EFFECT OF PATHOGEN-SUPPRESSING MODIFIED ATMOSPHERES ON STORED CUT FLOWERS

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1. INTRODUCTION

The first research on the use of modified atmospheres for storage of cut flowers was carried out in the 1930s (8, 14). Modified atmosheres containing increased CO_2 (5-15%) were said to prolong the life of flowers such as gladioli, snapdragons, cosmos, dahlias and carnations (Thornton, cited by Siegelman and Stoutmeyer (13)). According to Siegelman and Stoutmeyer (13), elevated CO_2 atmospheres were used subsequently for intercontinental flower shipments.

Subsequent work has also studied the effects on flowers of reduced-O_2 atmospheres, either alone or in combination with increased CO_2 (1, 5, 15, 16). The consensus of these reports is that modified atmospheres do not generally improve the storage and vase life of cut flowers. For example, while Uota and Garazsi (16) perceived an advantage of low O_2 atmospheres for carnations, Hanan (5) concluded that there were no commercial benefits over air storage. The diverse results obtained in investigations of the effects of modified atmosphere on cut flowers are summarized in review articles by Halevy and Mayak (4), Nowak and Rudnicki (9) and Rogers (11). Factors such as carbohydrate status, physiological age, cultivar differences and spore (pathogen) load possibly account for some apparently contradictory findings.

Other atmospheric modifications that have been investigated include the addition of SO_2 or H_2S (8), and the use of pure N_2 atmospheres (10). Halevy and Mayak (4) considered the successful and beneficial storage of daffodils in pure N_2 (10) to be in contrast to the generally inconclusive results obtained in modified atmosphere studies.

Staby *et al.* (12) indicated several problems with the application of modified atmosphere storage to cut flowers. These were: 1. a small safety margin with respect to phytotoxic responses, 2. cultivar specificity and the small volumes of individual cultivars marketed, and 3. the high cost of maintaining controlled atmospheres.

An advantage of modified atmospheres, apart from a prolonged postharvest life, can be the control of plant pathogens during storage (2). Goszczynska and Rudnicki (3) were able to store carnation buds for

long periods only when pathogen growth was controlled with appropriate fungicides. In low O_2 atmospheres, Uota and Garazsi (16) found that decay in carnations was reduced relative to the air control. Reduced O_2, increased CO_2, combinations of the two, and CO addition to modified atmospheres have all been found to reduce decay of stored plant material (2, 6, 7).

The present study was initiated to appraise the effects, on a range of cut flowers, of atmospheres considered likely to suppress pathogens. The approach taken was to screen a number of cut flower species for CO_2 tolerance, to compare four commercially important cut flowers under elevated CO_2 storage for up to 4 weeks, and to compare an elevated CO_2 atmosphere with 5 other atmospheres considered potentially effective against postharvest pathogens.

2. MATERIALS AND METHODS

2.1 Experiment 1.

The vase lives of twelve cut flower species were evaluated following storage for 7 days at 1 $\pm$2°C in the following atmospheres: air, and 7% CO_2, 14% CO_2 and 60% CO_2 added to air.

Ten species were supplied by a wholesale florist. These were Rosa 'Cara Mia', Rosa 'Sonia', Dianthus caryophyllus 'White Sim', Chrysanthemum morifolium 'Florida Marble', Alstroemeria 'Regina', Narcissus 'Paper White' (Narcissus), Narcissus pseudonarcissus 'King Alfred' (daffodil), Iris 'Blue Ribbon', Gypsophila paniculata 'Perfecta' and Lilium 'Enchantment'. The flowers were packed and cooled before transport to Davis. Glasshouse grown Cyclamen persicum and Gerbera were obtained locally. Flowers were grown under Winter (Jan. 1985) conditions.

The bases of the flower stems were cut under water, and the flowers pulsed overnight in a cold room (1 $\pm$2°C) in 1% w/v sucrose solution having an initial temperature of ca. 35°. The following day the flowers were transferred to 4 cylindrical modified atmosphere chambers (25 cm I.D., 750 cm long, 36.8 l volume). The chambers were made of PVC pipe with perspex end-plates secured over rubber seals. The flowers were stored dry. No appreciable wilt of any flowers was observed during storage. Condensation of water on the walls of the chambers was indicative of high humidity. Atmosphere flow rates were ca. 6 l per hr. CO_2 concentrations were monitored with a Carle Analytical Gas Chromatograph 111.

After 7 days the chambers were opened and the flowers removed. Stems were re-cut under water, and each flower was placed in a vase containing ca. 200 ml of 2% w/v sucrose plus 200 ppm hydroxyquinoline citrate (HQC). The flowers were held in a vase-life room at 20 $\pm$2°C, 50-70% R.H., and 1000 lux illuminance (13.5 $umol \cdot s^{-1} \cdot m^{-1}$ irradiance) from cool-white fluorescent lamps on a 12 hr photoperiod.

The condition of the flowers was examined daily, and vase-life was recorded as that stage at which a flower ceased to be attractive. The experiment was arranged as randomized complete block designs for each species. Replication was varied according to the number of flowers available for the different species; two-fold for cyclamen and Enchantment lily, four-fold for daffodil, and three-fold for all other species. Treatment effects were evaluated by two-way analysis of variance, and L.S.D. (p 0.05) values were computed to facilitate comparisons among treatment means when significant (p 0.05) variance ratios were obtained. The error mean squares and error degrees of freedom for each ANOVA are presented in the Appendix.

2.2 Experiment 2.

Four cut flower species were compared after storage at $1 \pm 2^{o}C$ in atmospheres of air, 10% CO_2, 15% CO_2 and 20% CO_2 for periods of 1, 2, 3 and 4 weeks.

Dianthus caryophyllus 'White Sim', Chrysanthemum morifolium 'Florida Marble', Rosa 'Sonia' and Iris 'Blue Ribbon' were supplied (March, 1985), prepared, and placed in modified atmosphere chambers as described above. The flow rate through the chambers was ca. 7 l per hr. Chambers were opened each week, and samples were withdrawn for vase-life determination. An initial sample for determination of unstored vase-life was placed in the vase-life room after the initial overnight pulsing in 1% sucrose. Because observations during Experiment 1 suggested that modified CO_2 atmospheres can differentially affect floral and foliar tissues, the quality of the leaves was also scored during evaluation of vase-life of the stored flowers.

Replication was four-fold, and a randomized complete block design was used for each species during vase-life assessment. The experiment was analyzed by three-way analysis of variance (Appendix). L.S.D (p 0.05) values for pairwise comparison of means were estimated.

2.3 Experiment 3.

Four cut flower species were stored for 3 weeks at $1 \pm 1^{o}C$ in the following atmospheres: air, 10% CO_2, 3% O_2, 3% O_2 + 10% CO_2, 3% O_2 + 5% CO and pure N_2. Where reduced oxygen atmospheres were used, nitrogen was the substituted gas.

Spring (April, 1985) grown flowers of Dianthus caryophyllus 'White Sim', Chrysanthemum morifolium 'Florida Marble', Rosa 'Sonia' and 3 cultivars of Alstroemeria, viz. 'Regina', 'Rosita' and 'Yellow King', were used as experimental material. The 3 cultivars of alstroemeria were divided among replications so that each was equally represented in each treatment. The experiment was conducted in the same fashion as described for experiments 1 and 2.

Vase-life for each species was determined in a completely

randomized design. Replication was six-fold. The experiment was analysed using one way analysis of variance (Appendix) and L.S.D. (p 0.05) values were computed for the comparison of treatment means.

3. RESULTS AND DISCUSSION

3.1 Response of various cut flowers to storage in CO_2 for 1 week (Expt. 1).

3.1.1 Effects of high (60%) CO_2

In contrast to many other horticultural crops, a number of flower species are quite tolerant of short-term exposure to very high concentrations of CO_2. No deleterious effects of 60% CO_2 were observed in lily, iris, carnation, gypsophila, daffodil and cyclamen on removal from the modified atmosphere chambers, and for lily, carnation and daffodil there were no pronounced effects of elevated CO_2 on vase-life (Fig. 1). The lily and carnation flowers appeared to be particularly tolerant of high CO_2 (60 %). However, the opening of buds of lilies stored in 60% CO_2 tended to be delayed. In addition, the leaves of lily stored in high CO_2 were somewhat darker green than those of flowers from the other treatments. The smaller mean vase-life of carnations stored in air or 60% CO_2 suggested a possible benefit from storage in 7 or 14% CO_2 atmospheres (Fig. 1). However, differences in carnation vase-lives were not significant (p 0.05). Daffodils stored well in all CO_2 concentrations used, but successive increments in the CO_2 concentration clearly repressed flower opening in the vase. One major difference between flowers and many other horticultural commodities is their surface/volume ratio, which may reduce the CO_2 injury caused by CO_2 accumulation in bulky organs exposed to similar concentrations of the gas.

Injury was noted in some flower species even immediately after removal from 60% CO_2. Chrysanthemum flowers had extensive petal browning and wilted leaves, alstroemeria leaves were also wilted, rose petals were discolored, and the narcissus flowers were paler yellow than those from lower CO_2 concentrations. Gerbera appeared to be the least CO_2-tolerant of the species screened. After storage in 60% CO_2 gerbera petals were completely brown and the scapes had collapsed. Even in flowers stored at 7% and 14% CO_2 the bases of the petals were brown.

Symptoms of CO_2 damage, other than those observed on removal of the flowers from storage, developed rapidly, and terminated the vase-life of iris, alstroemeria, cyclamen and gypsophila (Fig. 1) stored in 60% CO_2. Iris developed brown necrotic areas on the leaves and the petals darkened to a deeper blue. A general phenomenon noted in iris exposed to elevated CO_2 was bending of the stem, although this did not reduce vase-life. Flowers of alstroemeria removed from 60% CO_2 browned, as did the leaves. Cyclamen flowers from 60% CO_2 blued and shrivelled. In gypsophila an effect associated with high CO_2 was curling of the pedicels, in addition to early desiccation of individual flowers. High CO_2 (60%) inhibited bud opening in Sonia roses, and caused a light pink

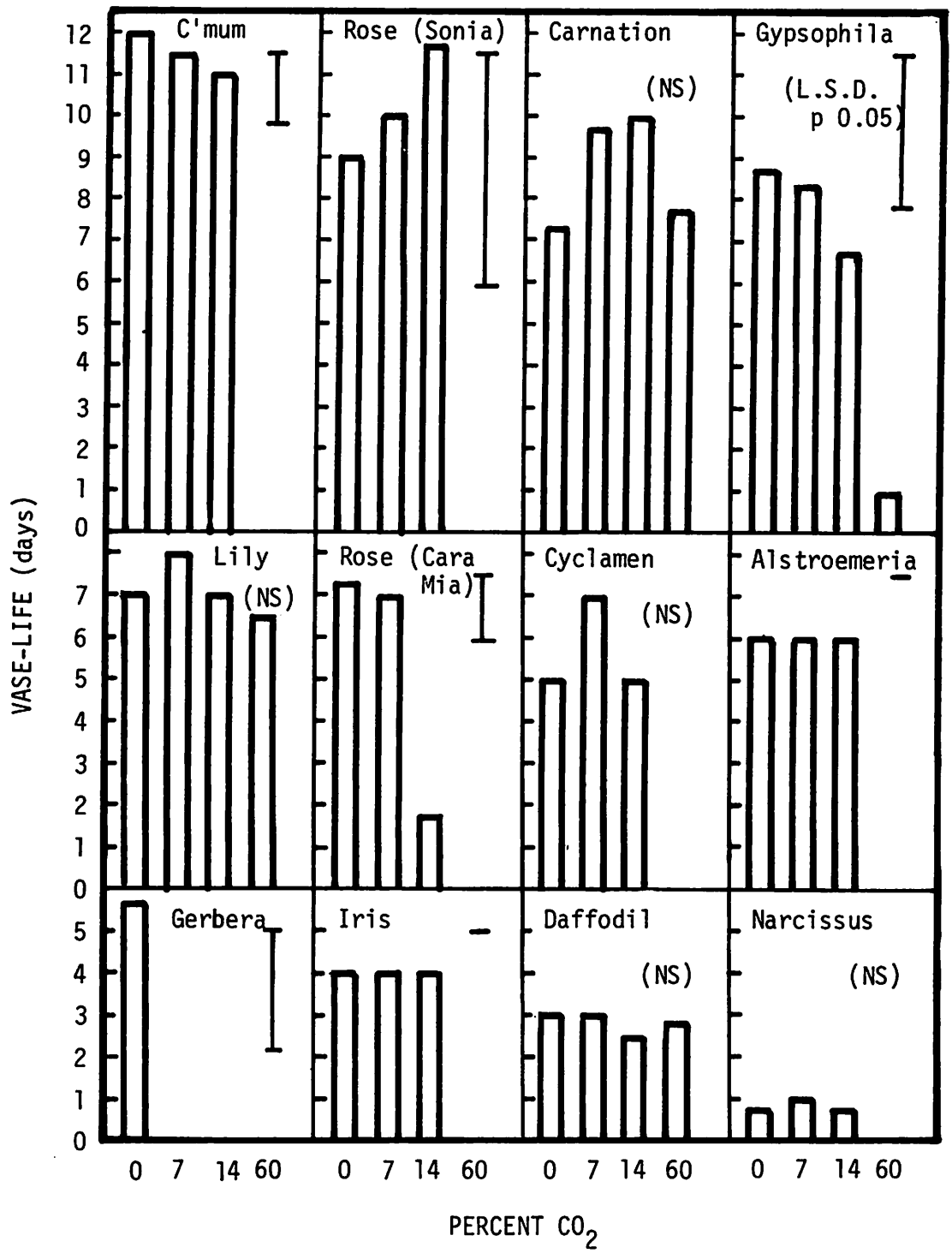

Fig. 1. The effect of elevated CO_2 (7, 14 and 60%) during 7 days storage at 1°C on the vase-life of 12 different species and cultivars of cut flowers. (NS indicates no significant differences).

discoloration which preceded browning and desiccation. In Cara Mia roses stored in 60% CO_2, the leaves desiccated, and blueing gave way, over time, to browning of the petals. In addition to the extensive browning of chrysanthemum petals noted on removal of these flowers from 60% CO_2, their wilted leaves desiccated rapidly in the vase-life room.

3.1.2 Effects of moderate (7% and 14%) CO_2

Although Sonia roses had normal vase life after storage in both 7% and 14% CO_2, the vase-life of Cara Mia roses was greatly reduced after storage in 14% CO_2. The reduced vase-life was primarily due to petal blueing, particularily around the margins. Gerbera, as noted above, was severely damaged by 7%, 14% and 60% CO_2. Chrysanthemum, like most species, tolerated 7% and 14% CO_2 relatively well. However, the leaves of chrysanthemum exposed to 14% CO_2 tended to be slightly darker green than those stored in air or 7% CO_2. None of the other species was deleteriously affected by cool storage for 1 week in 7% or 14% CO_2, suggesting some potential for high CO_2 storage as a means of pathogen control during long-term storage.

3.2 Long term storage of cut flowers in elevated CO_2 atmospheres (Expt. 2).

3.2.1 Chrysanthemums

The vase-life of stored chrysanthemum flowers and foliage decreased as the duration of storage increased, regardless of the storage atmosphere (Fig. 2). Twenty percent CO_2 caused browning of the petals, and the foliage of flowers stored in 15% and 20% CO_2 had a shorter vase-life after 2 and 3 weeks than that of foliage stored in air and, to a lesser extent, in 10% CO_2. Generally the foliage life was shorter than the flower life. Storage in CO_2 did not offer any consistent advantage over storage in air. After 4 weeks in 15% CO_2, unopened buds were more severely damaged than open flowers, suggesting a relationship between physiological age of the tissue and susceptibility to CO_2 damage.

3.2.2 Carnations

The vase life of carnation flowers stored in 20% CO_2 was consistently equal to or greater than that of flowers stored in other atmospheres (Fig. 2). Storage in 10% and 15% CO_2 atmospheres also tended to result in a longer vase-life than air storage - with the anomalous exception of flowers stored in 10% CO_2 for 1 week (Fig. 2). Fungal growth (probably Botrytis) commenced at 3 weeks and was substantial after 4 weeks in air-stored flowers, but was not noticeable on the flowers stored in CO_2-enriched atmospheres. Foliage life was not a factor limiting the vase-life of carnations (Fig. 2).

3.2.3 Iris

The vase-life of iris was short (2-3 days) (Fig. 3). In air

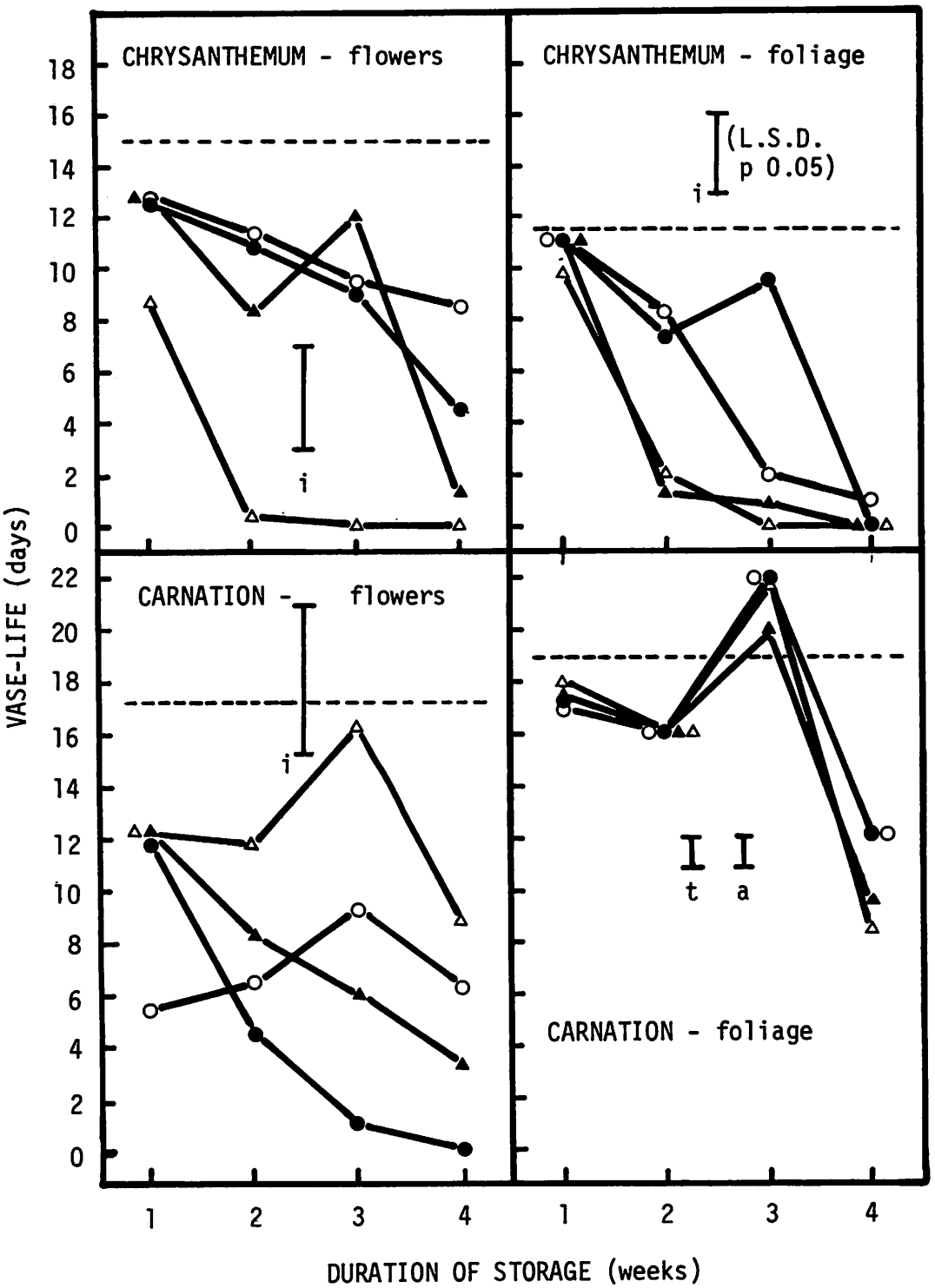

Fig. 2. The effect of elevated CO_2 (10 [○], 15 [▲] and 20% [△]), compared with air [●], during storage at 1°C on the vase-life of chrysanthemum and carnation flowers and foliage sampled from storage at weekly intervals over 4 weeks. (Dashed line indicates the vase-life of unstored material; L.S.D. bars are presented for the interaction (i) when significant, and for times (t) and for atmospheres (a) when the interaction was not significant (p 0.05)).

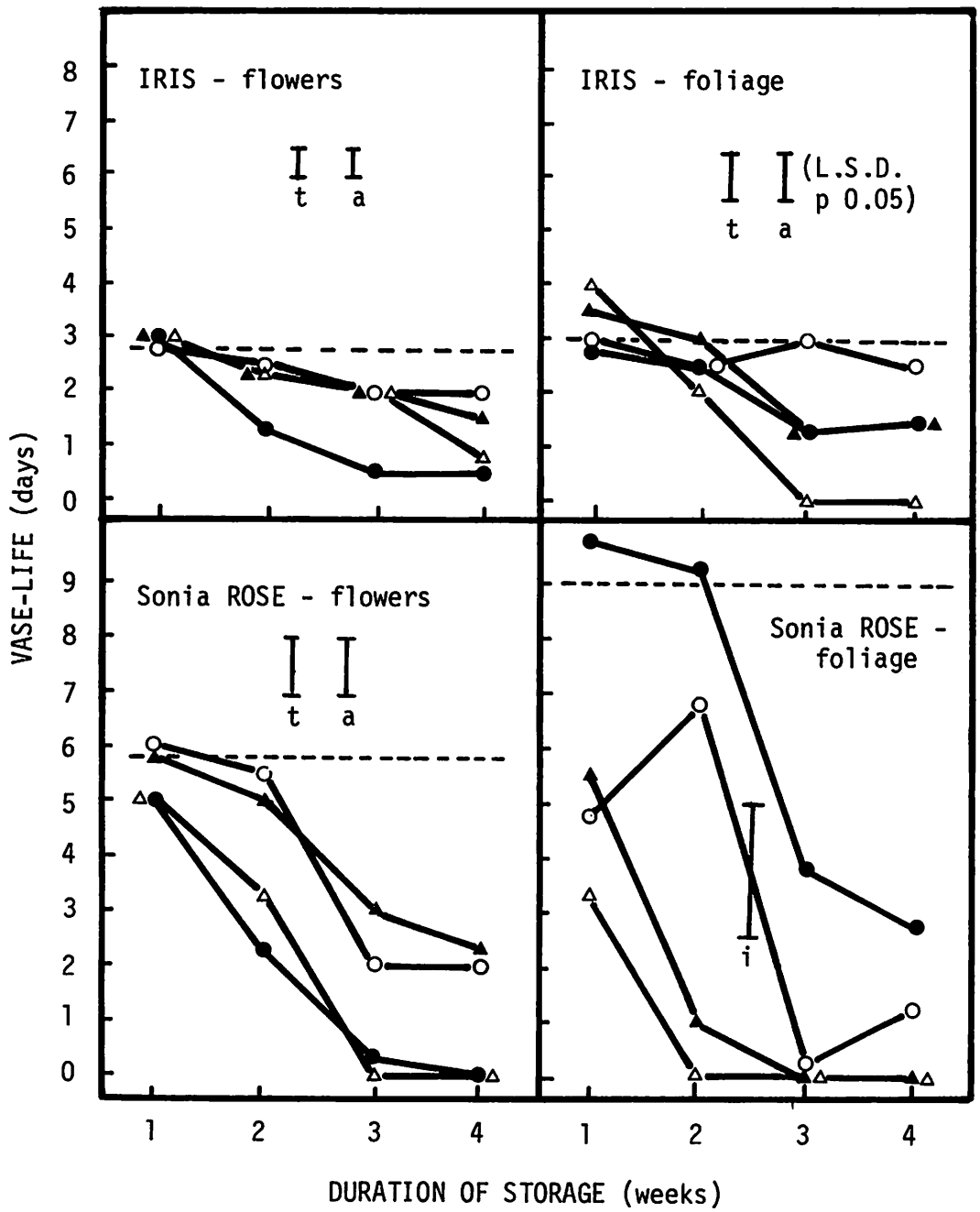

Fig. 3. The influence of elevated CO_2 (10 [○], 15 [▲] and 20% [△]), compared with air [●], during storage at 1°C on the vase-life of iris and Sonia rose flowers and foliage sampled from storage at weekly intervals over 4 weeks. (Dashed line indicates the vase-life of unstored material; L.S.D. bars are presented for the interaction (i) when significant, and for times (t) and atmospheres (a) when the interaction was not significant (p 0.05)).

storage, brown necrotic lesions, possibly associated with pathogen infection, were noted on some flowers stored for 2, 3 and 4 weeks. This disorder was responsible for the short vase-life of the air-stored flowers. Bending of iris stems occurred, although not consistently, in flowers stored in elevated CO_2 treatments. In some instances, bent stems preceded total collapse of the stems. Greenish discoloration of petals occurred at elevated CO_2 levels, but was not a serious problem. At 20% CO_2, foliage injury in the form of dark necrotic lesions terminated the vase life after 3 and 4 weeks storage (Fig. 3).

3.2.4 Rose

Sonia roses stored at 10% and 15% CO_2 had longer vase-lives than those stored in air or at 20% CO_2 (Fig. 3). Elevated CO_2 retarded flower bud opening, thus contributing to the longer vase-life. All CO_2 concentrations damaged the foliage, however, thus decreasing the effective vase-life of the flowers. At 7% CO_2 the foliage was bronzed. At higher concentrations, a transient bronzing was followed by twisting and desiccation of the leaves. At 20% CO_2, failure of the buds to open, drooping of the buds and light pink discoloration resulted in shortened (week 2) or no appreciable (weeks 3 and 4) vase-life.

Browning, probably a symptom of Botrytis infection, was a factor in the demise of all Sonia rose flowers. Only in the air treatment, however, was prolific pathogen growth observed during storage. This growth reduced the vase-life to nil at weeks 3 and 4. It was pathogen spread from Sonia roses in the air chamber which led to damage (infection) of iris and carnations in the same chamber. Browning of some outermost petals of Sonia rose was observed in the elevated CO_2 treatments, particularly by week 4, possibly indicating limited pathogen growth at 10, 15 and 20% CO_2.

3.3 Storage of cut flowers in a variety of modified atmospheres (Expt. 3).

3.3.1 Carnation

The vase life of carnations stored in pure N_2 was almost as long after 3 weeks of storage as that of unstored blooms (Fig. 4), over twice that of air-stored flowers. Those held in 5% CO perished, presumably owing to the ethylene-mimicking effect of CO (6). Storage in 10% CO_2 or 3% O_2 resulted in similar vase-lives to that of air-stored flowers. A somewhat longer vase-life obtained with 3% O_2 plus 10% CO_2 suggested that some benefit may accrue from standard controlled atmospheres.

3.3.2 Chrysanthemum

In contrast to carnations, chrysanthemums were severely damaged by the pure N_2 atmosphere (Fig. 4); the petals were extensively browned on removal from the chamber. Chrysanthemums stored as well in atmospheres considered likely to suppress pathogen growth as in air. Chrysanthemum

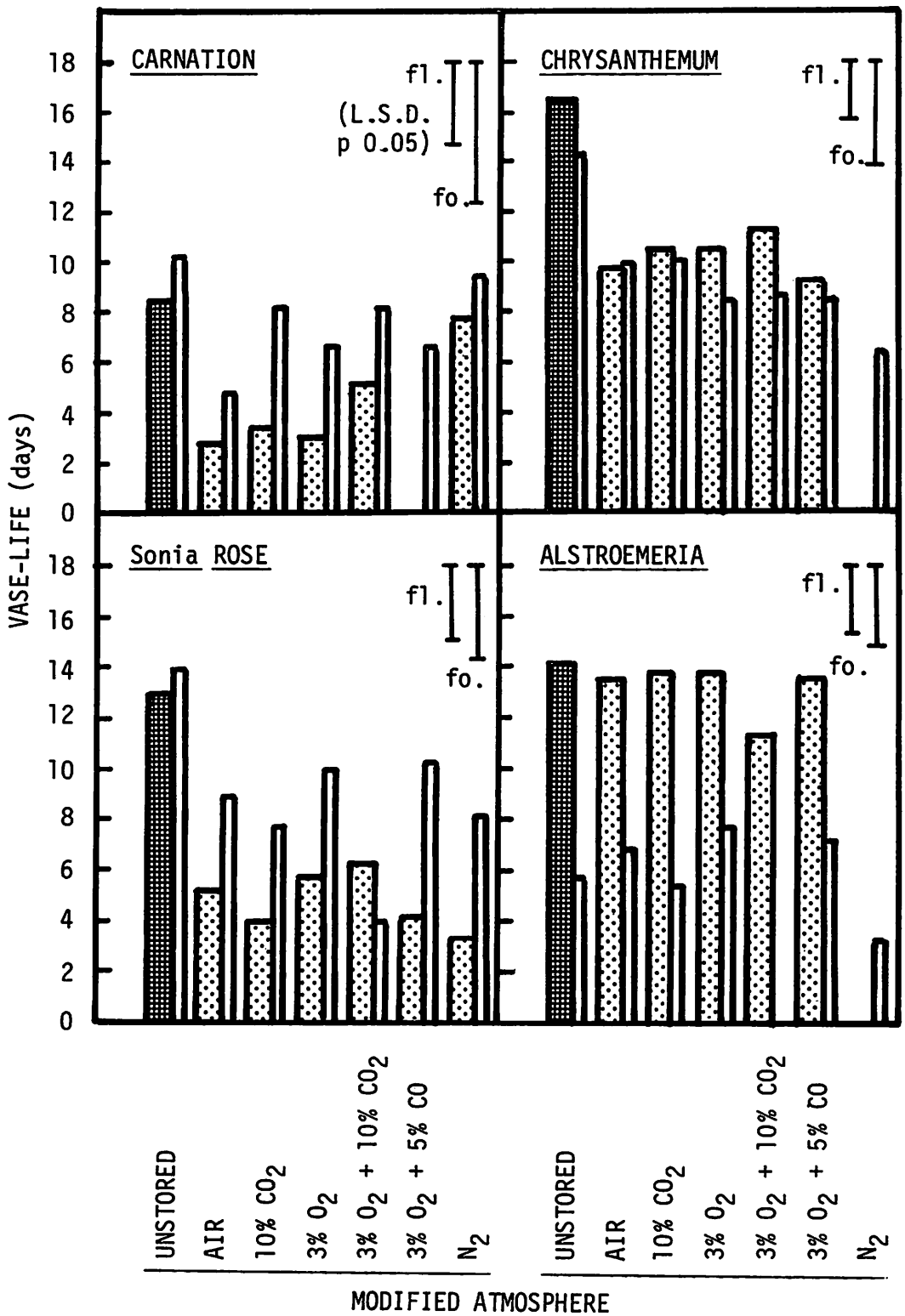

Fig. 4. The influence of 6 different modified atmospheres on the vase-life of carnation, chrysanthemum, Sonia rose and alstroemeria following storage for 3 weeks at 1°C. (Solid and dotted bars represent flowers, narrow hollow bars represent foliage; fl.=flowers, fo.=foliage).

foliage was generally found to become unattractive slightly before the flowers themselves were ready to be discarded (Fig. 4).

3.3.3 Rose

Unstored Sonia roses had a vase-life twice that of those held in modified atmospheres. The senescence of the unstored flowers followed the normal pattern of full bloom and subsequent petal fall and wilting. In contrast, the vase-life of flowers from all of the storage atmospheres was terminated by browning. This browning was believed to be caused by Botrytis. Although browning did not occur on flowers while in storage, we suspect that initial development of the pathogen in the high humidity environment of the storage chambers facilitated its rapid development during vase-life evaluation. None of the modified atmospheres averted this problem. Sonia rose foliage life generally exceeded flower life, with the exception of 3% O_2 and 10% CO_2. Leaves in this treatment developed bronzing followed by leaf curling and desiccation. While the foliage of Sonia roses stored in N_2 was satisfactory, black necrotic regions developed on their stems during storage, rendering them unsightly. In addition to apparent Botrytis infection, the rose buds held in N_2 failed to open and their petals were discolored.

3.3.4 Alstroemeria

Alstroemeria flowers stored well, their vase lives after removal from all atmospheres except pure N_2 and 3% O_2 plus 10% CO_2 being similar to that of unstored blooms (Fig. 4). Pure N_2 resulted in deformed flowers that browned and collapsed on removal from storage. The leaves rapidly developed dark lesions (Fig. 4). Such leaf damage occurred even more rapidly on alstroemeria stored in 3% O_2 plus 10% CO_2. In this atmosphere, however, the life of the flowers was not greatly reduced. A general problem noted with alstroemeria was that foliage became very chlorotic well before the flowers were judged unattractive (Fig.4).

4. CONCLUSIONS

Despite many years of research on the use of modified atmospheres for storage of cut flowers, this technique is not presently in commercial application. The data presented here indicate that some flower species are tolerant of atmospheres containing considerably higher concentrations of CO_2 than are normally considered safe with perishable horticultural crops. The indications of the beneficial effects of pure N_2 for carnations clearly warrant further investigation. The tolerance of a range of flowers to relatively high CO_2 concentrations indicates the potential for control of Botrytis growth during storage using this technique (2).

The differential response of two rose cultivars to the the atmospheres tested indicates the potential for selection of cultivars suited to long-term storage. Future research could profit from an examination of the physiological basis of these differences in attempting to under-

stand the nature of CO_2 tolerance in cut flowers. The difference in sensitivity of buds and open flowers of chrysanthemum might be an appropriate system for exploring mechanisms of plant responses to high CO_2.

5. SUMMARY

With the exception of gerbera, all of the flowers compared in this study were shown to store (for 7 days) as well in atmospheres containing elevated concentrations of CO_2 as in air (Experiment 1). Prolific growth of pathogenic organisms during storage was observed in an air chamber (Experiment 2), but not in any of those containing elevated CO_2.

Some cut flowers tolerate very high CO_2 concentrations (60%) without in-storage damage or an appreciable reduction in vase-life (e.g. enchantment lily, carnation and daffodil). Others (e.g. chrysanthemum, Sonia rose, alstroemeria and iris) fared well at a moderate CO_2 level (14%). Cara Mia roses were damaged by 14% CO_2, but blooms of Sonia were unaffected by this atmosphere.

General symptoms of CO_2-injury included browning and collapse of petals (e.g. chrysanthemum) and leaves (e.g. alstroemeria), petal discoloration (e.g. roses), repression of bud opening (e.g. roses) and bending of stems (e.g. iris). Buds of chrysanthemum were more susceptible to CO_2-injury than flowers.

The vase-life of stored carnations was improved when the storage atmosphere contained 20% CO_2 or pure N_2. An atmosphere containing 10% CO_2 appeared to improve the long-term storage of chrysanthemums.

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APPENDIX Parameters of the statistical analysis of modified atmosphere experiments with cut flowers.

Experiment 1. : Two way ANOVA (RCB design).

Flower	EMS	edf	VR	Flower	EMS	edf	VR
lily	0.458	3	1.73	narcissus	1.861	6	0.28
Cara Mia rose	0.639	6	65	carnation	6.472	6	0.86
chrysanthemum	0.750	6	124	cyclamen	5.335	3	3.34
gypsophila	3.472	6	10.9	iris	0.0006	6	19200
alstroemeria	0.0006	6	43200	gerbera	2.083	6	11.6
Sonia rose	7.778	6	9.27	daffodil	0.563	9	0.41

(EMS=error mean square, edf=error degrees of freedom, VR=variance ratio).

Experiment 2. : Three way ANOVA (RCB design).

Flower	EMS	edf	VR(t)	VR(a)	VR(i)
carnation	16.01	45	5.84	10.9	2.63
chrysanthemum	7.925	45	22.2	27.2	2.99
iris	0.402	45	22.2	7.92	1.87
rose	2.180	45	33.1	9.55	0.57
Foliage					
carnation	2.629	45	124	1.66	1.62
chrysanthemum	5.409	45	57.5	10.8	4.39
iris	1.505	45	9.48	2.95	1.73
rose	2.855	45	32.6	33.9	4.03

(t=treatments, a=atmospheres, i=interaction)

Experiment 3. : One way ANOVA (CR design).

Flower	EMS	edf	VR
carnation	7.628	30	5.35
chrysanthemum	4.150	30	26.2
rose	5.989	30	1.38
alstroemeria	5.433	30	32.7
Foliage	EMS	edf	VR
carnation	22.79	30	0.76
chrysanthemum	11.91	30	0.93
rose	9.594	30	3.10
alstroemeria	7.456	30	6.93