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

Robert S. Amoah, Sandra Landahl and Leon A. Terry*

Plant Science Laboratory, Cranfield University, Bedfordshire, MK 43 0AL, UK

*Corresponding author's e-mail address: l.a.terry@cranfield.ac.uk

Abstract

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

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

Nomenclature

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

EA	Ethylene, then air-flushed boxes
AA	Continuous air-flushed boxes
AE	Air, then ethylene-flushed boxes
Me	Ethylene gas distribution manifold
Me	Air distribution manifold
A	Hydrovane air compressor
C	Ethylene cylinder
OD	Outside diameter of tubing (mm)
ID	Inside diameter of tubing (mm)
RH	Relative humidity
HWD	Hot wire detector
FID	Flame ionisation detector
CO_2	Carbon dioxide
C1	Stock concentration of ethylene in nitrogen (5000 µl l^{-1})
C2	Target concentration of ethylene in air (µl l^{-1})
M1	Total mass flow rate of compressed air and ethylene stock (ml l^{-1})
M2	Target mass flow rate of ethylene in air (ml l^{-1})
E	Any given stock ethylene concentration (µl l^{-1})
Fe	Any given calibrated full-scale flow through the ethylene valve (SLPM)
F_a	Any given calibrated full-scale flow through the air valve (SLPM)
x	Percentage of the calibrated full-scale flow through the ethylene valve (%)
y	Percentage of the calibrated full-scale flow through the air valve (%)
z	Percentage of the calibrated full-scale flow through the control (pure air) valve (%)
Q1	Total gas flow rate through the ethylene/air mix tubing (ml l^{-1})
Q2	Gas flow rate through the pure air tubing (ml l^{-1})
χ^2	Chi-square

R^2	Correlation coefficient
RMSE	Root mean square error
df	Degrees of freedom
K*pred*	Model-predicted ethylene concentration
K*expt*	Experimentally obtained ethylene concentration

1 Introduction

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

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

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

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

2 Materials and methods

Controlled ethylene supplementation was accomplished using a custom-built continuous flow-through apparatus (Fig. 1). The flow rates of the mixing gases were regulated by connecting mass flow control valves (Sierra, The Netherlands: models 840-L-2-OV1-SV1-D-V1-S1 and 840-L-2-OV1-SV1-E-V1-S1) in line with the ethylene (Ve) and air streams (Va_1), respectively. A third mass flow control valve (Va_2) was connected in a parallel air stream for direct flushing of the control samples with pure air. The mass flow control valves were factory calibrated to deliver 0.03 and 13.5 standard litres per minute (SLPM) maximum flow rates of ethylene and air, respectively. All the valves were connected to a digital multi-channel MFC-4 control unit (Sable Systems, NV, USA) to regulate the flows. Certified ethylene (BOC, Surrey, UK) made inert with nitrogen against explosion and compressed (200 Nm^{-2}) into a 50 l capacity cylinder (C) was diluted with air from a Hydrovane Air Compressor (A) (HVO2, Bedfordshire, UK) at room temperature from the stock concentration 5000 µl l^{-1} to the target concentrations (10 µl l^{-1} in the test experiment). At each setting of the MFC-4, a regulated amount of ethylene from the cylinder was blended into a corresponding amount of air stream at a T-junction without a

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

Each storage box contained samples of vegetables (*ca*. 3.75 kg of sweet potato) and covered with water-sealed transparent lids. Six boxes were flushed with the ethylene/air mix. Another set of six control boxes containing the same weight of sample were flushed with the pure air. At dormancy break, three boxes each, previously flushed with either ethylene or air, respectively, were swapped from the ethylene to air treatment or vice-versa (Amoah et al., 2016). The gases were bubbled through bowls of water placed inside the boxes to maintain a relative humidity of *ca*. 70-95 %. The humidity and temperature in the storage boxes were regularly monitored using Gemini data loggers (Tiny-tag Ultra 2, 0-95 % RH, -25 ^{o}C to 85 ^{o}C, Part No. TGU-4500, West Sussex, UK). Exhaust tubing was connected to the opposite sides of the boxes relative to the inflow gas such that there was upward, diagonal flow-through stream to maintain the carbon dioxide (CO_2) concentration below 0.5 %. CO_2 levels in the boxes were regularly checked by injecting headspace gas samples into a gas chromatograph (GC model 8340, DP800 integrator, Carlos Erba Instruments, Herts, UK) fitted with a Hot Wire Detector (HWD), analytical column Porapak (2 m length, 6 mm O.D. and 4 mm I.D.) and calibrated with 10 % CO_2 standard (BOC, Surrey, UK). The exhaust gas was disposed-off through the room ventilation system. The store temperature was maintained at 25 ^{o}C.

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

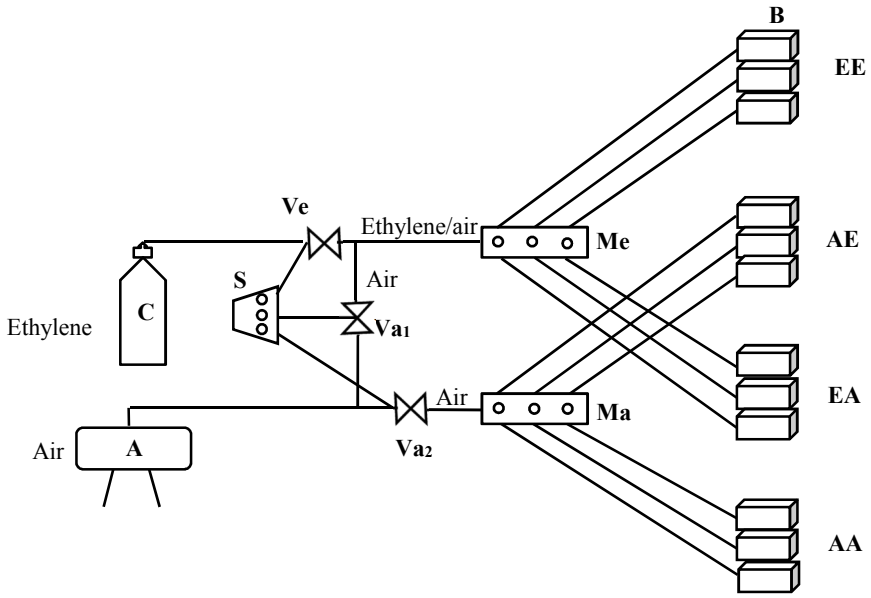

Fig. 1 A schematic diagram of the experimental setup

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

2.1 Theory: empirical model for supplemental ethylene concentration

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

Given that: *C1* is the stock concentration of ethylene in nitrogen (5000 µl l^{-1}), *C2* is the target concentration of ethylene in air, *M1* is the total mass flow of compressed air and ethylene stock, where the mass flow of ethylene stock is comparably negligible and *M2* is the target mass flow rate of ethylene in air.

Thus:

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

Therefore:

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

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

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

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

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

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

$$C2 = \frac{(x\ \%\ of\ Fe)\ X\ E}{(y\ \%\ of\ Fa)} \quad \text{.....Eq. (4)}$$

2.2 Flow setting for the gas valves

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

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

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

$$Q1\ =\ (x\ \%\ of\ Fe)\ +\ (y\ \%\ of\ Fa) \quad \text{..... Eq. (6)}$$

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

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

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

$$Q2 = z\,\%\,of\,Fa \quad \text{..... Eq. (8)}$$

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

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

$$Q1 = Q2 \quad \text{..... Eq. (9)}$$

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

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

$$z\,\% = \{(x\,\%\,of\,Fe) + (y\,\%\,of\,Fa)\} / (Fa) \quad \text{.....Eq. (11)}$$

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

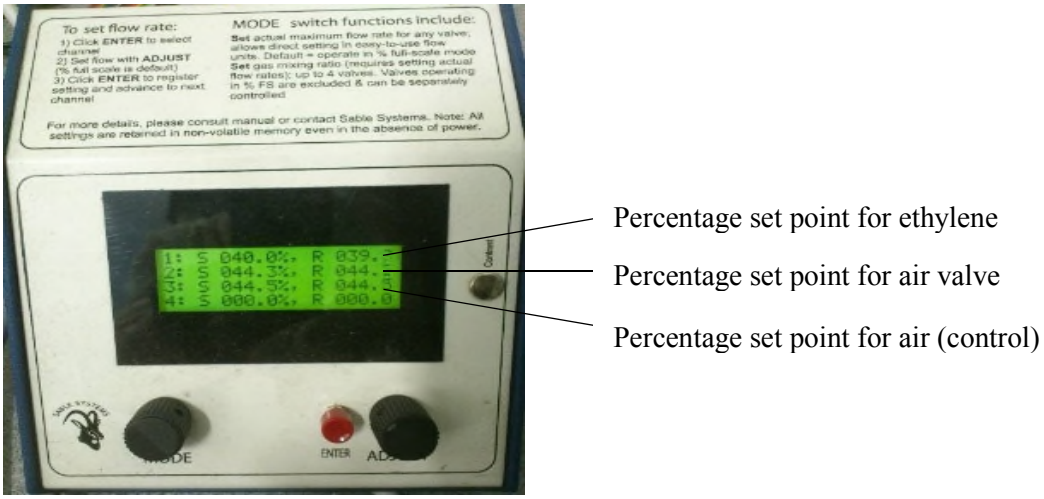

Fig. 2 Set points x, y and z on channels 1, 2 and 3 respectively, of the MFC-4 (Sable Systems)

2.3 Statistical analysis and model validation

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

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

3 Results and discussion

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

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

An inherent characteristic of MFC valves is that a linear relationship between the flow rate and the valve orifice opening is only valid between 10 % and 100 % set points of the calibrated full scale flows. Furthermore, at set points less than 2.5 % full-scale, the valves shut completely. This feature places minimum and maximum limits on the x and y set points. That in turn, sets limits to the flow rates and concentrations that could be achieved. With the given valves calibration (i.e. 0.03 and 13.5 SLPM for the ethylene and air valves, respectively), the maximum ethylene concentration that could be achieved was 100 $\mu l\ l^{-1}$ whilst the lowest concentration was 2 $\mu l\ l^{-1}$. To obtain higher concentrations and flow rates than what were 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 µl 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 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 ^{o}C, respectively, whilst the CO_2 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.

264

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

Run	df	*x* Set point (% FS of ethylene MFC)	*y* Set point (% FS of ethylene MFC)	Experimental concentration *Kexpt* (µl l^{-1})	Predicted concentration *Kpred* (µl l^{-1})	Chi-square χ^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

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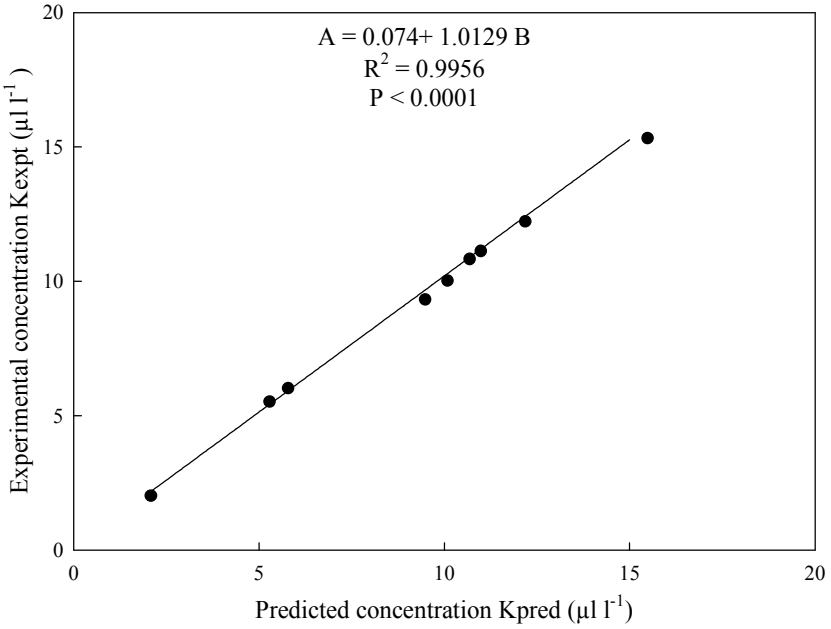

Fig. 3. Experimental and model-predicted ethylene concentrations at different inputs of x and y setpoints.

4 Conclusion

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

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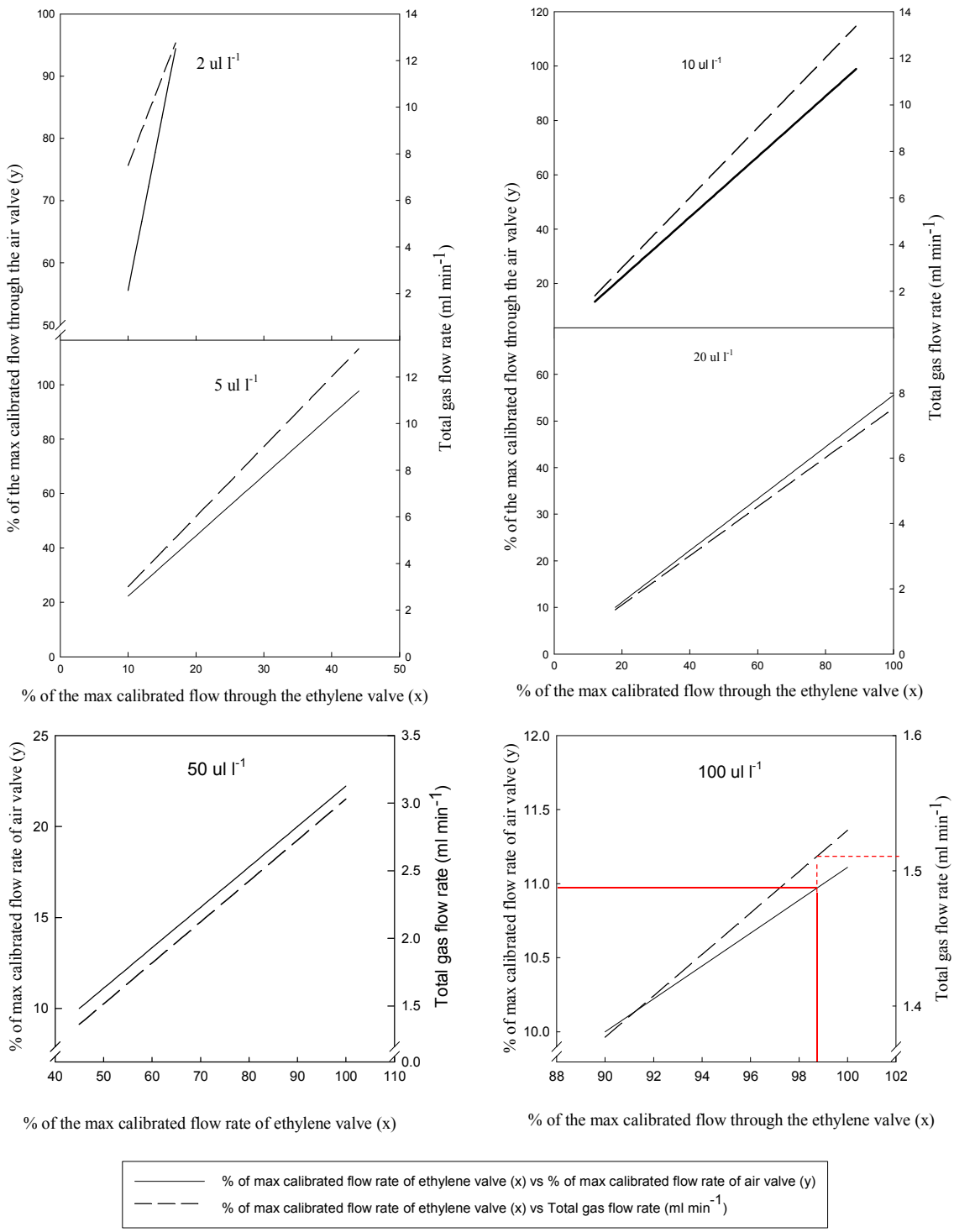

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3 Supplementary Fig. 1. Model-predicted ethylene concentrations (2, 5, 10, 20, 50 and 100 µl l-
4 1) and the corresponding flow rates at different x and y set points on the MFC-4 controller.
5 The red lines show how to read the percentage of maximum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

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