CHAPTER SIX

VASE LIFE AND BACTERIAL ENUMERATION STUDIES OF *BORONIA* spp.

6.1 INTRODUCTION

The flowers of *Boronia*, particularly the heavily scented species, such as *Boronia heterophylla* (red boronia) and *Boronia megastigma* (brown boronia), are grown commercially for cut flowers. However, *B. heterophylla* is reported to have a short vase life of only 2 d (Faragher 1989). Curiously, Lothian's (1953) preliminary vase life study found that the vase life of *B. heterophylla* was 6 to 8 d, although vase life assessment conditions were not mentioned. The vase life of *B. megastigma* is 10 d (Lothian 1953; Faragher 1989). Boronias flower from August to November (Elliott and Jones 1982). Therefore, they potentially offer a vibrant and fragrant start to spring for consumers who wish to purchase colourful flowers with fragrance. Daffodils, the popular alternative, have their place in providing brightness during late winter. However, they become rather commonplace when seen *en masse*, as they senesce in supermarket buckets marked "reduced for quick sale", probably because they were never treated against ethylene. Furthermore, daffodils have no fragrance when fresh, and emit a rather offensive odour as they senesce. Thus, boronias can potentially fill a niche market, being an alternative for the consumer seeking a bright and fragrant herald to spring. J. Faragher (pers. comm. 1991) said that, despite the short vase life of *B. heterophylla* (2 d), consumers buy it each spring, "get a few days out of it, throw it out, and forget and buy it again next spring". In order to develop a good reputation for a cut flower, growers and marketers cannot simply rely on the goodwill and forgetfulness of the public. Research aimed at improving the vase life of this popular crop is therefore imperative if its potential market value is to be realised.

Tija (1988) noted that *B. heterophylla*, a popular landscape specimen in New Zealand, had "tremendous potential" as an export cut flower crop. However, despite the popularity of *Boronia* spp. as cut flowers, to the author's knowledge, there is no published research investigating its vase life. Faragher (1989) listed most of the *Boronia* vase life research which has been performed, but none of it was published, all being in the form of "personal communications". From Joyce and Haynes (1989), it is known that *B. heterophylla* is ethylene-sensitive, and that STS delays water loss in the presence of ethylene. Lamont (1985) extended vase life of *B. serrulata* from 8 d (in tap water) to 11.2 d with sucrose (2%) and 8-HQC (8-hydroxyquinoline citrate at 200 mg L^{-1}). Sucrose is commonly added to vase solutions, and 8-

HQC has been considered an effective germicide (Zentmyer 1943; Larsen and Scholes 1965; Larsen and Cromarty 1967).

The aims of the experiments reported in this chapter were therefore to:

- examine the vase life response, including solution uptake, transpiration, net water loss, fresh weight and water content, of *Boronia heterophylla* to a range of vase solutions, including citric acid and 8-HQC, and to an STS pulse;

- determine the influence on vase life of the number of bacteria (cfu mL^{-1}) in vase solutions and stem segments and

- determine the effect of the most beneficial vase life treatment for *B. heterophylla* on the vase life of other boronias, two with bell-shaped flowers (*Boronia clavata*, *Boronia crassipes* × *Boronia heterophylla* 'Lipstick') and one with open-petalled flowers (*Boronia muelleri* 'Sunset Serenade').

6.2 MATERIALS AND METHODS

6.2.1 Plant material

Plant material used in this chapter came from the sources listed below.

Glasshouse grown plants

All *B. clavata* Paul G.Wilson, *B. crassipes* Bartling × *B. heterophylla* 'Lipstick', *B. heterophylla* F.Muell. and *B. muelleri* (Benth.) Cheel 'Sunset Serenade' material was obtained from nurseries. Plants were grown on in pots in a glasshouse at the Botany Department, UNE, Armidale. Native plant fertiliser (Osmocote®, Sierra Chemical Company, Castle Hill, New South Wales) was applied at intervals recommended by the manufacturer.

Four months prior to the spring (October) flowering of *B. heterophylla*, pots were moved from the glasshouse to the backyard of the author's home in Armidale, to provide the necessary cold requirement for flower bud initiation. Richards (1985) found that *B. heterophylla* required at least six weeks of minimum temperatures <6°C to promote floral initiation and development. Studies with *Boronia megastigma* Nees. revealed that flower bud initiation was greater, and less flower bud abortion occurred, at night temperatures of 6±2°C rather than 15±2°C (Roberts and Menary 1989). The average minimum temperatures during an Armidale winter, recorded over

29 seasons, ranged from 0.2°C to 1.9°C (Bureau of Meteorology 1988). In contrast, the minimum glasshouse temperature in winter was 12°C.

6.2.2 Vase solutions

Vase solutions used in the *B. heterophylla* vase life experiments were:

		pH
T1	8-HQC (250 mg L^{-1});	3.1
T2	distilled water (control);	5.3
T3	acidified distilled water (acid fied with HCl);	2.6
T4	citric acid (10 mol m^{-3});	2.6
T5	citric acid (10 mol m^{-3}) + sucrose (2% w/v);	2.5
T6	citric acid (10 mol m^{-3}) + chlorine (SDIC, 50 mg L^{-1});	2.5
T7	citric acid (10 mol m^{-3}) + sucrose (2% w/v) + chlorine (SDIC, 50 mg L^{-1});	2.5
T8	STS pulse (0.5 mol m^{-3} for 10.5 h), then into distilled water; and	5.7
T9	citric acid (300 mg L^{-1} = 1.43 mol m^{-3}).	3.0

The vase life experiment with other boronias (section 6.3.5) compared their vase life in distilled water (control) with that following an STS pulse (0.5 mol m^{-3} for 10.5 h) and placement into distilled water. Three species were used:

1a *Boronia muelleri* 'Sunset Serenade'[1], STS pulse (0.5 mol m^{-3} for 10.5 h), then into distilled water;
1b *B. muelleri* 'Sunset Serenade', distilled water;

2a *Boronia crassipes* × *Boronia heterophylla* 'Lipstick'[2], STS pulse (0.5 mol m^{-3} for 10.5 h), then into distilled water;
2b *B. crassipes* × *B. heterophylla* 'Lipstick', distilled water;

3a *Boronia clavata*, STS pulse (0.5 mol m^{-3} for 10.5 h), then into distilled water; and
3b *B. clavata*, distilled water.

Vase life parameters were measured as described previously (section 2.13).

6.2.3 Vase life evaluation of *Boronia* spp.

As is frequently the case with Australian native flora, there is a dearth of literature available, particularly on the stages of floral development for vase life assessment. Halevy and Mayak

---

[1] Hereafter referred to as *B. muelleri*.

[2] Hereafter referred to as *B.* 'Lipstick'.

(1979) lamented that longevity criteria for measurement of vase life were, at best, ill-defined. Some work has been done on boronia vase life (Lamont 1985; Faragher 1989; Joyce and Haynes 1989), but the criteria used to determine the end of vase life were unclear. Lamont (1985), in his vase life studies of *Boronia serrulata* Sm., harvested stems when at least three flowers were open in an inflorescence [usually consisting of six to nine flowers (Lamont 1989)]. He deemed the end of vase life to have occurred when three or more flowers in an inflorescence had wilted. Faragher (1989) noted that as boronias senesced, the flowers closed and faded and the stems became desiccated. Joyce and Haynes (1989) used the criterion of flower desiccation, resulting in an unattractive floral display, to indicate the end of vase life. However, they did not note the proportion of desiccated flowers (D. Joyce, pers. comm. 1994).

In the absence of specific published criterion/a to designate the end of vase life in *B. heterophylla*, the author elected to use the criterion that visual senescence had occurred when >50% of flowers on the leafy raceme nose inflorescence exhibited wilting. This criterion was discussed with Dr J. Faragher (pers. comm. 1991) and Mr G. Lamont (pers. comm. 1991). Both researchers considered it to be a reasonable criterion[3]. The criterion of >50% of wilted flowers defining the end of vase life, has been used previously as an index of racemose cut flower senescence (Kelly and Starman 1990; Dai and Paull 1991).

When *B. heterophylla* flowers senesce, the open bell-shaped flowers (Photograph 6.1) close. At this time their delightful fragrance disappears. The petals then fade from below the calyx and wilt (Photographs 6.2 to 6.3). Flower abscission may then occur. Petal colour changes from bright pink, through to pale pink, and eventually to white as senescence progresses. The flowers and leaves sometimes exhibit wilting and/or desiccation before the white colour stage is reached. During senescence, the leaves of *B. heterophylla* become desiccated, turn red and fall. However, leaf senescence usually only happens after petal wilting has occurred (i.e. after the flowers are considered unacceptable). Petal wilting, colour loss and desiccation appear to be dependent upon the vase solution used (section 6.3.2).

Boronia clavata, *B.* 'Lipstick' and *B muelleri* (Photographs 6.5 to 6.6) exhibit petal closure and wilting in a similar way to *B. heterophylla*, but only the hybrid 'Lipstick' exhibits petal fading as well, probably due to its *B. heterophylla* parentage. Leaf desiccation is a later symptom of senescence in all of the above boronias, as it is with *B. heterophylla*. Thus, the criterion to

---

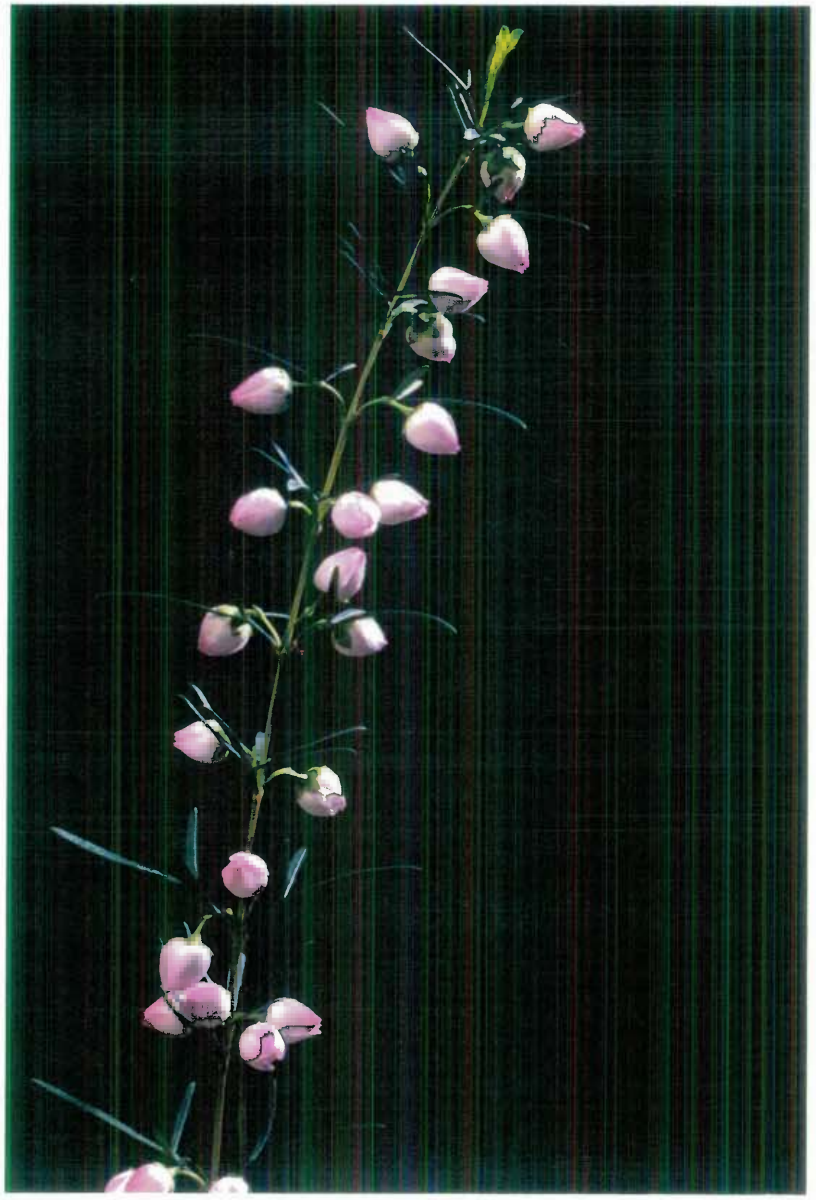
[3] Originally, it was intended to use the criterion that when >50% of flowers on the raceme exhibited >50% of petal fading, the end of vase life was reached, but the flowers were still attractive in their faded state (Photograph 6.4). The unanimous opinion of the many people asked in the Botany Department was that they would not have thrown the flowers out at this stage, indicating that the end of vase life had not been reached. It has since been pointed out (J. Truett, pers. comm. 1993) that Japanese consumers do not like the petal fading that occurs in *B. heterophylla*, so perhaps, in retrospect, the former criterion should have been used. However, another influence in the decision to change the criterion from petal fading to petal wilting, was that solution uptake was still proceeding at a rapid rate during this stage and no signs of desiccation were evident.

determine the end of vase life was the same as in *B. heterophylla*, i.e. when >50% of flowers on the inflorescence exhibited wilting, the end of vase life was reached.

Photograph 6.1. (**A**) The flowering and growth habit of *Boronia heterophylla*. Numerous flowers are produced by the plant. (**B**) A close-up of the bell-shaped flowers of *B. heterophylla*. The floriferous appearance of the plant is the result of single flowers which have formed in each axil of the leafy racemes. The flowers open acropetally.

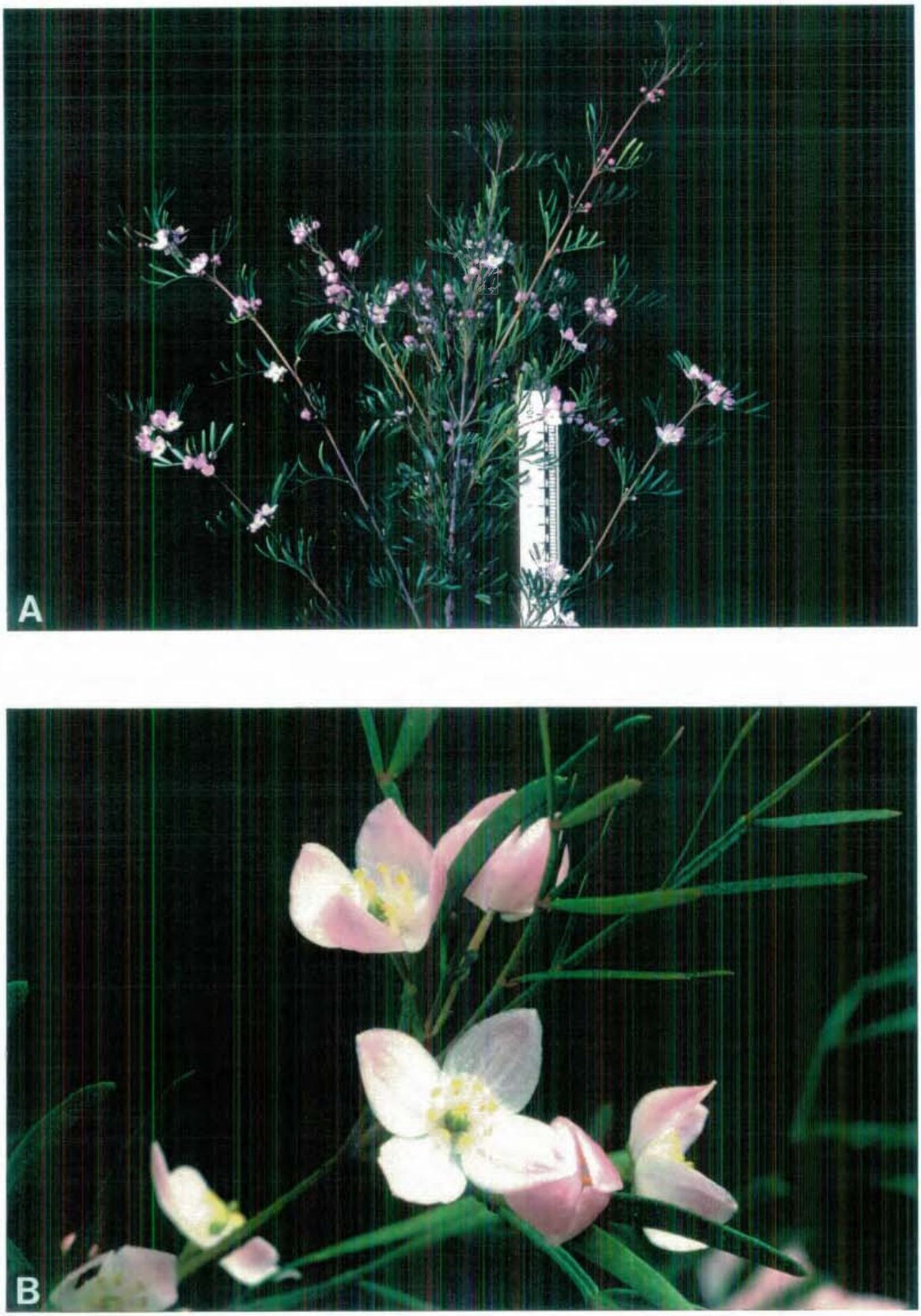
Photograph 6.2. (**A**) The stage of *B. heterophylla* floral development at which vase life experiment No. 1 was begun. Note that petal fading has begun, starting from below the calyx and then progressing down the corolla. (**B**) A close-up of the stage of *B. heterophylla* floral development at which vase life experiment No. 1 was begun. Note that all but the top flower has closed, and petal fading has begun.

Photograph 6.3. (**A**) An unwilted flower of *B. heterophylla* during vase life experiment No. 1. Note that fading of the corolla has progressed further from that shown in Photograph 6.2 b. (**B**) A *B. heterophylla* flower exhibiting the first visible signs of wilting. Note the slight puckering (P) evident on the corolla. (**C**) An extremely wilted *B. heterophylla* flower (i.e. well past the end of vase life). However, note that the leaves do not show any signs of desiccation. The colour of the corolla below the calyx has faded to white, and the remainder of the corolla is a pale pink colour.



Photograph 6.4. *B. heterophylla* flowering stem after 8 d in distilled water, prior to which it was pulsed with STS (0.5 mol m^{-3} for 10.5 h) (Treatment 8). Note that petal closure and fading has occurred, but no sign of petal wilting is evident.

Photograph 6.5. (**A**) A flowering stem of *Boronia clavata*, showing the sessile, bell-shaped flowers, which are produced singly in the axils of leafy racemes. (**B**) A close-up of a *B. clavata* flower which has begun to senesce, evidenced by flower closure and petal wilting (W).



Photograph 6.6. (**A**) The flowering and growth habit of *Boronia muelleri* 'Sunset Serenade'. The individual flower clusters have a cymose arrangement. (**B**) A close-up of the open-petalled flowers of *B. muelleri* 'Sunset Serenade'.

6.2.4 Preparation of agar media and dilution series

Two hours before it was required, the agar medium was prepared. It was mixed and dissolved in a microwave oven, before being autoclaved in a borosilicate screw topped bottle (Schott, Germany). Agar was maintained in a molten state at approximately 55°C in a water bath, then cooled to approximately 45°C before being dispensed. Any unused agar was discarded, and a fresh batch was made for subsequent cultures.

All microbial work was performed in a laminar flow cabinet, using aseptic techniques. A dilution series for vase solutions was prepared, whereby sterile distilled water (9 mL) was added to each McCartney bottle with a repetitive syringe (Becton, Dickinson & Co., USA). A 1 mL sample was taken of the vase water or stem segment suspension with a sterile repetitive pipette (Eppendorf, Germany). Samples were diluted and agitated between each serial dilution. Pour plates were made of several dilute suspensions per replicate by placing 1 mL of suspension onto petri dishes and adding approximately 10 mL of Plate Count Agar (Oxoid Ltd., Hampshire). The agar was added within 15 min of sample dilution in order to avoid growth, separation or death in the diluent (Harrigan and McCance 1976). The plates were mixed gently for approximately 5 s before being incubated for bacterial enumeration as described below (section 6.2.5).

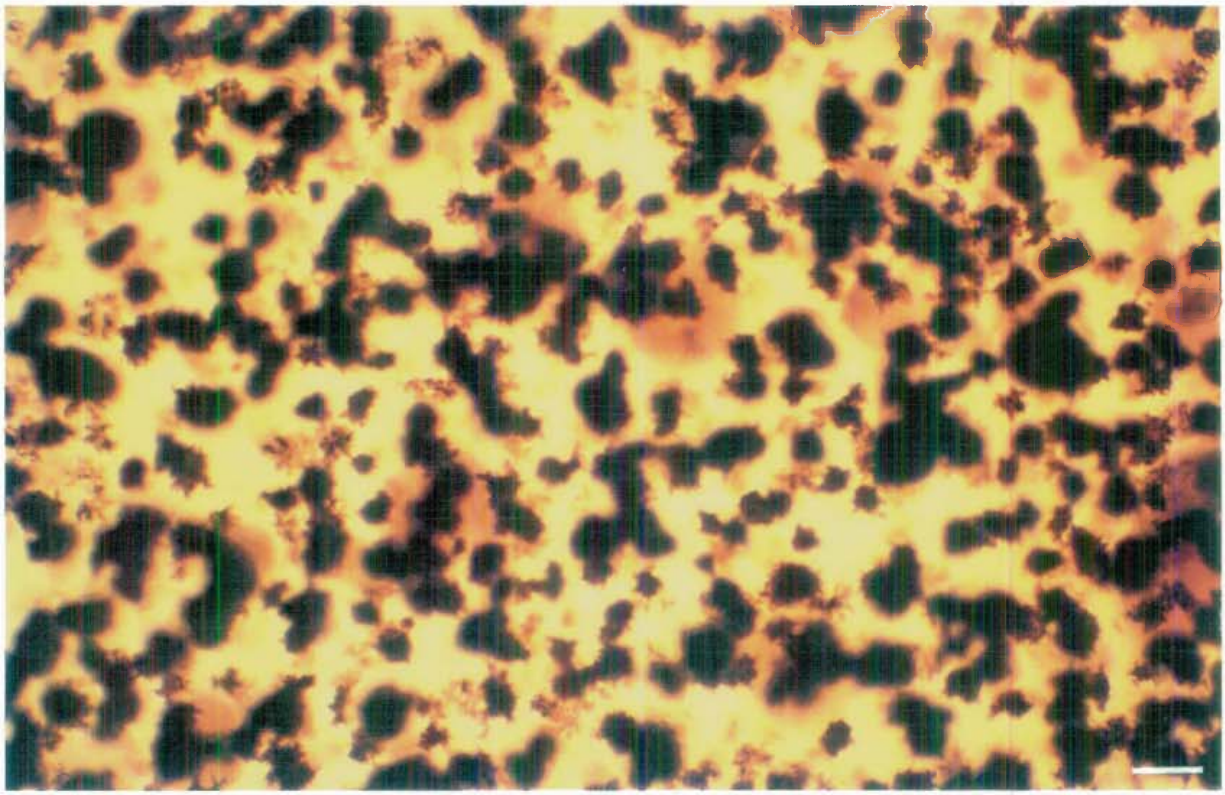
6.2.5 Determination of bacterial numbers in vase solutions and in stem segments

Vase solutions

The number of bacteria (cfu mL^{-1}) in vase solutions from *B. heterophylla* vase life study No. 1 was enumerated after 11 d of vase life; and after 0, 3, 6, 9 and 12 d of vase life in study No. 2. A 1 mL aliquot was removed aseptically from each vase and diluted (section 6.2.4) to give a maximum dilution of 10^4. Pour plates were made with Plate Count Agar (section 6.2.4). The plates were incubated at 30°C for 48 h, and counted with the aid of magnification from a colony counter (Model 3330, American Optical Company, New York). There were 10 replicates per treatment in vase life study No. 1, and five replicates per treatment in vase life study No. 2.

Ideally, at least one agar plate in a dilution series will grow between 30 and 300 bacterial colonies. This facilitates manual counting of colonies (Tortora *et al.* 1986) and eliminates overcrowding and microbial antagonism, which may reduce the count (Harrigan and McCance 1976). However, all dilutions in four vase solution treatments from *B. heterophylla* vase life study No. 1 produced too many colonies to be counted using the naked eye. Instead they were counted by determining the number of colonies in 10 random fields of view under a microscope

at either 100× or 200× magnification. Photograph 6.7 shows the vast number of bacterial agglomerations visible in a 10^4 dilution of 8-HQC at 40× magnification.



Photograph 6.7. Numerous bacterial colonies (black areas) from a 10^4 dilution of 8-HQC, as seen under light microscopy at 40× magnification. (bar = 100 µm)

Stem segments

Determination of the number of bacteria (cfu mL^{-1}) in *B. heterophylla* stem segments during vase life study No. 2 (see section 6.3.4) was based on the methods of van Doorn *et al.* (1989) and Urban and Lemattre (1991). The only exception to the method of van Doorn *et al.* (1989) was that a stomacher, used to remove bacteria from stems, was not available at the University. Thus, the modified method was as follows. After 18 d of vase life, leaves were removed from the stems and the basal 0 to 5 cm segment was cut and weighed. The basal stem segment was surface sterilised with 95% alcohol and cut aseptically into 0.5 cm segments in a sterile mortar. A sterile aliquot of NaCl solution (0.85% w/v), 10 times the fresh weight of the stem segment, was add to the mortar, and the stem material was ground into a pulp with a sterile pestle. Pour plates were made with the resultant solution and Plate Count Agar. This procedure was repeated with the next 5 cm stem segment, so that the number of bacteria in the basal 0 to 5 cm and 5 to 10 cm stem segments could be enumerated. The plates were incubated at 30°C for 48 h before being counted. There were five replicates of each stem length per treatment.

6.2.6 Analysis of data

Treatment means from *B. heterophylla* vase life experiment study no. 1 (section 6.3.1) were separated using Scheffé's test ($P < 0.05$) (StatView 4.0). Data from *B. heterophylla* vase life experiment study no. 2 (section 6.3.2) were not normally distributed and the variances were unequal and unable to be transformed satisfactorily. (The smallest value for Cochran's test for homogeneity of the variances, achieved using a reciprocal of x transformation, was 47, but a value of <0.35 was required.) It was therefore inappropriate to perform nonparametric statistical tests (see section 3.2.8). After discussions with a statistician at UNE, (I. Davies, pers. comm. 1995), it was decided to perform an ANOVA using the data which provided the best normality transformation. However, the test was performed at $P < 0.01$, which would be equivalent to $P < 0.05$ for homogeneous variances. The more rigorous $P < 0.01$ significance level reduces the probability of making a Type I error (I. Davies, pers. comm. 1995).

Means of bacterial numbers in *B. heterophylla* vase life experiment study no. 1 (section 6.3.3) were separated using Scheffé's test ($P < 0.05$) (StatView 4.0).

Results of the *B.* 'Lipstick' vase life experiment (section 6.3.5) exhibited non-normality and heterogeneity of the variances which were unable to be transformed (using conventional power law transformations from square root to the reciprocal of x). The control had a very small variance (0.27) because longevity ranged from only 6 to 7 d (Table 6.5) in all replicates. Therefore, these data were analysed using chi-square (χ^2) tests of independence (I. Davies, pers. comm. 1995). The vase life results for the other two *Boronia* spp. had normal distributions and homogeneous variances, so were able to be analysed using two group t-tests. All transformations used are indicated in Appendix C.

6.3 RESULTS

6.3.1 *Boronia heterophylla* vase life, solution uptake, transpiration, fresh weight, water content and net water loss, performed when flowers had started to senesce (study no. 1)

Longevity was significantly greater after stems were pulsed with STS (T8) than in all other treatments (Table 6.1). Pulsing with STS resulted in a more than 100% increase in vase life compared with distilled water alone (T2). Treatments containing chlorine (T6 and T7) had a detrimental effect on vase life, resulting in the shortest vase lives and premature senescence compared with the distilled water control (T2). Stems in citric acid at a concentration of 1.43 mol m^{-3} (T9) had the second longest vase life. This citric acid concentration was more beneficial to longevity than 10 mol m^{-3} (T4). The germicide, 8-HQC (T1), did not significantly extend

longevity over that of distilled water (T2). Acidifying the water (T3) to the same pH as citric acid (at 10 mol m^{-3}, T4) produced a slightly longer vase life than citric acid (T4), but it was not significantly different. The combination of citric acid and sucrose (T5) did not improve vase life over that of citric acid alone (at the same concentration, T4).

Table 6.1

Longevity of *B. heterophylla* in various treatment solutions, belated harvest maturity

Treatment	**pH**	**Vase life (d) ± SE◊**	**Range (d)**
T1: 8-HQC (250 mg L^{-1})	3.1	6.5^{cd*} ± 0.269	5 - 8
T2: Distilled water	5.3	6.3^{cd} ± 0.423	4 - 8
T3: Distilled water + HCl	2.6	7.5^{bc} ± 0.50	5 - 10
T4: Citric acid (10 mol m^{-3})	2.6	6.8^{bc} ± 0.20	6 - 8
T5: Citric acid + sucrose (2% w/v)	2.5	6.0^{cde} ± 0.258	5 - 7
T6: Citric acid + chlorine (50 mg L^{-1})	2.5	4.2^{e} ± 0.416	3 - 7
T7: Citric acid + sucrose + chlorine	2.5	4.6^{de} ± 0.40	3 - 6
T8: STS pulse, then distilled water	5.7	12.9^{a} ± 1.402	10 - 24
T9: Citric acid (1.43 mol m^{-3})	3.0	9.6^{ab} ± 0.340	8 - 11

◊ Vase life is the mean of 10 replicates per treatment; SE = standard error.

* Numbers followed by the same letter are not significantly different from each other ($P < 0.05$).

In general, the measured vase life parameters of solution uptake, transpiration, fresh weight, stem water content and net water loss (Figs. 6.1 to 6.5) reflected the trends evident in the vase life data (Table 6.1). Although uptake and transpiration varied during the first day of vase life (Figs. 6.1 and 6.2), from day 3 onward the treatments with the longest vase lives (T8, STS pulse and T9, 1.43 mol m^{-3} citric acid) had amongst the highest rates of uptake and transpiration. This trend was even more evident in changes in the fresh weight, stem water content and net water loss (Figs. 6.3 to 6.5). In those three graphs, T8 and T9 almost consistently produced the best results, indicating that the water balance was well maintained by those two treatment solutions. Curiously, 8-HQC (T1), which is known to close stomata and thereby reduce water loss (Stoddard and Miller 1962; Marousky 1969), was no more effective than most other treatments (Figs. 6.2 and 6.5). Treatments containing sucrose (T5 and T7) had low rates of uptake and transpiration, and also the lowest stem water content (Figs. 6.1, 6.2 and 6.4). However, fresh weight and net water loss (Figs. 6.3 and 6.5) were not lowest in treatments containing sucrose (T5 and T7). Similar results for sucrose were reported by Carter *et al.* (1989), and were attributed to decreased transpiration. Marousky (1969; 1971) observed that sucrose closed stomata, and thereby decreased water uptake, transpiration and water loss.

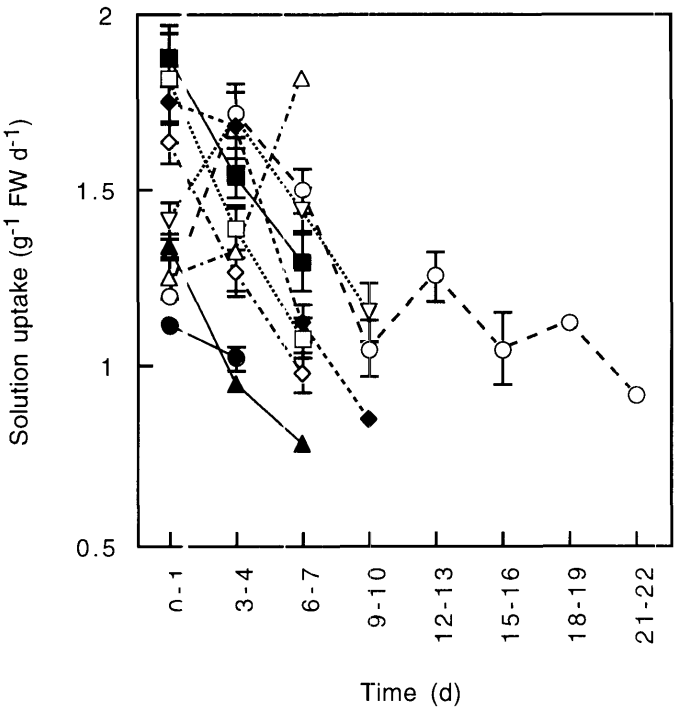


Fig. 6.1. The rate of solution uptake (expressed per g of fresh wt d^{-1}) during the vase life of *B. heterophylla* stems kept in nine treatment solutions (note: **T** = Treatmen). **T1** (■) 8-hydroxyquinoline citrate (250 mg L^{-1}); **T2** (□) distilled water; **T3** (◆) distilled water + HCl; **T4** (◇) citric acid (10 mol m^{-3}); **T5** (▲) C+S [citric acid (10 mol m^{-3}) and sucrose (2% w/v)]; **T6** (△) C+C [citric acid (10 mol m^{-3}) and chlorine (SDIC, 50 mg L^{-1})]; **T7** (●) C+S+C [citric acid (10 mol m^{-3}) and sucrose (2% w/v) and chlorine (SDIC, 5) mg L^{-1}]; **T8** (○) STS pulse (0.5 mol m^{-3}) for 10.5 h, then into distilled water; **T9** (▽) citric acid (1.43 mol m^{-3}). (W ere no error bar appears, the SE was smaller than the size of the symbol.)

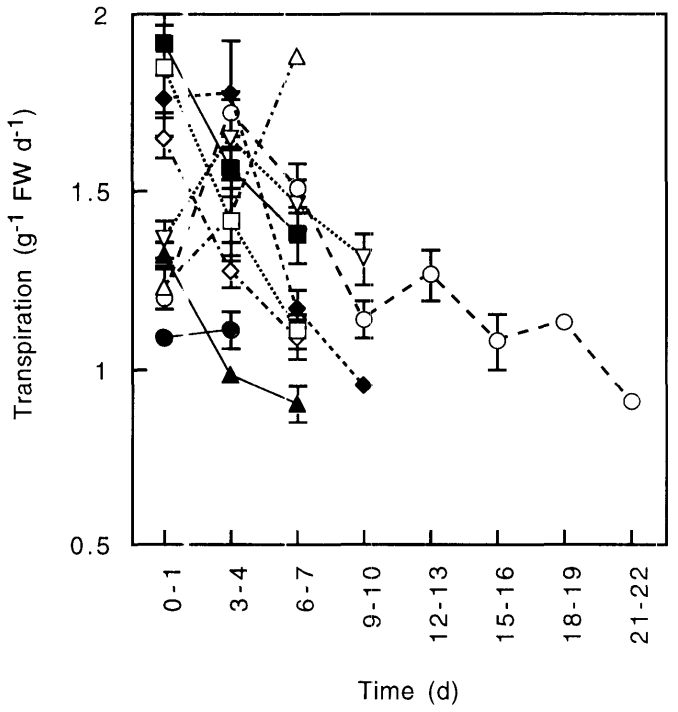


Fig. 6.2. The rate of transpiration (expressed per g of fresh wt d^{-1}) during the vase life of *B. heterophylla* stems kept in nine treatment solutions (note: **T** = Treatmen). **T1** (■) 8-hydroxyquinoline citrate (250 mg L^{-1}); **T2** (□) distilled water; **T3** (◆) distilled water + HCl; **T4** (◇) citric acid (10 mol m^{-3}); **T5** (▲) C+S [citric acid (10 mol m^{-3}) and sucrose (2% w/v)]; **T6** (△) C+C [citric acid (10 mol m^{-3}) and chlorine (SDIC, 50 mg L^{-1})]; **T7** (●) C+S+C [citric acid (10 mol m^{-3}) and sucrose (2% w/v) and chlorine (SDIC, 50 mg L^{-1})]; **T8** (○) STS pulse (0.5 mol m^{-3}) for 10.5 h, then into distilled water; **T9** (▽) citric acid (1.43 mol m^{-3}). (W ere no error bar appears, the SE was smaller than the size of the symbol.)

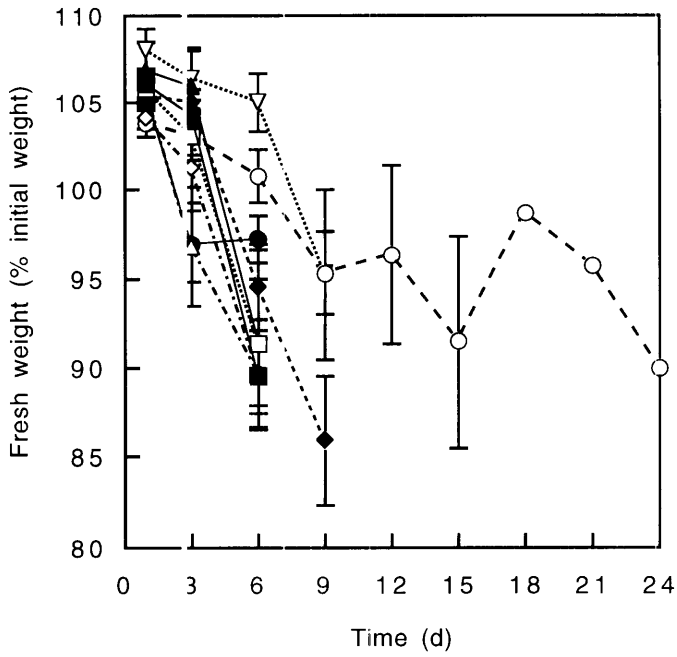


Fig. 6.3. The change in fresh weight (expressed as a % of the initial wt) during the vase life of *B. heterophylla* stems kept in nine treatment solutions (note: **T** = Treatment). **T1** (■) 8-hydroxyquinoline citrate (250 mg L^{-1}); **T2** (□) distilled water; **T3** (◆) distilled water + HCl; **T4** (◇) citric acid (10 mol m^{-3}); **T5** (▲) C+S [citric acid (10 mol m^{-3}) and sucrose (2% w/v)]; **T6** (△) C+C [citric acid (10 mol m^{-3}) and chlorine (SDIC, 50 mg L^{-1})]; **T7** (●) C+S+C [citric acid (10 mol m^{-3}) and sucrose (2% w/v) and chlorine (SDIC, 50 mg L^{-1})]; **T8** (○) STS pulse (0.5 mol m^{-3}) for 10.5 h, then into distilled water; **T9** (▽) citric acid (1.43 mol m^{-3}). (Where no error bar appears, the SE was smaller than the size of the symbol.)

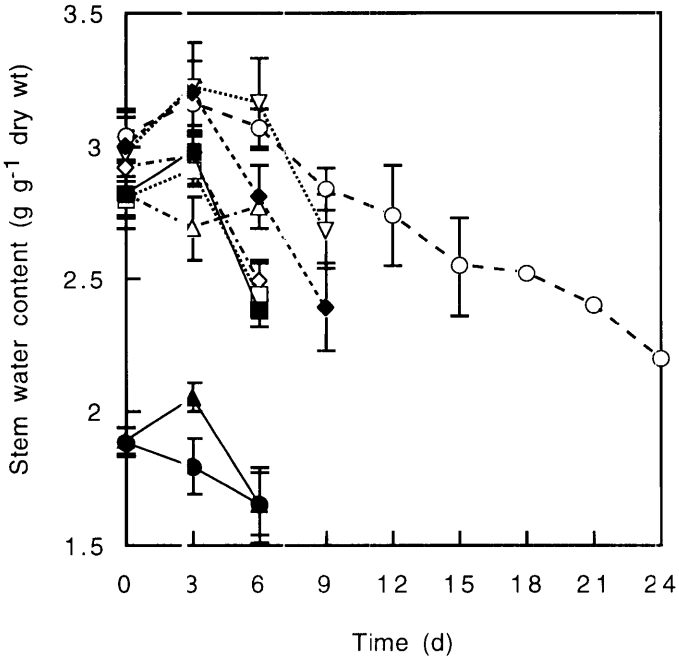


Fig. 6.4. The change in stem water content (expressed in grams of water per g of dry wt) during the vase life of *B. heterophylla* stems kept in nine treatment solutions (note: **T** = Treatment). **T1** (■) 8-hydroxyquinoline citrate (250 mg L^{-1}); **T2** (□) distilled water; **T3** (◆) distilled water + HCl; **T4** (◇) citric acid (10 mol m^{-3}); **T5** (▲) C+S [citric acid (10 mol m^{-3}) and sucrose (2% w/v)]; **T6** (△) C+C [citric acid (10 mol m^{-3}) and chlorine (SDIC, 50 mg L^{-1})]; **T7** (●) C+S+C [citric acid (10 mol m^{-3}) and sucrose (2% w/v) and chlorine (SDIC, 50 mg L^{-1})]; **T8** (○) STS pulse (0.5 mol m^{-3}) for 10.5 h, then into distilled water; **T9** (▽) citric acid (1.43 mol m^{-3}). (Where no error bar appears, the SE was smaller than the size of the symbol.)

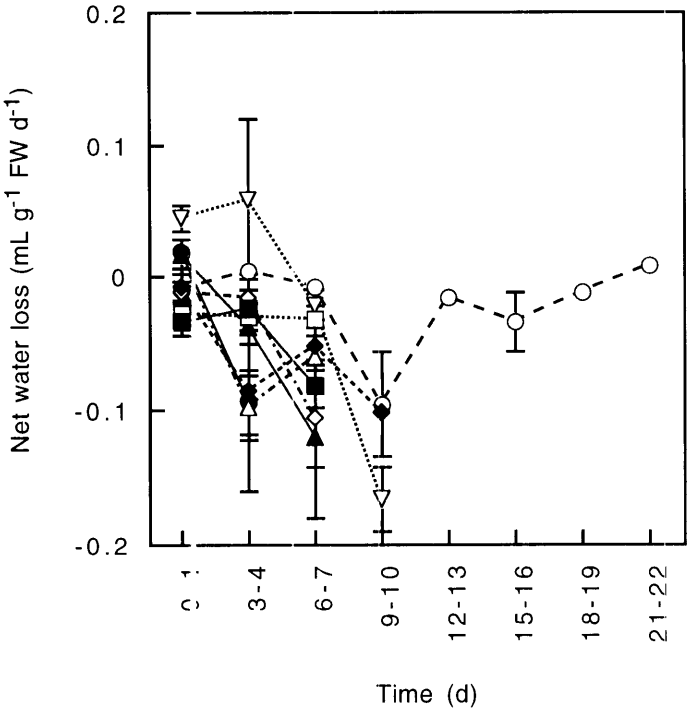


Fig. 6.5. The rate of net water loss (expressed in mL per g of fresh wt d^{-1}) during the vase life of *B. heterophylla* stems kept in nine treatment solutions (note: **T** = Treatment). **T1** (■) 8-hydroxyquinoline citrate (250 mg L^{-1}); **T2** (□) distilled water; **T3** (◆) distilled water + HCl; **T4** (◇) citric acid (10 mol m^{-3}); **T5** (▲) C+S [citric acid (10 mol m^{-3}) and sucrose (2% w/v)]; **T6** (△) C+C [citric acid (10 mol m^{-3}) and chlorine (SDIC, 50 mg L^{-1})]; **T7** (●) C+S+C [citric acid (10 mol m^{-3}) and sucrose (2% w/v) and chlorine (SDIC, 50 mg L^{-1})]; **T8** (○) STS pulse (0.5 mol m^{-3}) for 10.5 h, then into distilled water; **T9** (▽) citric acid (1.43 mol m^{-3}). (Where no error bar appears, the SE was smaller than the size of the symbol.)

6.3.2 *B. heterophylla* vase life, solution uptake, transpiration, fresh weight and net water loss when experiment was performed at optimal harvest maturity (study no. 2)

As expected, when the *B. heterophylla* vase life experiment was begun at optimal harvest maturity, flowers lasted longer (cf. Tables 6.2 and 6.1). This improvement occurred in all treatments, except citric acid (10 mol m^{-3}, T4), although the difference between the two citric acid results was minimal (from 6.8 to 6.4 d). Overall, the trends evident in Table 6.1 were repeated in Table 6.2. The STS pulse (T8) remained the most beneficial treatment, and citric acid + chlorine (T6) resulted in the shortest vase life. However, longevity in distilled water (T2) was markedly higher when flowers were harvested at an earlier maturity, increasing from 6.3 to 10.8 d (Tables 6.1 and 6.2). This result was reflected in the large longevity range (5 to 22 d), although the data were limited by the smaller sample size (n = 5). When citric acid was combined with sucrose (T5), longevity was more than doubled when flowers were harvested at optimal maturity. However, maturity at harvest was not affected by citric acid, whether supplied alone (T4, T9) or with chlorine (T6) (Tables 6.1 and 6.2). Increases in longevity were recorded for 8-HQC (T1), acidified water (T3) and citric acid + sucrose + chlorine (T7) after optimal

harvest maturity. Nevertheless, vase lives for these three treatments were still shorter than those obtained for the distilled water control (T2).

Table 6.2

Longevity of *B. heterophylla* in various treatment solutions, optimal harvest maturity

Treatment	**Vase life (d)*** ± **SE**◊	**Range (d)**
T1: 8-HQC (250 mg L^{-1})	7.8^{ab} ± 0.20	7 - 8
T2: Distilled water	10.8^{ab} ± 2.905	5 - 22
T3: Distilled water + HCl	10.4^{ab} ± 0.927	7 - 12
T4: Citric acid (10 mol m^{-3})	6.4^{b} ± 0.60	5 - 8
T5: Citric acid + sucrose (2% w/v)	12.4^{ab} ± 1.122	9 - 16
T6: Citric acid + chlorine (50 mg L^{-1})	5.8^{b} ± 0.374	5 - 7
T7: Citric acid + sucrose + chlorine	8.0^{ab} ± 1.00	5 - 11
T8: STS pulse, then distilled water	19.0^{a} ± 3.271	10 - 29
T9: Citric acid (1.43 mol m^{-3})	9.8^{ab} ± 0.860	7 - 12

◊ Vase life is the mean of 5 replicates per treatment; SE = standard error.

* Means were separated using Scheffé's test at the $P < 0.01$ level (equivalent to $P < 0.05$) because the data were not normally distributed and the variances were not homogeneous. (The log transformed data were used for the mean separation because they provided the best normality transformation. The untransformed data is shown here.)

The visible signs of *B. heterophylla* senescence in the vase solutions were:

T1, 8-HQC: Leaf drop;

T2, distilled water: Leaf drop;

T3, distilled water + HCl: Leaf drop;

T4, citric acid (10 mol m^{-3}): Leaf drop;

T5, citric + sucrose: Leaf and some bud drop. However, the petals held their colour and fragrance well (there was no fading from below the calyx, even at day 10);

T6, citric + chlorine: Leaf drop. End of vase life occurred when the petals wilted. This happened prematurely, i.e. before they lost < 50% colour;

T7, citric + sucrose + chlorine: Leaf drop. Petals held their colour and fragrance well (as in T7, C+S);

T8, STS pulse, then distilled water: Leaf drop. The flowers lasted a long time, but lost their colour. They faded to pale pink at the petal tips, and to white below the calyx;

T9, citric acid (1.43 mol m^{-3}): Leaf and bud drop.

Although leaf drop occurred in all treatment solutions, it was only minor and did not detract from the overall appearance of the cut flowers. As noted earlier (section 6.2.3), leaf drop and colour changes usually occur after petal wilting (and hence after the end of vase life).

The following graphs of solution uptake and transpiration (Figs. 6.6 and 6.7) reveal that, although vase life was significantly greater after pulsing with STS (T8), this treatment did not result in high rates of uptake and transpiration. Nevertheless, fresh weight (Fig. 6.8) and net water loss (Fig. 6.9) were well maintained after an STS pulse (T8). Stems in the solution giving the second longest vase life, citric acid + sucrose (T5), had consistently low rates for all of the water relations parameters measured. As mentioned previously (section 6.3.1), sucrose reduces uptake and transpiration by closing stomata (Marousky 1969; 1971). However, this did not result in a reduced rate of net water loss in treatments containing sucrose (T5 and T7) (Fig. 6.9). The results for the distilled water control (T2) were similar to those for the STS pulse (T8) in all parameters measured, except for change in fresh weight (Fig. 6.8). Fig. 6.8 shows that stems in distilled water (T2) lost weight more rapidly than stems treated with an STS pulse prior to placement in distilled water (T8). No graph was made of stem water content because all stems were ground up (the basal 10 cm) to determine bacterial numbers in the stems (section 6.3.4, ii).

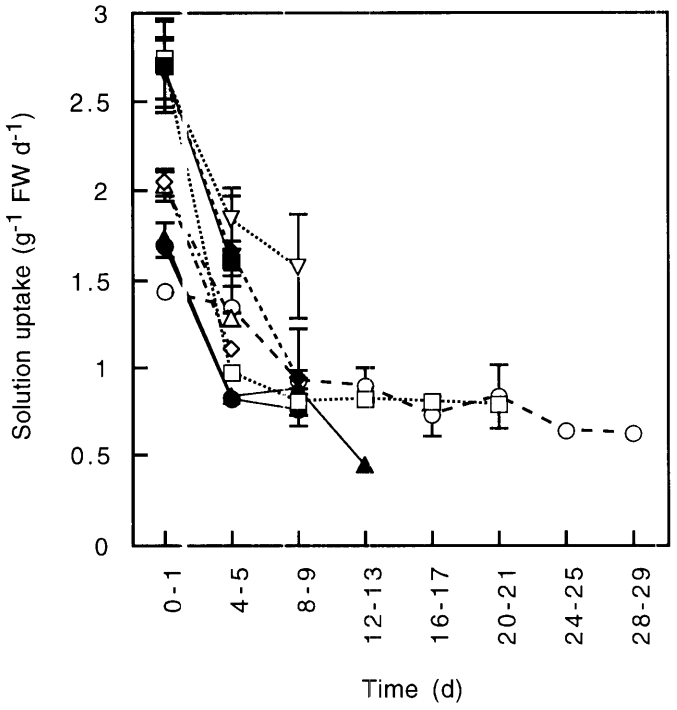


Fig. 6.6. The rate of solution uptake (expressed per g of fresh wt d^{-1}) during the vase life of *B. heterophylla* stems kept in nine treatment solutions (note: **T** = Treatmen). **T1** (■) 8-hydroxyquinoline citrate (250 mg L^{-1}); **T2** (□) distilled water; **T3** (◆) distilled water + HCl; **T4** (◇) citric acid (10 mol m^{-3}); T5 (▲) C+S [citric acid (10 mol m^{-3}) and sucrose (2% w/v)]; **T6** (△) C+C [citric acid (10 mol m^{-3}) and chlorine (SDIC, 50 mg L^{-1})]; **T7** (●) C+S+C [citric acid (10 mol m^{-3}) and sucrose (2% w/v) and chlorine (SDIC, 50 mg L^{-1})]; **T8** (○) STS pulse (0.5 mol m^{-3}) for 10.5 h, then into distilled water; **T9** (▽) citric acid (1.43 mol m^{-3}). (Where no error bar appears, the SE was smaller than the size of the symbol.)

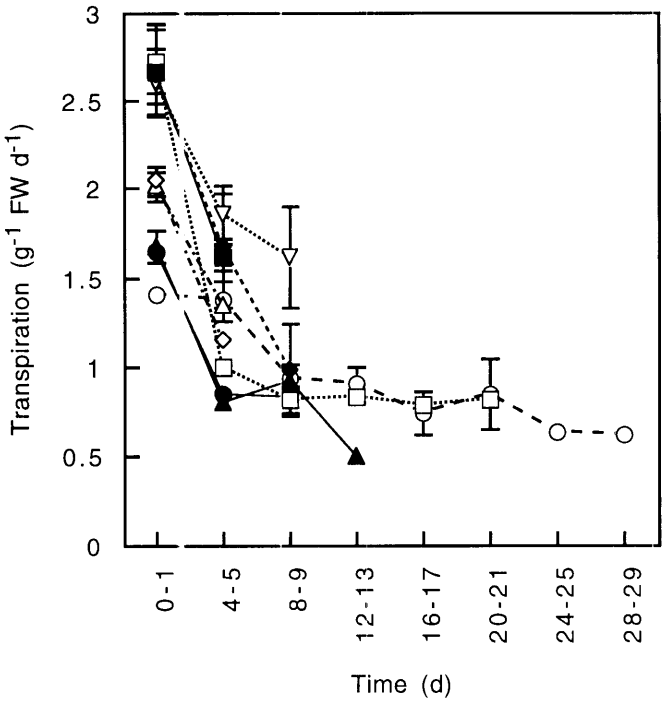


Fig. 6.7. The rate of transpiration (expressed per g of fresh wt d^{-1}) during the vase life of *B. heterophylla* stems kept in nine treatment solutions (note: **T** = Treatmen). **T1** (■) 8-hydroxyquinoline citrate (250 mg L^{-1}); **T2** (□) distilled water; **T3** (◆) distilled water + HCl; **T4** (◇) citric acid (10 mol m^{-3}); T5 (▲) C+S [citric acid (10 mol m^{-3}) and sucrose (2% w/v)]; **T6** (Δ) C+C [citric acid (10 mol m^{-3}) and chlorine (SDIC, 50 mg L^{-1})]; **T7** (●) C+S+C [citric acid (10 mol m^{-3}) and sucrose (2% w/v) and chlorine (SDIC, 50 mg L^{-1})]; **T8** (○) STS pulse (0.5 mol m^{-3}) for 10.5 h, then into distilled water; **T9** (▽) citric acid (1.43 mol m^{-3}). (Where no error bar appears, the SE was smaller than the size of the symbol.)

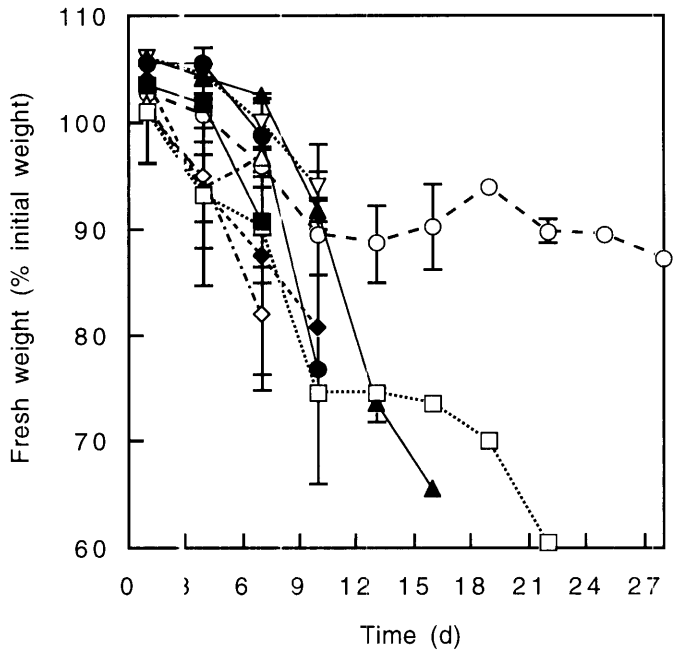


Fig. 6.8. The change in fresh weight (expressed as a % of the initial wt) during the vase life of *B. heterophylla* stems kept in nine treatment solutions (note: **T** = Treatment). **T1** (■) 8-hydroxyquinoline citrate (250 mg L^{-1}); **T2** (□) distilled water; **T3** (◆) distilled water + HCl: **T4** (◇) citric acid (10 mol m^{-3}); T5 (▲) C+S [citric acid (10 mol m^{-3}) and sucrose (2% w/v)]; **T6** (Δ) C+C [citric acid (10 mol m^{-3}) and chlorine (SDIC, 50 mg L^{-1})]; **T7** (●) C+S+C [citric acid (10 mol m^{-3}) and sucrose (2% w/v) and chlorine (SDIC, 50 mg L^{-1})]; **T8** (○) STS pulse (0.5 mol m^{-3}) for 10.5 h, then into distilled water; **T9** (▽) citric acid (1.43 mol m^{-3}). (Where no error bar appears, the SE was smaller than the size of the symbol.)

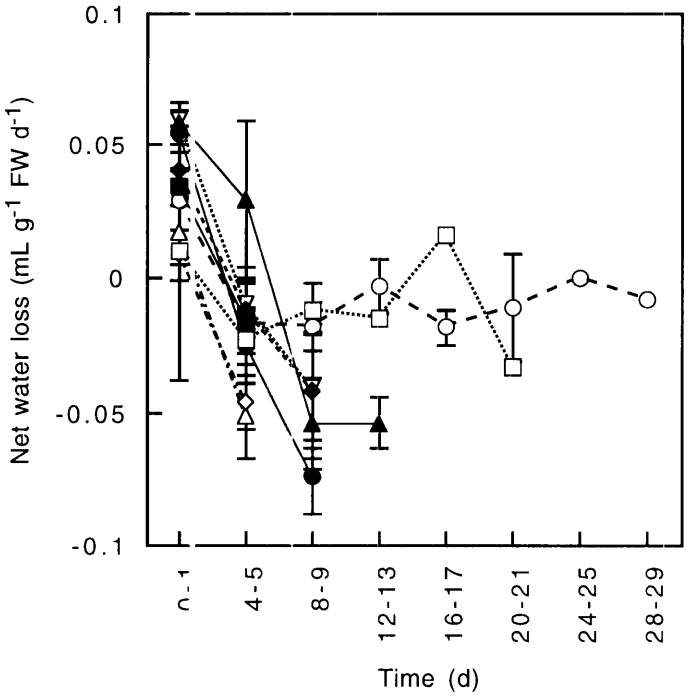


Fig. 6.9. The rate of net water loss (expressed in mL per g of fresh wt d^{-1}) during the vase life of *B. heterophylla* stems kept in nine treatment solutions (note: **T** = Treatment). **T1** (■) 8-hydroxyquinoline citrate (250 mg L^{-1}); **T2** (□) distilled water; **T3** (◆) distilled water + HCl; **T4** (◇) citric acid (10 mol m^{-3}); T5 (▲) C+S [citric acid (10 mol m^{-3}) and sucrose (2% w/v)]; **T6** (Δ) C+C [citric acid (1) mol m^{-3}) and chlorine (SDIC, 50 mg L^{-1})]; **T7** (●) C+S+C [citric acid (10 mol m^{-3}) and sucrose (2% w/v) and chlorine (SDIC, 50 mg L^{-1})]; **T8** (○) STS pulse (0.5 mol m^{-3}) for 10.5 h, then into distilled water; **T9** (▽) citric acid (1.43 mol n^{-3}). (Where no error bar appears, the SE was smaller than the size of the symbol.)

6.3.3 The number of bacteria (cfu mL^{-1}) in vase solutions after 11 d in *B. heterophylla* vase life study no. 1, and their influence on vase life

Curiously, the number of bacteria was highest in the solution that gave the significantly greatest longevity, the STS pulse (T8) (Fig. 5.10, Table 6.1). Vase solutions containing the germicide, 8-HQC (T1), had the next highest number of bacteria. Bacterial numbers in these two treatments were not significantly different (Fig. 6.10). The next highest bacterial levels occurred in solutions containing chlorine (T7 and T6). Distilled water (T2) was the solution with the lowest number of bacteria.

A scattergram of the number of bacteria (cfu mL^{-1}) and the pH of all vase solution treatments revealed that bacterial numbers were different for the same or similar pH levels (Fig. 6.11). This occurred in all treatments except T3 and T4, acidified distilled water, and citric acid (10 mol m^{-3}), respectively. The ineffectiveness of pH, *per se*, in influencing vase life and bacterial numbers was confirmed by different solutions with the same pH having significantly different vase lives (Table 6.1) and bacterial numbers (Fig. 6.11).

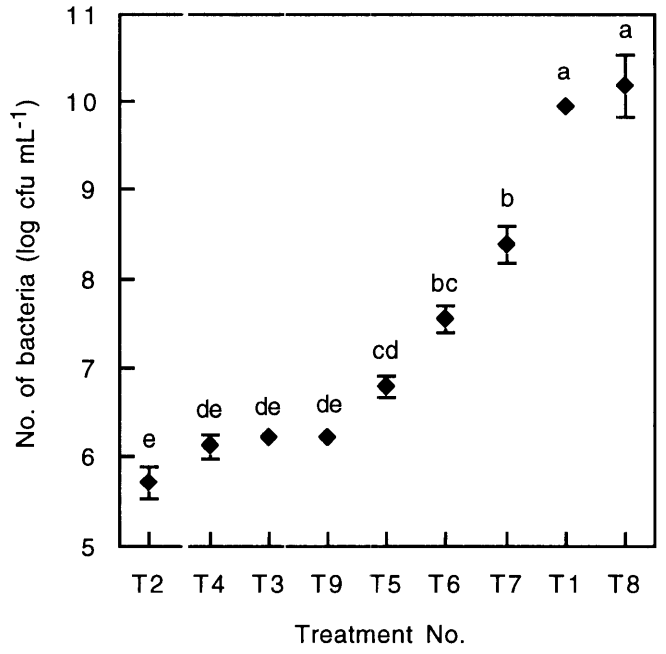


Fig. 6.10. Ranked numbers of bacteria (cfu mL^{-1}) per treatment in vase solutions after 11 d from *B. heterophylla* vase life experiment, study no. 1. Treatments with the same letter are not significantly different from each other ($P < 0.05$).

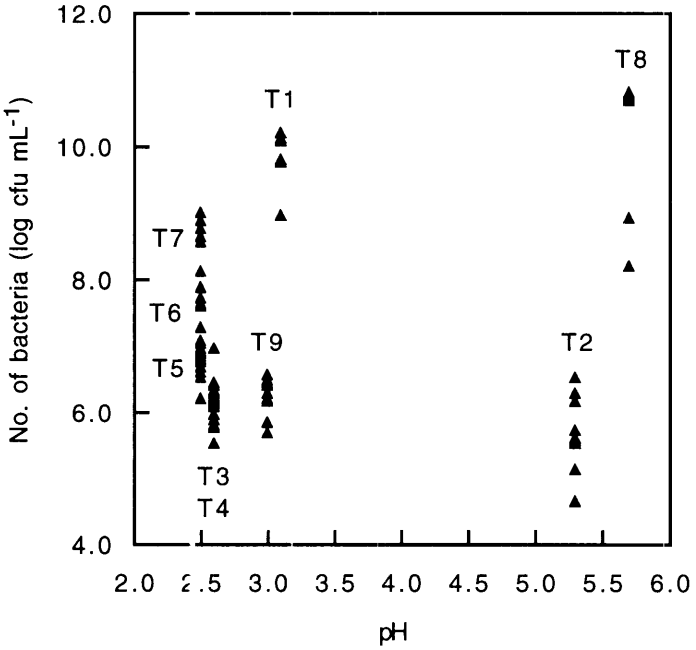


Fig. 6.11. Scattergram of the number of bacteria (cfu mL^{-1}) and the pH of all treatments in *B. heterophylla* vase life experiment, study no. 1. (Note: T = Treatment.) The number of bacteria for the same or similar pH levels differs in all Treatments except T3 and T4. Note that different solutions with the same pH have significantly different vase lives (see Table 6.1).

When the relationship between vase life and the number of bacteria (cfu mL^{-1}) was examined using simple linear regression analysis (Fig. 6.12), it was revealed that bacterial numbers had no significant influence on vase life, as reflected in the $\bar{R}^2$ (0.000) and the t-statistic (0.202) of the coefficient of x (bacterial number).

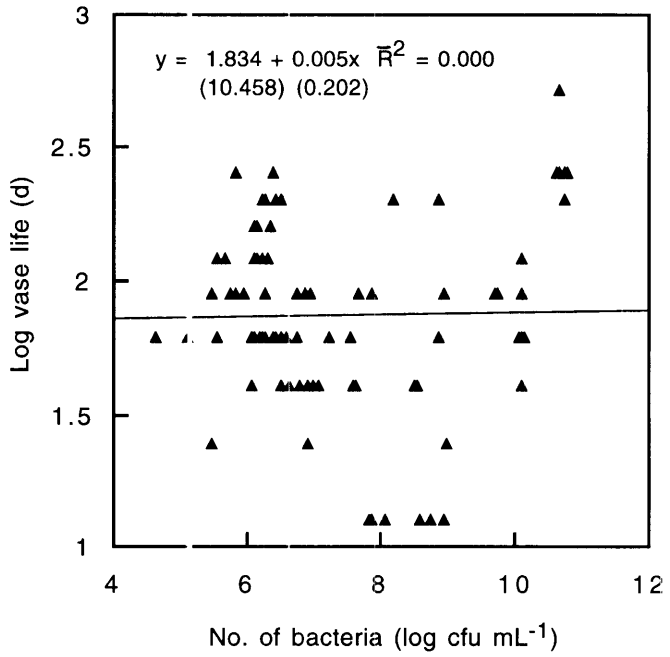


Fig. 6.12. Simple linear regression showing the relationship between vase life and the number of bacteria (cfu mL^{-1}) in the vase solutions of *B. heterophylla*, study no. 1. There was no relationship between bacterial numbers in the vase solution and floral longevity, as indicated by the adjusted R^2 (0.000) and the t-statistic (0.202) of the coefficient of *x*.

6.3.4 The number of bacteria (cfu mL^{-1}) in:
(i) vase solutions after 0, 3, 6, 9 and 12 d; and
(ii) stem segments after 18 d
in *B. heterophylla* vase life study no. 2, and their influence on vase life

(i) Vase solutions

Vase solution bacterial numbers (cfu mL^{-1}) during the vase life of *B. heterophylla* are shown in Table 6.3. No significant differences were found between treatments at day 0, but after that time, significant differences were detected. By day 3, the two citric acid solutions (T4 and T9) contained significantly higher numbers of bacteria. The number of bacteria in the STS pulse (T8) was in the second lowest statistical grouping on day 3. By day 6, the higher concentration of citric acid (10 mol m^{-3}, T4) had the significantly greatest number of bacteria. The germicidal solution, 8-HQC (T1) had the lowest number of bacteria, as curiously, did citric + sucrose (T5), and citric + sucrose + chlorine (T7) solutions. Bacterial numbers by day 9 were significantly greater in acidified distilled water (DW + HCl, T2) than in all other solutions. At day 9, all other treatments were statistically similar, except T1 (8-HQC), which had significantly lower bacterial numbers. By day 12, the solution containing citric + sucrose (T5) and the higher concentration of citric acid (10 mol m^{-3}, T4) had the significantly greatest number of bacteria.

Table 6.3

The number of bacteria (cfu mL^{-1}) in the vase solutions during *B. heterophylla* vase life experiment

Treatment	Bacterial numbers (cfu mL^{-1}) Time (d)				
	0	**3**	**6**	**9**	**12**
T1 8-HQC	4.40×10^3	1.00×10^{3c}	1.30×10^{5c}	1.78×10^{5b}	6.30×10^{5bc}
T2 DW	5.15×10^3	2.00×10^{4bc}	4.64×10^{5abc}	2.09×10^{5ab}	5.60×10^{5c}
T3 DW, HCl	6.40×10^3	4.20×10^{5b}	9.52×10^{5ab}	1.22×10^{6a}	1.43×10^{6b}
T4 cit (10 mol m^{-3})	7.10×10^3	3.17×10^{6a}	1.03×10^{6a}	7.60×10^{5ab}	3.40×10^{6a}
T5 CS	9.60×10^3	3.00×10^{3c}	3.12×10^{5c}	9.42×10^{5ab}	2.82×10^{6a}
T6 CC	1.02×10^4	2.60×10^{3c}	3.86×10^{5abc}	3.26×10^{5ab}	6.24×10^{5bc}
T7 CSC	7.00×10^3	7.00×10^{3bc}	2.96×10^{5c}	3.92×10^{5ab}	7.80×10^{5bc}
T8 STS, DW	9.70×10^3	1.20×10^{5bc}	3.83×10^{5bc}	5.56×10^{5ab}	6.64×10^{5bc}
T9 cit (1.43 mol m^{-3})	6.30×10^3	1.32×10^{6a}	4.62×10^{5abc}	7.26×10^{5ab}	1.23×10^{6bc}

Bacterial numbers are the means of 5 replicates per treatment.
Within each day, numbers followed by the same letter are not significantly different from each other ($P < 0.05$).

(ii) Stem segments

The number of bacteria (cfu g^{-1} fresh weight) in stem segments after 18 d is shown in Table 6.4. Bacterial numbers in stems were not determined prior to 18 d because the majority of stems in the vase life experiment were still alive. Accordingly, it was not possible to examine a relationship between longevity and bacterial numbers in stem segments. At least two agar plates from most treatments were so densely populated that they could not be counted, even under a microscope. Therefore, in calculating averages for those treatments, the highest figure recorded in all treatments was inserted, and those averages were preceded by the symbol, >. No mean separations were performed on these data because the true variances and distributions were unknown.

However, treatments with complete data sets were analysed by a paired sample t-test to determine whether there were significant differences between bacterial numbers in the basal 0 to 5 cm and 5 to 10 cm stem segments within each treatment. Significant differences were found in only two of the four treatments examined (Table 6.4). In T2 (distilled water) significantly more bacteria were found in the basal 0 to 5 cm stem segment than in the 5 to 10 cm segment, and in T9 (1.43 mol m^{-3} citric acid) bacterial numbers were also significantly greater in the basal 0 to 5 cm stem segment. Although several of the treatments could not be examined statistically, it can be seen (Table 6.4) that in all but one treatment (T5, citric + sucrose) bacterial numbers were higher in the basal 0 to 5 cm stem segments.

In the basal 0 to 5 cm stem segments, all vase solutions except 8-HQC (T1) and the STS pulse (T8) had ≥ 10^4 cfu g^{-1} fresh weight of bacteria. However, in the 5 to 10 cm stem segments, several solutions containing citric acid (10 mol m^{-3}: T4, T5 and T6) had high numbers of bacteria. Curiously, one of those solutions contained chlorine (T6). Bacterial numbers were very low (< 10^1 cfu g^{-1} fresh weight) in four solutions, 8-HQC (T1), distilled water (T2), citric + sucrose + chlorine (T7) and the STS pulse (T8). Yet, if the vase life of those treatments is compared with the bacterial count results (Tables 6.2 and 6.4), it is evident that low bacterial numbers inside stems does not mean that those stems will have a longer vase life.

A general indication of the number of bacteria inside stems, particularly in the basal 0 to 5 cm segments (Table 6.4), was obtained by bacterial counts in vase solutions (Table 6.3) on day 12. Thus, monitoring vase solution bacterial numbers throughout vase life may give a good indication of bacterial numbers inside stems without the need for destructive monitoring.

Table 6.4

The number of bacteria (cfu g^{-1} fresh weight) in stem segments after 18 d of *B. heterophylla* vase life experiment

Treatment	**Bacterial numbers (cfu g^{-1} FW) in stem segments**	
	Distance from base (cm)	
	0 to 5*	**5 to 10***
T1 8-HQC	2.03×10^{2ns}	1.60×10^{0ns}
T2 Distilled water	3.55×10^{4a}	4.60×10^{0b}
T3 Distilled water, HCl	$>1.40 \times 10^6$	3.67×10^1
T4 Citric (10 mol m^{-3})	$>1.39 \times 10^6$	$>1.31 \times 10^6$
T5 Citric, Sucrose	4.69×10^4	$>1.36 \times 10^6$
T6 Citric, Chlorine	$>1.75 \times 10^6$	$>1.31 \times 10^6$
T7 Citric, Sucrose, Chlorine	$>4.38 \times 10^5$	4.60×10^0
T8 STS pulse, Distilled water	2.58×10^{3ns}	6.00×10^{0ns}
T9 Citric (1.43 mol m^{-3})	1.05×10^{6a}	1.01×10^{4b}

* Data are the mean of 5 replicates per treatment.
The symbol, >, indicates that some or all bacterial colonies in those treatments were too numerous to be counted. Therefore, in calculating those means, the highest figure recorded in all treatments was inserted and the average was preceded by '>'. Statistical tests were not performed on those data.
Within each treatment, numbers followed by different letters indicate significant differences ($P < 0.05$) between stem segment bacterial numbers.
ns = not significantly different ($P < 0.05$).

Unfortunately, for study no. 2 it was not possible to regress vase life against bacterial numbers to determine whether there was any relationship between those two variables. This was not

feasible because the vase life data were not normally distributed and the variances could not be transformed satisfactorily (see section 6.2.6).

6.3.5 Vase life experiment with other *Boronia* species using vase solutions beneficial to *B. heterophylla*

There was a varied response in the vase lives of three other *Boronia* spp. (two with bell-shaped flowers, *B.* 'Lipstick' and *B. clavata*, and one with open-petalled flowers, *B. muelleri*) after treatment with an STS pulse (Table 6.5). As shown previously (sections 6.3.1 and 6.3.2), pulsing with STS was the most beneficial treatment in both *B. heterophylla* vase life experiments, irrespective of harvest maturity. In the present experiments, the only significant difference was found with the *B. heterophylla* hybrid, *B.* 'Lipstick'. In that hybrid, vase life increased from 6.5 to 12.6 d following an STS pulse (Table 6.5). No significant differences in longevity were found in *B. muelleri* and *B. clavata* after pulsing with STS (Table 6.5).

Table 6.5
Longevity of *B. muelleri* 'Sunset Serenade', *B. crassipes* × *B. heterophylla* 'Lipstick' and *B. clavata* with and without an STS pulse (0.5 mol m^{-3} for 10.5 h) treatment

***Boronia* species**	**Vase life (d) ± SE◊ (Range (d))**	
	Treatment	
	STS pulse	**Distilled water**
B. muelleri 'Sunset Serenade'	8.5 ± 1.232 (5 - 18)	8.8 ± 0.892 (4 - 12)
B. crassipes × *B. heterophylla* Lipstick'	12.6 ± 1.707a* (4 - 18)	6.5 ± 0.167b (6 - 7)
B. clavata	11.8 ± 1.397 (5 - 18)	10.2 ± 0.786 (6 - 14)

◊ Vase life is the mean of 10 replicates per treatment; SE = standard error.
* Treatments are significantly different (P < 0.05).

The visible signs of senescence in the three *Boronia* spp. were:

(i) *B. muelleri* 'Sunset Serenade'

Flowers closed and exhibited slight wilting when they senesced, however, no petal fading occurred. Buds which were immature at harvest (small and unopened) did not develop further [similar to the observation made by Faragher (1989) for other *Boronia* spp.]. Leaves did not desiccate until well after floral senescence had occurred.

(ii) *B. crassipes* × *B. heterophylla* 'Lipstick'

The following senescence symptoms occurred, irrespective of whether stems were treated with an STS pulse or not. Corolla fading occurred, beginning from below the calyx, and progressing towards the tip of the petals. The petal colour changed from bright pink to white (similar to *B. heterophylla*). However, not all the flowers closed as they senesced (as occurred in *B. heterophylla*). Flower closure appeared to be related to the stage of harvest. For example, buds which were immature at harvest (small and tightly closed) did not open (as noted by Faragher 1989), but rather, desiccated and changed to a blue-red colour. Buds which were nearly open at harvest did not open further, but did not desiccate prematurely. Open flowers remained open and faded eventually. However, if open flowers died prematurely, they remained bright pink but appeared desiccated. Foliage desiccation occurred well after flower vase life had ended.

(iii) *B. clavata*

Petal closure occurred, followed by wilting of the corolla (Photograph 6.5 b). The corolla did not change colour. The foliage did not drop, desiccate or change colour prior to the end of flower vase life. Observations were only made until the end of vase life, but it was likely that the foliage would have remained green and turgid until after flower vase life had ended.

No flower or foliage drop occurred in any of the three *Boronia* spp., irrespective of whether the stems were pulsed with STS or not.

The following sections show solution uptake, transpiration, fresh weight, stem water content and net water loss for the three *Boronia* spp. Stems were either pulsed with STS, or kept in distilled water without an STS pulse.

B. muelleri 'Sunset Serenade'

In this species, no significant differences were found between the treatments for any of the abovementioned water relations parameters. Solution uptake (Fig. 6.13) and transpiration (Fig. 6.14) were higher in the unpulsed stems for the first 7 d. However, the fresh weight (Fig. 6.15) was consistently higher in STS treated stems. The results for stem water content (Fig. 6.16) and net water loss (Fig. 6.17) were similar for both treatments.

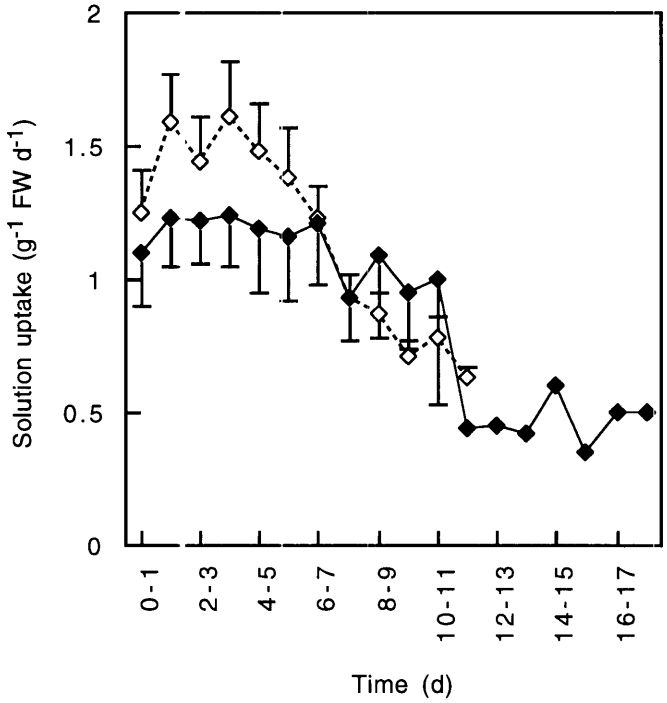


Fig. 6.13. The rate of solution uptake (expressed per g of fresh wt d^{-1}) during the vase life of *B. muelleri* 'Sunset Serenade'. The cut stems were either treated with an STS pulse (0.5 mol m^{-3} for 10.5 h) (◆) or kept in distilled water (◇). Within each day, there were no significant differences between treatments ($P < 0.05$). (Where no error bar appears, the SE was smaller than the size of the symbol.)

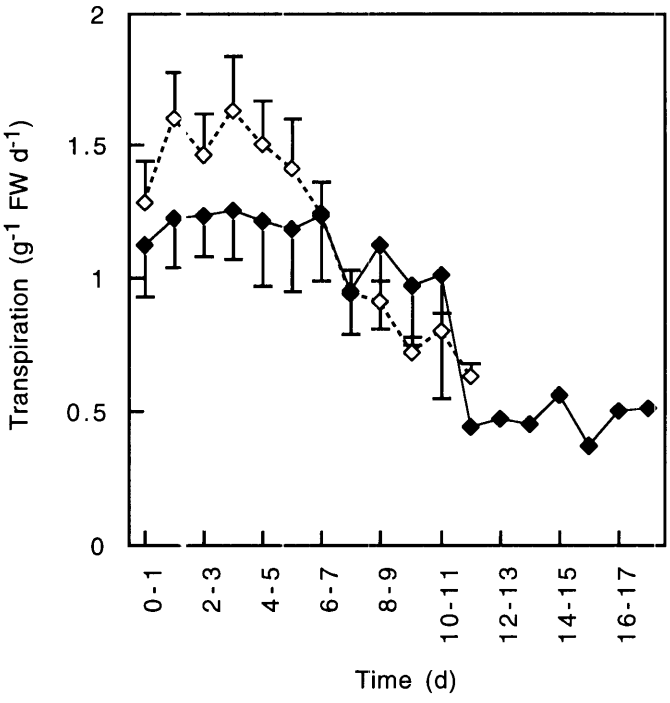


Fig. 6.14. The rate of transpiration (expressed per g of fresh wt d^{-1}) during the vase life of *B. muelleri* 'Sunset Serenade'. The cut stems were either treated with an STS pulse (0.5 mol m^{-3} for 10.5 h) (◆) or kept in distilled water (◇). Within each day, there were no significant differences between treatments ($P < 0.05$). (Where no error bar appears, the SE was smaller than the size of the symbol.)

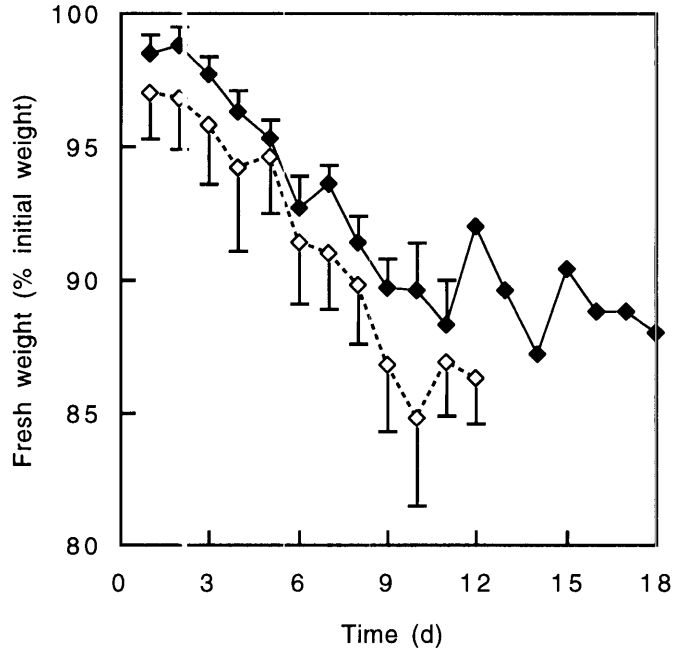


Fig. 6.15. The change in fresh weight (expressed as a % of the initial wt) during the vase life of *B. muelleri* 'Sunset Serenade'. The cut stems were either treated with an STS pulse (0.5 mol m^{-3} for 10.5 h) (◆) or kept in distilled water (◇). Within each day, there were no significant differences between treatments ($P < 0.05$). (Where no error bar appears, the SE was smaller than the size of the symbol.)

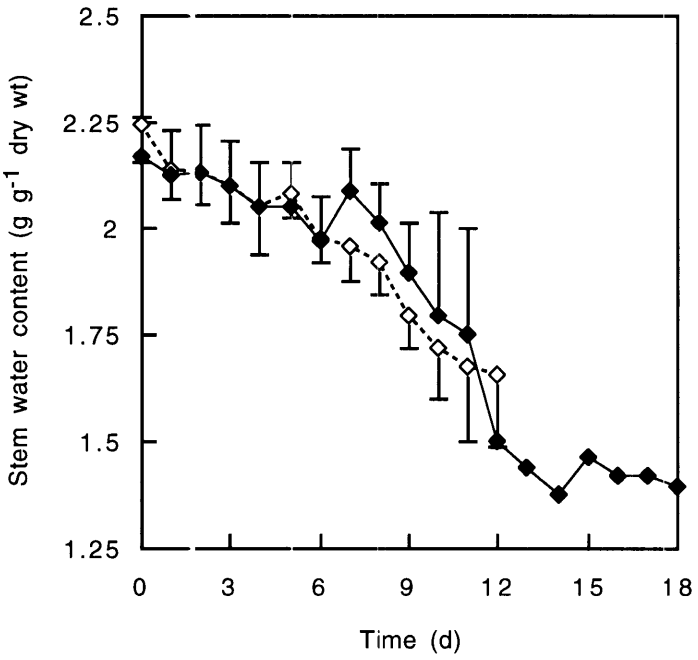


Fig. 6.16. The change in stem water content (expressed in grams of water per g of dry wt) during the vase life of *B. muelleri* 'Sunset Serenade'. The cut stems were either treated with an STS pulse (0.5 mol m^{-3} for 10.5 h) (◆) or kept in distilled water (◇). Within each day, there were no significant differences between treatments ($P < 0.05$). (Where no error bar appears, the SE was smaller than the size of the symbol.)

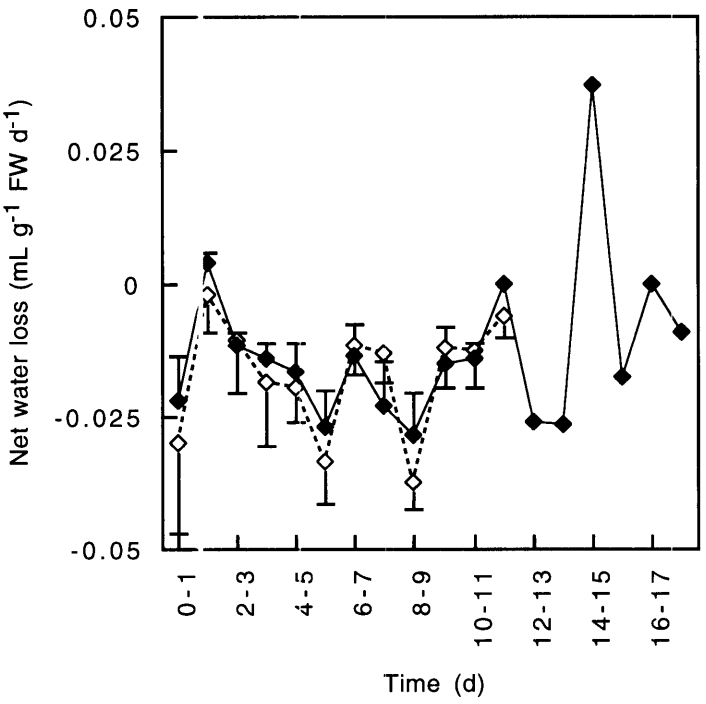


Fig. 6.17. The rate of net water loss (expressed in mL per g of fresh wt d^{-1}) during the vase life of *B. muelleri* 'Sunset Serenade'. The cut stems were either treated with an STS pulse (0.5 mol m^{-3} for 10.5 h) (◆) or kept in distilled water (◇). Within each day, there were no significant differences between treatments ($P < 0.05$). (Where no error bar appears, the SE was smaller than the size of the symbol.)

B. crassipes × *B. heterophylla* 'Lipstick'

In this species, numerous significant differences were recorded between the treatments in the water relations parameters measured. Overall, stems that were pulsed with STS had higher rates of solution uptake and transpiration (Figs. 6.18 and 6.19). Treatment with STS dramatically improved the fresh weight (Fig. 6.20) and water content (Fig. 6.21) of the flowering stems. Stems that had not been pulsed with STS, initially lost water at a faster rate than STS treated stems (Fig. 6.22), although the difference was minimal towards the end of vase life. No significant differences were recorded in the rate of net water loss for the two treatments (Fig. 6.22).

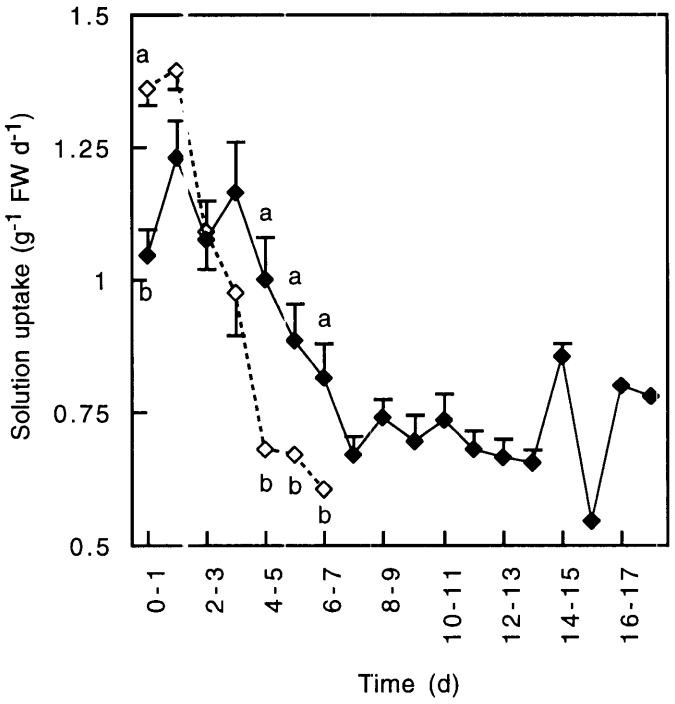


Fig. 6.18. The rate of solution uptake (expressed per g of fresh wt d^{-1}) during the vase life of *B. crassipes* × *B. heterophylla* 'Lipstick'. The cut stems were either treated with an STS pulse (0.5 mol m^{-3} for 10.5 h) (◆) or kept in distilled water (◇). Within each day, symbols followed by letters indicate significant differences between treatments ($P < 0.05$). (Where no error bar appears, the SE was smaller than the size of the symbol.)

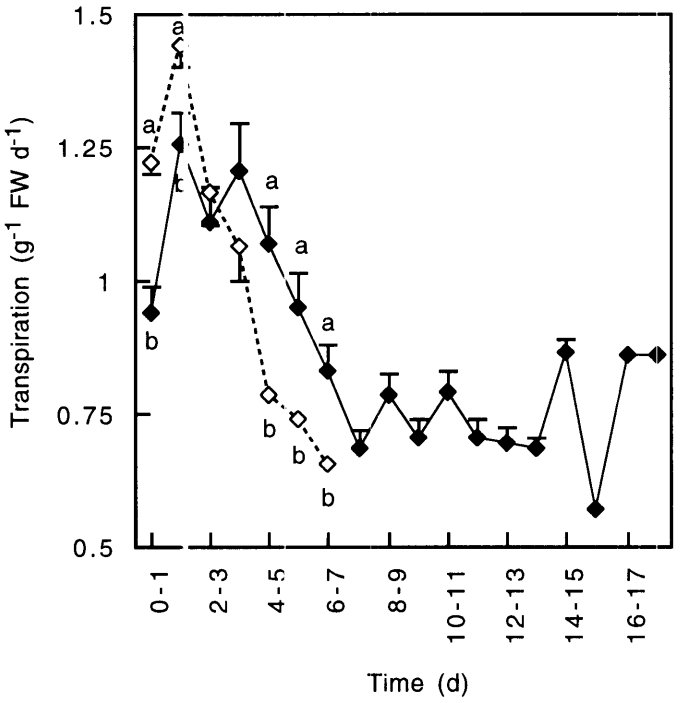


Fig. 6.19. The rate of transpiration (expressed per g of fresh wt d^{-1}) during the vase life of *B. crassipes* × *B. heterophylla* 'Lipstick'. The cut stems were either treated with an STS pulse (0.5 mol m^{-3} for 10.5 h) (◆) or kept in distilled water (◇). Within each day, symbols followed by letters indicate significant differences between treatments ($P < 0.05$). (Where no error bar appears, the SE was smaller than the size of the symbol.)

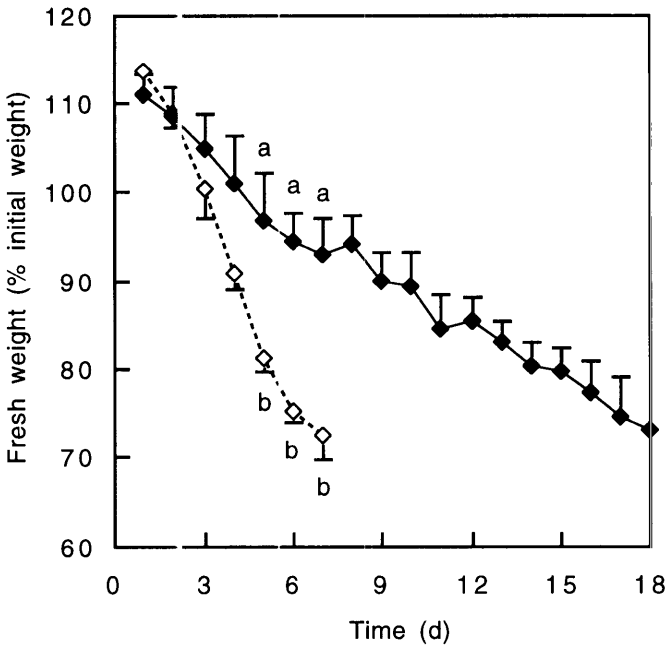


Fig. 6.20. The change in fresh weight (expressed as a % of the initial wt) during the vase life of *B. crassipes* × *B. heterophylla* 'Lipstick'. The cut stems were either treated with an STS pulse (0.5 mol m^{-3} for 10.5 h) (◆) or kept in distilled water (◇). Within each day, symbols followed by letters indicate significant differences between treatments ($P < 0.05$). (Where no error bar appears, the SE was smaller than the size of the symbol.)

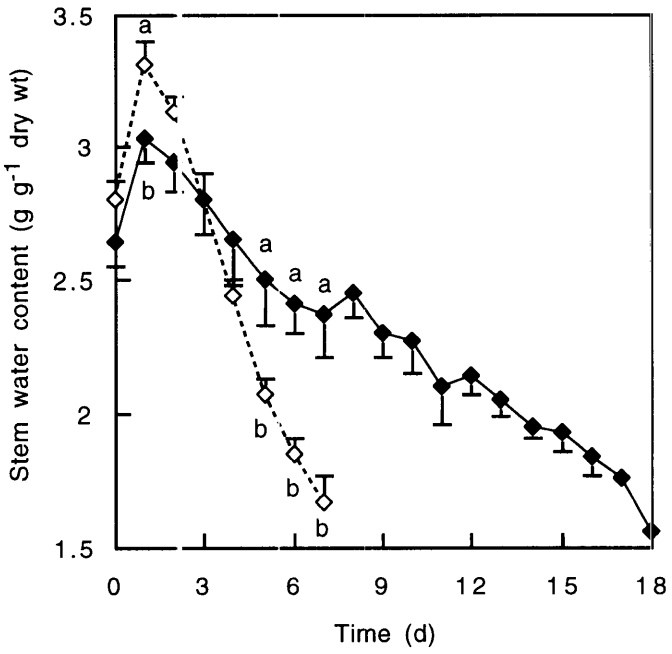


Fig. 6.21. The change in stem water content (expressed in grams of water per g of dry wt) during the vase life of *B. crassipes* × *B. heterophylla* 'Lipstick'. The cut stems were either treated with an STS pulse (0.5 mol m^{-3} for 10.5 h) (◆) or kept in distilled water (◇) Within each day, symbols followed by letters indicate significant differences between treatments ($P < 0.05$) (Where no error bar appears, the SE was smaller than the size of the symbol.)

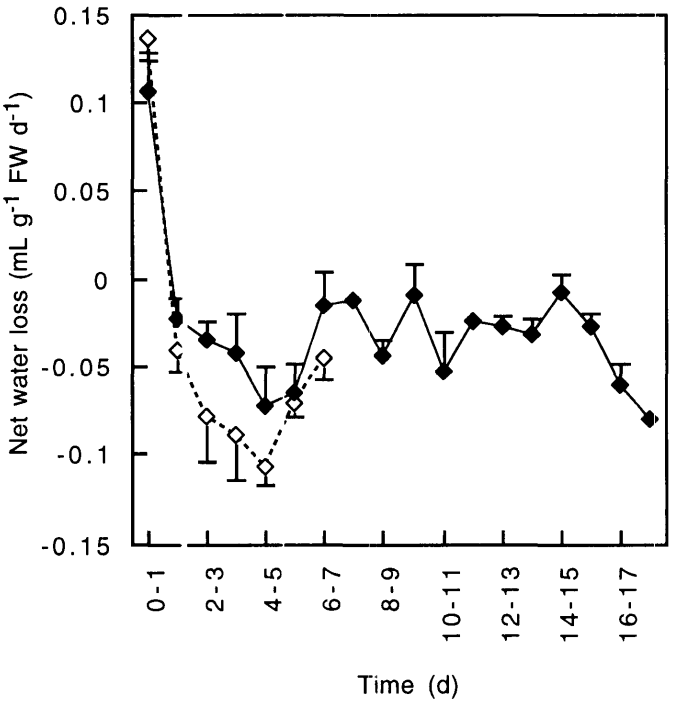


Fig. 6.22. The rate of net water loss (expressed in mL per g of fresh wt d^{-1}) during the vase life of *B. crassipes* × *B. heterophylla* 'Lipstick'. The cut stems were either treated with an STS pulse (0.5 mol m^{-3} for 10.5 h) (◆) or kept in distilled water (◇). Within each day, there were no significant differences between treatments ($P < 0.05$). (Where no error bar appears, the SE was smaller than the size of the symbol.)

B. clavata

In *B. clavata*, rates of solution uptake and transpiration were higher in stems pulsed with STS (Figs. 6.23 and 6.24).

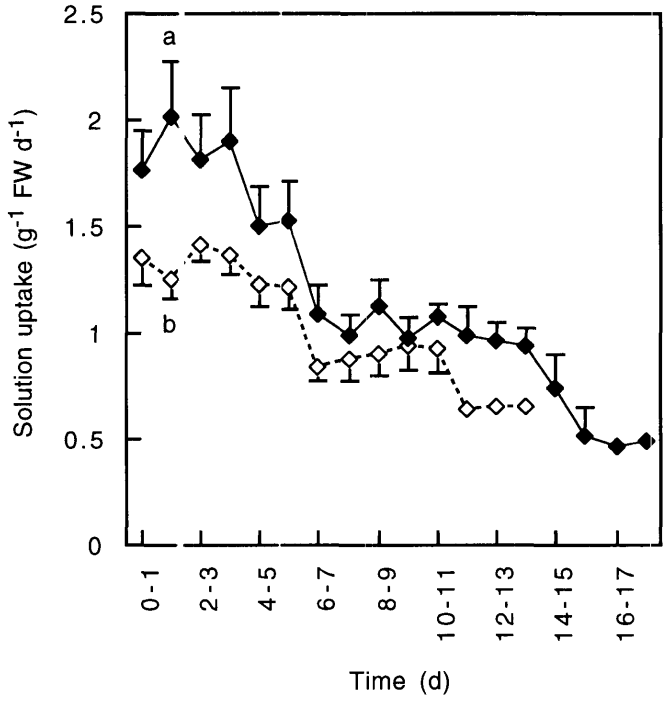


Fig. 6.23. The rate of solution uptake (expressed per g of fresh wt d^{-1}) during the vase life of *B. clavata*. The cut stems were either treated with an STS pulse (0.5 mol m^{-3} for 10.5 h) (◆) or kept in distilled water (◇). Within each day, symbols followed by letters indicate significant differences between treatments ($P < 0.05$). (Where no error bar appears, the SE was smaller than the size of the symbol.)

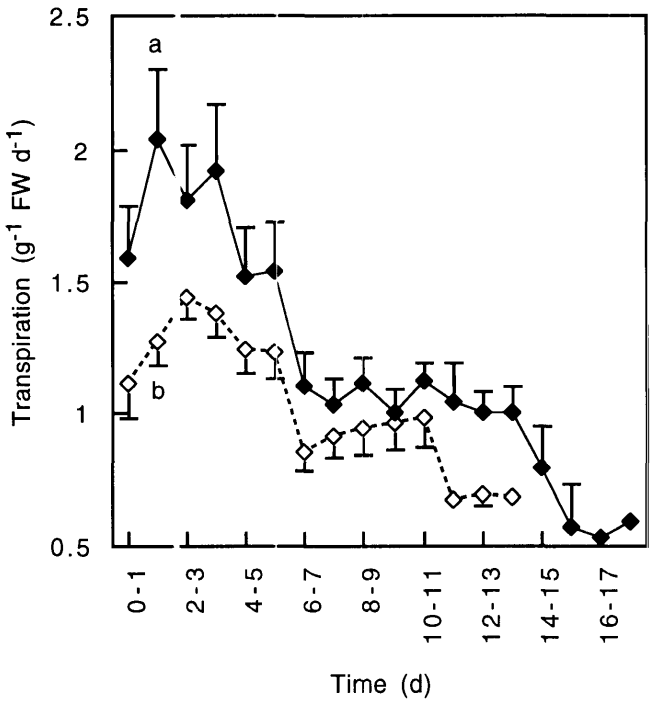


Fig. 6.24. The rate of transpiration (expressed per g of fresh wt d^{-1}) during the vase life of *B. clavata*. The cut stems were either treated with an STS pulse (0.5 mol m^{-3} for 10.5 h) (◆) or kept in distilled water (◇). Within each day, symbols followed by letters indicate significant differences between treatments ($P < 0.05$). (Where no error bar appears, the SE was smaller than the size of the symbol.)

Stem water content was also higher after stems were pulsed with STS (Fig. 6.26). However, fresh weight (Fig. 6.25) was higher in stems kept in distilled water. The rate of net water loss (Fig. 6.27) was similar for both treatments.

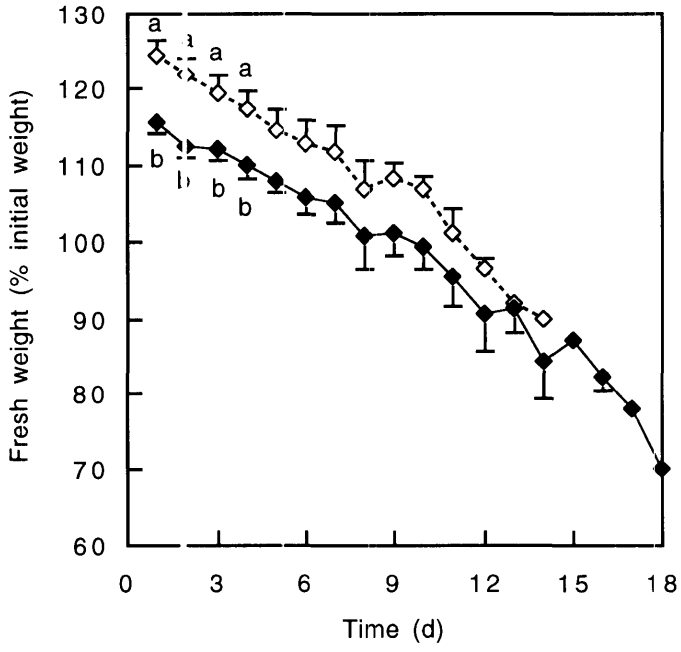


Fig. 6.25. The change in fresh weight (expressed as a % of the initial wt) during the vase life of *B. clavata*. The cut stems were either treated with an STS pulse (0.5 mol m^{-3} for 10.5 h) (◆) or kept in distilled water (◇). Within each day, symbols followed by letters indicate significant differences between treatments ($P < 0.05$). (Where no error bar appears, the SE was smaller than the size of the symbol.)

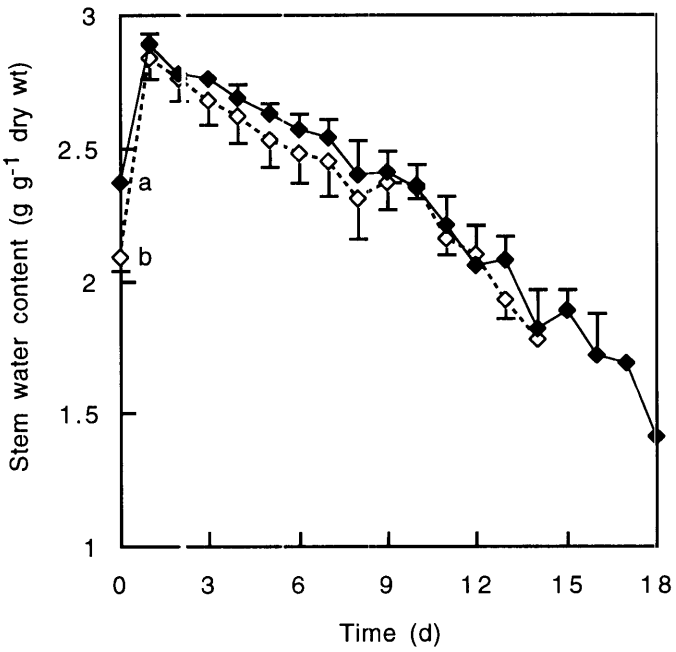


Fig. 6.26. The change in stem water content (expressed in grams of water per g of dry wt) during the vase life of *B. clavata*. The cut stems were either treated with an STS pulse (0.5 mol m^{-3} for 10.5 h) (◆) or kept in distilled water (◇). Within each day, symbols followed by letters indicate significant differences between treatments ($P < 0.05$). (Where no error bar appears, the SE was smaller than the size of the symbol.)

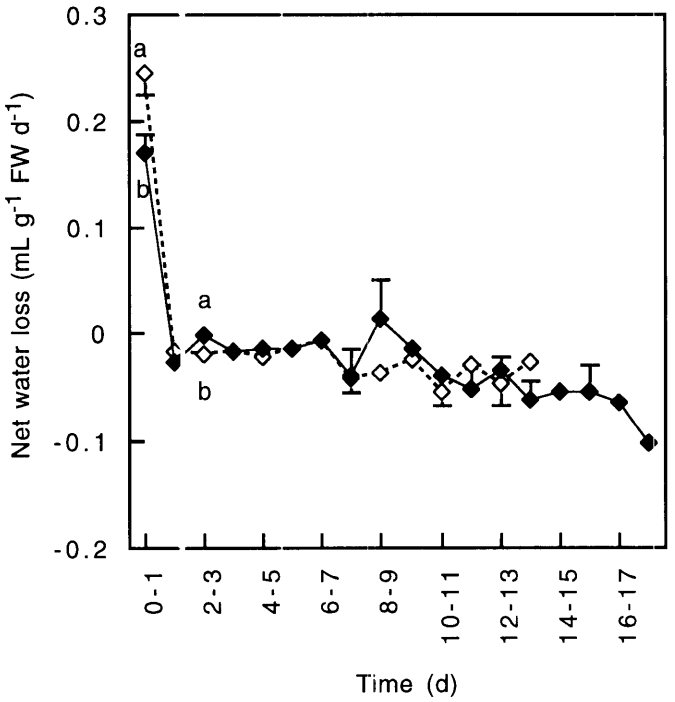


Fig. 6.27. The rate of net water loss (expressed in mL per g of fresh wt d^{-1}) during the vase life of *B. clavata*. The cut stems were either treated with an STS pulse (0.5 mol m^{-3} for 10.5 h) (◆) or kept in distilled water (◇). Within each day, symbols followed by letters indicate significant differences between treatments ($P < 0.05$). (Where no error bar appears, the SE was smaller than the size of the symbol.)

A summary of results for vase life and water relations parameters for the three *Boronia* spp. with and without an STS pulse treatment is shown in Table 6.6.

Table 6.6
Summary of results obtained for *B. muelleri* 'Sunset Serenade', *B. crassipes* × *B. heterophylla* 'Lipstick' and *B. clavata* with and without an STS pulse (0.5 mol m^{-3} for 10.5 h) treatment

	***Boronia* species**					
Parameter	***B. muelleri***		***B.* 'Lipstick'**		***B. clavata***	
measured	**+ STS**	**- STS**	**+ STS**	**- STS**	**+ STS**	**- STS**
Vase life (d)	8.5	8.8	12.6	6.5	11.8	10.2
Uptake	×	✓	✓	×	✓	×
Transpiration	×	✓	✓	×	✓	×
Fresh weight	✓	×	✓	×	×	✓
Water content	=	=	✓	×	✓	×
Net water loss	=	=	✓	×	=	=

Note: Stems were assigned to one of two treatments. The stems were either pulsed with STS (**+ STS**), or not (**- STS**). Within each species, the notation, ✓, indicates that the treatment resulted in an overall improvement in the parameter measured. The notation, ×, indicates that, overall, the treatment did not improve the parameter measured. The notation, =, indicates that the results for the parameter measured were similar for each treatment.

6.4 DISCUSSION

In the two *B. heterophylla* vase life experiments (sections 6.3.1 and 6.3.2), the STS/distilled water treatment (T8) resulted in a significantly greater vase life (Tables 6.1 and 6.2), irrespective of harvest maturity. *B. heterophylla* is known to be ethylene-sensitive (Joyce and Haynes 1989). The data presented in this chapter reveal that the most important factor responsible for increased longevity is blocking the effects of ethylene with an STS pulse. Sensitivity to ethylene is thought to be determined by the vase life reaction to pulsing with STS (Woltering and van Doorn 1988) compared with control flowers.

The different stages of floral development (maturity) at which the two vase life studies (sections 6.3.1 and 6.3.2) were initiated confirmed those treatments that were truly beneficial at increasing longevity. For example, treatments in which there was no obvious improvement in vase life between late and optimal harvest time (maturity) were clearly not beneficial. These were 8-HQC (T1), citric acid (10 mol m^{-3}, T4), citric acid + chlorine (T6) and citric acid (1.43 mol m^{-3}, T9). Treatments containing chlorine (T6 T7) resulted in premature senescence (Tables 6.1 and 6.2), probably because the concentration of 50 mg L^{-1} was too high. Tissue culture experiments at UNE have shown that *Boronia* is sensitive to chlorine, and a low concentration of 1 mg L^{-1} is used (A. Taji, pers. comm. 1992). Addition of sucrose (T5, Table 6.2) increased vase life over

that of distilled water. The STS pulse was by far the most effective treatment in prolonging vase life. However, it would be interesting to see whether this treatment plus a holding solution of sucrose (2% w/v) would increase vase life over that of the STS pulse alone. Furthermore, addition of sucrose prevented petal fading (section 6.3.2). Therefore, combining the sugar and STS treatments could give a long vase life with little or no petal fading. This achievement would make *B. heterophylla* more attractive on the Japanese market which, as mentioned earlier (section 6.2.3), dislikes the petal fading[4]. Australian native cut flowers exhibit varying responses when pulsed with 1 to 40% sucrose (w/v) (Carter *et al.* 1989; Manning *et al.* 1989), indicating that there is scope for the variation of sucrose concentration and exposure time in pulse treatments.

The two *B. heterophylla* vase life experiments performed at different harvest maturities (sections 6.3.1 and 6.3.2) provided interesting results in terms of the effectiveness of the timing of the STS pulse. It is generally accepted that for an STS pulse to be fully effective in ethylene-sensitive cut flowers, it must be applied as soon as possible after harvest (Sytsema 1980). Application at an early physiological age of the flower petals is also desirable because sensitivity to ethylene increases with age (Barden and Hanan 1972; Kende and Hanson 1976; Halevy and Mayak 1981). According to Sytsema (1980), "it seems the more difficult to stop this process the longer it has proceeded". Heins (1980) used ethanol to inhibit ethylene production, but found that it was not effective once climacteric ethylene production had begun. Thus, not only does ethylene production increase dramatically during senescence (Nichols 1966; Whitehead *et al.* 1984), but flowers exhibit an increased sensitivity to ethylene as they senesce (Camprubi and Nichols 1978; Whitehead *et al.* 1984; Borochov and Woodson 1989). The effectiveness of the STS results in both *B. heterophylla* vase life experiments at different stages of maturity is therefore surprising. It is possible that, in this species, increased ethylene production occurs at a later stage than <50% petal fading This notion is contrary to Akamine's (1963) findings of 97% fading producing peak ethylene production in *Vanda* orchids, but he removed the pollinia

---

[4] Although it was found that the fading which is characteristic of senescence in *B. heterophylla* and *B.* 'Lipstick' flowers could be stopped by the addition of sucrose to the vase solution, it may be awkward for the consumer to apply this treatment. Therefore, *Boronia molloyae* J.R.Drumm. may be a more suitable cut flower for the Japanese market than *B. heterophylla* and *B.* 'Lipstick' because it does not fade (Elliott and Jones 1982). Unfortunately, insufficient *B. molloyae* flowers were available to include them in a vase life experiment. Nevertheless, a recent catalogue of Australian flowers available for export to Japan (Sugiyama 1995) lists all of the above boronias, as well as *B. clavata* and *B. muelleri*.

Two new boronia cultivars, 'Moonglow' and 'Cameo' (Sunglow Flowers Pty. Ltd. 1990), which are both mutations of *B. heterophylla*, may have potential for development as cut flowers. 'Moonglow' has white bell-shaped flowers, and 'Cameo' has white bell-shaped flowers with a pink stripe on the outside of the petals. The fading problem that occurs in senescing *B. heterophylla* flowers could therefore be negated if these white flowers were accepted on the cut flower market. Both 'Moonglow' and 'Cameo' have the same fragrance as the common red form of *B. heterophylla* (P. Watkins, Sunglow Flowers Pty. Ltd., pers. comm. 1994). The cultivars flower from mid September to mid October (at Mundijong, Western Australia, 32°, 18' S; 115°, 59' E), which is similar to the spring flowering time of the red form of *B. heterophylla*. The vase life response of these two new cultivars is unknown, but if their response to an STS pulse was similar to that described in this chapter for the common red form of *B. heterophylla*, a potentially long-lasting cut flower could emerge on the international market.

to obtain his results. Furthermore, ethylene production rates are likely to be species-specific. Quantifying ethylene production in *B. heterophylla* during development and senescence may provide an explanation for the present results. Nevertheless, the absolute effectiveness of an STS pulse was surely demonstrated in the significant increase in vase life obtained for both floral maturities.

When the silver ion is combined with sodium thiosulphate to form the STS complex, it is known to be an ineffective germicide (Zagory and Reid 1986a; van Doorn, Zagory and Reid 1991). However, silver in the form of $AgNO_3$ has well established germicidal properties (Paull and Goo 1982; Goszczynska and Rucnicki 1988; van Doorn, Zagory and Reid 1991). The ineffectiveness of STS in preventing bacterial proliferation was further borne out in the present experiments. However, the silver concentration was very low in the pulse treatment and would have been minimal after replacement with distilled water.

In study no. 1, the STS pulse (T8) had the significantly greatest vase life (Table 6.1), but also the highest number of bacteria (Fig. 6.10). When the relationship between longevity and bacterial numbers was examined using linear regression analysis (Fig. 6.12), it was found that the number of bacteria had no significant influence on vase life, as reflected in the adjusted R^2 (0.000) and the t-statistic (0.202) of the coefficient of x. In study no. 2, T5 (citric + sucrose), had one of the significantly highest bacterial populations, but the second longest vase life (Tables 6.2 and 6.3). Distilled water (T2) had the lowest number of bacteria, and the third longest vase life (Tables 6.2 and 6.3). Therefore, although no statistical tests could be performed on the data from study no. 2, they would appear to confirm the result from study no. 1 that bacterial numbers do not influence the vase life of *B. heterophylla*. However, prevention of ethylene action with an STS pulse significantly increased vase life (Tables 6.1 and 6.2). Van Doorn, Zagory, de Witte and Harkema (1991) found that an STS pulse of carnations (cv. Scania) gave a significantly longer vase life than all other treatments (21 d cf. 9 d), including germicides. However, the number of bacteria in the basal 5 cm stem segments was higher in the STS treatment than in all others, except for a treatment of bacteria (10^7 cfu mL^{-1}) added to the vase solution. Yet these authors did not ascribe the increased longevity with STS treatment to protection against ethylene, although 'Scania' was previously found to be highly sensitive to ethylene (Woltering and van Doorn 1988). Rather, they concluded that bacteria did not affect longevity (van Doorn, Zagory, de Witte and Harkema 1991).

The solution pH did not influence the number of bacteria (Fig. 6.11), as bacterial numbers for the same or similar pH values differed in all cases except T3 and T4. Furthermore, the relative unimportance of pH is confirmed by the finding that different solutions with the same pH had significantly different vase lives (Table 6.1). Similarly, Zagory and Reid (1986b) observed that

there was no significant correlation between solution pH and vase life of vase water inoculated with bacteria.

Some authors (e.g. Put and Conway 1986; van Doorn, Buis and de Witte 1986; de Witte and van Doorn 1988; Put and Clerkx 1988; Clerkx *et al.* 1989) have indicated that once vase solution bacterial numbers reach high levels ($> 10^7$ cfu mL^{-1}), longevity will be decreased. The present results do not support such a proposition. The most beneficial treatment in terms of longevity, the STS pulse (T8, Table 6.1), was also the one with the highest number of vase solution bacteria (Fig. 6.10) (up to 10^{10} cfu mL^{-1}). However, it is possible that the type of bacteria are more important than the number of bacteria, a conclusion also reached by Zagory and Reid (1986b). They found that not all bacteria had the same effect on vase life (Zagory and Reid 1986b). Urban and Lemattre (1990) observed that a knowledge of bacterial numbers (inside stems) was not a reliable indicator of vase life. Accordingly, they advised against the setting of bacterial level limits at markets, noting an absence of any link between microbial numbers and vase life in cut roses.

In study no. 1, vase solutions containing the germicide, 8-HQC, had the second highest number of bacteria (Fig. 6.10). Agar plates on which 8-HQC vase solution diluents were grown were densely populated with bacteria of a similar appearance (Photograph 6.7). Van Doorn, de Witte and Perik (1990) found that stem segments kept in 8-HQC (250 mg L^{-1}) contained numerous bacterial colonies of a similar colour and shape. The bacterium was identified as *Pseudomonas fluorescens*. It is thus likely that 8-HQC was 'selecting' for certain types of bacteria because it is a known chelator, with a high affinity for the transition metals Cu, Mn, Fe, Cd, Co and Zn (Zentmyer 1943; Albert *et al.* 1947). If these elements are precipitated out of solution, they are not available for any micro-organism that relies upon them for growth. However, Albert *et al.* (1947) hypothesised that the chelating effect of 8-HQC was with an ionised metal on the bacterial surface, which inactivated a cellular enzyme. Nevertheless, irrespective of the mode of action, micro-organisms requiring those transition metals would be eliminated. Conversely, any microbe that did not require them would proliferate. The ability of *Ps. fluorescens* to grow and produce fluorescent pigments on iron-deficient media such as King's B medium (King *et al.* 1954) is commonly utilised as a diagnostic aid (Buchanan and Gibbons 1974). Many gram negative bacteria, including the genus *Pseudomonas*, are insensitive to 8-HQC, whereas gram positive bacteria are sensitive to its action and their growth is inhibited (Albert *et al.* 1947). *Pseudomonas* is the most common bacterial genus found in vase water (Taplin and Mertz 1973; McClary and Layne 1977; de Witte and van Doorn 1988; Put 1990), therefore inclusion of 8-HQC as a general vase solution germicide should not be recommended. Nevertheless, some researchers have found that bacterial growth was inhibited by 8-HQC (Larsen and Scholes 1965; Larsen and Cromarty 1967; van Doorn and Perik 1990), while others have not (Dansereau and Vines 1975; Jones and Hill 1993). Van Doorn, de Witte and Perik (1990) observed a variable

bacterial response to 8-HQC. Marousky (1980) noted that 8-HQC was not an effective germicide in cut flower solutions because its inhibitory action depended on the bacterial species. Marousky (1980) hypothesised that the varying bactericidal results obtained by researchers may be related to differences in water quality, particularly the presence or absence of the transition metals discussed by Zentmyer (1943) and Albert *et al.* (1947).

Bacterial numbers in vase solutions (Table 6.3) increased over time in all treatments. By day 6, all treatments (i.e. including those containing germicides) had bacterial concentrations of $\geq 10^5$ cfu mL^{-1}. Thus, no treatment limited bacterial growth markedly. The germicidal concentrations may not have been high enough to counteract the effect of nutrient leakage (Woltering 1987b) from the cut stems. However, as alluded to above (section 6.4), and as was found with roses (van Doorn, de Witte and Perik 1990), the balance between killing micro-organisms and killing cut flowers is often delicate. Bacterial numbers remained stable from day 6 until day 12 (the last day measured), probably because a stationary phase in bacterial growth was reached. This phase has been linked to several causes, including depletion of nutrients, accumulation of toxic waste products (Tortora *et al.* 1986), and/or competition between different microbial genera (Put 1990). Although the STS pulse treatment (T8) solution contained the greatest number of bacteria in study no. 1 (Fig. 6.10) it is uncertain why this was not the case in study no. 2 (Table 6.3). Nevertheless, the fact that vase life was significantly greater in STS pulsed stems in both experiments, regardless of the number of bacteria, reveals the irrelevance of bacterial numbers to the longevity of this species.

Bacterial numbers in the basal 5 cm stem segments were higher than in 5 to 10 cm segments in all but one treatment (Table 6.4). This result was similar to those of other researchers (Put and van der Meyden 1988; van Doorn *et al.* 1989; van Doorn and Perik 1990). The result is not surprising because bacterial cells are larger than pit apertures. Accordingly, they will colonise only those xylem conduits which are already open at the cut end. The percentage of conduits that remains open decreases with increasing distance from the cut end, a phenomenon which is exploited by the Milburn and Covey-Crump (1971) method of conduit length allocation (see Chapter 9).

Although it was not possible to determine whether there was a statistical relationship between vase life and the number of bacteria in stem segments (Tables 6.2 and 6.4), visual examination of the data show that T5 (citric + sucrose) had the second longest vase life and one of the highest bacterial populations in the stem segments. One of the shortest vase lives occurred in 8-HQC (T1), which had the lowest bacteria numbers in the stem segments. Van Doorn, Harkema and Otma (1991) found that there was no correlation between wilting in lilac (*Syringa vulgaris*) and the number of bacteria in the basal 5 cm stem segment.

Woltering and van Doorn (1988) tes ed the reaction to exogenous ethylene in 22 families and 93 species. They noted (i) that reaction s occurred at the family level; and (ii) that, generally, petals which wilted were insensitive to ethylene, whereas petals which abscissed were ethylene-sensitive. However, in all the *Boronia* spp. tested, senescence was characterised by petal wilting, irrespective of whether the spec es was ethylene-sensitive or not. Furthermore, sensitivity to ethylene did not occur at the family level because some species were ethylene-sensitive (*B. heterophylla*, *B. crass pes* × *B. heterophylla* 'Lipstick'), whereas others were not (*B. clavata* and *B. muelleri* 'Sunset Serenade').

Vase life responses of *B. muelleri*, *B.* 'Lipstick' and *B. clavata* (Table 6.5) revealed that only *B.* 'Lipstick' showed significantly increased longevity in response to an STS pulse. This selective result indicates that only *B.* 'Lipstick' is ethylene-sensitive, which is not surprising because one of its parents, *B. heterophylla*, is also sensitive to ethylene. When the vase lives of the three *Boronia* spp. are examined in conjunction with the graphs of solution uptake, transpiration, fresh weight, stem water content and net water loss (see Table 6.6 for summary), it appears that all of the above parameters need to be improved for longevity to be increased. No one parameter seemed to be more important than the other in promoting increased vase life.

In summary, *B. heterophylla and B* 'Lipstick' are ethylene-sensitive, and their senescence can be delayed by pulsing with STS. Blocking the effects of ethylene is far more important in promoting longevity in those species than either vase solution composition, or bacterial numbers in vase solutions or stems. In contrast, vase lives of *B. muelleri* and *B. clavata* are not improved by an STS pulse, indicating insensitivity to ethylene. Therefore, ethylene-sensitivity should not be assigned generally to plants at the family level on the basis of the reaction of a few species in the family.

Bacteria are not the most important factor causing decreased longevity in *Boronia*. The following chapter investigates the effect on *Acacia* of a bacterium which is known to decrease *Rosa* vase life.