The Effect of Elevated CO_2 on the Vegetative and Generative Growth of *Phalaenopsis*

J.A.M. Kromwijk, E. Meinen and T.A. Dueck
Wageningen UR Greenhouse Horticulture
Bleiswijk
The Netherlands

Keywords: crassulacean acid metabolism, flowering quality, leaf area, dry weight

Abstract

***Phalaenopsis* is a crassulacean acid metabolism (CAM) plant which absorbs and binds CO_2 as malate during the night. During daytime the stomata close and the CO_2 stored in the vacuole is released and used for photosynthesis. Because the CO_2 taken up by CAM plants was assumed to be unaffected by the CO_2 concentration in the air, additional CO_2 for increased growth was generally not supplied in *Phalaenopsis*. However, a literature study indicated that elevated CO_2 might have a positive effect in *Phalaenopsis*. To assess the effects of elevated CO_2 concentrations in *Phalaenopsis*, two experiments were performed with elevated CO_2. In the first experiment, 8 *Phalaenopsis* clones were induced to flower at 400 and 1000 ppm CO_2 in the greenhouse at day/night temperatures of 19.2/18°C. Two or more spikes were produced by 82% of the plants grown at 1000 ppm CO_2, and in 70% of the plants grown at 400 ppm CO_2. The mean number of spikes per plant was 9% higher and their dry weight was 12% higher. A second experiment was then designed to see if the effect of elevated CO_2 was limited to the flowering phase, or was effective during the vegetative phase as well. In this experiment, four *Phalaenopsis* clones were grown in climate controlled growth chambers at CO_2 concentrations of 400 or 1000 ppm. During the vegetative growth phase (30 weeks), the leaf area and root dry weight of plants grown at elevated CO_2 appeared to be higher than at low CO_2. At the end of the vegetative growth phase, plants from both CO_2 treatments were split into two groups: one group at low CO_2 and the other at elevated CO_2 for the generative growth phase, resulting in 4 treatment groups. Day/night temperatures were lowered to 19/18°C for 6 weeks to induce spiking followed by 12 weeks with day/night temperatures of 21/19°C during the growth and development of the spikes. Plants grown at elevated CO_2 during the generative growth phase yielded 15 and 10% more plants with multiple spikes and an improved flower stem quality, when grown at low and high CO_2 in the vegetative phase respectively. Elevated CO_2 during the vegetative growth phase had no significant effect on the number of spikes, but the trend indicates an increase in spike quality.**

INTRODUCTION

Phalaenopsis holds a leading position in the amount of pot plants sold annually in The Netherlands. The number of spikes per plant is an important quality aspect in *Phalaenopsis* when sold as a flowering pot plant. Plants with multiple spikes yield higher prices and thus *Phalaenopsis* growers look for ways to increase the number of spikes. *Phalaenopsis* is a crassulacean acid metabolism (CAM) plant which absorbs and binds CO_2 as malate during the night and utilize it for photosynthesis during the day. For many years growers assumed that the uptake of CO_2 by CAM plants was unaffected by the CO_2 concentration in the air, and thus additional CO_2 was not supplied in *Phalaenopsis* greenhouses. However, a literature study (Warmenhoven et al., 2003) indicated that additional CO_2 up to 1000 ppm might have a positive effect on photosynthesis and flowering in *Phalaenopsis*. Endo and Ikusima (1997) found more spikes (11% resp. 29%) and flowers (10% resp. 42%) as well as a higher spike fresh weight (13% resp. 58%) in cut flower *Phalaenopsis* when the CO_2 concentration was increased from mean ambient atmosphere (438 ppm) to 700 and 1000 ppm from sunset to sunrise. To assess the effects

of elevated CO_2 concentrations in growing *Phalaenopsis* pot plants under Dutch conditions, two experiments were performed to assess the effects of elevated CO_2 on flower quality. In the first experiment 8 *Phalaenopsis* clones were exposed to 400 and 800 ppm CO_2 during flower induction in the greenhouse. A second experiment was then designed to investigate whether the effect of elevated CO_2 on flower quality was limited to the flowering phase, or was effective during the vegetative phase as well.

MATERIALS AND METHODS

Experiment 1

Young plants (8 clones) were grown on a bark substrate for 29 weeks in a greenhouse at a constant day/night temperature (28°C) and 800 ppm CO_2 at a vapour pressure deficit under seven. The light sum was increased from 2-3 to 3.5 mol m^{-2} d^{-1} prior to flower induction. On 19 December, 70 plants of each cultivar were transferred to 2 separate greenhouses, one of which was maintained at 400 ppm (low) CO_2 and the other at 1000 ppm (elevated) CO_2 for 24 h/day. The plant density was set at 42 plants m^{-2} and flowering was induced at 19.5/18.5°C (day/night), while the light sum was increased to 6 mol $m^{-2}d^{-1}$ with a combination of sunlight and additional HPS lighting (100 µmol m^{-2} s^{-1}) during 16 h/day to February 7th and during 14 h/day thereafter. The humidity was controlled by spraying water mist when relative humidity was below 65%. The number of spikes with at least 3 flowers or buds, and the number of lateral branches were counted on 50 plants/cultivar/treatment. The number of flower buds (at least 5 mm), the fresh and dry weight of the leaves and spikes, and spike length was measured on 10 plants/cultivar/ treatment.

Experiment 2

Young *Phalaenopsis* plants (four clones) were grown on a bark substrate for 30 weeks in two climate rooms at a constant day/night temperature (28°C) at a relative humidity of 70%. Each growth chamber was filled with four groups of 108 plants per clone each. The plant density began at 75 plants m^{-2} and was decreased to 60-75 plants m^{-2} depending on the plant size per clone. One climate room received no additional CO_2 while the CO_2 concentration was maintained at 1000 ppm in the other room. Artificial light was supplied (Philips Master TL-D 50/840 HF), the light sum increasing from 3.1 mol $m^{-2}d^{-1}$ (first 24 weeks) to 3.9 mol $m^{-2}d^{-1}$ for the last 6 weeks prior to flower induction. The light intensities were 72 and 90 µmol m^{-2} s^{-1} during a 12 h/day.

At the end of the vegetative growth phase, plants from both the high and the low CO_2 treatments were split into four groups and placed in four growth chambers: two without additional CO_2 and the other two with 1000 ppm CO_2 for the duration of the generative growth phase. Thus plants grown at low CO_2 began flower induction at both low and high CO_2, and plants grown at high CO_2 also began flower induction at both low and high CO_2, resulting in four treatments. At the start of flowering induction plant density was 40-50 plants m^{-2} depending on the plant size per cultivar. Flowering was induced at 19/18°C (light/dark) and light intensity was increased to 139 µmol m^{-2} s^{-1} (6 mol m^{-2} d^{-1}). The relative humidity was set at 75% during the first five weeks and lowered to 70% in the sixth week after which the temperature was raised to 21/19°C (light/dark).

RESULTS AND DISCUSSION

Experiment 1

During the first 8 weeks of flower induction the measured mean CO_2 concentration was 410 and 921 ppm (Table 1), and slightly lower during the next 11 weeks: 398 and 805 ppm. The measured CO_2 concentration dropped slightly due to the higher ventilation as a result of warmer weather. In the second-last week of the generative phase the CO_2 concentration dropped to ambient outdoor CO_2-concentrations, due to

limited availability of CO_2. The number of spikes per plant and percentage of plants with multiple spikes in all 8 *Phalaenopsis* clones was higher in elevated CO_2 (Fig. 1). Two or more spikes per plant were produced by 82% of the plants grown in high CO_2 and in 70% of the plants grown in low CO_2 (Table 2). The mean number of spikes per plant was 9% higher, spike dry weight was 12% higher and the dry weight percentage was 5% higher in elevated CO_2 as well, but the mean number of lateral branches on the spikes was 14% lower on average per plant (Tables 2 and 3). No significant differences were found in spike length or number of flower buds (>5 mm) per plant and leaf dry weight between both treatments. Obviously the flowering quality in all clones increased as a result of elevated CO_2, while plant biomass was not influenced in the generative growth phase.

Experiment 2

Elevated CO_2 in the vegetative phase had no effect on the number of newly formed leaves (Table 4). Except for 'White Moon' where differences were very small, plants from the other clones grown in elevated CO_2 appeared to have a higher total leaf area than plants grown at low CO_2, an observation also noted by Kataoka (2004). The total leaf area averaged over four clones was 8% higher in elevated CO_2. The CO_2 level had no significant effect on leaf dry weight and total dry weight, but the root dry weight appeared to increase.

The supply of extra CO_2 during the vegetative phase had no effect on the number of spikes formed (Table 5), regardless of the CO_2 level applied during flower induction. On the other hand, elevated CO_2 during flower induction had a larger effect increasing the number of spikes, although the differences were not quite significant due to variation between clones and experimental conditions. Low CO_2 during the generative phase resulted in 68% plants with multiple spikes and elevated CO_2 in 80% of the plants with multiple spikes, which confirms the results of the first experiment. The increase in spike number differed between clones. The clone 'Brussels' produced multiple spikes quite easily without additional CO_2 and elevated CO_2 only resulted in a small increase in spike number and percentage of plants with multiple spikes. The other clones showed larger effects due to elevated CO_2. The percentage of plants with multiple spikes increased by 15% in 'Pink Twilight', 18% in 'Sacramento' and 19% in 'White Moon' in elevated CO_2 concentration during the generative phase (Table 6). Elevated CO_2 during the generative phase appeared to increase the spike dry weight by 11%, independent of the number of spikes/ plant (Table 7). In contrast to the first experiment, plants grown in elevated CO_2 in the second experiment appeared to produce more flower buds (Table 8). On average one additional flower bud was formed in elevated CO_2 during the generative phase compared to low CO_2. The CO_2 concentration had no effect on the number of lateral branches or spike length.

The results of these experiments confirm the results of Endo and Ikusima (1997), who found more spikes (11% resp. 29%) and flowers (10% resp. 42%) as well as a higher spike fresh weight (13% resp. 58%) in cut flower *Phalaenopsis* at increased CO_2 concentrations from 700 ppm resp. 1000 ppm from sunset to sunrise relative to ambient CO_2. The smaller effect found in this study might be due to variation between the clones used, and to differences in growth conditions which influence CO_2 uptake. Kromwijk et al. (2005), Ichihashi et al. (2008) and Dueck et al. (2011) found a higher CO_2 uptake at higher CO_2 concentrations in the air. Ichihashi et al. (2008) also showed that CO_2 uptake changed with temperature and relative humidity. CO_2 uptake was lower at increasing temperatures and CO_2 uptake was stimulated at 70% relative humidity and suppressed drastically at 30% relative humidity. The positive effect of a high humidity on the CO_2 uptake was confirmed by Dueck and Meinen (2008). The smaller effect of elevated CO_2 during the vegetative phase at 28°C might be explained by the negative effects of high temperatures on CO_2 uptake found by Ichihashi et al. (2008) and the lower CO_2 uptake in *Phalaenopsis* plants without spikes than in plants with spikes (Ota et al., 1991). No significant differences were found in spike length, but the trend observed in spike length, being 2 cm longer at elevated CO_2 during the generative phase, confirms results of

Kataoka (2004), who found an increase in spike length also. Elevated CO_2 in the vegetative growth phase appears to contribute more to the increase in plant biomass than to flower quality.

CONCLUSION

1. Elevated CO_2 (1000 ppm) during the generative phase increased the number of spikes per plant, the percentage of plants with multiple spikes (+12%) and spike dry weight in both experiments.
2. Elevated CO_2 (1000 ppm) during the generative phase appear to improve the number of flower buds per plant.
3. A trend was observed in the spike length, being 2 cm longer at elevated CO_2 (1000 ppm) during the generative phase.
4. Elevated CO_2 (1000 ppm) during the vegetative phase appear to increase leaf area and root dry weight, but had no effect on the number of new leaves.
5. Plants grown at elevated CO_2 during the vegetative phase appear to produce a higher spike biomass, but had no effect on the number of spikes or percentage of plants with multiple spikes.
6. The results of these experiments indicate that elevated CO_2 has the largest effect on flower quality when applied in the generative growth phase.

ACKNOWLEDGEMENTS

This research was financed by the Dutch Product Board of Horticulture. Young plants were supplied free-of-charge for both experiments by Anthura and Floricultura.

Literature Cited

Dueck, T. and Meinen, E. 2008. CO_2-opname bij *Phalaenopsis*. Rapport 191 Wageningen UR Glastuinbouw, Wageningen, The Netherands.

Dueck, T., Boer, P. de and Noort, F. van 2011. Teeltversnelling *Phalaenopsis* door klimaat optimalisatie tijdens op-en afkweek. Rapport GTB-1016A Wageningen UR Glastuinbouw, Wageningen, The Netherlands.

Endo, M. and Ikusima, I. 1997. Effect of CO_2 enrichment on yield and preservability of cut flowers in *Phalaenopsis*. J. Japan. Soc. Hort. Sci. 66:169-174.

Ichihashi, S., Higuchi, T., Shibayama, H., Tesima, Y., Nishiwaki, Y. and Ota, K. 2008. Aspects of CO_2 uptake in the crassulacean acid metabolism orchid *Phalaenopsis*. Acta Hort. 766:245-256.

Kataoka, K., Sumitomo, K., Fudano, T. and Kawase, K. 2004. Changes in sugar content of *Phalaenopsis* leaves before floral transition. Sci. Hort. 102:121-132.

Kromwijk, A., van Mourik, N., Schüttler, H., van, Wertwijn, R.O.P. and Schapendonk, A. 2005. Daglengte en lichtintensiteit bij *Phalaenopsis*. PPO-rapport 41717008.

Ota, K., Morioka, K. and Yamamoto, Y. 1991. Effects of leaf age, inflorescence, temperature, light intensity and moisture conditions on CAM photosynthesis in *Phalaenopsis*. J. Japan Soc. Hort. Sci. 60:125-132.

Warmenhoven, M.G., Marissen, N. and Kromwijk, J.A.M. 2003. Invloed van licht en CO_2 bij *Phalaenopsis*. PPO-rapport GT133017.

Tables

Table 1. Mean CO_2 concentration (ppm), temperature (°C), relative humidity (%) and light sum at plant height (mol m^{-2} day^{-1}) during the generative growth phase of *Phalaenopsis* in Experiment 1 (December 20, 2006 to May 3, 2007) and during the vegetative and generative growth phases in Experiment 2.

	CO_2 (ppm)		Temperature (°C)		Relative humidity (%)		Light sum* (mol m^{-2} d^{-1})	
	Low CO_2	High CO_2	Low CO_2	High CO_2	Low CO_2	High CO_2	Low CO_2	High CO_2
Experiment 1								
Week 1-8	410	921	19.2	19.2	58.4	58.0	5.1	5.2
Week 9-20	398	805	21.3	21.0	51.5	53.9	5.9	5.8
Experiment 2								
Week 1-30	433	1001	28.0	28.0	69.9	70.0	3.1/3.9	3.1/3.9
Week 30-36	433	1001	18.5	18.5	74.7	73.7	6.0	6.0
Week 36-48	440	1003	20.0	20.0	70.1	70.0	6.0	6.0

* Light sum during the first 24 weeks and the next 6 weeks in Experiment 2.

Table 2. Mean number of spikes per plant, number of lateral branches and mean proportion of plants with 0, 1, 2 or 3 spikes and with multiple spikes per plant in Experiment 1. Mean of eight *Phalaenopsis* clones (n=50 plants/cultivar/treatment).

CO_2 level (ppm)	Spikes $plant^{-1}$	Lateral branches $plant^{-1}$	0 spikes (%)	1 spike (%)	2 spikes (%)	3 spikes (%)	Multiple spikes (%)
400	1.73 a	1.88 b	0.0 a	30.4 b	66.0 a	3.5 a	69.6 a
1000	1.88 b	1.63 a	0.3 a	17.8 a	75.7 b	6.3 a	82.0 b

Different letters denote significant differences at $p<0.05$ (n=80).

Table 3. Mean number of flower buds per plant (>5 mm), spike length, dry weight and percentage dry weight of spikes and leaves in Experiment 1. Mean of 8 *Phalaenopsis* clones.

CO_2 level (ppm)	Number of flower buds	Spike length (cm)	DW spikes (g)	DW spikes (%)	DW leaves (g)	DW leaves (%)
400	14.5 a	58.8 a	3.5 a	10.0 a	6.1 a	6.2 a
1000	13.8 a	60.1 a	4.0 b	10.5 b	6.7 a	6.6 a

Different letters denote significant differences at $p<0.05$ (n=80).

Table 4. Mean number of new leaves, total leaf area (cm^2) and dry weight of leaves, roots and total dry weight (g) of *Phalaenopsis* plants grown at 400 and 1000 ppm CO_2 for 30 weeks at 28°C in Experiment 2.

CO_2 level (ppm)	Number of new leaves	Total leaf area (cm^2)	Leaf dry weight (g)	Root dry weight (g)	Total dry weight (g)
400	3.1	358	4.28±0.20	2.89±0.14	7.17±0.32
1000	3.0	385	4.16±0.16	3.37±0.20	7.52±0.34
No. of plants	40	40	24	24	24

Means of four clones ± SE.

Table 5. Mean number of spikes per plant in each of 4 *Phalaenopsis* clones grown at 400 and 1000 ppm CO_2 during the vegetative (Veg.) and generative (Gen.) growing phase in Experiment 2.

CO_2 Veg. week 1-30 (ppm)	CO_2 Gen. week 31-48 (ppm)	Brussels	Pink Twilight	Sacramento	White Moon	Total
400	400	1.97±0.06	1.45±0.09	1.67±0.08	1.57±0.22	1.67±0.08
400	1000	2.03±0.03	1.64±0.07	1.83±0.03	1.77±0.14	1.82±0.06
1000	400	2.06±0.00	1.32±0.04	1.80±0.03	1.65±0.03	1.71±0.10
1000	1000	2.09±0.03	1.79±0.10	1.79±0.11	1.72±0.11	1.85±0.06
No. of plants		34	29	30	35	127

Mean ± SE.

Table 6. Proportion of plants with multiple spikes in each of 4 *Phalaenopsis* clones grown at 400 and 1000 ppm CO_2 during the vegetative (Veg.) and generative (Gen.) growing phase in Experiment 2.

CO_2 Veg. week 1-30 (ppm)	CO_2 Gen. week 31-48 (ppm)	Brussels	Pink Twilight	Sacramento	White Moon	Total
400	400	94±5.9	49±8.6	65±8.1	56±22.2	66±8.2
400	1000	100±0.0	64±7.1	83±3.3	75±13.6	81±5.8
1000	400	100±0.0	32±3.6	80±0.0	68±2.9	70±9.4
1000	1000	94±0.3	83±9.8	75±11.2	69±7.7	80±4.8
No. of plants		34	29	30	35	127

Mean ± SE.

Table 7. Biomass of leaves, roots, spikes and total (g) of *Phalaenopsis* plants grown at 400 and 1000 ppm CO_2 during the vegetative (Veg.) and generative (Gen.) growing phase in Experiment 2.

CO_2 Veg. week 1-30 (ppm)	CO_2 Gen. week 31-48 (ppm)	Leaf dry weight	Root dry weight	Spike dry weight	Total dry weight
400	400	6.17±0.31	5.27±0.34	2.98±0.24	14.42±0.83
400	1000	6.23±0.23	5.74±0.29	3.30±0.24	15.27±0.70
1000	400	6.14±0.26	5.71±0.27	3.15±0.22	15.01±0.69
1000	1000	6.41±0.22	6.34±0.26	3.75±0.23	16.50±0.65

Means of data of 4 clones, n=24.

Table 8. Mean number of flower buds and lateral branches and spike length of *Phalaenopsis* plants grown at 400 and 1000 ppm CO_2 during the vegetative (Veg.) and generative (Gen.) growing phase in Experiment 2.

CO_2 Veg. week 1-30 (ppm)	CO_2 Gen. week 31-48 (ppm)	Flower buds	Lateral branches	Spike length (cm)
400	400	15.1	0.43	42.1
400	1000	16.1	0.41	42.7
1000	400	15.5	0.33	42.7
1000	1000	16.8	0.33	44.9

Means of data of 4 clones, n=127.

Figures

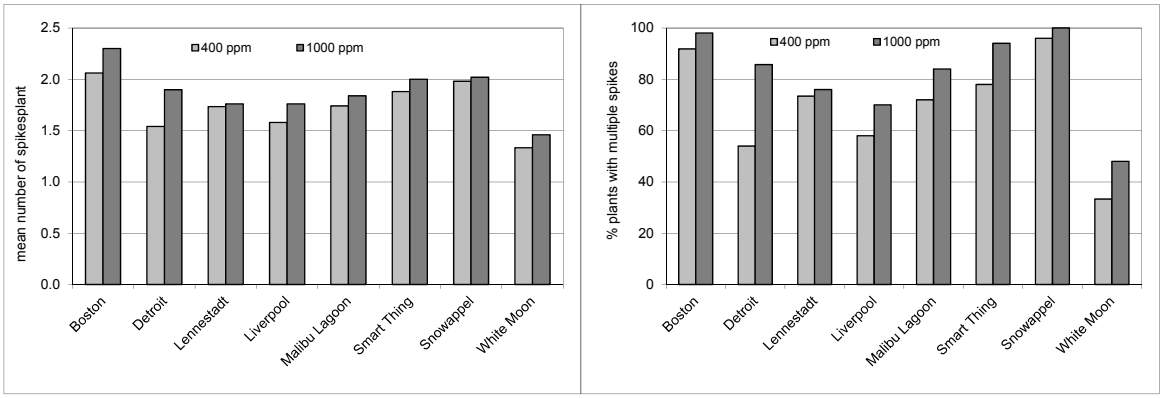

Fig. 1. Mean number of spikes per plant (left) and proportion of plants with multiple spikes (right) of eight *Phalaenopsis* clones grown at 400 or 1000 ppm CO_2 during the generative growth phase in a greenhouse.