APPENDIX A

PREPARATION OF SILVER THIOSULPHATE COMPLEX (from Joyce and Haynes 1989)

A. PREPARATION OF THE STS STOCK SOLUTION (40 mol m^{-3} Ag^+)

1. Dissolve 6.8 g of $AgNO_3$ in 500 mL of distilled water.

2. Dissolve 25.3 g of $Na_2S_2O_3$ in 500 mL of distilled water.

3. With vigorous stirring, slowly pour the $AgNO_3$ solution into the $Na_2S_2O_3$ solution to obtain a final volume of 1 L.

4. Store the 40 mol m^{-3} STS stock solution in a dark bottle (aluminium foil wrap) in a coldroom.

B. DILUTING THE STOCK SOLUTION FOR PULSING FLOWERS IN THE COLDROOM (0.5 to 2°C) OVERNIGHT (8 to 12 h)

1. Dilute 12.5 mL of 40 mol m^{-3} STS stock solution to 1,000 mL (by adding 987.5 mL of distilled water) to obtain a 0.5 mol m^{-3} STS pulsing solution.

APPENDIX B

SILVER THIOSULPHATE SOLUTION UPTAKE BY CUT FLOWERING STEMS IN THIS THESIS

The amount of silver thiosulphate (STS) taken up by cut flowering stems was estimated following the method described by Joyce (1992). This method involved weighing the STS pulsing solution before and after the pulse treatment, and weighing the stems before pulsing, and applying the following equation:

$$\text{STS uptake (mL)} = \frac{W1 - W2}{FW}$$

where W1 is the weight of solution before pulsing (g);

W2 is the weight of solution after pulsing (g); and

FW is the fresh weight of stems before pulsing (g).

Reid *et al.* (1980) found that cut stems did not take up STS differentially from water in the solution because the Ag concentration remained unchanged, even after 40% solution uptake. This allows for easy calculation of STS uptake by measuring the solution uptake before and after pulsing. According to Joyce (1992), using Geraldton wax (*Chamelaucium uncinatum*), the STS pulse (at the "overnight" concentration, i.e. 0.5 mol m^{-3} STS solution) has been effective if between 0.2 and 1.0 mL g^{-1} fresh weight (fw) has been taken up. This is equivalent to 0.1 µmol and 0.5 µmol of Ag g^{-1} of stem tissue respectively, and is calculated by multiplying the amount of STS solution absorbed by the stem by the STS (Ag) concentration (Reid *et al.* 1980).

However, Joyce (1992) indicated that the treatment was effective if either the above amounts were taken up, or if vase life was improved in the presence of ethylene. Whilst the above amounts were recommended levels for Geraldton wax, Joyce (D. Joyce, pers. comm. 1995) said that effective amounts for other cut flowers were not known. In the experiments performed in this thesis, some of the amounts of STS uptake were below the levels recommended by Joyce (1992). However, it is believed the STS treatment was effective as there was no difference in longevity between replicates with different STS uptake levels. The reason for the lower uptake reported in this thesis is probably because terminal shoots (20 cm long) were used and the stems had small diameters, and hence a lower capacity for solution uptake. The experiments described by Reid *et al.* (1980) and Joyce (1992) used respectively, cut stems 50 cm long, and bunches of stems presumably longer than 20 cm

The amounts of STS taken up by cut stems used in vase life experiments reported in this thesis are set out below according to chapter. (Note: T = Treatment; Rep = Replicate number.)

Chapter 3

Table 3.1: *A. subulata* exposed to ethylene

T3 (STS pulse, then into distilled water)

	Amount of STS uptake (mL g^{-1} fw)	Amount of Ag g^{-1} of tissue (µmol g^{-1} fw)
Rep 1	0.116	0.058
Rep 2	0.112	0.056
Rep 3	0.183	0.092
Rep 4	0.070	0.035
Rep 5	0.104	0.052
Rep 6	0.052	0.026
Rep 7	0.079	0.037
Rep 8	0.201	0.101
Rep 9	0.167	0.084

Table 3.2: *A. floribunda* exposed to ethylene

T3 (STS pulse, then into distilled water)

	Amount of STS uptake (mL g^{-1} fw)	Amount of Ag g^{-1} of tissue (µmol g^{-1} fw)
Rep 1	0.107	0.054
Rep 2	0.154	0.077
Rep 3	0.131	0.066
Rep 4	0.178	0.089
Rep 5	0.305	0.153
Rep 6	0.644	0.322
Rep 7	0.311	0.156
Rep 8	0.333	0.165
Rep 9	0.494	0.247

Table 3.3: *A. floribunda* vase life

T3 (STS pulse, then into distilled water)

	Amount of STS uptake (mL g^{-1} fw)	Amount of Ag g^{-1} of tissue (µmol g^{-1} fw)
Rep 1	0.475	0.238
Rep 2	0.275	0.138
Rep 3	0.203	0.102
Rep 4	0.314	0.157
Rep 5	0.124	0.062
Rep 6	0.126	0.063
Rep 7	0.186	0.093
Rep 8	0.141	0.071
Rep 9	0.150	0.075
Rep 10	0.162	0.081

T5 (SCC: STS pulse, then into citric acid and chloride)

	Amount of STS uptake (mL g^{-1} fw)	Amount of Ag g^{-1} of tissue (µmol g^{-1} fw)
Rep 1	0.148	0.074
Rep 2	0.168	0.084
Rep 3	0.152	0.076
Rep 4	0.168	0.084
Rep 5	0.221	0.111
Rep 6	0.220	0.110
Rep 7	0.156	0.078
Rep 8	0.144	0.072
Rep 9	0.148	0.074
Rep 10	0.163	0.082

Chapter 6

Table 6.1: *B. heterophylla* vase life (study no. 1)

T8 (STS pulse, then into distilled water)

	Amount of STS uptake (mL g^{-1} fw)	Amount of Ag g^{-1} of tissue (µmol g^{-1} fw)
Rep 1	0.138	0.069
Rep 2	0.135	0.068
Rep 3	0.096	0.048
Rep 4	0.135	0.068
Rep 5	0.132	0.066
Rep 6	0.187	0.094
Rep 7	0.127	0.064
Rep 8	0.106	0.053
Rep 9	0.186	0.093
Rep 10	0.255	0.128

Table 6.2: *B. heterophylla* vase life (study no. 2)

T8 (STS pulse, then into distilled water)

	Amount of STS uptake (mL g^{-1} fw)	Amount of Ag g^{-1} of tissue (µmol g^{-1} fw)
Rep 1	0.164	0.082
Rep 2	0.222	0.111
Rep 3	0.143	0.072
Rep 4	0.030	0.015
Rep 5	0.061	0.031

Table 6.5: Vase life of *B. muelleri* 'Sunset Serenade', *B. crassipes* × *B. heterophylla* 'Lipstick' and *B. clavata*

B. muelleri 'Sunset Serenade' (STS pulse, then into distilled water)

	Amount of STS uptake (mL g^{-1} fw)	Amount of Ag g^{-1} of tissue (µmol g^{-1} fw)
Rep 1	1.078	0.539
Rep 2	0.870	0.435
Rep 3	0.463	0.232
Rep 4	0.257	0.129
Rep 5	0.377	0.189
Rep 6	0.065	0.033
Rep 7	0.040	0.020
Rep 8	0.185	0.093
Rep 9	0.080	0.040
Rep 10	0.272	0.136

B. crassipes × *B. heterophylla* 'Lipstick' (STS pulse, then into distilled water)

	Amount of STS uptake (mL g^{-1} fw)	Amount of Ag g^{-1} of tissue (µmol g^{-1} fw)
Rep 1	0.393	0.197
Rep 2	0.536	0.268
Rep 3	0.561	0.281
Rep 4	0.584	0.292
Rep 5	0.492	0.246
Rep 6	0.215	0.108
Rep 7	0.206	0.103
Rep 8	0.197	0.099
Rep 9	0.237	0.119
Rep 10	0.176	0.088

B. clavata (STS pulse, then into distilled water)

	Amount of STS uptake (mL g^{-1} fw)	Amount of Ag g^{-1} of tissue (µmol g^{-1} fw)
Rep 1	1.243	0.622
Rep 2	0.681	0.341
Rep 3	0.569	0.285
Rep 4	0.947	0.474
Rep 5	0.890	0.445
Rep 6	0.468	0.234
Rep 7	0.330	0.165
Rep 8	0.353	0.177
Rep 9	0.339	0.170
Rep 10	0.300	0.150

APPENDIX C

ANOVA TABLES, t-VALUES, χ^2 CONTINGENCY TABLES AND REGRESSION RESULTS FROM DATA ANALYSED IN THIS THESIS

The presentation format for the following statistical tables and summaries uses the same table or figure numbers from each particular chapter to facilitate cross-examination of data.

Chapter 2

Table 2.1: t-values for osmotic potential of *A. amoena* xylem cell sap

Variable:	Xylem sap	Deionised H_2O Standard
Mean	53.0	50.0
Std Deviation	3.74	1.58
Observations	5	5
t-statistic	1.65	
Degrees of Freedom	8	
Significance	0.137	

The critical value of t for 8 df is 2.305 ($P< 0.05$), therefore there is no significant difference between treatments because the t-statistic (1.65) is < the critical t-value.

Chapter 3

Table 3.1: Chi-square (χ^2) test of independence

A 2 × 2 contingency table for the vase life of *A. subulata* in T1 (distilled water, + C_2H_4) and T2 (control: distilled water, no C_2H_4)
(Observed and expected frequencies - expected frequencies in brackets)

	Vase life (d) 3	>3	Totals
T1	3 (3)	6 (6)	9 (9)
T2	3 (3)	6 (6)	9 (9)
Totals	6 (6)	12 (12)	18 (18)

The χ^2 value = 0.000, which is < 3.84 (from the χ^2 distribution table, $P < 0.05$, df = 1) and therefore is not significant. However, because some expected values in the contingency table are <5, Fisher's Exact Test of Independence is also computed. The p-value for Fisher's Exact Test is > 0.9999, therefore H_0 that the data fit the theoretical distribution is accepted, and the two populations are not assumed to be significantly different.

Table 3.1 (cont'd): Chi-square (χ^2) test of independence

A 2 × 2 contingency table for the vase life of *A. subulata* in T2 (control: distilled water, no C_2H_4) and T3 (STS pulse, then distilled water, + C_2H_4)
(Observed and expected frequencies - expected frequencies in brackets)

	Vase life (d) 3	>3	Totals
T2	3 (4)	6 (5)	9 (9)
T3	5 (4)	4 (5)	9 (9)
Totals	8 (8)	10 (10)	18 (18)

The χ^2 value = 0.900, which is < 3.84 (from the χ^2 distribution table, P < 0.05, df = 1) and therefore is not significant. However, because some expected values in the contingency table are <5, Fisher's Exact Test of Independence is also computed. The p-value for Fisher's Exact Test is 0.3953, therefore H_0 that the data fit the theoretical distribution is accepted, and the two populations are not assumed to be significantly different.

Table 3.2: Chi-square (χ^2) test of independence

A 2 × 2 contingency table for the vase life of *A. floribunda* in T1 (distilled water, + C_2H_4) and T2 (control: distilled water, no C_2H_4)
(Observed and expected frequencies - expected frequences in brackets)

	Vase life (d)		
	≤3	>3	Totals
T1	6 (6.5)	3 (2.5)	9 (9)
T2	7 (6.5)	2 (2.5)	9 (9)
Totals	13 (13)	5 (5)	18 (18)

The χ^2 value = 0.277, which is < 3.84 (from the χ^2 distribution table, P < 0.05, df = 1) and therefore is not significant. However, because some expected values in the contingency table are <5, Fisher's Exact Test of Independence is also computed. The p-value for Fisher's Exact Test is > 0.9999, therefore H_0 that the data fit the theoretical distribution is accepted, and the two populations are not assumed to be significantly different.

Table 3.2 (cont'd): Chi-square (χ^2) test of independence

A 2 × 2 contingency table for the vase life of *A. floribunda* in T2 (control: distilled water, no C_2H_4) and T3 (STS pulse, then distilled water, + C_2H_4)
(Observed and expected frequencies - expected frequences in brackets)

	Vase life (d)		
	≤3	>3	Totals
T2	7 (8)	2 (1)	9 (9)
T3	9 (8)	0 (1)	9 (9)
Totals	16 (16)	2 (2)	18 (18)

The χ^2 value = 2.250, which is < 3.84 (from the χ^2 distribution table, P < 0.05, df = 1) and therefore is not significant. However, because some expected values in the contingency table are <5, Fisher's Exact Test of Independence is also computed. The p-value for Fisher's Exact Test is 0.2353, therefore H_0 that the data fit the theoretical distribution is accepted, and the two populations are not assumed to be significantly different.

Fig. 3.1: ANOVA tables for solution uptake in *A. floribunda*

Day 0-1 (log transformed data)

Source of variation	df	Sum of Squares	Mean Square	*F*-value	P-value
Treatment	7	14.430	2.061	32.837	<0.0001
Residual	72	4.520	0.063		

Day 1-2 (untransformed data)

Source of variation	df	Sum of Squares	Mean Square	*F*-value	P-value
Treatment	7	28.409	4.058	50.354	<0.0001
Residual	49	3.949	0.081		

Day 2-3 (untransformed data)

Source of variation	df	Sum of Squares	Mean Square	*F*-value	P-value
Treatment	4	4.928	1.232	9.556	0.0001
Residual	21	2.708	0.129		

Day 3-4 (untransformed data)

Source of variation	df	Sum of Squares	Mean Square	*F*-value	P-value
Treatment	2	0.128	0.064	1.550*	0.2519
Residual	12	0.494	0.041		

* Since 1.550 < 3.89 (from a table of critical values for *F*-distribution, P < 0.05), do not reject H_0 and do not proceed with a multiple comparisons procedure.

t-test of Day 4-5 (untransformed data)

Variable:	SDIC	SCC
Mean	1.37	0.96
Std Deviation	0.00	0.08
Observations	2	5
t-statistic	6.54	
Degrees of Freedom	5	
Significance	0.001	

The critical value of t for 5 df is 2.571 (P< 0.05), therefore there is a significant difference between treatments because the t-statistic (6.54) is > the critical t-value.

Fig. 3.2: ANOVA tables for transpiration in *A. floribunda*

Day 0-1 (reciprocal of the square root transformed data)

Source of variation	df	Sum of Squares	Mean Square	*F*-value	P-value
Treatment	7	1.631	0.233	45.306	<0.0001
Residual	72	0.370	0.005		

Day 1-2 (untransformed data)

Source of variation	df	Sum of Squares	Mean Square	*F*-value	P-value
Treatment	7	25.530	3.647	47.303	<0.0001
Residual	49	3.778	0.077		

Day 2-3 (untransformed data)

Source of variation	df	Sum of Squares	Mean Square	*F*-value	P-value
Treatment	4	4.425	1.106	10.120	<0.0001
Residual	21	2.296	0.109		

Day 3-4 (untransformed data)

Source of variation	df	Sum of Squares	Mean Square	*F*-value	P-value
Treatment	2	0.086	0.043	0.920*	0.4250
Residual	12	0.561	0.047		

* Since 0.920 < 3.89 (from a table of critical values for *F*-distribution, P < 0.05), do not reject H_0 and do not proceed with a multiple comparisons procedure.

t-test of Day 4-5 (untransformed data)

Variable:	SDIC	SCC
Mean	1.39	1.03
Std Deviation	0.01	0.06
Observations	2	5
t-statistic	7.46	
Degrees of Freedom	5	
Significance	0.001	

The critical value of t for 5 df is 2.571 (P< 0.05), therefore there is a significant difference between treatments because the t-statistic (7.46) is > the critical t-value.

Fig. 3.3: ANOVA tables for fresh weight in *A. floribunda*

Day 1 (untransformed data)

Source of variation	df	Sum of Squares	Mean Square	*F*-value	P-value
Treatment	7	5693.397	813.342	8.639	<0.0001
Residual	72	6778.351	94.144		

Day 2 (untransformed data)

Source of variation	df	Sum of Squares	Mean Square	*F*-value	P-value
Treatment	7	7645.500	1092.214	25.711	<0.0001
Residual	50	2123.996	42.480		

Day 3 (untransformed data)

Source of variation	df	Sum of Squares	Mean Square	*F*-value	P-value
Treatment	4	2994.330	748.582	14.730	<0.0001
Residual	22	1118.019	50.819		

Day 4 (untransformed data)

Source of variation	df	Sum of Squares	Mean Square	*F*-value	P-value
Treatment	2	41.011	20.506	1.296*	0.3093
Residual	12	189.879	15.823		

* Since 1.296 < 3.89 (from a table of critical values for *F*-distribution, P < 0.05), do not reject H_0 and do not proceed with a multiple comparisons procedure.

t-test of Day 5 (untransformed data)

Variable:	SDIC	SCC
Mean	91.85	85.07
Std Deviation	4.66	3.76
Observations	2	5
t-statistic	2.05	
Degrees of Freedom	5	
Significance	0.096	

The critical value of t for 5 df is 2.571 (P< 0.05), therefore there is no significant difference between treatments because the t-statistic (2.05) is < the critical t-value.

Fig. 3.4: ANOVA tables for stem water content in *A. floribunda*

Day 0 (reciprocal of *x* transformed data)

Source of variation	df	Sum of Squares	Mean Square	*F*-value	P-value
Treatment	7	0.343	0.049	12.424	<0.0001
Residual	72	0.284	3.948×10^{-3}		

Day 1 (untransformed data)

Source of variation	df	Sum of Squares	Mean Square	*F*-value	P-value
Treatment	7	4.203	0.600	14.496	<0.0001
Residual	72	2.982	0.041		

Day 2 (reciprocal of *x* transformed data)

Source of variation	df	Sum of Squares	Mean Square	*F*-value	P-value
Treatment	7	19.685	2.812	70.983	<0.0001
Residual	50	1.981	0.040		

Day 3 (untransformed data)

Source of variation	df	Sum of Squares	Mean Square	*F*-value	P-value
Treatment	4	1.093	0.273	9.886	<0.0001
Residual	22	0.608	0.028		

Day 4 (quadratic root transformed data)

Source of variation	df	Sum of Squares	Mean Square	*F*-value	P-value
Treatment	2	1.522×10^{-4}	7.609×10^{-5}	0.217*	0.8078
Residual	12	0.004	3.503×10^{-4}		

* Since 0.217 < 3.89 (from a table of critical values for *F*-distribution, P < 0.05), do not reject H_0 and do not proceed with a multiple comparisons procedure.

t-test of Day 5 (untransformed data)

Variable:	SDIC	SCC
Mean	1.12	0.92
Std Deviation	0.05	0.09
Observations	2	5
t-statistic	2.76	
Degrees of Freedom	5	
Significance	0.040	

The critical value of t fo 5 df is 2.571 (P< 0.05), therefore there is a significant difference between treati ients because the t-statistic (2.76) is > the critical t-value.

Fig. 3.5: ANOVA tables for net water loss in *A. floribunda*

Day 0-1 (untransformed data)

Source of variation	df	Sum of Squares	Mean Square	*F*-value	P-value
Treatment	7	0.550	0.079	8.281	<0.0001
Residual	72	0.683	9.491×10^{-3}		

Day 1-2 (untransformed data)

Source of variation	df	Sum of Squares	Mean Square	*F*-value	P-value
Treatment	7	0.609	0.087	34.795	<0.0001
Residual	49	0.122	2.498×10^{-3}		

Day 2-3 (reciprocal of *x* transformed data)

Source of variation	df	Sum of Squares	Mean Square	*F*-value	P-value
Treatment	4	6781.773	1695.443	6.011	0.0030
Residual	18	5076.623	282.035		

Day 3-4 (reciprocal of *x* transformed data)

Source of variation	df	Sum of Squares	Mean Square	*F*-value	P-value
Treatment	2	5365.594	2682.797	1.559*	0.2501
Residual	12	20652.464	1721.039		

* Since 1.559 < 3.89 (from a tabl of critical values for *F*-distribution, P < 0.05), do not reject H_0 and do not proceed with a multiple comparisons procedure.

t-test of Day 4-5 (untransformed data)

Variable:	SDIC	SCC
Mean	-0.02	-0.07
Std Deviation	0.01	0.03
Observations	2	5
t-statistic	2.36	
Degrees of Freedom	5	
Significance	0.065	

The critical value of t fo 5 df is 2.571 (P< 0.05), therefore there is no significant difference between treati ients because the t-statistic (2.36) is < the critical t-value.

Table 3.4: Chi-square (χ^2) test of independ nce

A 2 × 2 contingency table for the vase life of *A. amoena* phyllodes in T1 (distilled water) and T2 (citric acid) (Observed and expected frequencies - expected frequences in brackets)

	Vase life (d)		Totals
	<5	≥5	
T1	10 (5)	0 (5)	10 (10)
T2	0 (5)	10 (5)	10 (10)
Totals	10 (10)	10 (10)	20 (20)

The χ^2 value = 20.0, which is > 3.84 (from the χ^2 distribution table, P < 0.05, df = 1) and therefore is significant; the χ^2 p-value = < 0.0001. Th refore H_0 that the data fit the theoretical distribution is rejected, and the two populations are assumed to be signi icantly different.

Table 3.4 (cont'd): Chi-square (χ^2) test of independence

A 2 × 2 contingency table for the vase life of *A. amoena* flowers in T1 (distilled water) and T2 (citric acid) (Observed and expected frequencies - expected frequences in brackets)

	Vase life (d)		
	2	≥3	Totals
T1	6 (3)	4 (7)	10 (10)
T2	0 (3)	10 (7)	10 (10)
Totals	6 (6)	14 (14)	20 (20)

The χ^2 value = 8.571, which is > 3.84 (from the χ^2 distribution table, P < 0.05, df = 1) and therefore is significant; the χ^2 p-value = 0.0034. However, because some expected values in the contingency table are <5, Fisher's Exact Test of Independence is also computed. The p-value for Fisher's Exact Test is 0.018, therefore H_0 that the data fit the theoretical distribution is rejected, and the two populations are assumed to be significantly different.

Fig. 3.6: t-values for solution uptake in *A. amoena*

t-test of Day 0-1 (untransformed data)

Variable:	Distilled water	Citric acid
Mean	1.15	0.90
Std Deviation	0.34	0.20
Observations	10	10
t-statistic	1.95	
Degrees of Freedom	18	
Significance	0.067	

The critical value of t for 18 df is 2.101 (P< 0.05), therefore there is no significant difference between treatments because the t-statistic (1.95) is < the critical t-value.

t-test of Day 1-2 (untransformed data)

Variable:	Distilled water	Citric acid
Mean	0.50	0.91
Std Deviation	0.39	0.17
Observations	10	10
t-statistic	3.04	
Degrees of Freedom	18	
Significance	0.007	

The critical value of t for 18 df is 2.101 (P< 0.05), therefore there is a significant difference between treatments because the t-statistic (3.04) is > the critical t-value.

t-test of Day 2-3 (square root transformed data)

Variable:	Distilled water	Citric acid
Mean	0.37	0.94
Std Deviation	0.10	0.16
Observations	4	10
t-statistic	6.41	
Degrees of Freedom	12	
Significance	0.000	

The critical value of t for 12 df is 2.179 (P< 0.05), therefore there is a significant difference between treatments because the t-statistic (6.41) is > the critical t-value.

Fig. 3.7: t-values for transpiration in *A. anoena*

t-test of Day 0-1 (untransformed data)

Variable:	Distilled water	Citric acid
Mean	1.17	0.81
Std Deviation	0.30	0.19
Observations	10	10
t-statistic	3.12	
Degrees of Freedom	18	
Significance	0.006	

The critical value of t for 18 df is 2.101 (P< 0.05), therefore there is a significant difference between treatments because the t-statistic (3.12) is > the critical t-value.

t-test of Day 1-2 (square root transformed data)

Variable:	Distilled water	Citric acid
Mean	0.83	0.97
Std Deviation	0.21	0.09
Observations	10	10
t-statistic	2.01	
Degrees of Freedom	18	
Significance	0.060	

The critical value of t for 18 df is 2.101 (P< 0.05), therefore there is no significant difference between treatments because the t-statistic (2.01) is < the critical t-value.

t-test of Day 2-3 (untransformed data)

Variable:	Distilled water	Citric acid
Mean	0.39	0.99
Std Deviation	0.13	0.28
Observations	4	10
t-statistic	3.94	
Degrees of Freedom	12	
Significance	0.002	

The critical value of t for 12 df is 2.179 (P< 0.05), therefore there is a significant difference between treatments because the t-statistic (3.94) is > the critical t-value.

Fig. 3.8: t-values for fresh weight in *A. anoena*

t-test of Day 1 (cubic root transformed data)

Variable:	Distilled water	Citric acid
Mean	4.53	4.70
Std Deviation	0.21	0.08
Observations	10	10
t-statistic	2.35	
Degrees of Freedom	18	
Significance	0.030	

The critical value of t for 18 df is 2.101 (P< 0.05), therefore there is a significant difference between treatments because the t-statistic (2.35) is > the critical t-value.

t-test of Day 2 (untransformed data)

Variable:	Distilled water	Citric acid
Mean	76.19	104.98
Std Deviation	14.68	7.77
Observations	10	10
t-statistic	5.48	
Degrees of Freedom	18	
Significance	0.000	

The critical value of t for 18 df is 2.101 (P< 0.05), therefore there is a significant difference between treatments because the t-statistic (5.48) is > the critical t-value.

t-test of Day 3 (untransformed data)

Variable:	Distilled water	Citric acid
Mean	65.11	96.90
Std Deviation	6.95	10.03
Observations	4	10
t-statistic	5.74	
Degrees of Freedom	12	
Significance	0.000	

The critical value of t fo · 12 df is 2.179 (P< 0.05), therefore there is a significant difference between treatments because the t-statistic (5.74) is > the critical t-value.

Fig. 3.9: t-values for stem water content in *A. amoena*

t-test of Day 0 (untransformed data)

Variable:	Distilled water	Citric acid
Mean	1.19	1.19
Std Deviation	0.12	0.13
Observations	10	10
t-statistic	0.11	
Degrees of Freedom	18	
Significance	0.915	

The critical value of t fo · 18 df is 2.101 (P< 0.05), therefore there is no significant difference between treatments because the t-statistic (0.11) is < the critical t-value.

t-test of Day 1 (square root transformed data

Variable:	Distilled water	Citric acid
Mean	1.06	1.17
Std Deviation	0.13	0.04
Observations	10	10
t-statistic	2.73	
Degrees of Freedom	18	
Significance	0.014	

The critical value of t fo · 18 df is 2.101 (P< 0.05), therefore there is a significant difference between treatments because the t-statistic (2.73) is > the critical t-value.

t-test of Day 2 (untransformed data)

Variable:	Distilled water	Citric acid
Mean	0.65	1.29
Std Deviation	0.26	0.06
Observations	10	10
t-statistic	7.44	
Degrees of Freedom	18	
Significance	0.000	

The critical value of t fo · 18 df is 2.101 (P< 0.05), therefore there is a significant difference between treatments because the t-statistic (7.44) is > the critical t-value.

t-test of Day 3 (untransformed data)

Variable:	Distilled water	Citric acid
Mean	0.41	1.11
Std Deviation	0.13	0.10
Observations	4	10
t-statistic	10.97	
Degrees of Freedom	12	
Significance	0.000	

The critical value of t fo · 12 df is 2.179 (P< 0.05), therefore there is a significant difference between treatments because the t-statistic (10.97) is > the critical t-value.

Fig. 3.10: t-values for net water loss in *A. amoena*

t-test of Day 0-1 (untransformed data)

Variable:	Distilled water	Citric acid
Mean	-0.02	0.09
Std Deviation	0.14	0.06
Observations	10	10
t-statistic	2.34	
Degrees of Freedom	18	
Significance	0.031	

The critical value of t for 18 df is 2.101 (P< 0.05), therefore there is a significant difference between treatments because the t-statistic (2.34) is > the critical t-value.

t-test of Day 1-2 (untransformed data)

Variable:	Distilled water	Citric acid
Mean	-0.22	-0.04
Std Deviation	0.08	0.04
Observations	10	10
t-statistic	6.80	
Degrees of Freedom	18	
Significance	0.000	

The critical value of t for 18 df is 2.101 (P< 0.05), therefore there is a significant difference between treatments because the t-statistic (6.80) is > the critical t-value.

t-test of Day 2-3 (untransformed data)

Variable:	Distilled water	Citric acid
Mean	-0.25	-0.08
Std Deviation	0.11	0.04
Observations	4	10
t-statistic	4.55	
Degrees of Freedom	12	
Significance	0.001	

The critical value of t for 12 df is 2.179 (P< 0.05), therefore there is a significant difference between treatments because the t-statistic (4.55) is > the critical t-value.

Chapter 4

Table 4.1: ANOVA table for RWC of *A. amoena* stems at end of cavitation experiments

(untransformed data)

Source of variation	df	Sum of Squares	Mean Square	*F*-value	P-value
Treatment	3	15440.590	5146.863	34.358	<0.0001
Residual	33	4943.373	149.799		

Table 4.2: ANOVA tables for number of bacteria (cfu mL^{-1}) in solutions during cavitation experiments

Day 1 (log transformed data)

Source of variation	df	Sum of Squares	Mean Square	*F*-value	P-value
Treatment	2	8.976	4.488	104.262	<0.0001
Residual	27	1.162	0.043		

Day 3 (log transformed data)

Source of variation	df	Sum of Squares	Mean Square	*F*-value	P-value
Treatment	2	22.275	11.138	42.615	<0.0001
Residual	27	7.057	0.261		

Day 5 (quadratic root transformed data)

Source of variation	df	Sum of Squares	Mean Square	*F*-value	P-value
Treatment	2	3025.348	1512.674	32.818	<0.0001
Residual	27	1244.517	46.093		

Table 4.3: Regression results for relationship between AAE and number of bacteria (cfu mL^{-1}) in solutions

ANOVA table [AAE -v- bacteria (cfu mL^{-1})

Distilled water Day 1

Source of variation	df	Sum of Squares	Mean Square	*F*-value	P-value
Regression	1	131951.391	131951.391	0.851	0.3833
Residual	8	1240791.009	155098.876		

Regression coefficients [AAE -v- bacteria (cfu mL^{-1})]

Variable	Coefficient	Std error	Std coefficient	t-value	P-value
Intercept	715.140	263.631	715.140	2.662	0.0287
cfu mL^{-1}	-0.027	0.029	-0.310	-0.922	0.3833

Distilled water Day 3

Source of variation	df	Sum of Squares	Mean Square	*F*-value	P-value
Regression	1	12703818.326	12703818.326	0.823	0.3909
Residual	8	123532744.174	15441593.022		

Regression coefficients [AAE -v- bacteria (cfu mL^{-1})]

Variable	Coefficient	Std error	Std coefficient	t-value	P-value
Intercept	2740.498	1656.114	2740.498	1.655	0.1366
cfu mL^{-1}	0.007	0.008	0.305	0.907	0.3909

Distilled water Day 5

Source of variation	df	Sum of Squares	Mean Square	*F*-value	P-value
Regression	1	146368.289	146368.289	0.773	0.5409
Residual	1	189362.378	189362.378		

Regression coefficients [AAE -v- bacteria (cfu mL^{-1})]

Variable	Coefficient	Std error	Std coefficient	t-value	P-value
Intercept	1273.982	728.992	1273.982	1.748	0.3309
cfu mL^{-1}	-0.005	0.005	-0.660	-0.879	0.5409

Citric acid Day 1

Source of variation	df	Sum of Squares	Mean Square	*F*-value	P-value
Regression	1	49934.439	49934.439	6.160	0.0421
Residual	7	56745.561	8106.509		

Regression coefficients [AAE -v- bacteria (cfu mL^{-1})]

Variable	Coefficient	Std error	Std coefficient	t-value	P-value
Intercept	524.950	117.370	524.950	4.473	0.0029
cfu mL^{-1}	-0.115	0.046	-0.684	-2.482	0.0421

Citric acid Day 3

Source of variation	df	Sum of Squares	Mean Square	*F*-value	P-value
Regression	1	1225679.450	1225679.450	18.148	0.0037
Residual	7	472776.550	67539.507		

Regression coefficients [AAE -v- bacteria (cfu mL^{-1})]

Variable	Coefficient	Std error	Std coefficient	t-value	P-value
Intercept	400.750	101.409	400.750	3.952	0.0055
cfu mL^{-1}	0.001	0.000177	0.849	4.260	0.0037

Citric acid Day 5

Source of variation	df	Sum of Squares	Mean Square	*F*-value	P-value
Regression	1	63456.377	63456.377	0.916	0.3754
Residual	6	415498.498	69249.750		

Regression coefficients [AAE -v- bacteria (cfu mL^{-1})]

Variable	Coefficient	Std error	Std coefficient	t-value	P-value
Intercept	500.188	124.150	500.188	4.029	0.0069
cfu mL^{-1}	0.00004411	0.00004608	0.364	0.957	0.3754

DICA (Chlorine) Day 1

Source of variation	df	Sum of Squares	Mean Square	*F*-value	P-value
Regression	1	342225.806	342225.806	9.546	0.0149
Residual	8	286800.194	35850.024		

Regression coefficients [AAE -v- bacteria (cfu mL^{-1})]

Variable	Coefficient	Std error	Std coefficient	t-value	P-value
Intercept	743.065	202.618	743.065	3.667	0.0063
cfu mL^{-1}	-1.661	0.538	-0.738	-3.090	0.0149

DICA (Chlorine) Day 3

Source of variation	df	Sum of Squares	Mean Square	*F*-value	P-value
Regression	1	1052467.787	1052467.787	32.811	0.0004
Residual	8	256614.613	32076.827		

Regression coefficients [AAE -v- bacteria (cfu mL^{-1})]

Variable	Coefficient	Std error	Std coefficient	t-value	P-value
Intercept	-47.960	84.520	-47.960	-0.567	0.5860
cfu mL^{-1}	0.166	0.029	0.897	5.728	0.0004

DICA (Chlorine) Day 5

Source of variation	df	Sum of Squares	Mean Square	*F*-value	P-value
Regression	1	144347.975	144347.975	0.620	0.4538
Residual	8	1863132.125	232891.516		

Regression coefficients [AAE -v- bacteria (cfu mL^{-1})]

Variable	Coefficient	Std error	Std coefficient	t-value	P-value
Intercept	955.138	307.133	955.138	3.110	0.0144
cfu mL^{-1}	-0.059	0.075	-0.268	-0.787	0.4538

Chapter 5

Table 5.1: ANOVA table for *A. amoena* vase life (study no. 1)

(untransformed data)

Source of variation	df	Sum of Squares	Mean Square	*F*-value	P-value
Treatment	2	32.267	16.133	11.869	0.0002
Residual	27	36.700	1.359		

Fig. 5.1: ANOVA tables for solution uptake in *A. amoena*

Day 0-1 (untransformed data)

Source of variation	df	Sum of Squares	Mean Square	*F*-value	P-value
Treatment	1	3.303	3.303	0.005	0.9464
Residual	18	12773.570	709.643		

Day 1-2 (untransformed data)

Source of variation	df	Sum of Squares	Mean Square	*F*-value	P-value
Treatment	1	8171.363	8171.363	19.547	0.0003
Residual	18	7524.606	418.034		

Day 2-3 (quadratic root transformed data)

Source of variation	df	Sum of Squares	Mean Square	*F*-value	P-value
Treatment	1	4.440	4.440	40.629	<0.0001
Residual	18	1.967	0.109		

Day 3-4 (untransformed data)

Source of variation	df	Sum of Squares	Mean Square	*F*-value	P-value
Treatment	1	1006.242	1006.242	2.961	0.1133
Residual	11	3738.287	339.844		

Day 4-5 (untransformed data)

Source of variation	df	Sum of Squares	Mean Square	*F*-value	P-value
Treatment	1	58.864	58.864	2.429	0.1578
Residual	8	193.898	24.237		

Fig. 5.2: ANOVA tables for transpiration in *A. amoena*

Day 0-1 (untransformed data)

Source of variation	df	Sum of Squares	Mean Square	*F*-value	P-value
Treatment	2	1525.058	762.529	1.649	0.2110
Residual	27	12486.052	462.446		

Day 1-2 (cubic root transformed data)

Source of variation	df	Sum of Squares	Mean Square	*F*-value	P-value
Treatment	2	2.772	1.386	8.208	0.0016
Residual	27	4.559	0.169		

Day 2-3 (untransformed data)

Source of variation	df	Sum of Squares	Mean Square	*F*-value	P-value
Treatment	2	9450.408	4725.204	15.714	<0.0001
Residual	27	8118.954	300.702		

Day 3-4 (untransformed data)

Source of variation	df	Sum of Squares	Mean Square	*F*-value	P-value
Treatment	2	1390.808	695.404	3.337	0.562
Residual	20	4167.818	208.391		

Day 4-5 (untransformed data)

Source of variation	df	Sum of Squares	Mean Square	*F*-value	P-value
Treatment	2	98.917	49.459	2.173	0.1507
Residual	14	318.710	22.765		

Day 5-6 (square root transformed data)

Source of variation	df	Sum of Squares	Mean Square	*F*-value	P-value
Treatment	1	8.317	8.317	38.962	<0.0001
Residual	10	2.135	0.213		

Day 6-7 (untransformed data)

Source of variation	df	Sum of Squares	Mean Square	*F*-value	P-value
Treatment	1	164.873	164.873	3.795	0.1232
Residual	4	173.756	43.439		

Fig. 5.3: ANOVA tables for time series analysis of dissolved oxygen concentration in *A. amoena* vase life expt

Two-factor repeated measures, days 1-3, all treatments:

Source of variation	df	Sum of Squares	Mean Square	*F*-value	P-value
Treatments (A)	2	89.602	44.801	27.586	0.0001
Subjects w.groups	27	43.848	1.624		
Repeated Measure (B)	2	6.635	3.317	7.842	0.001
AB	4	21.456	5.364	12.681	0.0001
B x subjects w.groups	54	22.842	0.423		

Treatments (A) are significantly different. Time (B) is significant. The interaction of (A) and (B) is significant.

ANOVA tables for differences in oxygen concentration between treatments:

Day 1 (untransformed data)

Source of variation	df	Sum of Squares	Mean Square	*F*-value	P-value
Treatment	2	46.483	23.242	69.062	0.0001
Residual	27	9.086	0.337		

Day 2 (untransformed data)

Source of variation	df	Sum of Squares	Mean Square	*F*-value	P-value
Treatment	2	38.097	19.049	31.388	0.0001
Residual	27	16.386	0.607		

Day 3 (untransformed data)

Source of variation	df	Sum of Squares	Mean Square	*F*-value	P-value
Treatment	2	26.477	13.239	8.672	0.0012
Residual	27	41.218	1.527		

ANOVA table (oxygen concentration and longevity)

Source of variation	df	Sum of Squares	Mean Square	*F*-value	P-value
Regression	1	16.672	16.672	8.926	0.006
Residual	28	52.295	1.868		

Regression coefficients (oxygen concentration and longevity)

Variable	Coefficient	Std error	Std coefficient	t-value	P-value
Intercept	7.705	0.950	7.705	8.111	0.000
Average O_2 conc.	-0.636	0.213		-2.988	0.006

Longevity = 7.705 (8.111) - 0.636 (-2.988) (ave O_2 conc). Adjusted $R^2 = 0.215$.

Fig. 5.5: Stomatal diffusive resistance in light

Time 4 h:

Source of variation	df	Sum of Squares	Mean Square	*F*-value	P-value
Regression	1	182.125	182.125	0.404	0.5429
Residual	8	3608.431	451.054		

Time 28 h:

Source of variation	df	Sum of Squares	Mean Square	*F*-value	P-value
Regression	1	60899.954	60899.954	28.812	0.0001
Residual	18	38046.815	2113.712		

Time 53 h:

Source of variation	df	Sum of Squares	Mean Square	*F*-value	P-value
Regression	1	22219.577	22219.577	71.6	0.0001
Residual	8	2482.639	310.33		

Time 74 h:

Source of variation	df	Sum of Squares	Mean Square	*F*-value	P-value
Regression	1	12339.164	12339.164	15.929	0.004
Residual	8	6197.131	774.641		

Fig. 5.6: Stomatal diffusive resistance in darkness

Time 5 h:

Source of variation	df	Sum of Squares	Mean Square	*F*-value	P-value
Regression	1	191.526	191.526	0.231	0.6438
Residual	8	6639.235	829.904		

Time 27.5 h:

Source of variation	df	Sum of Squares	Mean Square	*F*-value	P-value
Regression	1	45396.997	4536.997	0.877	0.3614
Residual	18	931636.211	51757.567		

Time 55 h:

Source of variation	df	Sum of Squares	Mean Square	*F*-value	P-value
Regression	1	372.398	372.398	1.611	0.2400
Residual	8	1848.886	231.111		

Time 77 h:

Source of variation	df	Sum of Squares	Mean Square	*F*-value	P-value
Regression	1	72.641	72.641	0.049	0.8305
Residual	8	11874.770	1484.346		

Table 5.2: *A. amoena* xylem water potentials at end of diffusive resistance experiments

Light condition (distilled water and citric acid, 10 mol m^{-3})

Source of variation	df	Sum of Squares	Mean Square	*F*-value	P-value
Regression	1	11.078	11.078	13.152	0.0067
Residual	8	6.738	0.842		

Darkness condition (distilled water and citric acid, 10 mol m^{-3})

Source of variation	df	Sum of Squares	Mean Square	*F*-value	P-value
Regression	1	16.577	16.577	52.593	<0.0001
Residual	8	2.521	0.315		

Fig. 5.9: Distance travelled by paint particles in distilled water and in citric acid (10 mol m^{-3})

Unpaired t-test

df	Unpaired t-value	Probability (1-tail)
8	-5.687	0.0002

Chapter 6

Table 6.1: ANOVA table for *B. heterophylla* vase life (study no. 1)

(log transformed data)

Source of variation	df	Sum of Squares	Mean Square	*F*-value	P-value
Treatment	8	9.592	1.199	26.173	<0.0001
Residual	81	3.710	0.046		

Table 6.2: ANOVA table for *B. heterophylla* vase life (study no. 2)

(log transformed data)

Source of variation	df	Sum of Squares	Mean Square	*F*-value	P-value
Treatment	8	4.773	0.597	7.240	<0.0001
Residual	36	2.967	0.082		

Fig. 6.10: ANOVA table for number of bacteria in *B. heterophylla* vase solutions after 11 d (study no. 1)

(log transformed data)

Source of variation	df	Sum of Squares	Mean Square	*F*-value	P-value
Treatment	8	212.739	26.592	99.018	<0.0001
Residual	79	21.216	0.269		

Fig. 6.12: Regression results for relationship between vase life and number of bacteria in vase solutions, *B. heterophylla* (study no. 1)

ANOVA table (log vase life -v- log cfu mL ^{1})

Source of variation	df	Sum of Squares	Mean Square	*F*-value	P-value
Regression	1	0.005	0.005	0.041	0.8406
Residual	86	10.817	0.126		

Regression coefficients (log vase life -v- log cfu mL^{-1})

Variable	Coefficient	Std error	Std coefficient	t-value	P-value
Intercept	1.834	0.175	1.834	10.458	<0.0001
cfu mL^{-1}	0.005	0.023	0.022	0.202	0.8406

Table 6.3: ANOVA tables for bacterial numbers in vase solutions during *B. heterophylla* vase life experiment (study no. 1)

Day 0 (untransformed data)

Source of variation	df	Sum of Squares	Mean Square	*F*-value	P-value
Treatment	8	1637.550	204.694	0.067*	0.9996
Residual	9	27393.030	3043.670		

* Since 0.067 < 3.23 (from a table of critical values for *F*-distribution, P < 0.05), do not reject H_0 and do not proceed with a multiple comparisons procedure.

Day 3 (cubic root transformed data)

Source of variation	df	Sum of Squares	Mean Square	*F*-value	P-value
Treatment	8	89756.745	11219.593	38.874	<0.0001
Residual	36	10390.033	288.612		

Day 6 (quadratic root transformed data)

Source of variation	df	Sum of Squares	Mean Square	*F*-value	P-value
Treatment	8	665.619	83.202	10.080	<0.0001
Residual	36	297.165	8.255		

Day 9 (quadratic root transformed data)

Source of variation	df	Sum of Squares	Mean Square	*F*-value	P-value
Treatment	8	836.453	104.557	4.519	0.0007
Residual	36	832.850	23.135		

Day 12 (square root transformed data)

Source of variation	df	Sum of Squares	Mean Square	*F*-value	P-value
Treatment	8	6766396.081	845799.510	34.637	<0.0001
Residual	36	879073.068	24418.696		

Table 6.4: t-values from paired t-tests of bacterial numbers in stem segments, *B. heterophylla* vase life experiment (study no. 2)

Treatment 1, 8-HQC (reciprocal of the square root transformed data)

Variable:	0 to 5 cm segment	5 to 10 cm segment
Mean	0.43	0.60
Std Deviation	0.52	0.36
Paired observations	5	
t-statistic	-0.45	
Degrees of Freedom	4	
Significance	0.675	

The critical value of t for 4 df is 2.776 (P< 0.05), therefore there is no significant difference between stem segment lengths because the t-statistic (0.45) is < the critical t-value.

Treatment 2, Distilled water (log transformed data)

Variable:	0 to 5 cm segment	5 to 10 cm segment
Mean	10.06	1.47
Std Deviation	1.24	0.39
Paired observations	5	
t-statistic	13.32	
Degrees of Freedom	4	
Significance	0.000	

The critical value of t for 4 df is 2.776 (P< 0.05), therefore there is a significant difference between stem segment lengths because the t-statistic (13.32) is > the critical t-value.

Treatment 8, STS pulse, then into distilled water (reciprocal of the square root transformed data)

Variable:	0 to 5 cm segment	5 to 10 cm segment
Mean	0.18	0.42
Std Deviation	0.28	0.08
Paired observations	3	
t-statistic	-1.98	
Degrees of Freedom	2	
Significance	0.186	

The critical value of t for 2 df is 4.303 (P< 0.05), therefore there is no significant difference between stem segment lengths because the t-statistic (1.98) is < the critical t-value.

Treatment 9, citric acid (1.43 mol m^{-3}) (square root transformed data)

Variable:	0 to 5 cm segment	5 to 10 cm segment
Mean	1018.02	76.94
Std Deviation	135.22	72.30
Paired observations	5	
t-statistic	11.67	
Degrees of Freedom	4	
Significance	0.000	

The critical value of t for 4 df is 2.776 (P< 0.05), therefore there is a significant difference between stem segment lengths because the t-statistic (11.67) is > the critical t-value.

Table 6.5: t-values and chi-square (χ^2) test of independence for *Boronia* spp. vase life

t-values for *B. muelleri* 'Sunset Serenade' vase life

(untransformed data)

Variable:	+ STS pulse	Distilled water only
Mean	8.5	8.8
Std Deviation	3.89	2.82
Paired observations	10	10
t-statistic	-0.20	
Degrees of Freedom	18	
Significance	0.846	

The critical value of t for 18 df is 2.101 (P< 0.05), therefore there is no significant difference between treatments because the t-statistic (0.20) is < the critical t-value.

A 2 × 2 contingency table for the vase life of *B. crassipes* × *B. heterophylla* 'Lipstick' in T1 [STS pulse (0.5 mol m^{-3} for 10.5 h), then into distilled water], and T2 (control: distilled water only)
(Observed and expected frequencies - expected frequences in brackets)

	Vase life (d)		
	<7	≥7	Totals
T1	2 (3.5)	8 (6.5)	10 (10)
T2	5 (3.5)	5 (6.5)	10 (10)
Totals	7 (7)	13 (13)	20 (20)

The χ^2 value = 1.978, which is < 3.84 (from the χ^2 distribution table, P < 0.05, df = 1) and therefore is not significant. However, because some expected values in the contingency table are <5, Fisher's Exact Test of Independence is also computed. The p-value for Fisher's Exact Test is 0.3498, therefore H_0 that the data fit the theoretical distribution is rejected, and the two populations are assumed to be significantly different. (Fisher's Exact Test is a more accurate measure than the χ^2 value, I. Davies, pers. comm. 1995.)

t-values for *B. clavata* vase life

(untransformed data)

Variable:	+ STS pulse	Distilled water only
Mean	11.80	10.20
Std Deviation	4.42	2.49
Paired observations	10	10
t-statistic	1.00	
Degrees of Freedom	18	
Significance	0.331	

The critical value of t for 18 df is 2.101 (P< 0.05), therefore there is no significant difference between treatments because the t-statistic (1.00) is < the critical t-value.

Chapter 7

Table 7.2: ANOVA table for RWC of *A. amoena* stems at end of all cavitation experiments

(untransformed data)

Source of variation	df	Sum of Squares	Mean Square	*F*-value	P-value
Treatment	5	17413.212	3482.642	24.099	<0.0001
Residual	51	7370.132	144.512		

Chapter 9

Fig. 9.4: t-values for hydraulic conductivity of *A. amoena* stems in either distilled water or citric acid (10 mol m^{-3}). Separate t-tests were performed for data at each 12 h period.

Variable:	Citric, 12 h	Distilled H_2O, 12 h
Mean	2.7375E-7	2.0431E-7
Std Deviation	1.8788E-7	6.6547E-8
Observations	5	5
t-statistic	0.78	
Degrees of Freedom	8	
Significance	0.458	

The critical value of t for 8 df is 2.305 (P< 0.05), therefore there is no significant difference between treatments because the t-statistic (0.78) is < the critical t-value.

Variable:	Citric, 24 h	Distilled H_2O, 24 h
Mean	1.8943E-7	9.4754E-8
Std Deviation	1.2022E-7	4.458E-8
Observations	5	5
t-statistic	1.65	
Degrees of Freedom	8	
Significance	0.137	

The critical value of t for 8 df is 2.305 (P< 0.05), therefore there is no significant difference between treatments because the t-statistic (1.65) is < the critical t-value.

Square root transformed data:

Variable:	Citric, 36 h	Distilled H_2O, 36 h
Mean	0.00039049	0.00026533
Std Deviation	0.00010432	4.5202E-5
Observations	5	5
t-statistic	2.46	
Degrees of Freedom	8	
Significance	0.039	

The critical value of t for 8 df is 2.305 (P< 0.05), therefore there is a significant difference between treatments because the t-statistic (2.46) is > the critical t-value.

Variable:	Citric, 48 h	Distilled H_2O, 48 h
Mean	1.3727E-7	5.9891E-8
Std Deviation	8.2051E-8	3.0804E-8
Observations	5	5
t-statistic	1.97	
Degrees of Freedom	8	
Significance	0.084	

The critical value of t for 8 df is 2.305 (P< 0.05), therefore there is no significant difference between treatments because the t-statistic (1.97) is < the critical t-value.

Variable:	Citric, 60 h	Distilled H_2O, 60 h
Mean	1.2921E-7	6.0894E-8
Std Deviation	6.6969E-8	2.5507E-8
Observations	5	5
t-statistic	2.13	
Degrees of Freedom	8	
Significance	0.066	

The critical value of t for 8 df is 2.305 (P< 0.05), therefore there is no significant difference between treatments because the t-statistic (2.13) is < the critical t-value.

Square root transformed data:

Variable:	Citric, 72 h	Distilled H_2O, 72 h
Mean	0.00031996	0.00023733
Std Deviation	9.4757E-5	3.4974E-5
Observations	5	5
t-statistic	1.83	
Degrees of Freedom	8	
Significance	0.105	

The critical value of t for 8 df is 2.305 (P< 0.05), therefore there is no significant difference between treatments because the t-statistic (1.83) is < the critical t-value.

Variable:	Citric, 84 h	Distilled H_2O, 84 h
Mean	1.1281E-7	6.6358E-8
Std Deviation	5.7374E-8	2.8986E-8
Observations	5	5
t-statistic	1.62	
Degrees of Freedom	8	
Significance	0.145	

The critical value of t for 8 df is 2.305 (P< 0.05), therefore there is no significant difference between treatments because the t-statistic (1.62) is < the critical t-value.

Variable:	Citric, 96 h	Distilled H_2O, 96 h
Mean	1.1271E-7	6.5579E-8
Std Deviation	5.7168E-8	2.8503E-8
Observations	5	5
t-statistic	1.44	
Degrees of Freedom	8	
Significance	0.187	

The critical value of t for 8 df is 2.305 (P< 0.05), therefore there is no significant difference between treatments because the t-statistic (1.44) is < the critical t-value.

Variable:	Citric, 108 h	Distilled H_2O, 108 h
Mean	1.1432E-7	7.4432E-8
Std Deviation	5.2576E-8	4.1014E-8
Observations	5	5
t-statistic	1.19	
Degrees of Freedom	8	
Significance	0.267	

The critical value of t for 8 df is 2.305 (P< 0.05), therefore there is no significant difference between treatments because the t-statistic (1.19) is < the critical t-value.

Variable:	Citric, 120 h	Distilled H_2O, 120 h
Mean	1.0777E-7	7.5432E-8
Std Deviation	5.5939E-8	3.7212E-8
Observations	5	5
t-statistic	0.96	
Degrees of Freedom	8	
Significance	0.368	

The critical value of t for 8 df is 2.305 (P< 0.05), therefore there is no significant difference between treatments because the t-statistic (0.96) is < the critical t-value.

APPENDIX D

INTEGRATOR REPORTS FROM ETHYLENE DETECTION IN *ACACIA* USING FLAME IONISATION DETECTION GAS CHROMATOGRAPHY

The reports of ethylene analysed by a gas chromatograph integrator (Model HP 3396A, Hewlett-Packard Company, Avondale, PA, USA) appear below. These reports are from vase life experiments of *Acacia* exposed to exogenous ethylene for 24 h (described in Chapter 3). The reports have been produced as "Area %" reports, which is the area of a peak expressed as a percentage of the total area accumulated during the run. Abbreviations which appear in the report and their explanations are listed below (from HP 3396A Operating Manual, Hewlett-Packard Company, Avondale, PA, USA, 1987).

RT — The retention time/s of the peak/s in the run, in order of increasing retention time. Retention time is the unique identifier for a peak.

AREA — The area in counts for each peak. One count is 0.125 μV-sec. The area given is the final area after baseline determination and/or correction have been done.

TYPE — Up to four characters which indicate how the peak detection and quantitation processed the peak. The TYPE provides information about baseline construction. All the HP 3396A peak type codes encountered are listed below.

WIDTH — During the run, the integrator computes the width of every peak in the run and reports the results. Width is computed in units of decimal minutes using the determined height and area. Width = Area/(Height × 60).

AREA% — The area of a peak as a percentage of the total height accumulated during the run.

Codes for Peak Types, Baseline Codes (B, V, P, H):

Baseline codes indicate how a peak starts and ends and how the chromatographic baseline is constructed.

B (Baseline) — Peak begins or ends on baseline.

V (Valley point) — A valley point occurs when a peak begins before the previous peak ends by returning to baseline.

P (Baseline penetration) — Baseline is penetrated, then reset to the lowest point at the beginning or end of the peak.

H (Horizontal baseline) — Baseline is horizontal and extends from the last declared baseline point.

I (Incomplete) — The peak ends prematurely, after its apex but before the signal returned to baseline, valley, or tangent points.

The settings used on the chart recorder were:
Attenuation 1; Chart Speed 1.0; Area Reject 100; Threshold 0; Peak Width 0.04.

Test for retention time and detection of pure ethylene (50 μL L^{-1})

```
*  RUN #     3       JAN   1, 1901   00:08:45
START
                  1.390
                  STOP


RUN#      3             JAN   1, 1901   00:08:45

AREA%
        RT        AREA TYPE   WIDTH        AREA%
      .390       13468   PB    .210   100.00000

TOTAL AREA=   13468
MUL FACTOR=1.0000E+00
```

Test for ethylene detection in an empty sealed experimental jar to determine whether plastic lids and seals emitted detectable ethylene

Time 0 h

```
*  RUN #    11       JAN   1, 1901   01:05:28
START
STOP


RUN#     11             JAN   1, 1901   01:05:28

NO RUN PEAKS STORED
```

Time 24 h

```
*  RUN #    12       JAN   1, 1901   03:29:34
START
STOP


RUN#     10             JAN   1, 1901   00:29:34

NO RUN PEAKS STORED
```

Test for ethylene detection in senescing carnation flowers (*Dianthus caryophyllus*) to determine whether ethylene could be detected in senescing flowers known to produce ethylene

Time 0 h

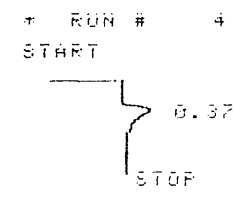
RUN# 3 JAN 1, 1901 00:50:19

AREA%

RT	AREA	TYPE	WIDTH	AREA%
.388	.262	BV	.204	100.00000

TOTAL AREA= .262
MUL FACTOR=1.0000E+00

Time 24 h



RUN# 4 JAN 1, 1901 00:13:37

AREA%

RT	AREA	TYPE	WIDTH	AREA%
.379	4145	PV	.181	100.00000

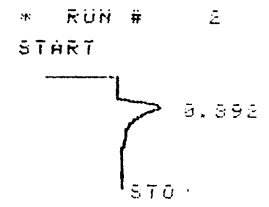
TOTAL AREA= 4145
MUL FACTOR=1.0000E+00

Sensitivity of *Acacia subulata* to 50 µL L^{-1} exogenous ethylene
(Note: T = Treatment; R = Replicate; representative samples from each treatment only are shown)

T1: Distilled water; in an ethylene atmosphere of 50 µL L^{-1};
T2: Distilled water; without added ethylene; and
T3: STS pulse (0.5 mol m^{-3}) for 12 h, then distilled water; in an ethylene atmosphere of 50 µL L^{-1}.

Time 0 h

T1, R1



RUN# 2 JAN 1, 1901 00:37:34

AREA%

RT	AREA	TYPE	WIDTH	AREA%
.392	82.4	PB	.232	100.00000

TOTAL AREA: 82.4
MUL FACTOR:1.0000E+00

T2, R1

RUN# 8 JAN 1, 1901 00:56:37

NO RUN PEAKS STORED

T3, R1

```
*  RUN #     5      JAN  1, 1901  00:47:20
START
          0.360
STOP


RUN#      5            JAN  1, 1901  00:47:20

AREA%
       RT        AREA TYPE  WIDTH       AREA%
     .360        8126   PV   .211   100.00000

TOTAL AREA=    8126
MUL FACTOR=1.0000E+00
```

Time 24 h

T1, R1

```
*  RUN #     1      JAN  1, 1901  00:03:25
START
          0.390
STOP


RUN#      1            JAN  1, 1901  00:03:25

AREA%
       RT        AREA TYPE  WIDTH       AREA%
     .390        5547   VV   .232   100.00000

TOTAL AREA=    5547
MUL FACTOR=1.0000E+00
```

T2, R1

```
*  RUN #     2      JAN  1, 1901  00:20:04
START


STOP


RUN#      2            JAN  1, 1901  00:20:04

NO RUN PEAKS STORED
```

T3, R1

```
*  RUN #     4       JAN  1, 1901  00:11:21
START
                  0.393
                  STOP


RUN#      4            JAN  1, 1901  00:11:21

AREA%
       RT       AREA TYPE  WIDTH       AREA%
     .393       4394   BV   .223   100.00000

TOTAL AREA=    4394
MUL FACTOR=1.0000E+00
```

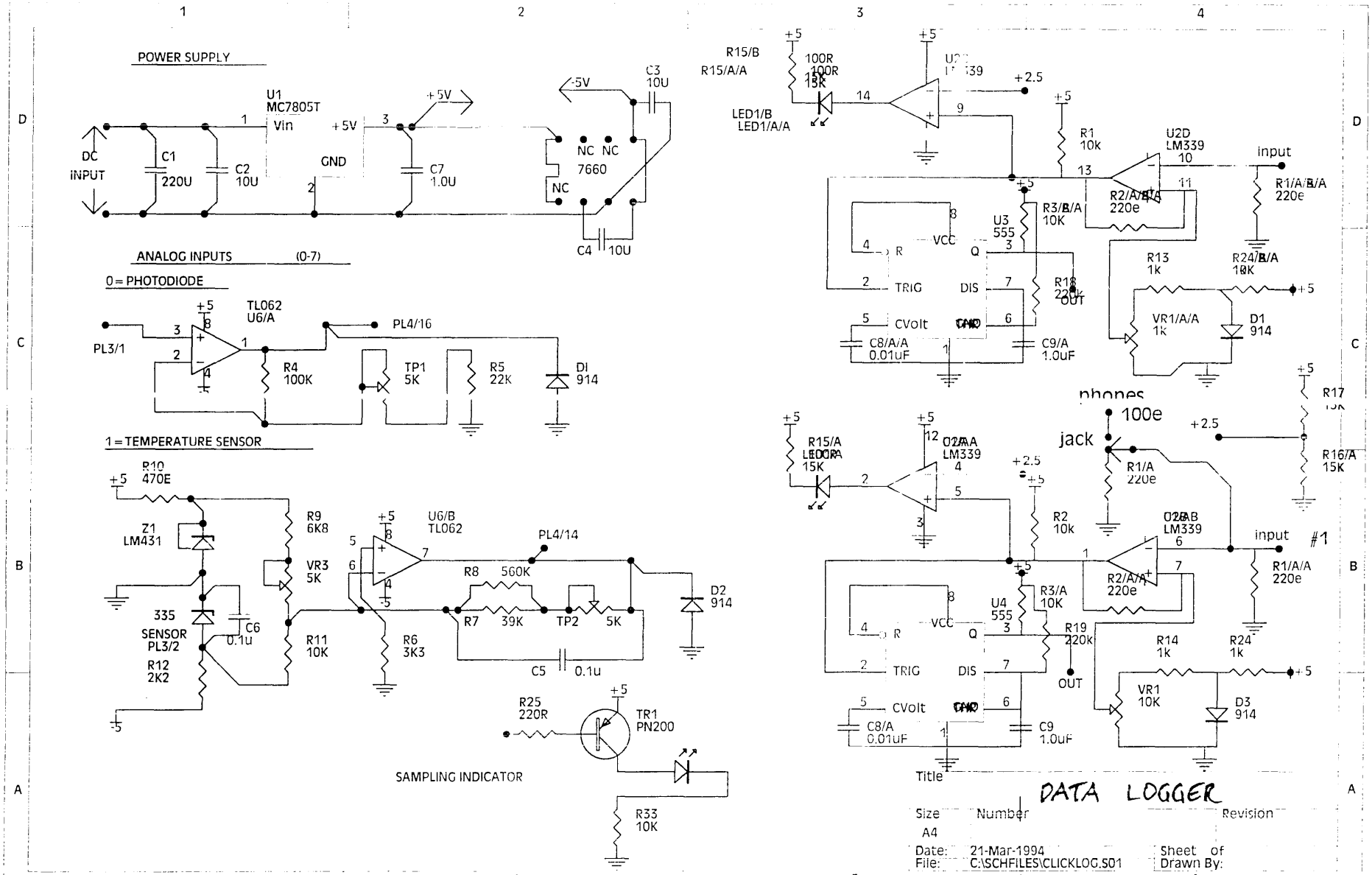
Test for retention time and detection of pure ethylene (500 µL L^{-1})

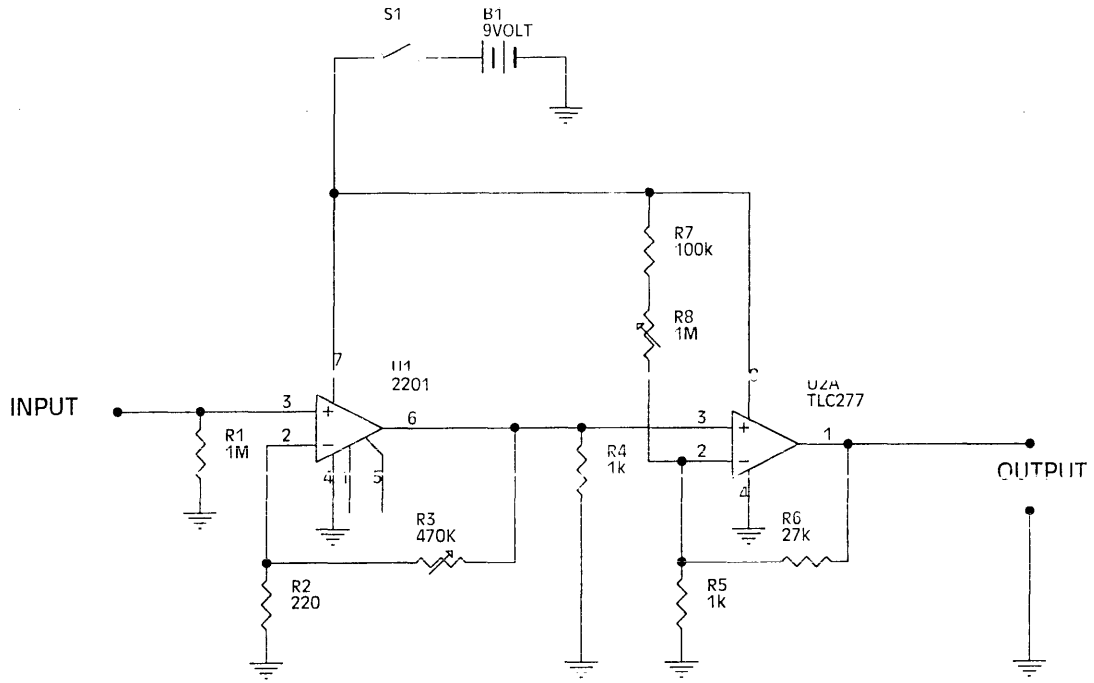
```
*  RUN #     5       JAN  1, 1901  01:08:30
START
                  0.392
                  STOP


RUN#      5            JAN  1, 1901  01:08:30

AREA%
       RT       AREA TYPE  WIDTH       AREA%
     .392      41152   PV   .230   100.00000

TOTAL AREA=   41152
MUL FACTOR=1.0000E+00
```

The integrator reports from the sensitivity of *Acacia floribunda* to 500 µL L^{-1} exogenous ethylene experiment are similar to those already shown above for *A. subulata*, except that the ethylene peaks are higher and consequently encompass a larger area. To save space, the reports are not shown here, but are available from the author upon request.

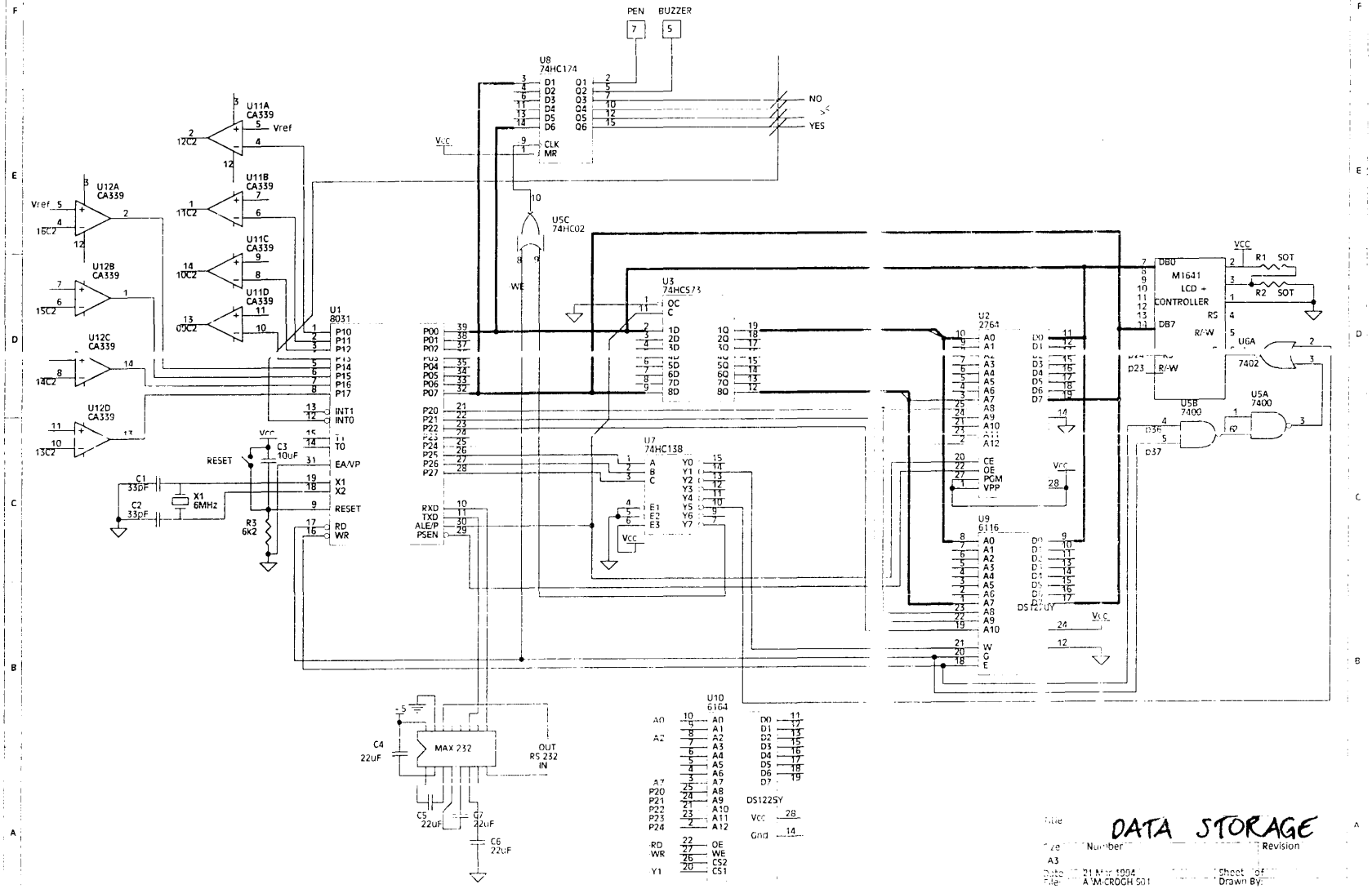
APPENDIX E

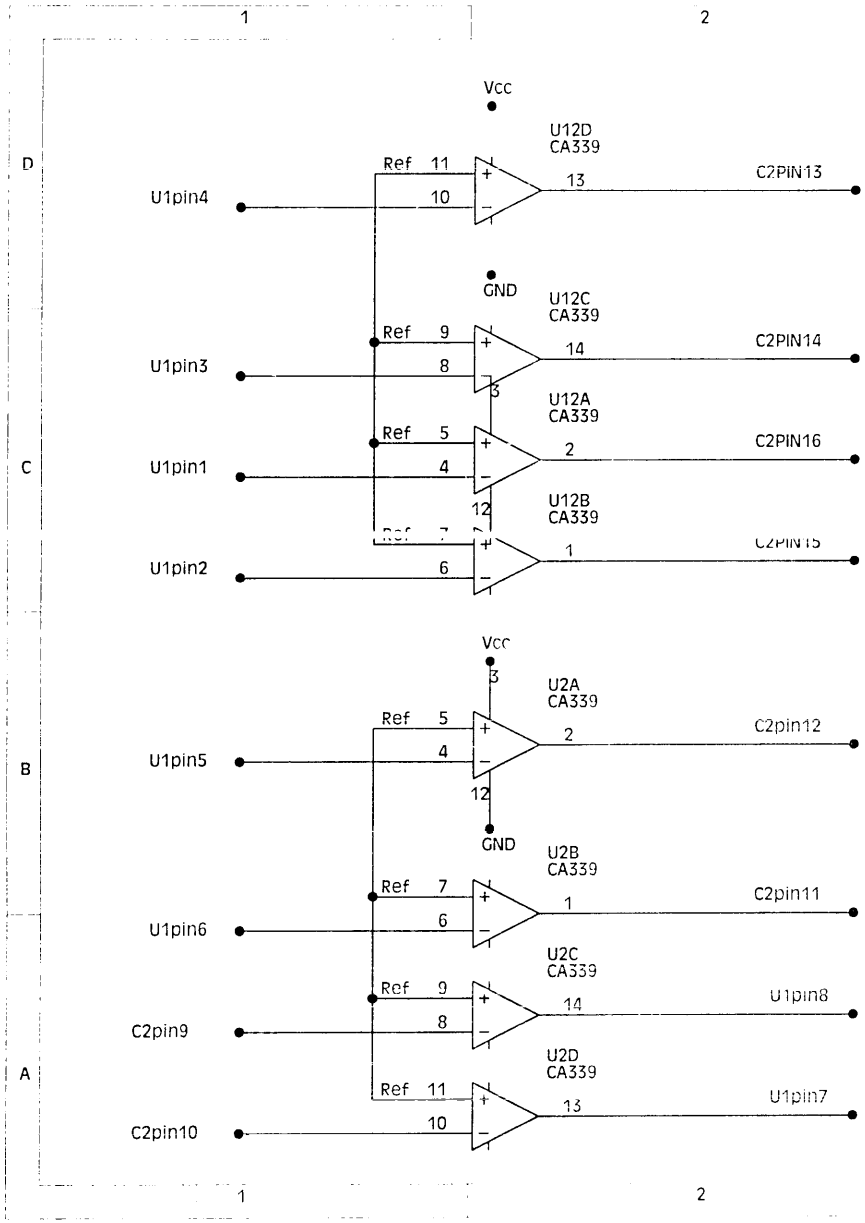
CIRCUIT DIAGRAMS OF DATA LOGGER AND PREAMPLIFIERS


POWER SUPPLY
U1
MC7805T
Vin
+5V
GND
DC
INPUT
C1
220U
C2
10U
C3
10U
-5V
NC NC
7660
NC
C4
10U
C7
1.0U
ANALOG INPUTS (0-7)
0 = PHOTODIODE
TL062
U6/A
PL3/1
PL4/16
R4
100K
TP1
5K
R5
22K
DI
914
1 = TEMPERATURE SENSOR
R10
470E
Z1
LM431
R9
6K8
VR3
5K
335
SENSOR
PL3/2
R12
2K2
C6
0.1u
R11
10K
U6/B
TL062
R8
560K
R7
39K
TP2
5K
R6
3K3
PL4/14
D2
914
C5
0.1u
R25
220R
TR1
PN200
R33
10K
SAMPLING INDICATOR
R15/B
R15/A/A
LED1/B
LED1/A/A
100R
15K
U2
LM339
+2.5
R1
10k
U2D
LM339
10
input
R1/A/B/A
220e
R3/B/A
10K
U3
555
R2/A/B/A
220e
R13
1k
R24/B/A
10K
R18
220k
OUT
VR1/A/A
1k
D1
914
C8/A/A
0.01uF
C9/A
1.0uF
phones
100e
jack
R17
15K
R16/A
15K
R15/A
15K
U2AA
LM339
R1/A
220e
R2
10k
U2AB
LM339
input
#1
R1/A/A
220e
R3/A
10K
U4
555
R2/A/A
220e
R19
220k
R14
1k
R24
1k
VR1
10K
D3
914
C8/A
0.01uF
C9
1.0uF
Title
DATA LOGGER
Size
A4
Number
Revision
Date: 21-Mar-1994
File: C:\SCHFILES\CLICKLOG.SCH
Sheet of
Drawn By:



Title	BLACK BOX	
Size A4	Number	Revision
Date: 21-Mar-1994	Sheet 4 of	
File: A:\BLACKBOX.S01	Drawn By:	


PEN
BUZZER
U8
74HC174
NO
YES
U11A
CA339
Vref
U11B
CA339
U11C
CA339
U11D
CA339
U12A
CA339
U12B
CA339
U12C
CA339
U12D
CA339
U5C
74HC02
WE
U3
74HC573
U1
8031
P10
P11
P12
P14
P15
P16
P17
INT1
INT0
T0
EA/VP
X1
X2
RESET
RD
WR
RXD
TXD
ALE/P
PSEN
C1
33pF
C2
33pF
X1
6MHz
C3
10uF
R3
6k2
U7
74HC138
U2
2764
U9
6116
DS1225Y
M1641
LCD +
CONTROLLER
DB0
DB7
RS
R/W
E
R1 50T
R2 50T
U6A
7402
U5B
7400
U5A
7400
D36
D37
P23
R/W
U10
6164
DS1225Y
MAX 232
C4
22uF
C5
22uF
C7
22uF
C6
22uF
OUT
RS 232
IN
Vcc
Gnd
DATA STORAGE
Number
Revision
A3
Sheet of
Drawn By:



Title DATA STORAGE

Size A4 Number Revision

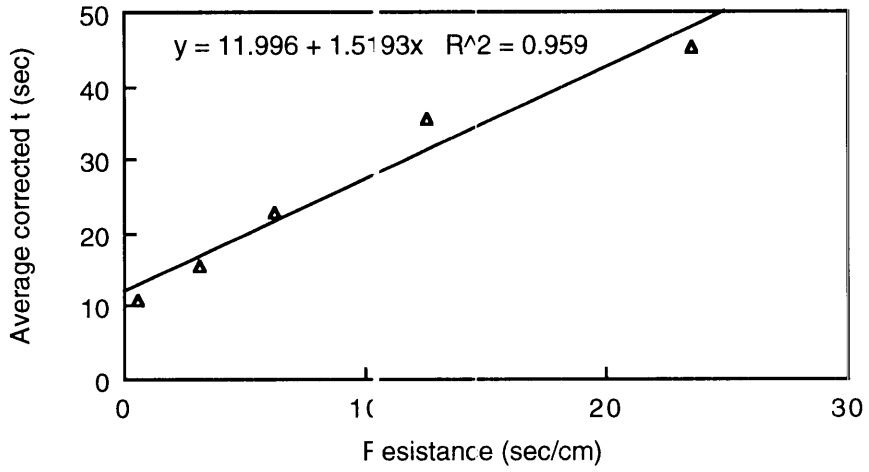
Date: 21-Mar-1994 Sheet of

File: A:\MICRO1_8.S01 Drawn By:

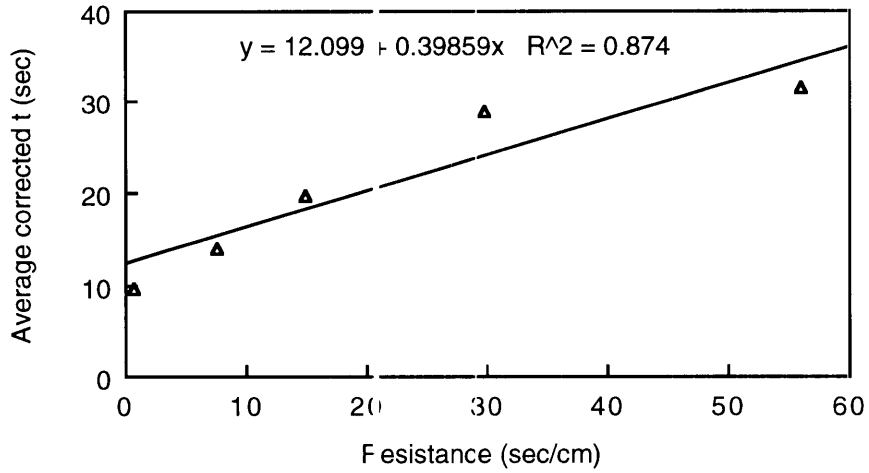
APPENDIX F

CALIBRATION CURVES FOR DIFFUSIVE RESISTANCE POROMETRY

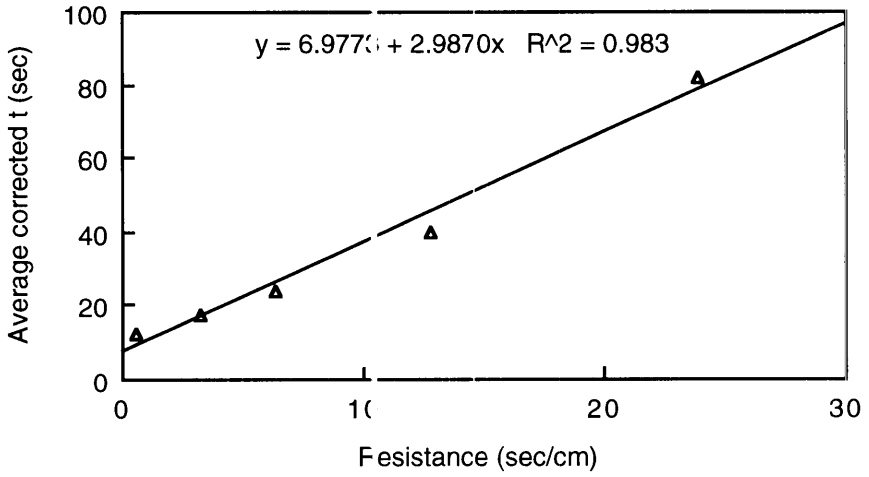
Calibration in darkness, 11.3.93:



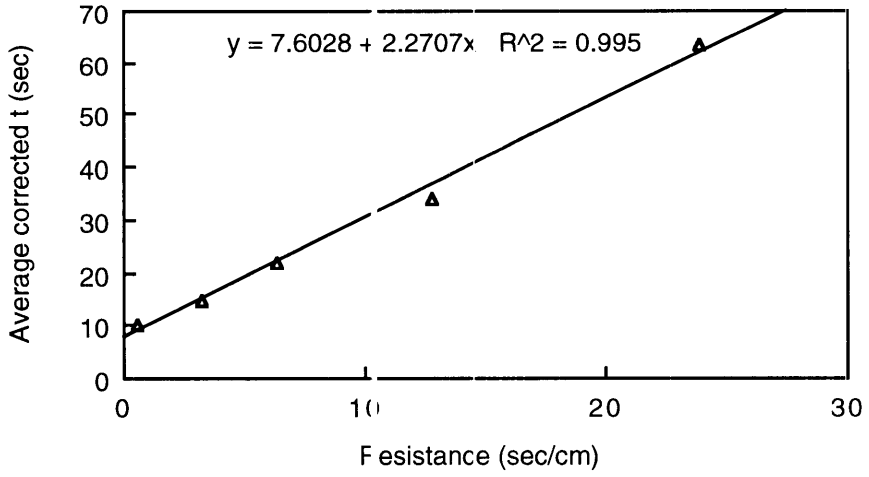
Calibration in darkness, 12.3.93:



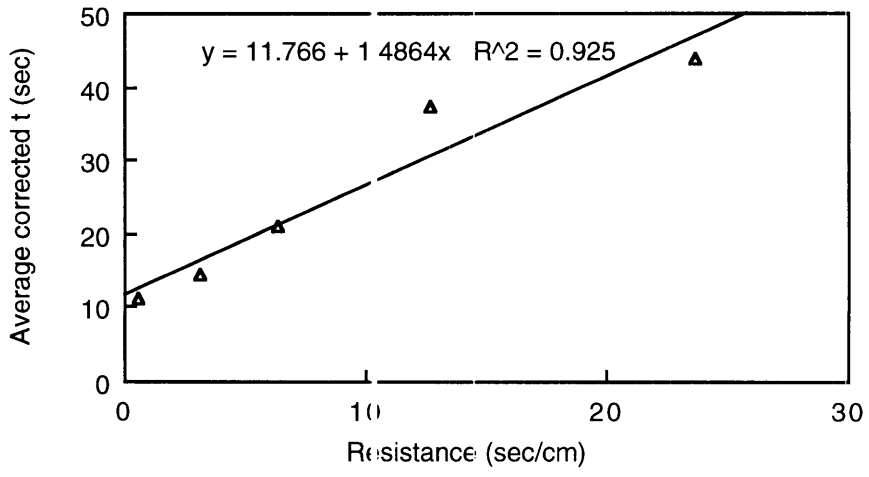
Calibration in darkness, 13.3.93:



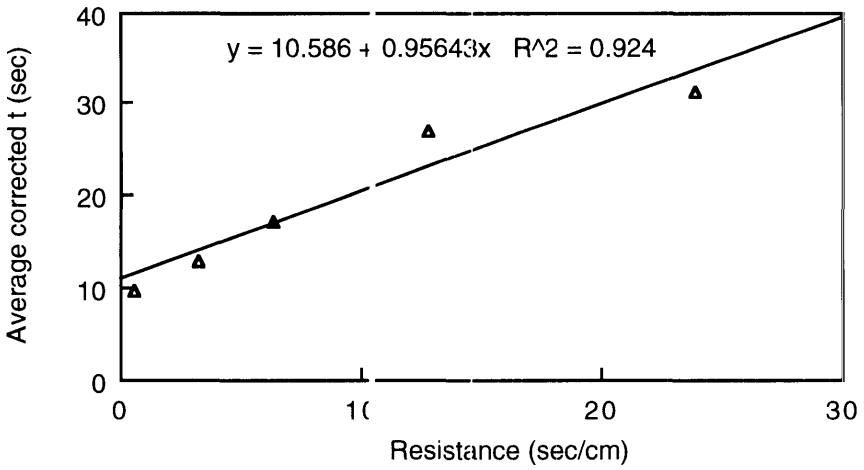
Calibration in darkness, 14.3.93:



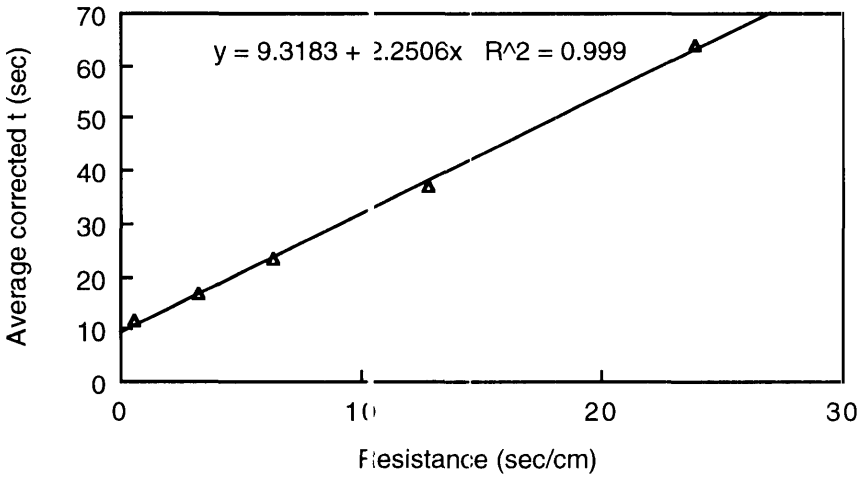
Calibration in light, 11.3.93:



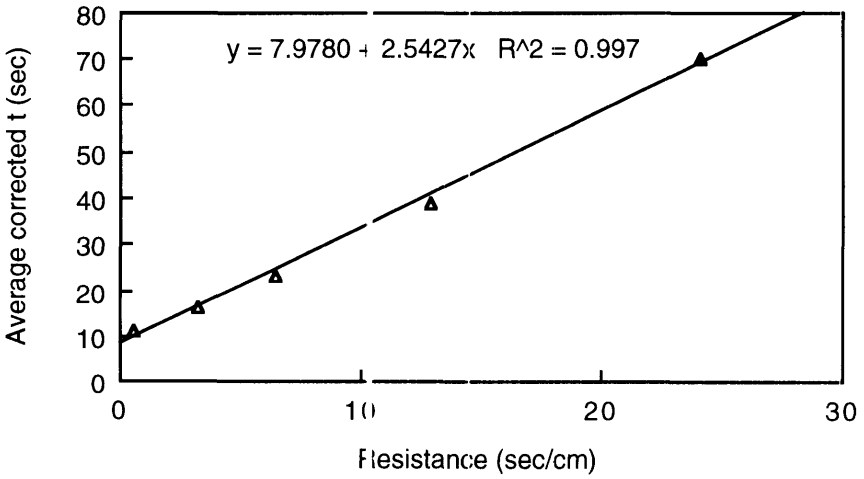
Calibration in light, 12.3.93:



Calibration in light, 13.3.93:



Calibration in light, 14.3.93:



APPENDIX G

RESULTS OF FIRST- AND SECOND-STAGE BACTERIAL IDENTIFICATION TABLE TESTS

Bacteria were isolated from either the vase water or the stems of cut *Rosa hybrida* L. 'Sonia' on days 3 and 6 of vase life. Listed below are results of the first- and second-stage identification table tests (Cowan 1974) performed by the author. The results in bold are the ones in which the isolates differ from each other. The preliminary identification available from the tables is given, however, a final and more accurate identification was obtained through fatty acid analysis (see Appendix H).

Note: + is a positive reaction
- is a negative reaction

SPECIMEN 1 (DAY 3 STEM)

From the test results below, this specimen was preliminarily identified as either *Pseudomonas fluorescens* or *Pseudomonas putida.*

Gram	negative rod
Catalase	+
Oxidase	+
Motility	+
Slime production	+
Fluorescence in uv light	+
Growth at 5° C	+
Growth at 42° C	-
Citrate utilisation as C source	**+**
Nitrate reduced to nitrite	**-**
Gelatin hydrolysis	**+**
Lysine decarboxylase	-
Ornithine decarboxylase	-
O-F test	O+
CHOs, acid from:	
Glucose	+
Lactose	-
Maltose	-
Mannitol	-
Salicin	-
Sucrose	-
Xylose	**+**
Trehalose	-
Casein hydrolysis	**+**

SPECIMEN 2 (DAY 3 VASE)

From the test results below, this specimen was preliminarily identified as either *Pseudomonas fluorescens* or *Pseudomonas putida.*

Gram	negative rod
Catalase	+
Oxidase	+
Motility	+

Slime production	+
Fluorescence in uv light	+
Growth at 5° C	+
Growth at 42° C	-
Citrate utilisation as C source	**-**
Nitrate reduced to nitrite	**-**
Gelatin hydrolysis	**+**
Lysine decarboxylase	-
Ornithine decarboxylase	-
O-F test	O+
CHOs, acid from:	
Glucose	+
Lactose	-
Maltose	-
Mannitol	-
Salicin	-
Sucrose	-
Xylose	**-**
Trehalose	-
Casein hydrolysis	**+**

SPECIMEN 3 (DAY 6 STEM)

From the test results below, this specimen was preliminarily identified as *Pseudomonas putida*. The specimen was separated from *Ps. fluorescens* on the basis of the gelatin hydrolysis result: *Ps. putida* never hydrolyses gelatin, whereas *Ps. fluorescens* does (Stanier *et al.* 1966).

Gram	negative rod
Catalase	+
Oxidase	+
Motility	+
Slime production	+
Fluorescence in uv light	+
Growth at 5° C	+
Growth at 42° C	-
Citrate utilisation	**+**
Nitrate to nitrite	**+**
Gelatin hydrolysis	**-**
Lysine decarboxylase	-
Ornithine decarboxylase	-
O-F test	O+
CHOs, acid from:	
Glucose	+
Lactose	-
Maltose	-
Mannitol	-
Salicin	-
Sucrose	-
Xylose	**-**
Trehalose	-
Casein hydrolysis	**-**

SPECIMEN 4 (DAY 6 VASE)

From the test results below, this specimen was preliminarily identified as either *Pseudomonas fluorescens* or *Pseudomonas putida*.

Gram	negative rod
Catalase	+
Oxidase	+
Motility	+
Slime production	+
Fluorescence in uv light	+
Growth at 5° C	+
Growth at 42° C	-
Citrate utilisation	**-**
Nitrate to nitrite	**-**
Gelatin hydrolysis	**+**
Lysine decarboxylase	-
Ornithine decarboxylase	-
O-F test	O+
CHOs, acid from:	
Glucose	+
Lactose	-
Maltose	-
Mannitol	-
Salicin	-
Sucrose	-
Xylose	**-**
Trehalose	-
Casein hydrolysis	**-**

APPENDIX H

BACTERIAL IDENTIFICATION USING FATTY ACID ANALYSIS

The results of the fatty acid bacterial identification using the Hewlett-Packard 5898A Microbial Identification System (Hewlett-Packard Company, Avondale, Pennsylvania, U.S.A.) were performed by the Biological and Chemical Research Institute at Rydalmere, Sydney, and are detailed in the photocopied letter shown on the next two pages. When interpreting the results, the following sentence (from the last paragraph on p. 8-6 of the HP Microbial Identification System Operating Manual - also included in Appendix H) should be borne in mind: "A similarity index of 0.6 to 1.0 is an excellent match (1.0 is the highest possible)."

Thus, the isolate from the rose stem at 3 days was too close to identify accurately; the vase isolate at 3 days was too low to be considered an accurate match; but the rose stem isolate at 6 days was a high match with *Pseudomonas putida* biotype A (= *Ps. putida* biovar A - see 7.4 (Discussion) re preferred name of infrasubspecific rank), and the vase isolate at 6 days was a high match with *Pseudomonas chlororaphis* (formerly *Pseudomonas fluorescens* biovar D prior to the 8th edition (1974) of Bergey's Manual). Therefore, only the vase and stem isolates at 6 days were used as they were the two bacteria identified with certainty.

A brief explanation of the fatty acid bacterial identification method appears in this Appendix after the photocopied letter from the Biological and Chemical Research Institute.

Biological
and Chemical
Research Institute

NSW Agriculture

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jb:gd (B1039)

3 June 1993

Dear Ms Williamson,

RE: FATTY ACID BACTERIAL IDENTIFICATION

The four bacterial isolates from *Rosa hybrida* 'Sonia' and vase water have been identified by fatty acid analysis as follows:-

Rose Stem 3 days	Pseudomonas fluorescens biotype B	0.664
	P. fluorescens biotype A	0.631
	P. marginalis	0.634
	P. putida biotype A	0.582
Vase 3 days	P. putida A	0.392
Stem 6 days	P. putida A	0.827
	P. syringae savastanoi pv. nerium	0.621
	P. marginalis	0.567
Vase 6 days	P. chlororaphis	0.898

To assist you in interpreting the results I have attached an extract from the HP5898A Microbial Identification System - Operating Manual No. 19298--90100.

From the results I would consider the identification for Rose stem 3 days too close to separate by fatty acid analysis and require further tests to give a positive identification.

Vase 3 days has a single low match to *Pseudomonas putida* biotype A.

Stem 6 days has a high match to *Pseudomonas putida* biotype A. It is unlikely to be a *Pseudomonas syringae* pathovar as you have already tested it to be oxidase +ve.

Vase 6 days identifies with an excellent match to *Pseudomonas chlororaphis*.

For further information on the identification of your isolates I suggest you refer to "Bergey's Manual of Systematic Bacteriology" Volume 1 Publ Williams and Wilkins 1984 pps 161–172 (photocopy included). It is important to perform the tests as they would have been performed originally to set up the tables. These tests and methods can be found in Bergey's Eighth Edition.

To store your isolates I suggest the best method is freeze drying using a suspension of 10% sucrose and 5% peptone. Secondly store at −80°C in turbid 10% sucrose solution, or under liquid nitrogen. Thirdly store on slopes of Glucose Yeast Calcium Carbonate agar or Peptone yeast Extract Agar (see Oxoid Manual for recipe). This last method will keep bacteria for two to three month periods at least prior to resubbing.

Hope this information is helpful.

Yours sincerely,

JEFFREY BRADLEY
for DR P C FAHY
Senior Plant Pathologist (Bacteriology)

The following three pages are from the Hewlett-Packard 5898A Microbial Identification System - Operation Manual, May 1987 (Hewlett-Packard Company, Route 41, Avondale, Pennsylvania 19311, U.S.A.).

8.3 Library Search Report

Once a microorganism has been properly cultured, processed, and analyzed by the MIS, its fatty acid composition can be matched with those of known organisms which are stored in the MIS library. The computer compares the composition of unknown organisms with those in its memory, taking into account strain to strain and experimental variation. The computer has been trained to recognize those acids which may be related to other acids in an extract. If one acid is a precursor of another acid in a bacterium, the computer will account for a decrease in one acid and an increase in the other.

The library search is rapid; the result, printed below the fatty acid composition report, is available within one minute of the completion of the gas chromatographic analysis. The MIS library search prints the most likely matches to the unknown composition and a similarity index for each match.

Figure 8-4
Library Search Report

```
ISBA [Rev 2.0]   Pseudomonas . . . . . 0.956 (P. fluorescens D)
                   P. chlororaphis . . . . . 0.956 (P. fluorescens D)
                   P. fluorescens . . . . . 0.794
                     P. f. A . . . . . 0.794
                     P. f. C . . . . . 0.719
                     P. f. G . . . . . 0.715
                   P. putida . . . . . 0.553
                     P. p. biovar B . . . . . 0.553
```

Interpreting the Library Search

If the search results in more than one possible match, the suggested identities are listed in descending probability. The most probable genus identification is printed first. The next line identifies the species match, preceded by an abbreviation of the genus. Where applicable, sub-species level identifications are printed next, preceded by both an abbreviated genus and species name. Other likely matches, at each level, are listed by decreasing similarity index values. It is possible that a species of another genus will have a higher similarity index than another sub-species entry of the closest match species.

Similarity Index

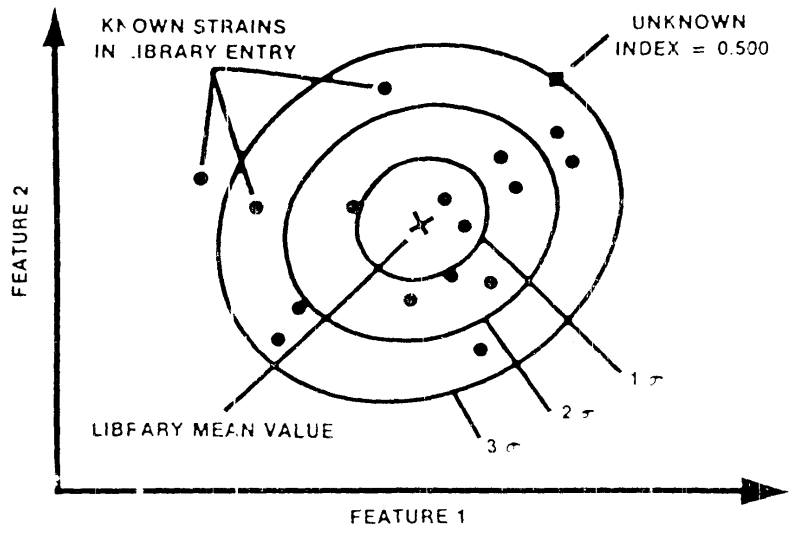
The similarity index is a numerical value which expresses how closely the composition of an unknown isolate compares with the fatty acid composition of the library matches. This index value is a calculation of the unknown's distance in n-dimensional space, from the mean profiles of the closest library entries.

A similarity index of 0.6 to 1.0 is an excellent match (1.0 is the highest possible). The figure below illustrates a library entry built from a group of known strains (only 2 dimensions of a n-dimensional space shown). The contour lines represent the distance from the mean value in standard deviation units (sigma). An unknown strain nearly equivalent to the outlying strain (3 sigma away from the mean) would be reported with a 0.500 similarity index.

Single Match

An MIS identification giving only one match with a similarity index greater than 0.500 has a strong likelihood of being correct. Single-match identifications with similarities less than 0.300 may indicate an organism not in the library, but related to the MIS match. (Low similarity indices may also be a result of not following procedures or system malfunctions. See Section 11, Trouble Shooting.)

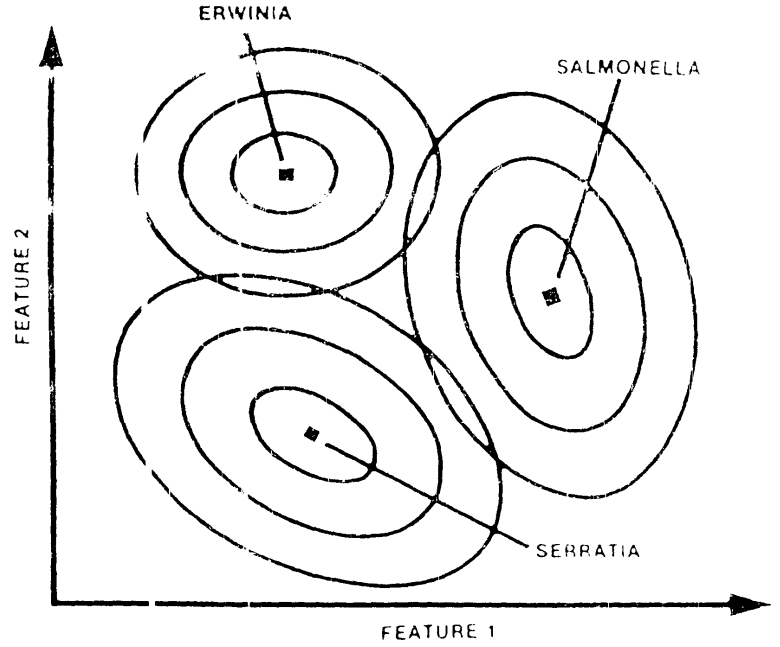
Figure 8-5
Typical Library Entry
2-Dimensions Shown



Multiple High Matches

It is possible with organisms having extremely similar fatty acid compositions, such as the Enterobacteriaceae to have a library search resulting in matches all with similarities greater than 0.500. The figure below illustrates (in two dimensions) how this occurs. Often, a few simple auxiliary (biochemical or serological) tests can easily differentiate or confirm the match.

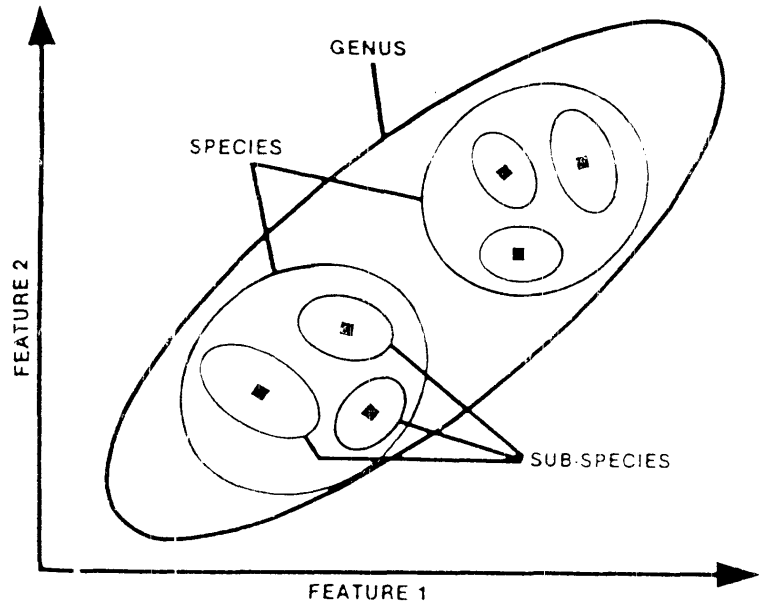
Figure 8-6
Overlapping Entries
2-Dimensions Shown



Sub-species Grouping

The MIS identifies organisms at the lowest possible entry level since library entries made at the sub-species level have smaller variance than at species or genus levels. The figure below illustrates how higher level identifications are comprised of the summation of several smaller, lower level compositions. The ability of the MIS to identify microorganisms is heavily influenced by current taxonomy. Those microorganisms that are presently grouped together, yet are quite diverse (such as the Corynebacteria) are difficult to identify since the high variance in fatty acid compositions (reflecting the diversity of the group) of the strains used for training provides a poor model for pattern recognition.

Figure 8-7
Genus, Species, & Sub-Species Shown in 2-Dimensions



Shown below is a Library Search Report with sub-species level matching.

Figure 8-8
Library Search to the Sub-Species Level

```
TSBA (Rev 2.0)   Pseudomonas . . . . . 0.956 (P. fluorescens 0)
                   P. chlororaphis . . . . . 0.956 (P. fluorescens 0)
                   P. fluorescens . . . . . 0.794
                     P. f. A . . . . . 0.794
                     P. f. C . . . . . 0.719
                     P. f. G . . . . . 0.719
                   P. putida . . . . . 0.553
                     P. p. biovar B . . . . . 0.553
```

The material in this document (except for section 10) is copied from the HP 5898A Microbial Identification System - Operation Manual (May 1987) *, the Microbial Identification System, Library Generation Software - Users Manual (April 1988) ** and the M.I.S. Training Study Material Manual **.

* Hewlett-Packard Company, Route 41, Avondale, PA 19311, USA

** Microbial ID, Inc., 115 Barksdale Professional Center, Newark, Delaware 19711, USA

APPENDIX I

PREPARATION OF MATERIAL FOR SCANNING ELECTRON MICROSCOPY USING THE CRITICAL POINT DRIED METHOD

Fixation:	In 3% glutaraldehyde in 0.1 M Sörenson's phosphate buffer, pH 7.0, for 24 h at 4°C
Buffer Wash:	In four changes (15 min each change) of 0.1 M Sörenson's phosphate buffer (total of 1 h).
Post-Fixation:	In 1% OsO_4 in 0.1 M Sörenson's phosphate buffer for 15 h.
Buffer Wash:	In four changes (15 min each change) of 0.1 M Sörenson's phosphate buffer (total of 1 h).
Dehydration:	In 30, 50 70, 80, 90 and 95% ethanol (10 min each change), then two changes in 100% dried ethanol of 15 min each.
Critical Point Drying:	The specimens were transferred to thimbles in a boat of 100% dried ethanol and critical point dried in a Polaron E 3000 Critical Point Dryer (Polaron, England), using an ethanol/CO_2 system.
Mounting:	Dried specimens were mounted onto aluminium stubs with Supa Glue® (Selleys, Australia).
Coating:	Specimens were sputter coated for 4 min with 20 nm gold.

APPENDIX J

JEOL JSM35 MANUAL, COOLING STAGE INSTRUCTIONS

I N S T R U C T I O N S

3 5 - C S 2

C O O L I N G S T A G E 2

No. IEP35-CS2

1. GENERAL

The 35-CS2 Cooling Stage is designed for observation of frozen specimens. By using this stage specimens can be observed without the need for vacuum evaporation or chemical treatment (fixation or dehydration). Thus, specimens can be observed in an almost-natural state, making the 35-CS2 Cooling Stage ideal for the study of biological specimens. It is also effective for observing hydrates and specimens that are easily affected by heat.

2. SPECIFICATIONS

o Specimen cooling temperature:	Approx. -130°C
o Temperature measurement:	Copper-constantan ~~Chromel-alumel~~ thermocouple
o Refrigerant tank capacity:	180cc
o Refrigerant:	Liquid nitrogen
o Specimen exchange:	Airlock mechanism
o Specimen size:	10mm(in dia) × 5mm(in thickness)
o Range of specimen observation:	X, Y directions: ±5mm Z direction: ±1.5mm Rotation: ±20° Tilt: 0° ~ 45° at a working distance of 39mm, untiltable at a working distance of 15mm
o Working distance:	15mm and 39mm
o Specimen absorbed current:	Detectable

5. OPERATION

1. Depress the PUMP DOWN switch to evacuate the column.
2. Fill the refrigerant tank with refrigerant (use funnel) and allow the stage to cool. (It takes approx. 90min. for the stage to reach -130°C.)
   Note: Gasification of the refrigerant is quite rapid at first, so be sure to replenish as necessary until the evaporation rate becomes normal.
3. After mounting the specimen on the specimen pedestal (when using the pin type pedestal, impale the specimen on the pin), place the pedestal in the specimen holder and secure the pedestal by tightening the retaining screw (Fig. 5).
4. Screw the specimen exchange rod into the specimen holder, and submerge the holder in the refrigerant (Fig. 6) to freeze the specimen (allow about one minute for boiling to cease). When the specimen is frozen, use tweezers to place the cap over the specimen, while keeping the holder submerged in the refrigerant (Fig. 6).
5. Insert the specimen holder into the specimen exchange chamber and depress the vacuum control button to rough the chamber (the red lamp lights up when evacuation starts).
6. After evacuation has been completed and the lamp has gone out, invert the holder so as to allow the cap to drop to the bottom of the exchange chamber.
7. Open the airlock valve (withdraw the airlock knob), reorient the holder so that the specimen faces upwards and mount the holder on the cooling stage.

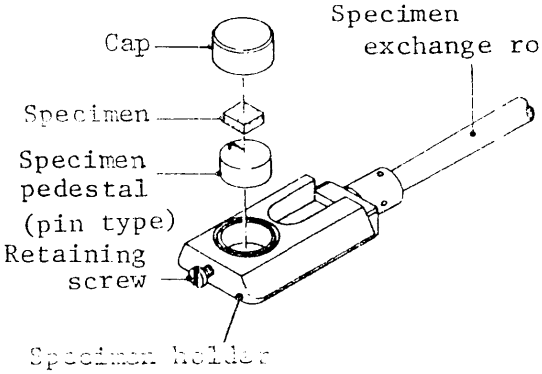


Fig. 5 Loading the specimen

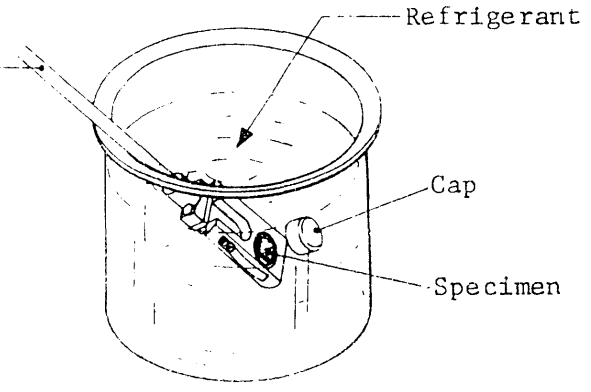


Fig. 6 Freezing the specimen

8. Unscrew the specimen exchange rod from the specimen holder, return it to the exchange chamber and close the chamber (push in the airlock knob).
9. Obtain a scanning image in accordance with the procedure given in the JSM-35 instruction manual.

Note: An adequate accelerating voltage is 5 to 10kV. Specimen observation can be carried out within the range specified in Chap. 2, Specifications.

Caution: 1. Prior to removing the CS2 or exposing the specimen chamber to the atmosphere, be sure to drain off any refrigerant remaining in the refrigerant tank via the drain pipe. Wait for the stage to return to room temperature.

2. Before removing the CS2, be sure to detach the cooling braided wire and the thermocouple connector.

APPENDIX K

INSTRUCTIONS FOR USING THE IMAGE ANALYSER: SETTING UP AND MEASUREMENT OF OBJECTS

Equipment and connections:

Microscope:

Leitz Laborlux S (Wild Leitz GMBH, Type 020-505.030, Leitz, Portugal).

Video camera:

The video camera, Ikegami CCD (Model ICD-42E, Ikegami Tsushinki Co. Ltd., Japan), is screwed into the top of the microscope.

The silver connector plugs into the **Video out** plug. This black cord connects to silver adaptor connectors connecting to a thinner grey cord marked **O**, which plugs into the TV monitor.

TV monitor:

Sony Trinitron Colour Video Monitor (Model PVM-1371QM, Sony Corporation, Japan).

The TV monitor switches (inside the front cover of TV) should be on: **AFC** Slow, **Scan Mode** Normal, **Sync** Int, **Secam switch** is not selected, **RGB** is selected.

TV monitor connections at back are: the black connector from the video camera, after connecting to the grey cord, splits into three **Red**, **Green** and **Blue** connectors, marked (with tape) respectively 1, 2, 3. Plug them into the corresponding TV monitor plugs marked (with tape) 1, 2, 3. A short black cord is also plugged in at the back of the TV monitor leading from **G out** to **EXT SYNC** in. Switches at the back of the TV monitor are on: **R out**, **G out**, **B out**, **Line A Video on**, **Line B Video on**, **EXT SYNC off**. Nothing is connected to **VTR CMPTR** or **AUDIO (RGB)**.

Computer:

NEC PowerMate/MultiSync II (Model APC-H4371, NEC Corporation, Japan).

Switches at the back of the computer are on: **Manual Mode** is switched to on, **Colour** switched to colour, **Mode** switched to 8.

Hard disk:

NEC PowerMate 1, APC IV (Model APC-H2020E, NEC Corporation, Japan).

Connections at the back of the hard disk are: The flat grey cord with four sections (which is the RGB connection from the video camera and TV monitor) is plugged into the top black male plug marked **4**. The silver plug with attached cream cord is plugged into the top right plug marked **1** and connected to the **Signal input** plug of the computer screen. The black plug and cord is plugged into **Com** and connects with the sketch pad and a black transformer (Model 62-1006-202, Sino-American). (The transformer also needs to be plugged into the power.) The cream keyboard cord is plugged into the plug marked **Keyboard**. There is no attachment to the **Printer**. The three grey plugs marked **C**, **V** and **M** remain unconnected.

Sketch pad:

SummaSketch® Plus (Model MM 201, Summagraphics Corporation, Fairfield CT, USA). Use the yellow key (**1**) of the mouse

Menu selection:

(Instructions for this section are also contained in Casanova, M.T. 1993. The ecology of charophytes in temporary and permanent wetlands: an Australian perspective. Ph.D. thesis, University of New England, Armidale.)

Note: Enter = Return key.

Selecting and naming data sets:

Switch on power at wall; once the computer has booted, after the beep prompt strike the **F1** key to continue, then at the **Fixed Disk Menu** select **(MEG) BQ Meg IV** by typing **<6>**, **<Enter>**. Select **Templates and Extant Data** with the mouse. Insert a 5-1/4" disk in the drive, choose the **Select** pull down menu and while holding down the mouse choose **Select Data Volume**. [If you have not already created a data volume, instead of choosing the **Select** pull down menu, choose the **Options** pull down menu and while still holding down the mouse choose **Create New Data Volume**. Delete the string of numbers in the **Sample Name for New Data Volume** box by backspacing from the last right hand character. Type a new name in the box, press **<Enter>**, and click **OK** with the mouse. To see the newly created data volume, go back to the **Select** pull down menu and while holding down the mouse, choose **Select Data Volume**. If the volume does not show in the left hand **Available volumes** box, click on **Volume search** in the right hand box. Select the desired volume in the left hand **Available volumes** box (it will become highlighted), then click on **Select Data Volume** in the right hand **Current Data Volume** box.] If you have already created data set/s in the selected volume, they will appear on the next screen in the left hand **Extant data sets** box. Click on the extant data set you want (it will become highlighted), and then click on **Select data set** in the right hand box. (If you have not already created a data set, instead of choosing the **Select** pull down menu, choose **Options** and while still holding down the mouse choose **Create New Data Set**. Choose a template from the left hand box, e.g. **basic template**. Delete the string of numbers in the right hand **Sample Name for New Data Set** box, type a name you want in the box, press **<Enter>**, and click **Select Template** with the mouse. The sample name you typed will come up on the next screen. Click on **Create Data Set**. In order to use the newly created data set, you will have to go to the pull down **Procedures** menu and while holding the mouse select **Begin Again**. Go through the above instructions until you have selected the required data set.

Selecting measurement parameters:

Choose the **Procedures** pull down menu and while holding down the mouse choose **Measurement**. Two messages will briefly appear on the screen: (1) **Loading Data Set** you've chosen; and **Loading Procedure Option DIGITIZE**. Choose the **Select** pull down menu and while holding down the mouse choose **Add / Assoc**. Click on (e.g.) **Area** in the left hand **Associations Setup** box (it will become highlighted) and then click on **OK→select**. Delete any unwanted selections in the next screen by clicking on (highlighting) (e.g.) **L1** in the right hand **Selections** box, click **Delete**, then click **OK**. Choose the pull down menu **Modes** to select the **Magnification** and **Units of Measurement** you want. If you are making several measurements under the microscope at the same magnification, the magnification can be automatically set to that level for all measurements by clicking to the left of the 'check box' BEFORE selecting the magnification. When you've finished choosing the magnification and units of measurement, select the **MEGx** pull down menu and while holding down the mouse choose **Execute MEGx**. Two messages will come up telling you (1) the data will be saved to your selected file; and (2) **Loading Procedure Option ADVANCED**. (If you are making more than one measurement in any session, the computer will invariably crash at

this point. It should take only 5 sec to load the program. If the program doesn't load and the screen "freezes", switch off the power at the wall and go through the above procedures again.)

Measurement of objects:

After selecting the **Execute MEGx** command and the advanced procedure option is loaded, the screen will show four square windows. Open the four windows by clicking once on each of the following: **Edit, Calibrate, Control** and **Window**. (You only need to open the **Window** window once because once you've made a screen size choice, e.g. **Full Screen**, it will be remembered.)

Obtaining an image:

To obtain a suitable image from the microscope to the TV monitor, perform the following sequence: **Display real image, Store** (the TV screen will turn red), **Display real image** (the microscope field of view will come back), **Set threshold** and hold the mouse down while dragging across the sketch pad until the desired threshold is reached, **Store image to ...** (Background # 1 is the default and is fine), **Display real image, Store, Display background**.

When setting the threshold, use **Threshold 2** to measure bright objects (e.g. xylem conduits) on a dark background. **Threshold 1** is used for dark objects on a bright background.

Annotating the image:

You are now ready to annotate the image on the TV monitor. To move from the computer screen to the TV monitor, choose any of the commands from the top left window. Usually the commands that are used are: **Cut, Draw, Fill, Erode, Dilate**. To move from the TV monitor to the computer screen, you need to press the **<Esc>** key on the keyboard. Basically, you will need to remove the unwanted highlighted areas from the TV monitor so that only the objects you want to measure appear red on the TV monitor. Setting the threshold is usually a trade-off between highlighting wanted and unwanted objects. A usual sequence is to select **Draw**, **<Esc>**, **Fill**, **<Esc>**, **Cut**, **<Esc>**, etc. until only the objects you want to measure remain highlighted. When drawing from the edge of the monitor, start beyond the limit of the sketch pad and slowly move the mouse so that a yellow line appears on the TV monitor. Keep holding down the mouse until you have finished drawing off the edge of the monitor. Press **<Esc>** and then select **Fill**, click the cursor inside the partitioned drawn area that you want to remove. The chosen area will fill with red, press **<Esc>**, select **Cut** and position the cursor on the filled red area. Either keep doing this or cutting individual objects until the unwanted objects are removed. When drawing around an area on the monitor, to ensure that the line is complete, retrace over the initial part of the line and finish off by bringing the line towards the area to be filled.

Frequently, despite careful drawing with the mouse, after the **Fill** command is selected the whole screen becomes red. There is nothing that can be done about this (unfortunately, there is no "undo" command). All that you can do is begin annotating the image again by pressing the **<Esc>** key, selecting **Clear, Display real image, Store** and starting annotation again.

Use the **Dilate** command to see small highlighted (i.e. red) areas on the TV monitor. Often the small areas are easier to cut and see when they are not just tiny, red dots. However, be careful that you don't keep choosing to **Dilate** objects further and further because they may not **Erode** back to a previously seen suitable size. Rather, view the measured objects under **Options** (see *To view measurements of counted objects* below) and delete unwanted measurements.

To check what highlighted (red) objects on the TV monitor need deleting, either select **Display real image**, or obtain a temporary view by choosing **Cut** and positioning the cursor on an unhighlighted (i.e. not red) area of the monitor.

When you are ready to count (i.e. measure) the highlighted areas, make sure **Display background** is selected, then click the **Objects** command in that box.

After you have finished counting the objects on that section of the monitor, before you move to the next area of the slide ENSURE that **Display real image** is selected, note some reference points and move to the next area. If you forget what you've measured, clicking on **Display real image** again will show the previously counted screen (only the previously stored screen is kept in memory).

Quitting the program:

When you have finished measuring objects, ENSURE that **Display real image** is selected, then select **Exit** from all the open windows and then select **Exit MEGx**. Then choose the **Procedures** pull down menu and while holding down the mouse either choose **Begin Again** or **Exit To D.O.S.**. If you have finished, after selecting **Exit To D.O.S.**, a prompt will appear at the bottom of the screen **Exiting To DOS: (Y/N).** Type <**Y**>. At the **Fixed Disk Menu**, type <**RETURN** (= Enter)> to exit. You are now back to the **C:\ >** prompt, open the disk drive and switch off at the power point.

To view measurements of counted objects:

After the objects have been counted, yellow plus signs appear on all counted objects. To ensure no small unwanted and undetected areas were counted, choose the **Options** pull down menu and while holding down the mouse choose **List/Change Data**. Scroll through the data using the arrow keys and delete any unwanted values by double clicking on them. (For example, in my measurements, any unwanted objects measured 0.003. You can check this by physically counting the number of highlighted objects before measurement and then checking how many were actually counted by the computer. Sometimes small pixels on the TV monitor remain undetected by the eye, even after the **Dilate** command is used.)

Obtaining totals of measured objects:

Once measurements have been completed, the total of all individual measurements for that data set can be obtained. ENSURE that you have selected **Display real image** before selecting **Exit MEGx**. Under the pull down **Procedures** menu choose **Statistics** while holding down the mouse. Under the pull down **Statistics** menu choose **Proceed Status Quo** while holding down the mouse. To get out of this screen choose the **Procedures** pull down menu and while holding down the mouse choose **Begin Again** or **Exit To D.O.S.**.

Transfer of data to other disks and programs:

Data can be saved in Ascii format and transferred to other programs or 3-1/2" disks (see Casanova 1993).

APPENDIX L

HYDRAULIC CONDUCTION EQUATIONS

The Hagen-Poiseuille equation (1839;1840) describes laminar water flow through an ideal (smooth) capillary (from Zimmermann and Brown 1971):

$$\text{Volume } (m^3\ s^{-1}) = \frac{\pi}{8\eta} \times \frac{\Delta p}{\ell} \times t \times \Sigma r^4 \qquad \text{(L.1)}$$

where
- η = viscosity of the liquid (kg m^{-1} s^{-1});
- $\Delta p/\ell$ = pressure gradient along the capillary (Pa);
- ℓ = length of capillary (i.e. stem segment) (m);
- t = time (s); and
- r = radius of the capillary (m).

Dimond (1966) modified the Hagen-Poiseuille equation from:

$$p = \frac{(8\ell\eta v)}{\pi r^4} \qquad \text{(L.2)}$$

to:

$$p = \frac{(8\ell\eta v)}{\pi\Sigma(nr^4)} \qquad \text{(L.3)}$$

to account for water flow in stems, which contain many conduits of differing radii,

where

$\Sigma(nr^4)$ = $n_1r_1^4 + n_2r_2^4 + \ldots + n_nr_n^4$, are the number of xylem conduits having a radius of $r_1, r_2, r_3, \ldots r_n$.

Thus, if Dimond's (1966) equation (L.3) is solved for v (volume), it becomes:

$$v = \frac{\pi\Sigma(nr^4)p}{8\ell\eta} \qquad \text{(L.4)}$$

where
- v = volume (m^3 s^{-1});
- r = radius of capillary (m);
- p = pressure (Pa);
- η = viscosity of the liquid (kg m^{-1} s^{-1});
- ℓ = length of capillary (i.e. stem segment) (m).

The above equation (L.4) calculates the theoretical flow of a liquid through tubes per second. However, observed rates of flow in *Acacia amoena* stems [two treatments, distilled water and citric acid (10 mol m^{-3}), n = 5 per treatment] were determined over 12 h periods. Thus, the theoretical volume per second was multiplied by 12 h (43200 seconds = SI unit). This figure was then used to standardise observed volumes between treatments.

Constants used in the above equation were:

π = 3.142;

p = 64 mm Hg/750 = 0.0853 bar = 85330 Pa;

ℓ = 0.2 m;

η = viscosity of H_2O at 20°C = 0.01002 poise = 0.001002 kg m^{-1} s^{-1};

η = viscosity of citric acid (10 mol m^{-3}) at 20°C = 0.0101276 poise = 0.001013 kg m^{-1} s^{-1}

APPENDIX M

THE RESIDUAL SUM OF SQUARES METHOD FOR DETERMINING THE OPTIMAL ALLOCATION OF CONDUIT LENGTH CLASSES (MILBURN AND COVEY-CRUMP 1971)

The technique recommended by the Milburn and Covey-Crump (1971) method of conduit length class allocation is to calculate the total residual sum of squares (RSS) for each possible conduit length grouping, using simple linear regression analysis. The grouping with the smallest total residual sum of squares is then chosen, because it represents the best possible distribution of conduit lengths.

A. INDIAN INK CONDUIT LENGTH DATA

The first possible allocation of conduit lengths to be tested was:

Distance from cut end: 0-2 cm

Simple Regression X 1 : Length (cm) Y 1 : % of initial filled

Count:	R:	R-squared:	Adj. R-squared:	RMS Residual:
3	.899	.808	.617	25.476

Analysis of Variance Table

Source	DF:	Sum Squares:	Mean Square:	F-test:
REGRESSION	1	2737.874	2737.874	4.218
RESIDUAL	1	649.045	649.045	p = .2885
TOTAL	2	3386.919		

Residual Information Table

SS[e(i)-e(i-1)]:	e ≥ 0:	e < 0:	DW test:
1947.135	2	1	3

Distance from cut end: 3-5 cm

Simple Regression X 1 : Length (cm) Y 1 : % of initial filled

Count:	R:	R-squared:	Adj. R-squared:	RMS Residual:
3	.997	.994	.989	.8

Analysis of Variance Table

Source	DF:	Sum Squares:	Mean Square:	F-test:
REGRESSION	1	111.288	111.288	173.677
RESIDUAL	1	.641	.641	p = .0482
TOTAL	2	111.929		

Residual Information Table

SS[e(i)-e(i-1)]:	e ≥ 0:	e < 0:	DW test:
1.922	2	1	3

Distance from cut end: 6-8 cm

Simple Regression X 1 : Length (cm) Y 1 : % of initial filled

Count:	R:	R-squared:	Adj. R-squared:	RMS Residual:
3	.939	.881	.762	.244

Analysis of Variance Table

Source	DF:	Sum Squares:	Mean Square:	F-test:
REGRESSION	1	.44	.44	7.408
RESIDUAL	1	.059	.059	p = .2242
TOTAL	2	.499		

Residual Information Table

SS[e(i)-e(i-1)]:	e ≥ 0:	e < 0:	DW test:
.178	1	2	3

Distance from cut end: 9-11 cm

Simple Regression X 1 : Length (cm) Y 1 : % of initial filled

Count:	R:	R-squared:	Adj. R-squared:	RMS Residual:
3	.327	.107	-.786	.29

Analysis of Variance Table

Source	DF:	Sum Squares:	Mean Square:	F-test:
REGRESSION	1	.01	.01	.12
RESIDUAL	1	.084	.084	p = .7877
TOTAL	2	.094		

Residual Information Table

SS[e(i)-e(i-1)]:	e ≥ 0:	e < 0:	DW test:
.252	1	2	3

Therefore, the total residual sum of squares for the first allocation is 649.829.

Alternative allocations of conduit length groups which were tested are shown below:

Distance from cut end: 0-3 cm

Simple Regression X 1 : Length (cm) Y 1 : % of initial filled

Count:	R:	R-squared:	Adj. R-squared:	RMS Residual:
4	.853	.728	.592	23.95

Analysis of Variance Table

Source	DF:	Sum Squares:	Mean Square:	F-test:
REGRESSION	1	3068.776	3068.776	5.35
RESIDUAL	2	1147.219	573.61	p = .1468
TOTAL	3	4215.995		

Residual Information Table

SS[e(i)-e(i-1)]:	e ≥ 0:	e < 0:	DW test:
2574.536	2	2	2.244

However, as the 0-3 cm allocation has a larger RSS than 0-2 cm, it must be discarded and so 0-2 cm is the preferable allocation.

Distance from cut end: 3-6 cm

Simple Regression X 1 : Length (cm) Y 1 : % of initial filled

Count:	R:	R-squared:	Adj. R-squared:	RMS Residual:
4	.976	.953	.93	2.071

Analysis of Variance Table

Source	DF:	Sum Squares:	Mean Square:	F-test:
REGRESSION	1	175.022	175.022	40.81
RESIDUAL	2	8.577	4.289	p = .0236
TOTAL	3	183.6		

Residual Information Table

SS[e(i)-e(i-1)]:	e ≥ 0:	e < 0:	DW test:
17.401	2	2	2.029

However, as the 3-6 cm allocation has quite a large RSS compared with, say, 3-5 cm, it is unlikely to be the best allocation.