reliable ethylene  
80 supplementation regimes to uniquely suit individual crops and storage conditions.

81 Postharvest applications of ethylene are predominantly in the gaseous phase and come from  
82 the catalytic decomposition of ethanol in ethylene generators or from cylinders of the  
83 compressed gas which are diluted with air to the required concentrations (Blankenship and  
84 Sisler, 1991). The latter application method is more suited to laboratory studies. With many  
85 laboratory-based designs for storage trials, however, only fixed concentrations and flow rates  
86 are possible. Facilities and techniques to flexibly manipulate the gas flow parameters such as  
87 the concentration, flow rate and timing of exposure to the crops poses a major challenge.

88 Gas mixing systems are employed for analytical research to blend two or more different gases  
89 in precise proportions to achieve target concentrations delivered at specified flow rates. In static  
90 mixing systems, the individual gases, measured gravimetrically, volumetrically or  
91 manometrically are combined whilst in dynamic gas mixing techniques, streams of gases with  
92 known flow rates are mixed (Degn and Lundsgaard, 1980). The individual flow rates of the  
93 mixing gases are adjusted by integrated mass flow controllers (MFCs). Achieving the target  
94 concentration and flow rate, however, may require iterative settings of the MFCs, followed by  
95 sampling for validation in a suitable instrument such as the gas chromatograph. This process  
96 can be time consuming and inconvenient especially, in experiments which require rapid  
97 stabilisation of the target concentration. Innovative designs that allow for automatic adjustment  
98 of the gas mixture to achieve precise concentrations and flow rates are commercially available  
99 (Dansensor Co., 2014) but they are industrial in scale and require significant pressures (above  
100  $5 \text{ Nm}^{-2}$ ) and flows ( $1500 \text{ l min}^{-1}$ ) which are too great for down-scaled laboratory applications.  
101 This research note describes a modular laboratory design which permits the simultaneous  
102 flushing of multi-storage chambers with adjustable ethylene levels at any time during  
103 prolonged storage.

104

## 105 **2 Materials and methods**

106 Controlled ethylene supplementation was accomplished using a custom-built continuous flow-  
107 through apparatus (Fig. 1). The flow rates of the mixing gases were regulated by connecting  
108 mass flow control valves (Sierra, The Netherlands: models 840-L-2-OV1-SV1-D-V1-S1 and  
109 840-L-2-OV1-SV1-E-V1-S1) in line with the ethylene ( $V_e$ ) and air streams ( $V_{a1}$ ), respectively.  
110 A third mass flow control valve ( $V_{a2}$ ) was connected in a parallel air stream for direct flushing  
111 of the control samples with pure air. The mass flow control valves were factory calibrated to  
112 deliver 0.03 and 13.5 standard litres per minute (SLPM) maximum flow rates of ethylene and  
113 air, respectively. All the valves were connected to a digital multi-channel MFC-4 control unit  
114 (Sable Systems, NV, USA) to regulate the flows. Certified ethylene (BOC, Surrey, UK) made  
115 inert with nitrogen against explosion and compressed ( $200 \text{ Nm}^{-2}$ ) into a 50 l capacity cylinder  
116 (C) was diluted with air from a Hydrovane Air Compressor (A) (HVO2, Bedfordshire, UK) at  
117 room temperature from the stock concentration  $5000 \mu\text{l l}^{-1}$  to the target concentrations ( $10 \mu\text{l l}^{-1}$   
118  $^1$  in the test experiment). At each setting of the MFC-4, a regulated amount of ethylene from  
119 the cylinder was blended into a corresponding amount of air stream at a T-junction without a

120 mixing chamber. The blended gases were supplied directly into the crop storage boxes (EE,  
121 EA, AA, AE in Fig. 1). A simple mathematical model was derived to permit real-time variation  
122 of the ethylene concentration and flow rates based on digital settings of appropriate percentages  
123 of the calibrated maximum flows. The ethylene/air mix and the pure air (for control treatments)  
124 were flushed through individual storage boxes (100 l propylene boxes) via multi-channel gas  
125 distribution manifolds (Me and Ma) (HNL Engineering Ltd., Durham, UK). All gas flow  
126 channels consisted of equal diameter tubing (Product No. NXM06/04 Super-flexible nylon  
127 pipes 204805 6 mm O.D. and 4 mm I.D., Air Equipment, Flitwick, Bedfordshire, UK).

128

129 Each storage box contained samples of vegetables (*ca.* 3.75 kg of sweet potato) and covered  
130 with water-sealed transparent lids. Six boxes were flushed with the ethylene/air mix. Another  
131 set of six control boxes containing the same weight of sample were flushed with the pure air.  
132 At dormancy break, three boxes each, previously flushed with either ethylene or air,  
133 respectively, were swapped from the ethylene to air treatment or vice-versa (Amoah *et al.*,  
134 2016). The gases were bubbled through bowls of water placed inside the boxes to maintain a  
135 relative humidity of *ca.* 70-95 %. The humidity and temperature in the storage boxes were  
136 regularly monitored using Gemini data loggers (Tiny-tag Ultra 2, 0-95 % RH, -25 °C to 85 °C,  
137 Part No. TGU-4500, West Sussex, UK). Exhaust tubing was connected to the opposite sides of  
138 the boxes relative to the inflow gas such that there was upward, diagonal flow-through stream  
139 to maintain the carbon dioxide (CO<sub>2</sub>) concentration below 0.5 %. CO<sub>2</sub> levels in the boxes were  
140 regularly checked by injecting headspace gas samples into a gas chromatograph (GC model  
141 8340, DP800 integrator, Carlos Erba Instruments, Herts, UK) fitted with a Hot Wire Detector  
142 (HWD), analytical column Porapak (2 m length, 6 mm O.D. and 4 mm I.D.) and calibrated  
143 with 10 % CO<sub>2</sub> standard (BOC, Surrey, UK). The exhaust gas was disposed-off through the  
144 room ventilation system. The store temperature was maintained at 25 °C.

145 Periodically, the headspace gas in each box was analysed as previously described by Terry *et*  
146 *al.* (2007). A 60 ml plastic syringe was used to withdraw and inject samples (*ca.* 10 ml per  
147 injection) into the gas chromatograph described above, but fitted with flame ionisation detector  
148 (FID, 250 °C) and analytical column (Porapak, 2 m length, 6 mm O.D. and 4 mm I.D.) to  
149 ascertain the gas concentrations in the ethylene- and air-flushed boxes. The calibration standard  
150 for ethylene was 10.3 µl l<sup>-1</sup> ethylene. Using the derived model, the MFC-4 was adjusted  
151 accordingly until the appropriate concentrations in the respective boxes were achieved.

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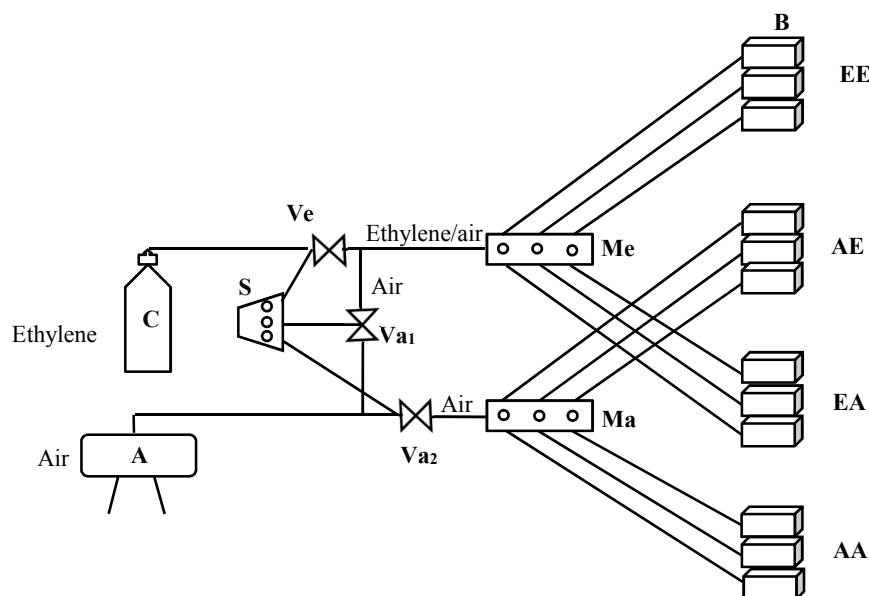


Fig. 1 A schematic diagram of the experimental setup

**A.** Hydrovane air compressor **C.** Ethylene cylinder **Ve.** Ethylene mass flow control valve  
**Va.** Air mass flow control valve **S.** MFC-4 **Me.** Ethylene distribution manifold **Ma.**  
 Air distribution manifold **B.** Crop storage boxes **EE.** Continuous ethylene-flushed  
 boxes **EA.** Ethylene, then air-flushed boxes **AE.** Air, then ethylene-flushed boxes  
**AA.** Continuous air-flushed boxes

161

## 162 2.1 Theory: empirical model for supplemental ethylene concentration

163 The rule of proportion was used to calculate the mass flows of the gases in order to obtain the  
 164 targeted mix by mass fraction blending. Martinez-Flores et al. (2012) adopted this approach  
 165 for different dilutions of ethylene with nitrogen to derive calibration curves for assessing the  
 166 evolution of biologically important gases for the postharvest studies of horticultural crops.

167 Given that:  $C1$  is the stock concentration of ethylene in nitrogen ( $5000 \mu\text{l l}^{-1}$ ),  $C2$  is the target  
 168 concentration of ethylene in air,  $M1$  is the total mass flow of compressed air and ethylene stock,  
 169 where the mass flow of ethylene stock is comparably negligible and  $M2$  is the target mass flow  
 170 rate of ethylene in air.

171 Thus:

$$172 \quad \frac{M1}{M2} = \frac{C1}{C2} \dots\dots\dots \text{Eq. (1)}$$

173 Therefore:

$$174 \quad C2 = C1 \frac{M2}{M1} \dots\dots\dots \text{Eq. (2)}$$

175 To obtain the target ethylene concentration ( $C2$ ) for gassing the storage boxes, metered  
 176 amounts of the ethylene and air flows were mixed by inputting appropriate percentage gas  
 177 flows on the MFC-4 (Sable Systems, Fig. 2). The full-scale mass flow rate of ethylene valve  
 178 was 0.03 SLPM and the full-scale mass flow rate of air valve was 13.5 SLPM.

179 Given that  $x$  and  $y$  are the percentage settings of the calibrated full-scale (FS) flows of the  
 180 ethylene and air flow control valves, respectively, then Eq. (2) becomes:

$$181 \quad C2 = \frac{(x \% \text{ of } 0.03) \times 5000}{(y \% \text{ of } 13.5)} \dots\dots\dots \text{Eq. (3)}$$

182 With the optimal set points  $x$  and  $y$  on the MFC4-4, it was possible to obtain the desired  
 183 ethylene concentrations and flow rates.

184 With any given initial ethylene concentration  $E$ , calibrated full-scale air flow rate  $F_a$  and  
 185 calibrated full-scale ethylene flow rate  $F_e$ , the generalised model for the diluted ethylene  
 186 concentration would be given by

$$187 \quad C2 = \frac{(x \% \text{ of } F_e) \times E}{(y \% \text{ of } F_a)} \dots\dots\dots \text{Eq. (4)}$$

188

## 189 **2.2 Flow setting for the gas valves**

190 The total gas flow rate  $Q1$  through the ethylene/air mix line is the sum of the individual gas  
 191 flow rates through the ethylene and air valves, respectively, and was expressed as:

192

$$193 \quad Q1 = (x \% \text{ of } 0.03) + (y \% \text{ of } 13.5) \dots\dots\dots \text{Eq. (5)}$$

194 Given a calibrated maximum air flow rate  $F_a$  and a calibrated maximum ethylene flow rate  $F_e$ ,  
 195 the generalised model for the total gas flow rate would be given by:

$$196 \quad Q1 = (x \% \text{ of } F_e) + (y \% \text{ of } F_a) \dots\dots\dots \text{Eq. (6)}$$

197 The flow rate of the pure air stream was also given as:

$$198 \quad Q2 = z \% \text{ of } 13.5 \dots\dots\dots \text{Eq. (7)}$$

199 Thus for a calibrated full-scale air flow rate  $F_a$ , a generalised model for the flow rate would be  
 200 given by:

$$201 \quad Q_2 = z \% \text{ of } F_a \dots\dots\dots \text{Eq. (8)}$$

202 Where z is the percentage set point of the calibrated full-scale reading of the second air valve.

203 But for equal gas flows through the ethylene-treated and the air control boxes:

$$204 \quad Q_1 = Q_2 \dots\dots\dots \text{Eq. (9)}$$

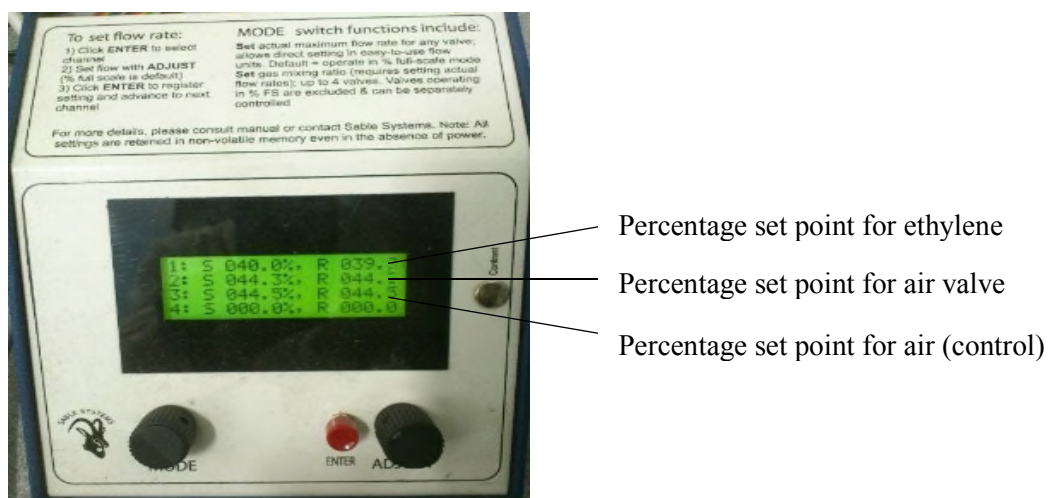
$$205 \quad \text{Therefore } z \% = \{(x \% \text{ of } 0.03) + (y \% \text{ of } 13.5)\} / (13.5) \dots\dots\dots \text{Eq.(10)}$$

206 Thus the generalised model a for the flow rate given calibrated maximum air flow rate  $F_a$  and  
 207 calibrated maximum ethylene flow rate  $F_e$ , would be given by:

$$208 \quad z \% = \{(x \% \text{ of } F_e) + (y \% \text{ of } F_a)\} / (F_a) \dots\dots\dots \text{Eq. (11)}$$

209 Equation (10) was used to determine the set point z of the pure air valve on the MFC-4. A  
 210 simple Excel macro was created to facilitate real-time variation of the ethylene concentration  
 211 and the corresponding gas flow rates.

212



213

214 **Fig. 2** Set points x, y and z on channels 1, 2 and 3 respectively, of the MFC-4 (Sable Systems)

215

### 216 **2.3 Statistical analysis and model validation**

217 The empirical model (Eq. 3) was validated by plotting experimental data sets of ethylene  
 218 concentrations (obtained from the input of different x and y set points) against the model  
 219 predicted values and regression analysis performed to assess the model performance. Fitness  
 220 of the model was evaluated by the chi-square ( $\chi^2$ ) test for goodness of fit, the statistical values



221 of the F-Test (i.e. P-value at 5 % probability level), the correlation coefficient ( $R^2$ ) and the root  
222 mean square error (RMSE). The RMSE has been used as a standard statistical metric for  
223 evaluation of model performance in meteorology, air quality, and climate research studies  
224 (Chai and Draxler, 2014).

225

### 226 **3 Results and discussion**

227 A high correlation coefficient ( $R^2 = 0.9956$ ,  $P < 0.0001$ ) and low RMSE value (0.149) were  
228 obtained between the concentrations of the experimental and model-predicted datasets (Table  
229 1; Fig. 3). Furthermore, comparison of the chi-square values ( $\chi^2$ ) to the p-values shows that  
230 there were no significant differences between the experimental concentrations and the  
231 predicted values and that the differences could only be due to unavoidable experimental errors.  
232 This authenticates the model efficiency for predicting ethylene concentration of the mixed gas.  
233 In addition, the apparatus permitted different pairs of x and y set points on the MFC-4 to  
234 achieve the same ethylene concentration at different gas flow rates (See Supplementary Fig.  
235 1). Conversely, it was also possible to input different set points of x and y to achieve different  
236 concentrations at the same flow rate. This permits flexibility in the way the ethylene may be  
237 consumed and can be matched with the ventilation requirements of the commodity in the  
238 storage boxes.

239 There was a reciprocal effect between the flow rate and the corresponding ethylene  
240 concentration whereby higher ethylene concentrations were associated with lower flow rates  
241 or vice-versa. The final ethylene concentration was thus inversely proportional to the total gas  
242 flow rate.

243 An inherent characteristic of MFC valves is that a linear relationship between the flow rate and  
244 the valve orifice opening is only valid between 10 % and 100 % set points of the calibrated full  
245 scale flows. Furthermore, at set points less than 2.5 % full-scale, the valves shut completely.  
246 This feature places minimum and maximum limits on the x and y set points. That in turn, sets  
247 limits to the flow rates and concentrations that could be achieved. With the given valves  
248 calibration (i.e. 0.03 and 13.5 SLPM for the ethylene and air valves, respectively), the  
249 maximum ethylene concentration that could be achieved was  $100 \mu\text{l l}^{-1}$  whilst the lowest  
250 concentration was  $2 \mu\text{l l}^{-1}$ . To obtain higher concentrations and flow rates than what were  
251 achieved in the present study, the MFC valves will need to be calibrated with higher full-scale

252 flows. Maintaining a constant temperature is very important as freezing temperatures could  
 253 lead to water vapour condensation and blockage of the tubing, which could affect gas flow.

254 A consistent ethylene concentration of *ca.* 10.1  $\mu\text{l l}^{-1}$  was achieved in the trial experiment at *x*  
 255 and *y* set points 40.0 % and 44.3 %, respectively (Fig. 2). This produced a steady gas flow rate  
 256 of *ca.* 6.0  $\text{ml min}^{-1}$  for flushing 6 boxes of fresh produce over 3 months of storage. The mean  
 257 relative humidity and temperature in the boxes, as measured with the data loggers, were 60 -  
 258 95 % and 24.8 °C, respectively, whilst the CO<sub>2</sub> concentrations in the headspace was *ca.* 0.1 %.  
 259 When tubing to the ethylene and air-flushed boxes were swapped to the air and ethylene  
 260 supplies, respectively, the concentrations equilibrated at the new gas concentrations within 30  
 261 min. The apparatus and model described herein, thus provided an easy means for achieving  
 262 target ethylene concentrations with minimum delays during optimisation of the flow  
 263 parameters using the MFC4-unit.

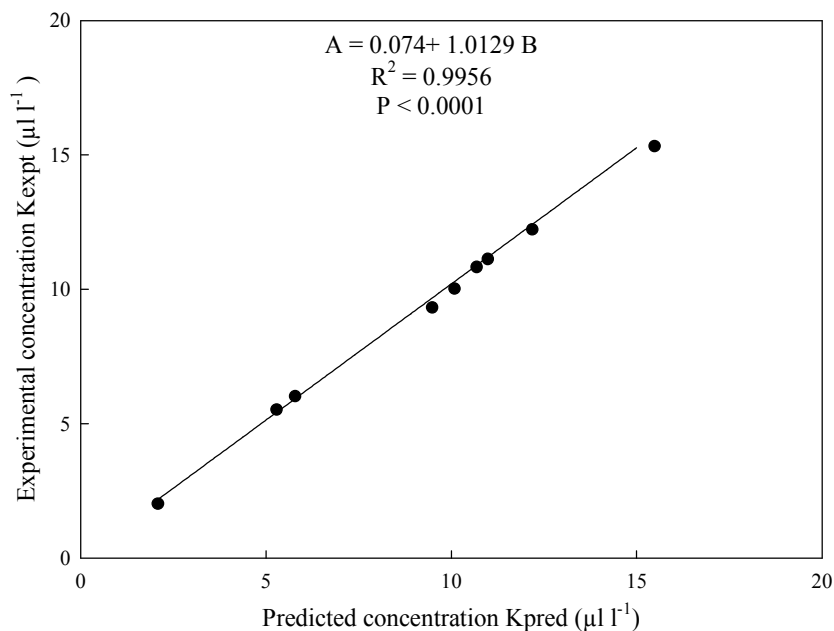
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265 Table 1. Experimental and model-predicted values of ethylene concentrations

Run	df	<i>x</i> Set point (% FS of ethylene MFC)	<i>y</i> Set point (% FS of ethylene MFC)	Experimental concentration $K_{expt}$ ( $\mu\text{l l}^{-1}$ )	Predicted concentration $K_{pred}$ ( $\mu\text{l l}^{-1}$ )	Chi- square $\chi^2$	P- values (0.05)
1	2	14.3	80.0	2.1	2.0	0.0050	5.991
2	2	29.4	62.1	5.3	5.5	0.0073	5.991
3	2	30.1	56.0	5.8	6.0	0.0067	5.991
4	2	36.0	42.0	9.3	9.5	0.0042	5.991
5	2	40.0	44.3	10.1	10.0	0.0010	5.991
6	2	42.4	46.9	10.7	10.8	0.0009	5.991
7	2	44.3	44.5	11.0	11.1	0.0009	5.991
8	2	50.3	45.9	12.2	12.2	0.0000	5.991
9	2	62.9	45.6	15.5	15.3	0.0026	5.991

266 Values for  $K_{expt}$  and  $K_{pred}$  are the means of three readings; df is the degree of freedom

267



268

269 Fig. 3. Experimental and model-predicted ethylene concentrations at different inputs of x and  
 270 y setpoints.

271

## 272 4 Conclusion

273 The growing interest in ethylene research has brought in its wake the need for suitable  
 274 laboratory facilities for generating precise concentrations and flow rates for empirical studies.  
 275 The customised design for ethylene supplementation of fresh produce as described in this work  
 276 provides an easy-to-manage and low cost facility for in-situ ethylene research. With optimised  
 277 settings of the respective gas flows, a steady ethylene concentration could be achieved for  
 278 prolonged periods. In addition, the apparatus as operated with the derived model permits real-  
 279 time variation of the concentration and flow rate as required. Also, customised calibration of  
 280 the mass flow control valves would allow a wide range of gas flow rates and concentrations to  
 281 be delivered.

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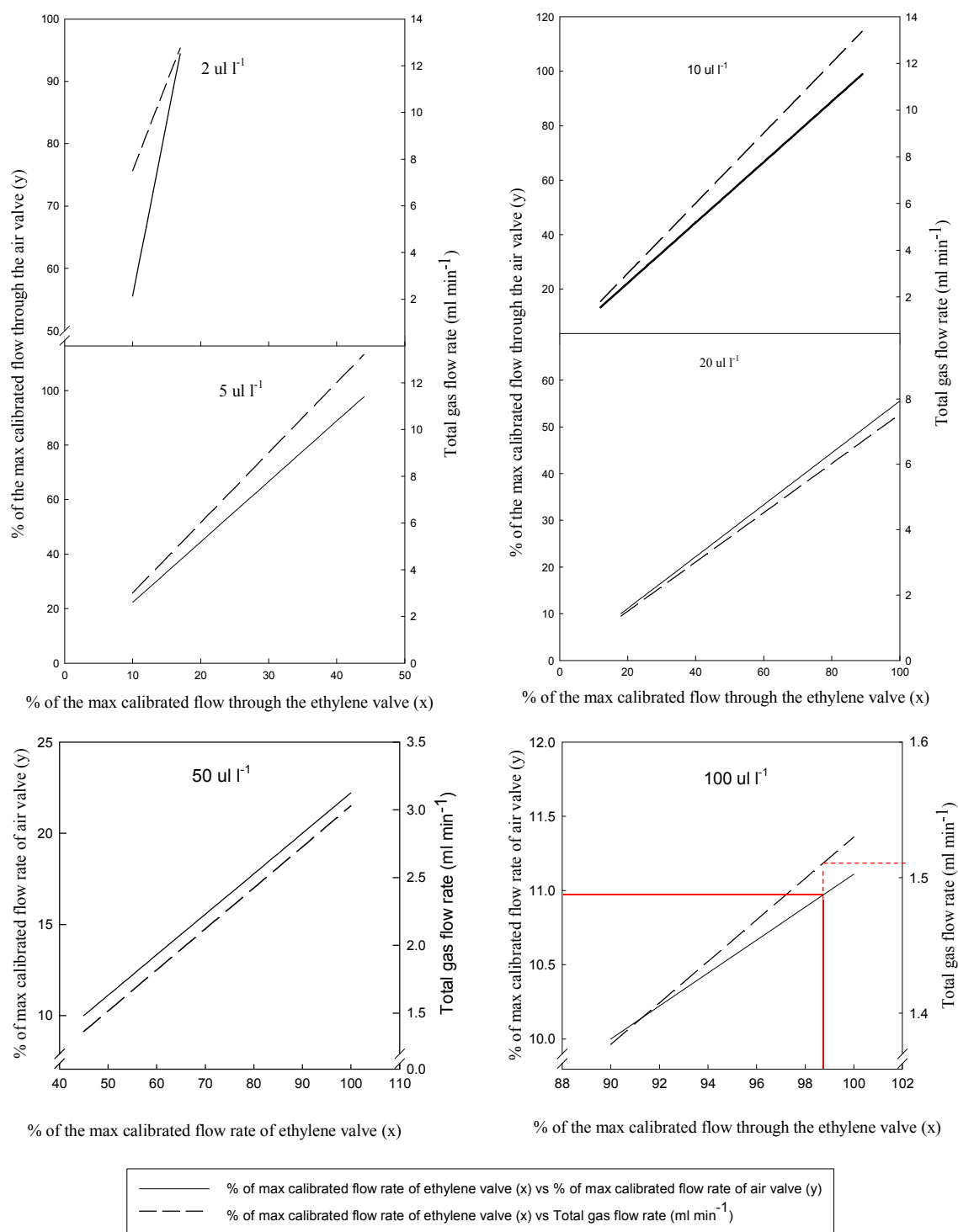
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2

3 Supplementary Fig. 1. Model-predicted ethylene concentrations (2, 5, 10, 20, 50 and 100  $\mu\text{l l}^{-1}$ ) and the corresponding flow rates at different x and y set points on the MFC-4 controller.  
 4  $\text{l}^{-1}$ ) and the corresponding flow rates at different x and y set points on the MFC-4 controller.  
 5 The red lines show how to read the percentage of maximum calibrated ethylene flow (abscissa)  
 6 against percentage maximum calibrated air flow (ordinate) and total gas flow rate.

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