Project Title: **Robust Product Design and Prediction for Post-Harvest Pot Plant Quality and Longevity**

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CONTENTS

Page
1. GROWER / RETAILER SUMMARY 1
    Headlines 1
    Background and deliverables 2
    Summary of the project and main conclusions 4
    Action points 24
2. OBJECTIVES AND MILESTONES 25
3. SCIENCE SECTION 28
    3.1. Executive summary 28
    3.2. Introduction 30
    3.3. Production and treatment regimes 32
        3.3.1. Transport and home life treatments – Year 1 and 2 32
        3.3.2. Production treatments – Year 3 and 4 33
    3.4. Development of objective quality models (Objective 1) 40
        3.4.1. Objective quality scores 40
        3.4.2. Model fitting for poinsettia 42
        3.4.3. Model fitting for begonia 46
        3.4.4. Conclusions 50
    3.5. Robust product design methodology: the effects of home-life and supply chain treatments on plant quality (Objective 3) 51
        3.5.1. Modelling quality loss 51
        3.5.2. Effects of home-life and supply chain factors 53
        3.5.3. Conclusions 55
    3.6. Utility of robust product design methodology (Objective 4) 63
        3.6.1. Robust product design methodology 63
        3.6.2. Production effects on poinsettia robust home-life quality 65
        3.6.3. Production effects on begonia robust home-life quality 67
        3.6.4. Conclusions 71
    3.7. Development of chlorophyll fluorescence (CF) screening techniques (Objective 2) 81
        3.7.1. Theory and experimental details 81
        3.7.2. Monitoring CF activity 83
        3.7.3. Leaf greenness effects 85
        3.7.4. Conclusions 86
    3.8. Predictive utility of chlorophyll fluorescence (Objective 5) 93
        3.8.1. Predictions of plant death in poinsettia 93
        3.8.2. Predictive power of CF for batch effects in poinsettia 95
        3.8.3. Predictive power of CF for batch effects in begonia 95
        3.8.4. Statistics required for PI batch data sampling plans 96
        3.8.5. Conclusions 99
4. TECHNOLOGY TRANSFER 103
5. REFERENCES 105
6. APPENDICES 107
    6.1. Generalised estimating equations (GEE) 107

1. GROWERS / RETAILER SUMMARY

Headlines

- A method has been developed to quantify poinsettia and begonia plant quality in an objective way, taking account of all relevant plant characters.

- Objective quality models developed during this project have enabled the effects of particular physiological variables on overall quality to be quantified. Major factors determining quality in poinsettia included cyathia, leaf and bract drop, leaf and bract paling and bract edge necrosis (BEN). Major factors determining quality in begonia included total bud and flower count, bud and flower drop, and numbers of damaged leaves.

- Objective quality models devised in the course of this project can be used to judge relative quality in past experiments where physiological characters were scored but quality estimates were not made!

- The effects of transport treatments on the subsequent decline in quality during home life were modelled and showed that cold exposure during transport was extremely deleterious to subsequent home-life quality for poinsettia. Begonia appeared less affected by normal marketing stress but, nevertheless, marketing had a measurable effect on reducing begonia quality.

- Low nitrogen (N) during production slowed the subsequent rate of quality decline during home life. Effects in poinsettia were slight compared to the effects of some home-life factors but the effects for begonia were potentially much greater. However, the problem of raising the initial quality of low nitrogen plants remains outstanding.

- High potassium (K) during production helped reduce begonia flower drop in high temperature home-life environments and made the plants more robust against home-life environments

- Consumer assessments of quality showed a considerable level of agreement between consumers both for poinsettia and for begonia. However, there were systematic differences between the consumer assessments and the expert assessments suggesting that consumer preferences can differ significantly from those of a grower expert.

- Chlorophyll fluorescence (CF) proved to be an objective indicator of plant quality so long as scoring was done under carefully defined conditions.

- CF measured at de-sleeve could be weakly predictive of post-harvest quality later in home life. However, a strong statistical relationship was found between CF and flower drop for batches of begonia plants subjected to normal marketing stress and CF appears better suited to batch prediction than to individual plant prediction.

Background and deliverables

UK pot plant producers recognise that their products must be of the highest visual quality and must have the potential for long home life if they are to compete successfully with foreign imports. It is now well established that pot plants that are visually similar at the time of marketing can show great variation in subsequent, home-life longevity. For example, researchers at the Westland Flower Auction showed that the 'effective' home life of poinsettia 'Angelika' varied between four and ten weeks depending on previous growing history.

Factors determining differences in the post-harvest longevity of pot plants remain largely uncertain. However, one factor affecting subsequent longevity appears to be the transport chain, and studies have shown that, at worst, transport can reduce subsequent longevity by 66%. Recent reviews have also concluded that another important factor is nutrition during production. High levels of fertilizer appear, in general, to be deleterious to post-harvest quality, and high nitrogen (N) levels appear particularly damaging to subsequent longevity. Pot plant species reported to benefit from low N include begonia, campanula, cyclamen, New Guinea impatiens, poinsettia, rose and schefflera.

Post-harvest longevity has traditionally been determined by testing plants in a single post-harvest environment intended to simulate 'normal' home-life conditions. However, in practice, pot plants may be exposed to a wide range of 'normal' home-life conditions (watering, illumination, temperature etc), and robustness to withstand varied conditions is, potentially, a most important component defining pot plant quality. This type of scenario has long been recognised in manufacturing industry and there is an extensive bibliography concerning industrial robust product design methodology aimed at producing products robust against varied conditions during use.

UK grown pot plants can be expected to have higher potential post-harvest quality and longevity than imported produce, since they should be fresher and should have been subjected to a much shorter marketing chain. However, the two products are likely to appear similar at the time of delivery to the retailer, and there is currently no objective method to determine the potential post-harvest quality and longevity of pot plants. A possible way of doing this is to utilise chlorophyll fluorescence (CF). Light energy is absorbed by chlorophyll molecules and used in photosynthesis. However, some of the absorbed light is re-emitted as CF, and the amount re-emitted can be an effective indicator of the impact of stress factors such as low temperature, air pollution or drought. van Kooten, working in the Netherlands, has written, "The methodology is suitable for judging potential plant longevity, but the relationship is not simple and it takes a few precautions to make sure you are not measuring unwanted effects (personal communication).

This project has aimed to take account of all of these factors in order to improve the competitiveness of UK pot plant producers and to benefit consumers (and retailers) by facilitating the production and delivery of more consistent and longer-lived pot plants to the point of sale. Specific aims have been:

- **To identify and to quantify key production and supply chain factors influencing post-harvest quality and longevity in a wide range of home-life environments and, by adapting robust product design procedures used in manufacturing industry, to identify protocols giving enhanced post-harvest product robustness to withstand varied post-harvest environmental and handling treatments.**

- **To evaluate the usefulness of CF in order to provide objective criteria of potential post-harvest quality and longevity**

The project has focused on poinsettia and begonia as 'model' plants because they account for about 27% of the value of the UK pot plant sector.

The deliverables from this work include:

- Mathematical methods that can be used for the objective determination of quality in any ornamental plant species

- Mathematical methods that can be used to determine the relative importance of the various plant characteristics perceived to be important by expert assessors versus consumer assessors

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- Recommendations regarding temperature conditions that must be met during the transporting of poinsettia to avoid potential damage to home-life longevity.

- Recommendations on ideal home-life environments for poinsettia and begonia for incorporation into customer care information.

- Recommendations for nutritional treatments to be used during the production of poinsettia and begonia to maximize home-life quality.

- Recommendations regarding test conditions needing to be met for effective CF assessment of quality.

- A regression model for begonia batch flower drop during home-life based on CF measurements during marketing.

Summary of the project and main conclusions

Experimental overview

Years 1 and 2 of the project used commercially grown poinsettias and begonias. These were subjected to varying levels of marketing stress, and were then tested for quality and longevity in a wide range of simulated home-life conditions at Efford. Years 3 and 4 of the project used poinsettias and begonias produced experimentally at Efford in a range of nutritional regimes. These were also tested for quality and longevity in a similar range of home-life conditions to those used for the commercial plants.

Measured physiological characters and 'expert' quality scores were recorded weekly during simulated home life conditions (Fig. G.1). This enabled quality in poinsettia and begonia to be modelled and defined objectively in terms of the measured physiological variables **(Objective 1)**. These models were then used to assess the effects of marketing and home-life treatments on quality loss in home life (**Objective 3**) and the effects of nutritional regimes on home-life quality and longevity (**Objective 4**). In parallel with the quality assessments, screening techniques were developed using chlorophyll fluorescence (CF) to monitor 'stress' in poinsettia and begonia **(Objective 2).** The utility of CF was then determined as a means of predicting potential home-life longevity at the point of dispatch or sale **(Objective 5)**.

Fig. G.1 Expert assessment in progress

Three levels of marketing stress were imposed:

- *Minimum stress*: plants were transported over the short distance from New Milton to Efford as quickly as possible, ensuring that the minimum temperature did not fall below 14-15ºC. These plants were then de-sleeved and held at 15ºC with 1,000 lux lighting (14 hours/day) for 48 hours until the start of a simulated retail phase.

- *Commercial marketing stress*: plants were transported from New Milton, via commercial depots, to retail outlets close to Efford using either the Sainsbury or the Safeway chain. These plants were collected from the local retail outlet, transported to Efford, and then subjected to a simulated retail phase at the same time as the minimum stress plants.

- *Cold-transport stress*: Pots were rapidly transported to Efford and then cold-stored, still sleeved and boxed, at 7ºC for 2 days. These pots then entered the simulated retail phase, along with pots from the other marketing treatments.

The simulated retail phase was at 18ºC and 1,000 lux lighting (14 hours/day). Pots from the minimum stress treatment were unsleeved but pots from the other two marketing treatments remained sleeved. After 48 hours, all pots were de-sleeved and home-life treatments were started. Home-life treatments were imposed for 6-8 weeks and comprised all combinations of two contrasting temperatures (16ºC or 21ºC) given in separate controlled-temperature rooms, two lighting levels for 14 hours/day (600 lux or 300 lux) achieved by the use of 50% mesh shading and two watering regimes. In the case of poinsettia, these were standard or wet, with the wet treatment achieved by standing the pots in saucers that held approximately 1 cm of water at all times. In the case of begonia, these were standard or fluctuating, with the fluctuating treatment achieved by allowing pots to dry out almost to the wilting point before re-watering

Nutritional treatments during production in Years 3 and 4 comprised various levels of feed nitrogen (N) and potassium (K). In Year 3, there were 2 levels of each nutrient, low and high, combined in all combinations (see Tables 1 - 4, Science Section). In the case of poinsettia, starting levels of N (2 weeks after potting) were 225 and 335 ppm, but these were gradually and proportionally reduced during crop growth to final levels of 150 and 225 ppm. Levels of K were 175 and 350 ppm and these remained fixed during production. The combination of low N and low K was regarded as the commercial standard. In the case of begonia, levels of N in Year 3 were 100 and 300 ppm, reducing to a final 50 and 150 ppm. Levels of K were 100 and 300 ppm, also reducing to 50 and 150 ppm. The low nutrient levels were rather lower than current commercial practice, whilst the high levels were rather higher than current commercial practice.

For poinsettia in Year 4, four levels of N were combined with two levels of K (see Table 2, Science Section). Initial levels of N ranged from 150 to 335 ppm, gradually reducing to a range from 100 to 225 ppm. Levels of K were 175 and 350 ppm, remaining fixed during production. For begonia in Year 4, the nitrogen feed levels were increased relative to the nitrogen feed levels in Year 3, as it was thought that the lowest nitrogen levels in Year 3 gave excessively pale leaf colour. The feed periods in the two experiments were of different lengths and were not strictly comparable. However, during the later part of the production period, the lowest N feed level used in the Year 4 experiment was roughly intermediate between the two N feed levels used in Year 3 (see Table 4). Unfortunately, the begonia experiment in Year 4 had to be destroyed due to a notifiable disease but a repeat experiment was performed in the next year using additional funding supplied by DEFRA.

Modelling pot plant quality (Objective 1)

Mathematical procedures were developed and refined over the course of the project to enable poinsettia and begonia pot plant quality to be objectively quantified by summing 'weighted' measures of several measured physiological characters. These procedures were based on analyses made of observed relationships between physiological scores and measures of perceived quality recorded at the same time. The parameters of the models represent the contributions of each of the physiological variates to overall plant quality, and this approach has enabled the influence of particular physiological characters on perceived quality to be quantified.

In the case of poinsettia, the model constructed in Year 2 indicated that:

In Sonora

- quality was markedly reduced by cyathia and bract drop, by pale leaves and bracts, and by bract edge necrosis (BEN)
- there was a significant interaction between the proportion of cyathia present and the presence of pale leaves, indicating that a low cyathia count reduced quality to a lesser extent when pale leaves were also present on the plant

- there was a significant interaction between pale bracts and BEN, indicating that the detrimental effects of pale bracts lessened when plants also showed evidence of BEN.

In Spotlight
- quality was markedly reduced by cyathia, bract and leaf drop, pale leaves and BEN
- there was a significant interaction indicating that the effects of pale leaves on plant quality were less marked when a plant had BEN than when it did not.

There was no further data for the poinsettia model in Year 3 as the expert scores of overall plant quality were missed. However, quality score data was collected in Year 4 and the poinsettia model based on the Year 4 data compared well with the poinsettia model based on the Year 2 data. One key feature of the Year 4 data was that there was no improvement in model fit due to the inclusion of a cultivar term and this indicated that observed differences in quality scores between the two cultivars could be well described by differences in the measured physiological characters.

In the case of begonia, the model constructed in Year 2 indicated that:

In Balli
- a high flower and/or bud count markedly improved plant quality
- quality was markedly reduced by flower and/or bud drop, and damaged leaves
- there was a significant interaction between flower drop and bud drop indicating that flower drop had less detrimental effect on plant quality when bud drop was also high
- a significant interaction between flower drop and damaged leaf count indicated that the effects of flower drop on plant quality were less marked when there was also high bud drop.

In Batik
- a high flower and/or bud count markedly improved plant quality
- quality was markedly reduced by flower and/or bud drop, and damaged leaves
- there was a significant interaction between flower drop and bud drop indicating that flower drop had less detrimental effect on plant quality when bud drop was also high.

The model constructed for begonia in Year 3 indicated that:

- high flower counts increased plant quality in cultivar Mariette, but not in Batik where flower counts remained high throughout home life
- high flower or bud drop reduced quality
- high damaged leaf count reduced quality
- the presence of pale leaves reduced quality
- the effects on quality of individual physiological attributes were lessened when more than one symptom of home-life stress was observed on a plant.

The model constructed for begonia in Year 3 indicated that:

- Blitz was given a lower quality score than Batik, irrespective of the recorded physiological characteristics
- High flower drop reduced plant quality

- High bud drop reduced plant quality
- The presence of pale upper canopy leaves reduced plant quality
- The presence of pale flowers reduced plant quality
- The significant interaction terms in the model suggested that the effects on quality of the individual physiological attributes became smaller when more than one symptom of home-life stress was observed for a plant.

For both poinsettia and begonia, the objective quality score models that were developed for this project could also be valuable in allowing relative quality to be judged in experiments carried out in the past when physiological characters were scored, but quality estimates were not made!

Effects of marketing and home-life factors on post-harvest quality (objective 3)

The decline in objective quality scores during home life was modelled for both poinsettia and begonia, and quality loss curves were fitted. These enabled comparisons to be made of the effects of transport chain and home-life factors on post-harvest quality and longevity. Fig G.2 shows the decline in objective quality scores in two contrasting treatments for poinsettia in Year 2. The upper line (Sonora, minimum marketing stress, 16ºC home-life temperature, 600 lux and standard watering) shows no obvious decline in quality until after week 3 of home life. In contrast, quality declined progressively through to week 5 for the lower line (Spotlight, cold transport, 21ºC home-life temperature, 300 lux and wet watering regime), when quality decline became more gradual. Clearly, the upper line represented superior home-life quality and longevity throughout home-life compared with the lower line.

This modelling approach clearly demonstrated that cold transport had an adverse effect on subsequent home-life quality in poinsettia, even though damage was not apparent at the point of simulated retail sale (see Fig. G.3). Effects became clearly apparent after only one week in home life and generally persisted throughout the remainder of home life. Analysis of the effects of cold transport on individual physiological characters showed that cold transport promoted bract loss, particularly during the first two weeks after de-sleeve (Fig. G.4). Cold stress also exacerbated leaf loss, increased cyathia loss (Year 2 only) and gave paler bracts. A sub-set of 16 plants subjected to simulated cold transport in Year 4 confirmed that cold-stress greatly reduces potential longevity (and, presumably, customer satisfaction) by increasing post-marketing bract and leaf loss, and increasing loss of bract and leaf colour. In contrast, cold-temperature transport had no obvious, specific detrimental effects on subsequent longevity in begonia.

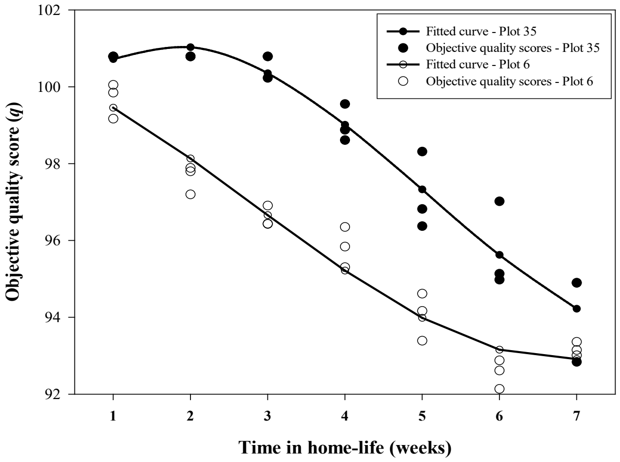


Fig. G.2. Modelled decline in objective quality during home life for poinsettia. Upper line, Sonora, minimum marketing stress, 16°C home-life temperature, 600 lux and standard watering; lower line, Spotlight, cold transport, 21°C home-life temperature, 300 lux and wet watering regime.

Fig. G.3. Effects of simulated cold transport treatment in poinsettia 'Spotlight' after 7.5 weeks of home life in Year 2: left, minimum stress treatment; right, cold transport treatment

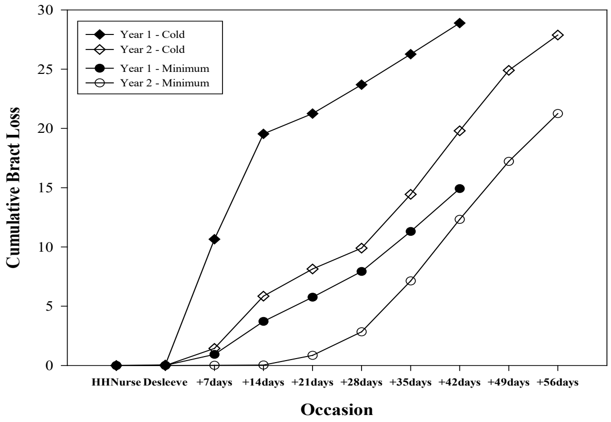


Fig. G.4. Effects of simulated cold transport on cumulative bract loss in poinsettia

Commercial chain plants generally showed a reduced home-life quality compared with minimum chain plants. In Year 1 for poinsettia, the commercial chain plants responded rather similarly to the cold-stressed plants, but this was almost certainly because temperatures were allowed to fall to about 10°C during delivery to the supply depot (see Fig. 1 Science Section). Other, unknown, factors also reduced the home-life potential of the commercially transported poinsettia and in Year 4, for example, the commercial chain plants showed a small, but significant, reduction in longevity of 2.8 days relative to minimum-chain plants (see Fig 5b, Science Section). Commercially transported begonia plants also showed a reduced home-life potential compared to minimum chain plants (see Figs 6c, 6d, 7b and 8b Science Section). This most clearly manifested itself in reduced flower numbers in the transported plants compared to the minimum chain plants.

The combination of cool temperature (16°C) and high light (600 lux) proved beneficial to home-life quality in both species. This is shown for poinsettia (Year 2) in Fig. G.5. In particular, cool temperature reduced cyathia loss and retained bract colour better than the higher temperature, whilst high light reduced bract and leaf loss compared to low light.

Fig. G.5. Effects of home-life treatments in poinsettia ‘Spotlight’ after 7.5 weeks of home life in Year 2 (minimum stress chain, control watering): left, 16°C and 600 lux; right, 21°C and 300 lux.

Cool temperature reduced flower and bud drop in begonia (see Fig. G.6) and helped retain flower and leaf colour, resulting in higher quality scores during home life for low versus high temperature (see Fig. 7d, Science Section). High light promoted bud opening and slowed the decline in flower and foliage colour. Cool temperature together with high light was particularly beneficial, and the effects for begonia are shown in Year 1 in Fig. G.7.

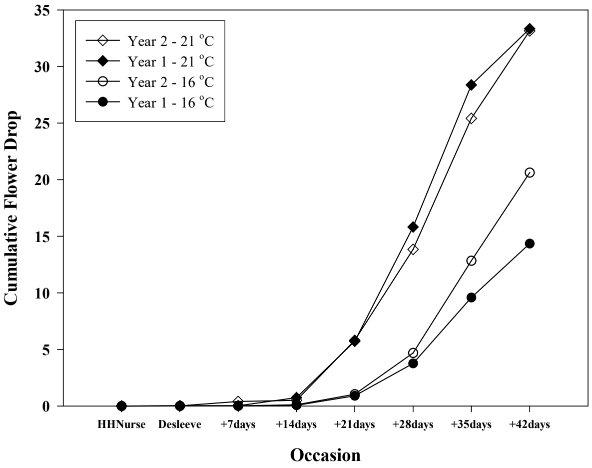


Fig. G.6. Effects of home-life temperature on cumulative flower drop in begonia in Year 1 and 2

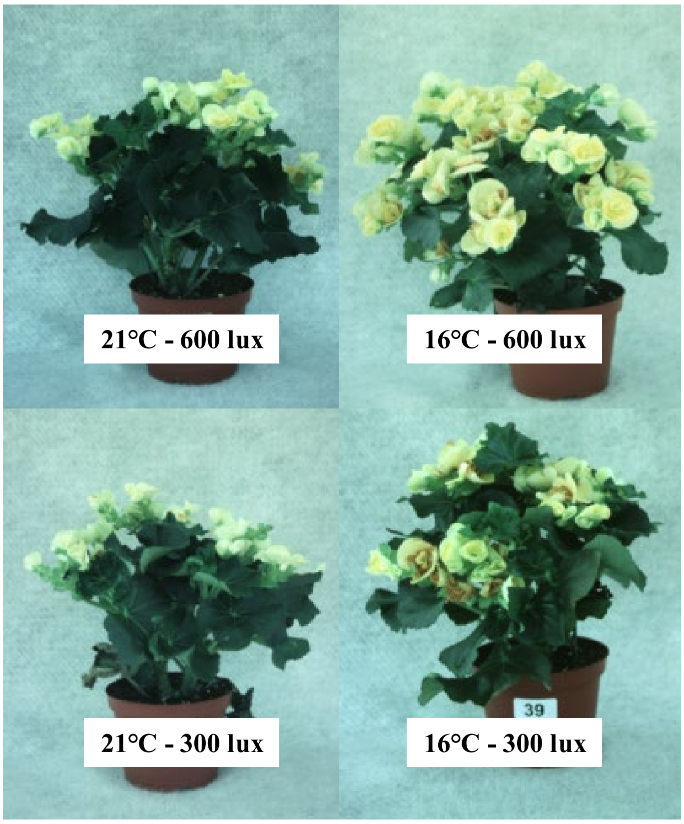


Fig. G.7. Effects of home-life temperature and light on begonia cultivar, Balli, six weeks after the start of home life (Year 1).

Effects of nutritional factors during production on post-harvest quality (objective 4)

Mean objective quality, the rate of quality decline and longevity during home-life (home-life duration to the point where plant quality was assessed as 'good' rather than 'excellent' or 'very good') were used as performance measures of pot plant quality during home life. These measures enabled comparisons to be made of the effects of nutritional factors during production on subsequent post-harvest quality. Key findings were that low N during production helped slow the rate of quality decline during home life in poinsettia and begonia and that high K during production reduced flower drop in begonia, especially in high home-life temperature environments.

Poinsettia

In the case of poinsettia, all nutritional treatments gave plants that met retail quality specifications at marketing in both years. However, low N increased plant height and diameter at marketing relative to high N (Fig. G.8).

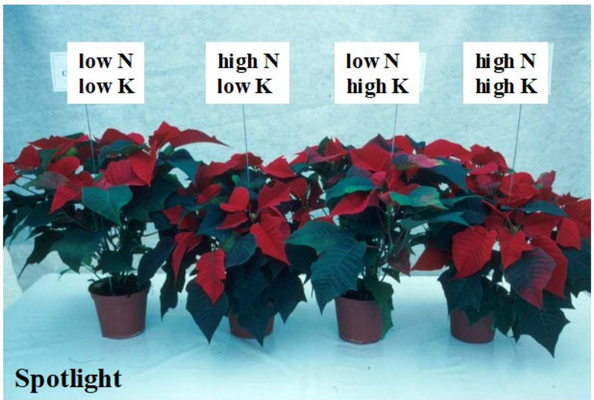


Fig. G.8. Effects of nutritional treatments on poinsettia cv. Spotlight at marketing in year 3

There were subsequent effects of N and K on post-harvest quality, but these were rather small. In Year 3, N had no effect on bract loss, but this was consistently increased by high K. High N slightly increased cyathia loss, but K had no effect on this character. The most marked effects of N and K were on bract fading and BEN (Fig. G.9). Low N plants showed slower bract fading than high N plants, whilst levels of BEN were increased by high K but little affected by N. This latter effect was possibly due to low levels of calcium (Ca) which were found to be associated with high K during production. However, low Ca was also associated with low nitrogen, presumably due to the use of calcium nitrate to manipulate nitrogen levels, and as low N was not associated with BEN the possible relationship between BEN and low Ca is not straightforward.

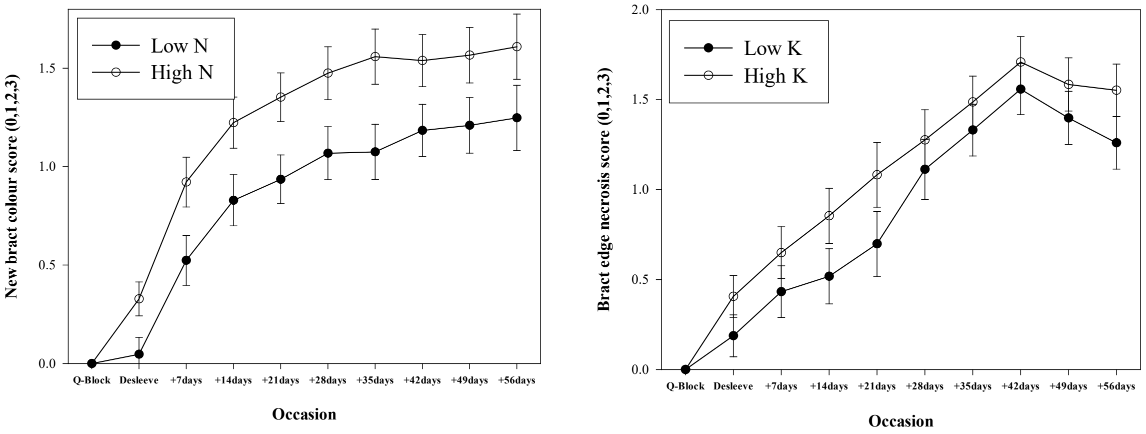


Fig. G.9. Left – effects of nitrogen (N) on bract paling (high score = high paling); right – effects of potassium (K) on bract edge necrosis (high score = high BEN)

In Year 4, high K again increased bract drop in Sonora (but not Spotlight) and increased the incidence of BEN. Mean quality was significantly higher in the low K treatment than in the high K treatment for the commercial chain, but not in the minimum stress chain. In contrast to the findings of Year 3, low N had no consistent effect on bract colour. In spite of this, the rate of objective quality decline during home life was slower in the two low N treatments than in the two high N treatments, at least for Sonora (Fig. G.10). This converted to an average increase in longevity due to low N of about 3½ days. This was rather similar to findings in Year 3, but was rather slight compared to, for example, the increase in longevity of about 2 weeks due to the combined effect of cool home-life temperature and high light (relative to high home-life temperature and low light).

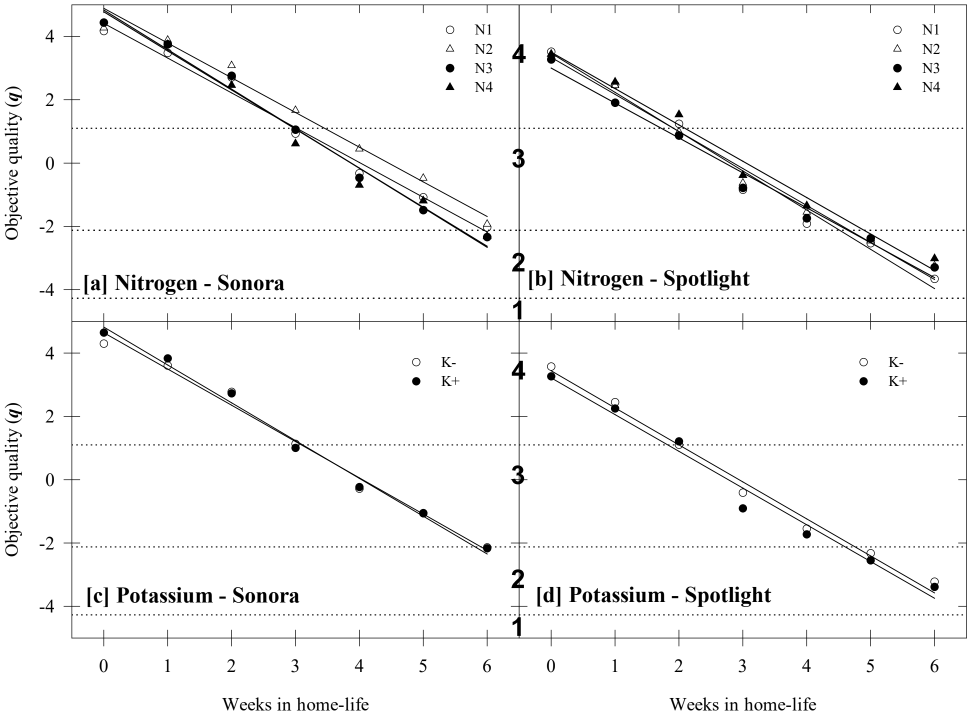


Fig. G.10. Effects of N and K during production on subsequent objective quality of poinsettia during home life in Year 4 (4 = Excellent, 3 = Very Good, 2 = Good and 1 = Poor or Very Poor)

Begonia

Low N during production strongly reduced the rate of quality decline in begonia in Year 3 as shown in Fig. G.11. However, this effect was not commercially useful and did not translate into increased longevity because the low N feed severely reduced objective quality at marketing by giving very pale leaves (Fig. G.12). The Year 4 begonia trial was designed to improve leaf colour by using a higher minimum level nitrogen feed to avoid the problem of pale leaves observed in Year 3 and the graphs in Fig G.13 show the nitrogen and potassium feed effects on foliar colour. Although there were small differences between the treatments, all the plants were of good saleable quality. It is interesting to note that the rate of fading of foliar colour of the low N treatment was reduced relative to the other N treatments. However, as the low N foliar colour was initially lower, there was no increase in foliar colour longevity due to low N over the time course of the experiment.

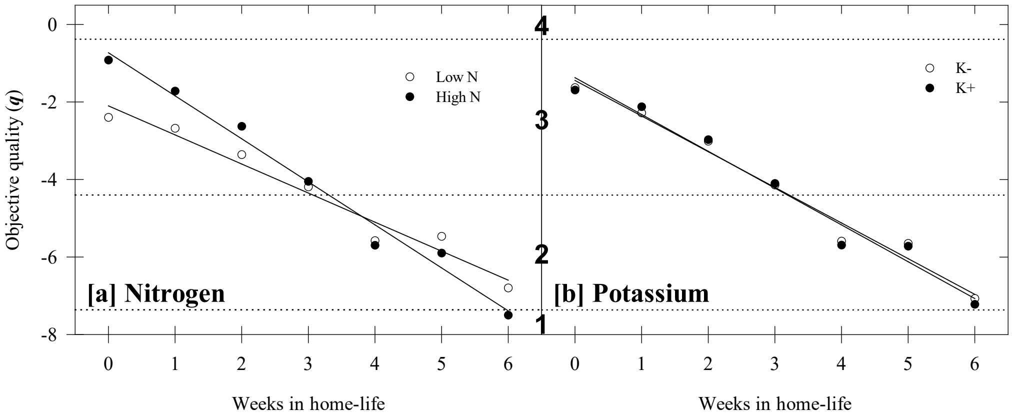


Fig. G.11. Main effects of N and K during production on subsequent objective quality of begonia during home life in Year 3 (4 = Excellent, 3 = Very Good, 2 = Good and 1 = Poor or Very Poor)

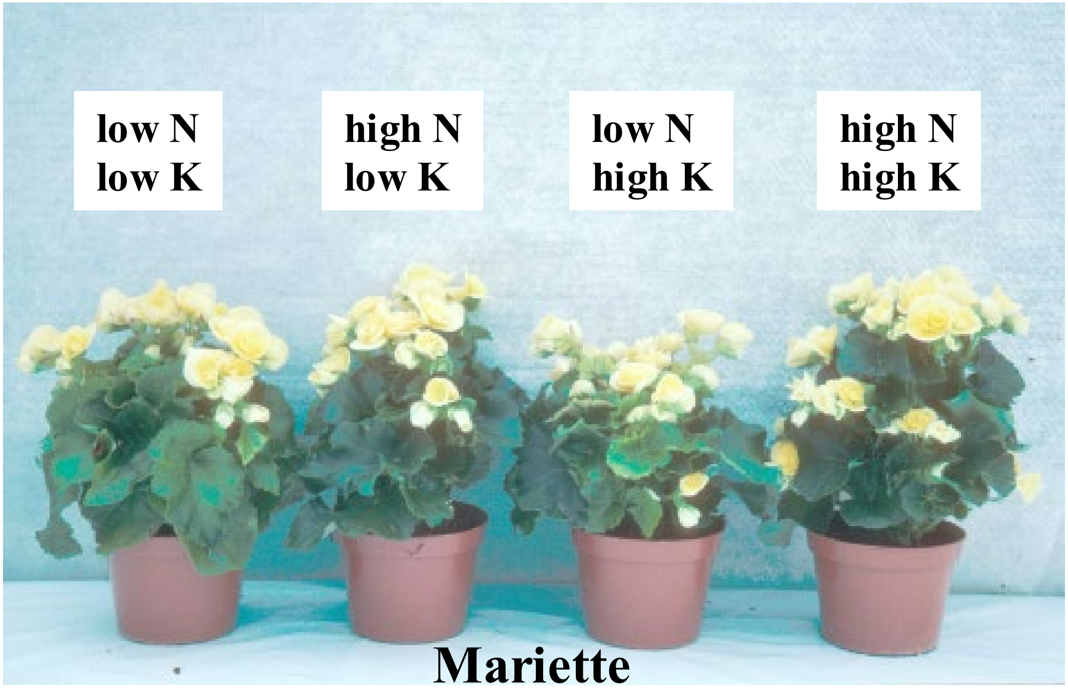


Fig. G.12. Effects of nutritional treatments on begonia Mariette at marketing in Year 3.

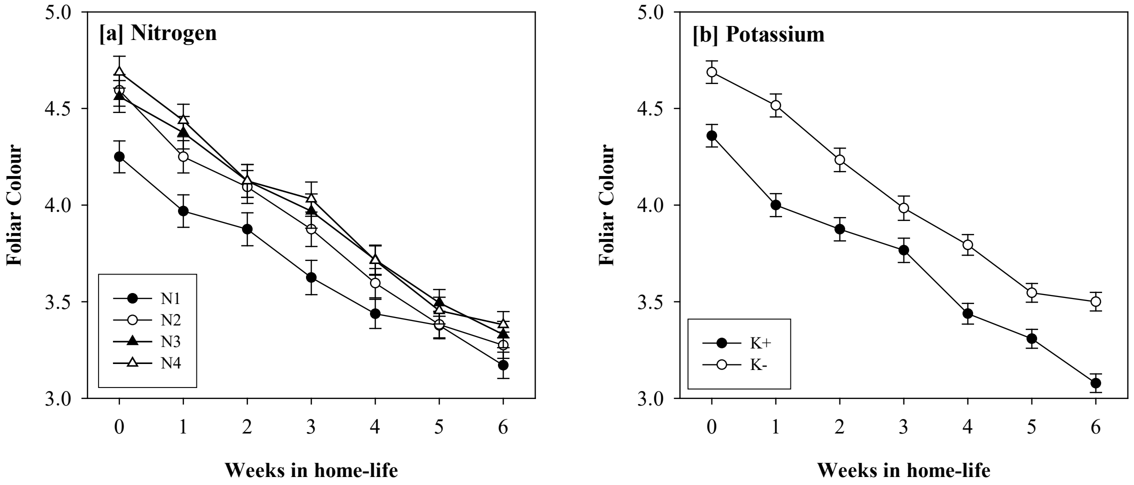


Fig G13[a-b]: Foliar colour score (1-5; 5 = dark green & 1 = severe paling) for begonia plants in Year 4, for nitrogen [a] and potassium treatments [b] during production

Fig G.14a shows the effects of the nitrogen feeds on observed plant quality during home life in Year 4. All the plants were of high quality at the beginning of home life but there was very little evidence of any effect of the nitrogen feed treatments on subsequent home-life quality or longevity. Either the nitrogen feed treatments in Year 4 were chosen at too high a level to show any beneficial effect, or it is not possible to find a compromise level of nitrogen feed that gives both high initial plant quality and extended home life.

Fig G.14b shows the effects of potassium feed on home life quality and there was clear evidence that the high potassium treatment reduced the average rate of decline of begonia quality during home-life, albeit from a slightly reduced initial quality level due to slightly paler leaves.

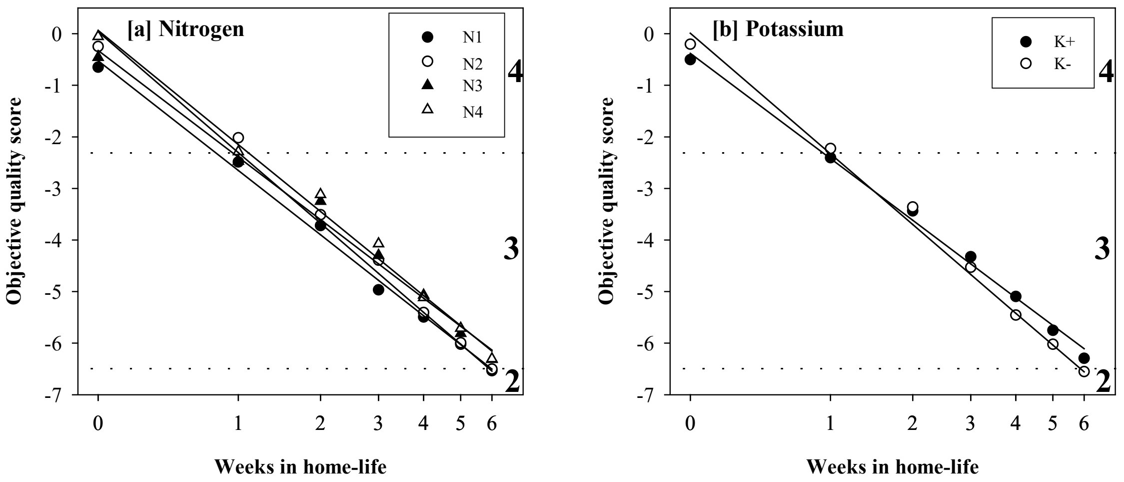


Fig. G.14. Main effects of N and K during production on subsequent objective quality of begonia during home life in Year 4 (4 = Excellent, 3 = Very Good, 2 = Good and 1 = Poor or Very Poor)

Flower drop in begonia

The effect of K on the rate of quality decline in begonia was examined in detail by examining the effects of K on the individual determinants of plant quality. Figs G15a-d show the effects of K feed levels on percentage cumulative flower drop for the two varieties in each of Years 3 and 4. There was a very strong contrast between the high and the low temperature plants, with the high temperature plants showing accelerated flower drop relative to the low temperature plants. There was also very good evidence that the high K feed treatment gave increased resistance to flower drop relative to the low K feed treatment at the high home-life temperature, especially during early home-life.

The strongest response was seen in Year 4 (Figs G15c-d) where there was good evidence that high K reduce high temperature flower drop, especially for Batik but also for Blitz. The evidence from Year 3 (Figs G15a-b) was less strong but there was some evidence that high K reduced flower drop in Mariette in the high temperature regime, especially in the critical first two weeks of home life. It is unlikely that a purchaser would tolerate flower drop in newly purchased plants therefore increased robustness against flower drop in high-temperature home life environments could be very important commercially.

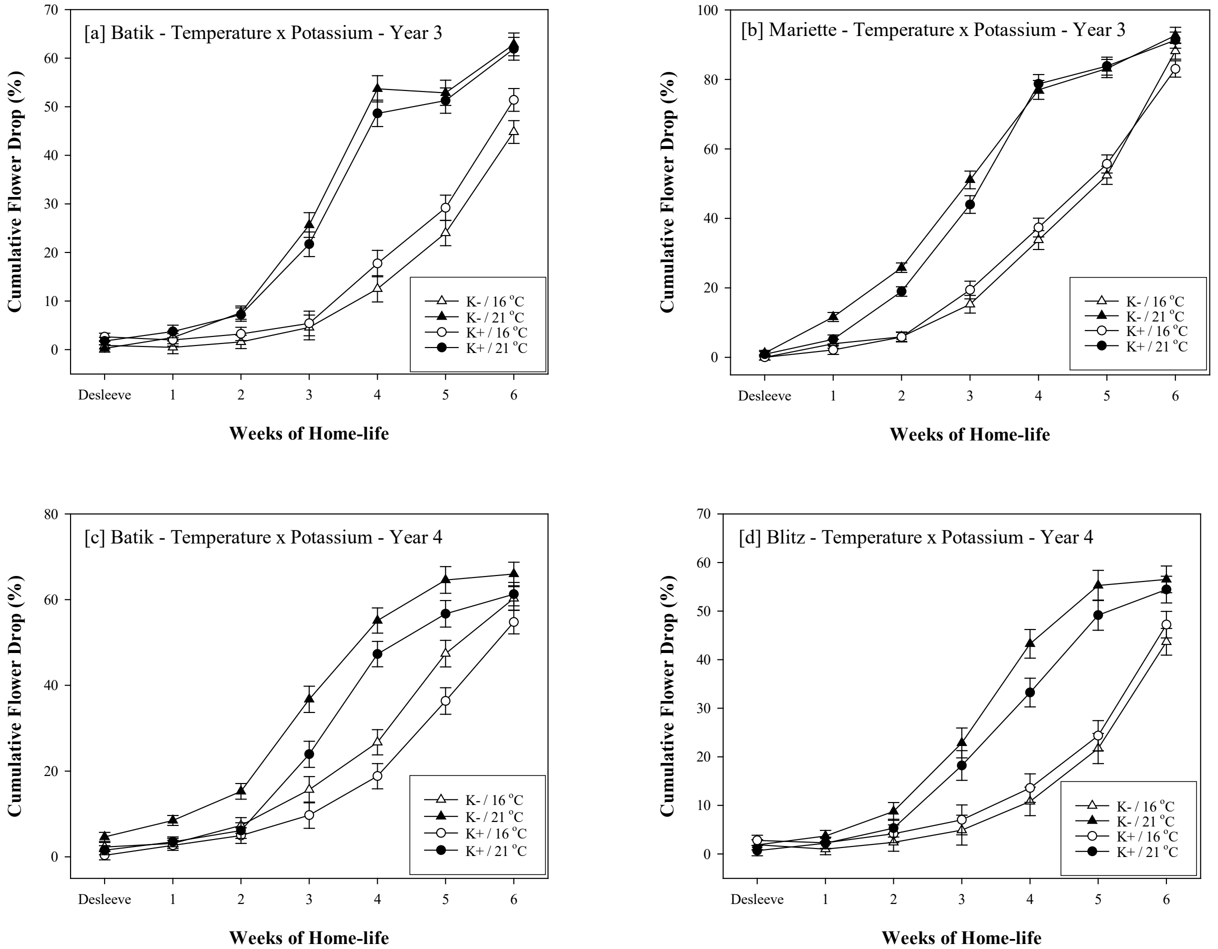


Fig G15[a-d]: Effects of potassium treatments on percentage cumulative flower drop in begonia at two home-life temperatures for [a] Batik Year 3, [b] Mariette Year 3, [c] Batik Year 4, [d] Blitz Year 4.

Consumer perception of quality

When the project was initiated, it was decided that one specialist expert would score poinsettia quality and a different specialist expert would score begonia quality. This was done deliberately to maximise comparability between the different experiments in the different years of the project. However, during the course of the project, the need to compare the expert assessments of quality with consumer assessments of quality became apparent. For this reason, DEFRA agreed to fund a short-term project HH1529SPC to enable five additional 'consumer' estimates of quality to be made on the Year 4 poinsettia trial by non-science members of Efford staff in parallel with the expert quality scores. HDC subsequently funded an exactly similar set of consumer scores on the corresponding Year 4 begonia trial.

The analysis of the Year 4 poinsettia trial showed that although there was considerable variation between the five additional assessors, there was some evidence that the consumer assessors perceived quality differently from the expert. Fig G.16 shows the mean quality profiles over time in home life for the four N feed treatments as determined by the five consumer assessors and by the expert assessor. The most striking feature of these plots is that the five consumer assessors all ranked the lowest N treatment (N1) best or equal best throughout home life. However, the expert assessor gave the highest ranking to the N2 treatment regime.

The analysis of the Year 4 begonia trial showed equally interesting comparisons between the consumers and the expert assessor. Fig G17 shows the mean quality profiles over time in home life for the four N feed treatments as determined by the five consumer assessors and by the expert assessor. It is striking that although the expert assessor recorded virtually no differences between the four nitrogen feed treatments, the consumer assessors showed a distinct preference for the low N feed plants.

Taken together, these results show the importance of using consumer assessors in addition to expert assessors when scoring ornamental plant quality. Expert scores are not always fully consistent with consumer preferences and the choice of an N feed production regime for optimising post-harvest quality must be conditioned on the preferences of the consumer, which will not necessarily coincide with those of the expert grower (or retailer). Further work on this aspect of plant quality could be of great importance to the horticulture industry.

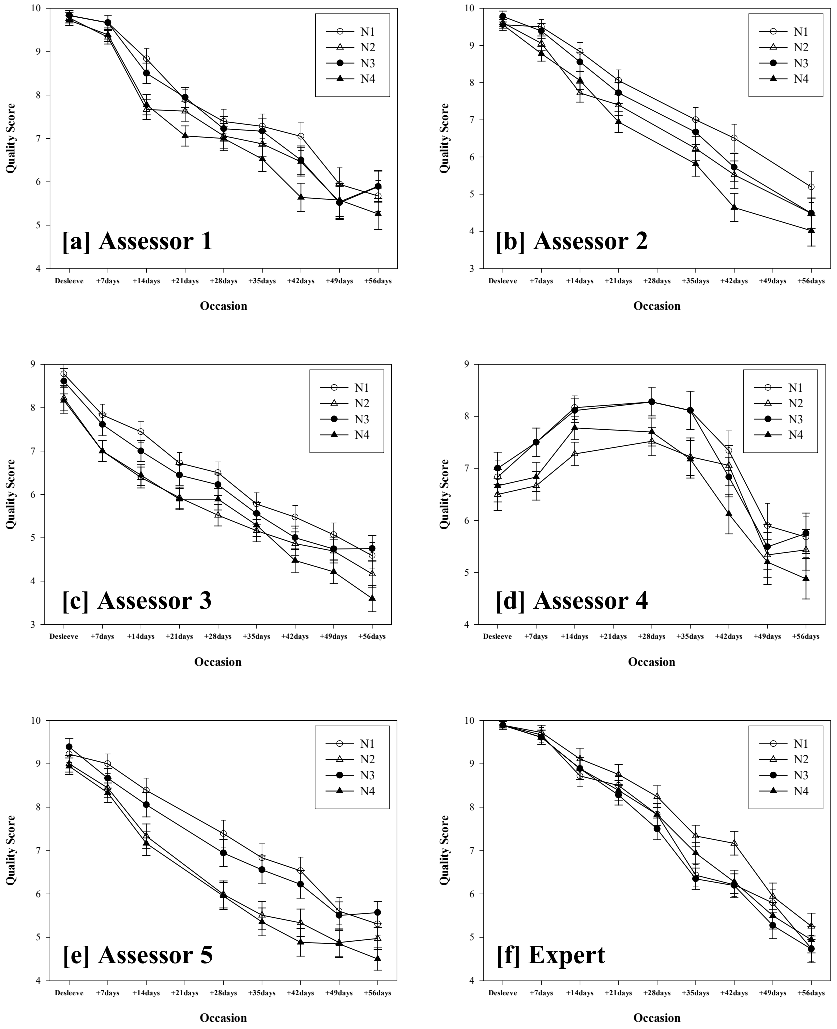


Fig. G.16. Pairwise plots showing mean quality scores for the four nitrogen production treatments in the Year 4 poinsettia trial as assessed by the five consumer assessors and by the expert assessor

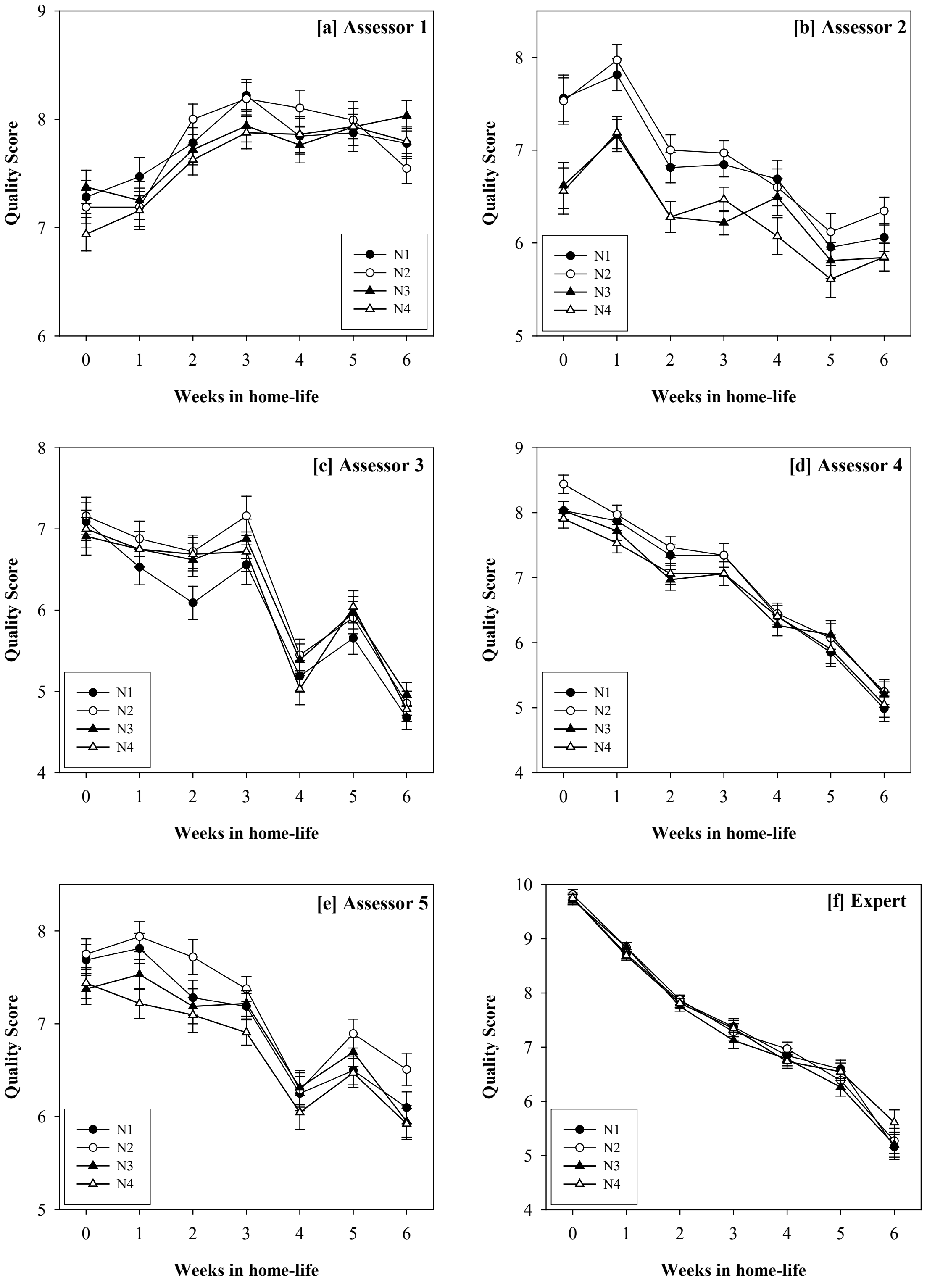


Fig. G.17 Mean quality scores for four nitrogen feed treatments in the Year 4 begonia trial as assigned by the five consumer assessors and the expert assessor

Use of chlorophyll fluorescence (CF) as an indicator of stress (Objective 2)

Two types of fluorimeter were tested in this work, both manufactured and made available by Hansatech Ltd. Of the two, the most useful proved to be the Plant Efficiency Analyser (PEA) and this was the instrument used throughout the trial. As it was found that the environmental conditions under which the readings were taken had a marked effect on CF scores, all plants were put into uniform test conditions well before readings were taken. Leaf clips were attached to appropriate leaves at least 1 hour prior to measurement to give sufficient time for dark adaptation (see Fig. G.18). In begonia, another important factor was leaf position corresponding to leaf age. In both poinsettia and begonia, CF score was markedly influenced by cultivar

Fig. G.18. Leaf clips attached to lower leaves of begonia plants prior to measurement of CF

Of the several procedures and measures of CF tested, 'first hit' Performance Index (PI), devised by Professor Strasser of the University of Geneva, was shown to be the most effective, with PI essentially mirroring objective plant quality decline over time in home life. The PI decline over time clearly showed the effects of deleterious factors such as cold transport and the beneficial effects of factors such as cool home-life temperature.

Fig. G.19, for example, shows the decline in PI over time in poinsettia for the three marketing treatments in Year 2, together with a graph showing the decline in objective quality score over the same period. These graphs show that CF had the potential to detect the deleterious effects of cold transport at de-sleeve, at which time there was no obvious visual effect of cold stress on quality. In addition, CF scores for the cold-stressed plants were lower than for other plants throughout home life, whereas objective scores showed differences only during the first three weeks of home life.

Fig. G.20 similarly shows the respective decline in PI score and in objective quality score associated with home-life temperature in Year 2 for begonia. The two graphs show marked similarities, with both graphs showing the beneficial effects of cool temperature throughout home life. Again, the graphs lead to the conclusion that CF is effective as an objective indicator of plant quality at the time that the CF measurements were made.

It is important to note that although these results showed that CF was an effective indicator of plant stress at the time the CF measurements were made, they did not necessarily show the effectiveness of CF for predicting future plant quality. The predictive power of CF for future plant quality will be examined in the next section.

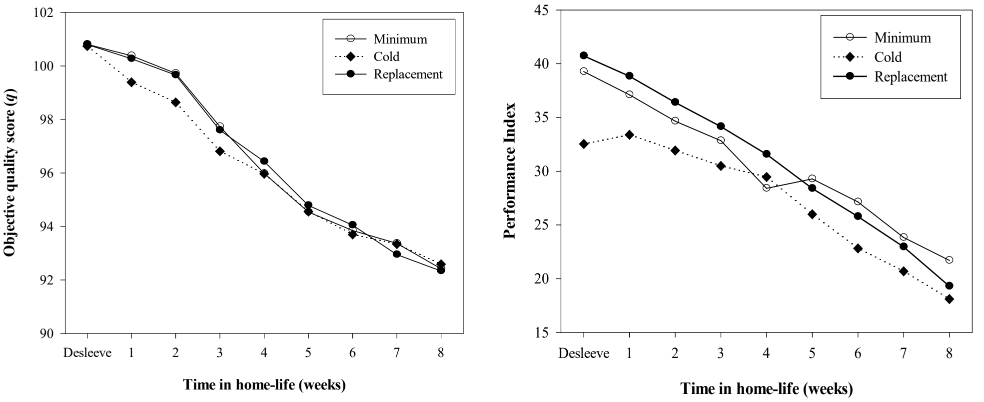


Fig. G.19. Decline in CF score (Performance Index) (Right) and in Objective Quality Score (Left) with time in home life for poinsettia subjected to contrasting marketing stresses in Year 2

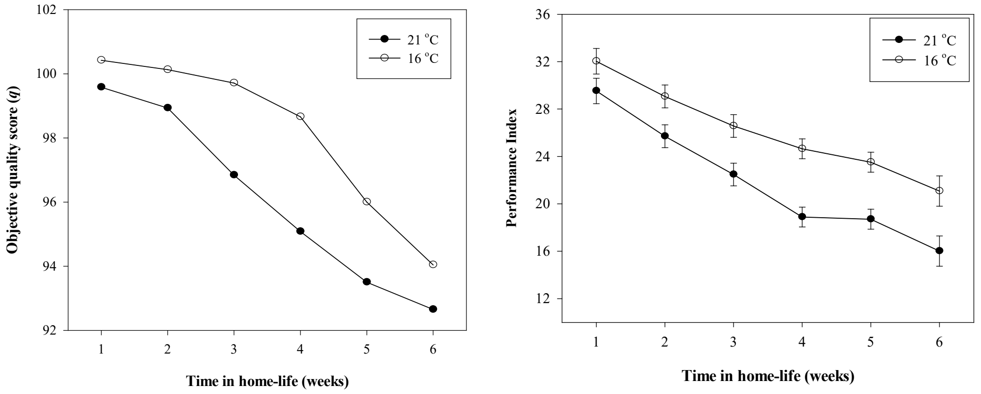


Fig. G.20. Decline in CF score (Performance Index) (Right) and in Objective Quality Score (Left) with time in home life for begonia subjected to contrasting home-life temperatures in Year 2

Predictive value of chlorophyll fluorescence (CF) (Objective 5)

Although CF was routinely shown throughout the project to be an accurate indicator of pot plant quality at the time of measurement, additional analyses were carried out to test whether CF scores could be predictive of potential longevity. An unplanned opportunity to test predictive potential in poinsettia occurred in Year 3 when a large number of plants deteriorated so rapidly in home life that they had to be removed before the end of the trial, showing almost total leaf wilt. The cause of the decline was not identified but could have been due to a pathogenic agent affecting individual plants. Subsequent analysis showed that CF was a potentially useful predictor of plant death, but only when recorded one week prior to plant death. There was also considerable variability, and predictions of subsequent plant death, although significant, were statistically very weak. For this reason, it was concluded that CF had greater potential value for batch screening.

The potential value of CF for batch screening appeared to be confirmed in Year 2 by a clear association between bract drop recorded for contrasting marketing chains in week 2 of home life and PI score at de-sleeve (high bract drop = low PI score). However, the relationship could not be confirmed in Year 4 when bract drop due to simulated cold transport treatment was at least as great as in Year 2 (see Fig. G.21). It was concluded that CF did not have the predictive power necessary to act as a reliable indicator of potential quality at the point of sale for poinsettia.

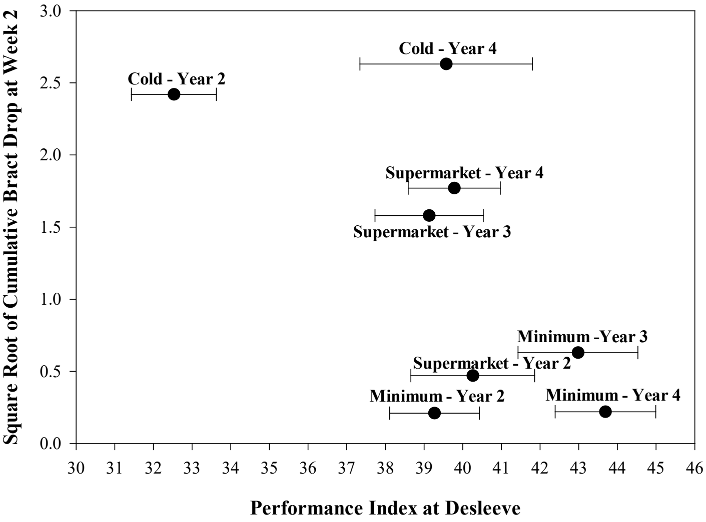


Fig. G.21. Mean performance index (PI) at de-sleeve and square root of cumulative bract drop two weeks after de-sleeve for poinsettia supply chain treatments in Years 2, 3 and 4

The predictive value of CF scores, recorded at de-sleeve, on subsequent quality in home life was tested for individual begonia plants in Year 3. This showed that CF (as PI) at de-sleeve could be predictive of subsequent plant quality for the first two weeks of home life. However, as with poinsettia, the individual data values showed considerable scatter and it seemed therefore that the potential for batch screening was probably greater than the potential for screening individual plants. Fig G.22 shows the combined mean batch data for all transport batch treatments in Years 1 to 4 and shows that CF can be used at marketing as a predictor of flower drop over the two weeks following marketing. A simple linear relationship between batches showed that the PI could explain over 60% of the batch variability in flower drop measured at de-sleeving. The Year 1 plots fall slightly below the regression line but this was possibly due to the choice of leaves for PI measurement. The Year 1 data points in Fig G.22 are based on only two systematically chosen canopy leaves whereas all the other points are based on three. It is possible that the two leaves chosen for the Year 1 data systematically underestimated PI compared with the three leaves chosen for the other years of data.

Overall, there is good evidence that the relationship in G.22 is well determined and this shows that there is substantial potential for using chlorophyll fluorescence for monitoring begonia batch quality during marketing.

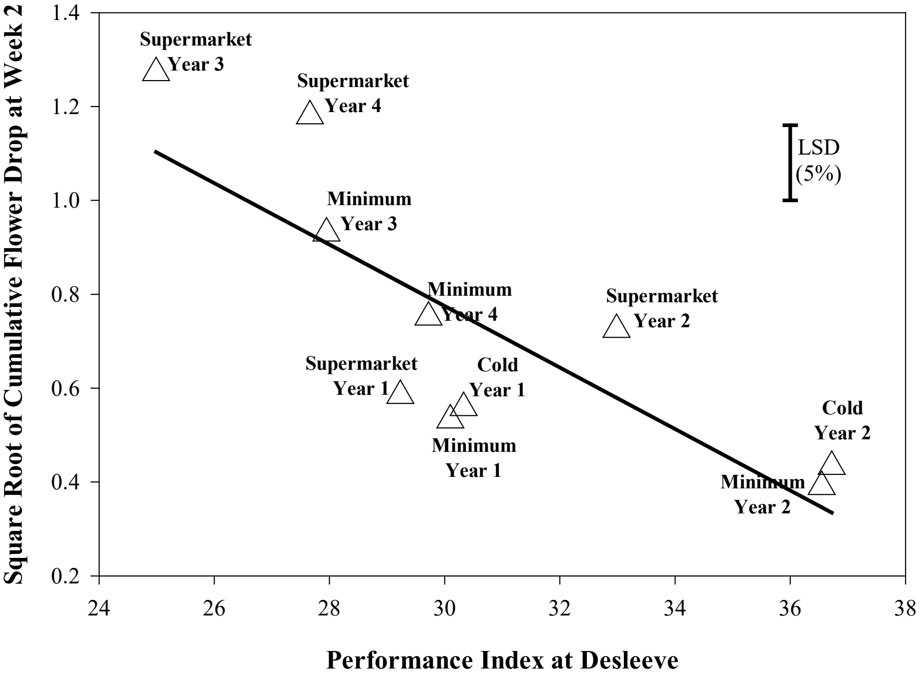


Fig. G.22. Mean performance index (PI) at de-sleeve and square root of cumulative flower drop two weeks after de-sleeve for begonia supply chain treatments in Years 1, 2, 3 and 4

Action points for growers and retailers

- Low temperature (below 14°C) transport promotes subsequent quality loss in poinsettia and markedly reduces home-life longevity. In particular cold stress increases leaf, bract and cyathia loss.

  Growers and retailers should avoid using low temperature transport (below 14°C) for poinsettia wherever possible. The use of data loggers for transport chain records is recommended.

  Customers should be advised to avoid keeping poinsettia plants in cold conditions after purchase, and to get the plants home as quickly as possible.

- Whilst begonia is less affected by marketing stress than poinsettia, cold conditions during marketing will lead to quality loss, particularly a reduction in flower number.

  Growers and retailers should avoid using low temperature transport (below 10°C) for begonia.

- Both poinsettia and begonia had the best home-life performance when held at a cooler temperature (16°C) and good light levels (600 lux), in contrast to high temperatures (21°C) and low light levels (300 lux).

  Growers and retailers should utilise this information on optimum home life conditions to update customer care labels for poinsettia and begonia

- Experimental work on reducing N levels below those used in current commercial practice has indicated some potential for improved longevity of poinsettia and, to a lesser extent, of begonia. Increased levels of K were beneficial in reducing flower drop in begonia, especially in high temperature (21°C) home-life environments.

  Growers might take note of these nutritional effects to improve the home life performance of pot plants. However, caution must be exercised against using very low N levels, particularly at the end of the production phase as the plants may not attain marketable quality.

- Chlorophyll fluorescence has the potential to provide a predictive indication of marketing and post-harvest quality of pot plants and other ornamentals. However, further research is needed to develop and refine a tool that could be used by growers and retailers.

- The main factors determining quality in poinsettia include leaf and bract drop, leaf and bract paling, cyathia loss and bract edge necrosis (BEN). Major factors determining quality in begonia include total bud and flower count, bud and flower drop, and numbers of damaged leaves.

  Growers should utilize the above quality characteristics when developing home-life tests for quality assurance work for customers, and to target the quality characteristics needed in new varieties and selections of poinsettia and begonia.

2. OBJECTIVES AND MILESTONES

The scientific objectives of this project were:

1. **To develop statistical quality loss response functions for measuring the post-harvest quality of commercially grown poinsettia and begonia plants over a range of simulated home-life environments defined by temperature, lighting and irrigation supply factors.**

2. **To develop effective chlorophyll fluorescence screening techniques for the prediction of longevity and robustness of poinsettia and begonia using the same plants and range of home-life environments used for objective 1.**

3. **To adapt robust product design methodology from industry for investigating the effects of source, cultivar, lighting, nutrition and supply chain factors on the quality and robustness of poinsettia and begonia using the range of home-life environments developed for objective 1.**

4. **To quantify the utility of robust product design methodology for improving the robustness and longevity of poinsettia and begonia, and to identify specific production factors that affect post-harvest longevity.**

5. **To quantify the utility of chlorophyll fluorescence for routine market prediction of robustness and longevity of commercial poinsettia and begonia produced and marketed under a range of conditions.**

<u>Original Milestones</u>

The numbering of milestones relates to the Objectives given above. Dates indicate when the milestones were completed. The symbol ☐ means completed milestone.

Year 1 (October 1998 - September 1999)

1.1	**May 1999**	**Establish quality loss functions for poinsettia ☐**
2.2	**Jul 1999**	**Relate chlorophyll fluorescence parameters in poinsettia to quality loss functions ☐**
1.2	**Aug 1999**	**Establish quality loss functions for begonia ☐**
2.3	**Sep 1999**	**Relate chlorophyll fluorescence parameters in begonia to quality loss functions ☐**

Year 2 (October 1999 - September 2000)

1.3	**May 2000**	**Validate quality loss functions for poinsettia ☐**

2.4	**Jul 2000**	**Determine the utility of chlorophyll fluorescence to predict potential longevity in commercial poinsettia ☐**
1.4	**Aug 2000**	**Validate quality loss functions for begonia ☐**
2.5	**Sep 2000**	**Determine the utility of chlorophyll fluorescence to predict potential longevity in commercial begonia ☐**

Year 3 (October 2000 - September 2001)

3.1/4.1	**Jun 2001**	**Develop and quantify statistical methods for the estimation of post-harvest robustness of poinsettia ☐**
5.1	**Jul 2001**	**Make preliminary assessments of the power of chlorophyll fluorescence to predict post-harvest longevity in poinsettia ☐**
3.2/4.2	**Aug 2001**	**Develop and quantify statistical methods for the estimation of post-harvest robustness of begonia ☐**
5.2	**Sep 2001**	**Make preliminary assessments of the power of chlorophyll fluorescence to predict post-harvest longevity in begonia ☐**

Year 4 (October 2001 - September 2002)

5.3	**Jun 2002**	**Quantify chlorophyll fluorescence procedures for predicting post-harvest longevity in poinsettia ☐**
3.3/4.3	**Jul 2002**	**Complete evaluation of robust product design methodology for improving longevity in poinsettia and quantify the effects of production factors ☐**
5.4	**Aug 2002**	**Quantify chlorophyll fluorescence for predicting post-harvest longevity in begonia ☐**
3.4/4.4	**Sep 2002**	**Complete evaluation of robust product design methodology for improving longevity in begonia and quantify the effects of production factors ☐**

Comments on objectives and milestones

Objectives 1 and 2, comprising phase one of the project, together with associated milestones, were met by the end of Year 2 (September 2000). This was affirmed at the Consortium meeting on 6 November 2000. At its earlier meeting on 21 March, the Consortium agreed that no changes were needed to Objectives 3, 4 and 5, phase two of the project, but that the two experimental production factors for poinsettia would both be nutritional (levels of N and K). The Consortium made a similar decision for begonia at its meeting on 6 November. Objectives 3, 4 and 5, together with associated milestones, were met for poinsettia by the end of Year 4 (September 2002). This was affirmed at the Consortium meeting on 11 November 2002. However, the infection of begonia plants with *Fusarium foetens* in Year 4 at Efford meant that objectives 3, 4 and 5, associated with begonia, were not met in full within the original agreed time frame of the project. Given the importance of this trial to the overall concept of growing pot plants in such a way as to increase robustness to withstand marketing and home-life environments, Defra agreed to fund a repeat begonia trial in the following year.

<u>New milestones for new Year 4 (October 2002 – September 2003)</u>

4/5	**Feb 2003**	**Finalize arrangements (financial and scientific) for carrying out a repeat of the Year 4 begonia trial ☐**
4.5/5.5	**Mar 2003**	**Begin nutritional treatments on 'bought in' begonia plants at Efford ☐**
4.6/5.6	**Jun 2003**	**Complete the home-life phase of the repeat begonia trial, having collected all physiological data (including chlorophyll fluorescence and leaf greenness), expert score data and consumer score data (new) ☐**
4.7	**Aug 2003**	**Complete the biometric analysis of nutritional effects on the robustness of begonia in home life☐**
5.7	**Aug 2003**	**Quantify the value of chlorophyll fluorescence for predicting post-harvest longevity batch effects in begonia☐**

3. SCIENCE SECTION

3.1. Executive summary

- Pot poinsettias and begonias were subjected to varying levels of marketing stress and were then tested for quality and longevity in a wide range of simulated home-life environments. Physiological plant characteristics and expert quality scores were recorded at weekly intervals throughout home life. This information was used to relate plant quality to measured physiological variables **(Objective 1)**.

- Objective quality scores, determined as a linear combination of plant physiological characteristics, were used to provide models of integrated plant quality. The parameters of the model represented the contributions of each of the physiological variates to overall plant quality. The model fitting procedure was based on a general estimating equations (GEE) approach **(Objective 1)**.

- The effects of physiological characters on perceived plant quality were quantified. Major factors determining quality in poinsettia included cyathia drop, leaf and bract drop, leaf and bract paling and bract edge necrosis (BEN). Major factors determining quality in begonia included total bud and flower count, bud and flower drop, and numbers of damaged leaves. **(Objective 1)**.

- The decline in objective quality scores over time in home life was modelled and quality loss curves were fitted. These were used to quantify the effects of transport chain and home-life factors on post-harvest quality and longevity (**Objective 3**). Cold-temperature transport had large adverse effects on longevity for poinsettia but not for begonia. The combination of cool home-life temperature (16°C) and high light (600 lux) was particularly beneficial to home-life quality.

- Low N during production reduced quality decline in home life in both poinsettia and begonia **(Objective 4).** In poinsettia, the maximum increase in longevity due to low N was 3½ days (cv Sonora). In begonia, quality loss in home life due to low N was substantially reduced in Year 3 but quality at marketing was also reduced. In Year 4, a higher minimum level of N feed was used in the begonia trial and this gave higher quality plants at the beginning of home life. However, there was then very little evidence of any N effect on subsequent home-life.

- High K feed during begonia production reduced begonia flower drop in the first two weeks in the high temperature home-life environment **(Objective 4).** There was very little flower drop in the low temperature home-life environment therefore high K specifically improved robustness against high temperature home-life environments. This showed that high K feed during production increases begonia robustness against home-life environments.

- Chlorophyll fluorescence (CF) screening was tested using the Hansatech Plant Efficiency Analyser (PEA). to monitor 'stress' in poinsettia and begonia **(Objective 2)**. CF was measured either as F_v/F_m or as 'Performance Index' (PI) devised by Professor Reto Strasser (consultant to the project). Starting in Year 2, ‘second hit’ CF values were compared with ‘first hit’ CF values. The second hit values measured photosynthetic efficiency after the imposition of a first-hit machine-induced stress (saturating light). CF parameters were recorded in parallel with the collection of plant quality and plant physiological data. **(Objective 2)**.

- 'First hit’ PI was shown to be the most effective plant stress measure, with scores essentially mirroring objective plant quality assessments. CF effectively mirrored the observed immediate plant quality in home life and clearly showed the effects of deleterious factors such as cold transport. The most useful CF predictors were the first-hit performance index, PI, and the second-hit ratio F_v/F_m. **(Objective 2)**.

- A number of factors needed to be standardized for comparative CF including environment factors such as temperature and light at the time of measurement. Another important factor that affected CF scores in begonia was leaf position (age). In both poinsettia and begonia, the CF score was markedly influenced by cultivar **(Objective 2)**.

- CF measured at marketing had weak predictive power for home-life quality but did not appear useful for predicting individual plant performance **(Objective 5)**. CF did appear to be a potentially useful predictor of individual plant death in the Year 3 poinsettia trial, but only for CF recordings taken one week prior to plant death. It was concluded that CF prediction, as tested in this project, was not sufficiently consistent to provide a reliable indicator of potential quality for individual poinsettia or begonia at the point of sale.

- CF appeared to have potential for batch screening in the Year 2 poinsettia trial when a clear association was found for cold-stressed plants between high bract drop during home life and low PI score at de-sleeve. However, this relationship could not be confirmed in Year 4, presumably due to confounding by other effects. **(Objective 5)**.

- CF batch means measured at marketing were used to predict begonia mean batch flower drop during home life **(Objective 5)**. A simple linear relationship showed that PI measured at de-sleeving could explain over 60% of between-batch flower-drop variability. Flower drop was an important quality characteristic **(Objective 1)** and this strong predictive model showed good evidence that PI could be used at marketing to predict mean home-life batch quality of begonia.

- In the final year of the trial, five sets of consumer quality scores for each of poinsettia and begonia were compared against the expert quality scores. There was good agreement between the consumer assessors but there were some consistent differences from the experts. These results show that consumers have consistent quality preferences and that expert assessments of quality need to be calibrated against consumer preferences. Further work on the consumer assessment of quality could be of substantial commercial importance. **(Objectives 1, 3 and 4)**.

3.2. Introduction

The pot plant industry is a major sector of UK horticulture, with a farm gate value of about £61m (DEFRA provisional statistics for 2000). However, competition from imports is fierce (valued at about £87m in 2000) and is intensifying. UK growers recognize that they will successfully compete with overseas producers only if their products are of the highest visual quality at the point of sale and also have the potential for long home life. Failure to match consumer expectation in terms of product quality and longevity results in consumer disappointment and lost repeat sales. This is also recognized by the UK multiple retailers who have begun introducing guarantees of product longevity.

It is now well established that pot plants that are visually similar at the time of marketing can show great variation in subsequent home-life longevity. For example, van Dijk and Barendse (1991) working for the Westland Flower Auction, showed that the 'effective' home life of poinsettia 'Angelika' varied between four and ten weeks depending on previous growing history. Transport simulation generally reduced subsequent ‘keeping quality’, but the effects of this also varied greatly, depending on prior growing history. At worst, transport reduced subsequent longevity by up to 66%.

Production factors determining differences in the post-harvest longevity of pot plants remain largely uncertain. However, recent reviews (Nell *et al.* 1995; Hendriks, 2001) have concluded that important factors include the growing temperature, light and fertilizer use. Low levels of tissue calcium (Ca) have been implicated as a primary cause of the disorder, bract edge necrosis (BEN), in poinsettias (Woltz and Harbaugh, 1986). Edmondson (1996) has speculated that high levels of applied potassium (K) during the production of begonias have a deleterious effect on post-harvest longevity by competitively inhibiting Ca uptake. High levels of fertilizer appear, in general, to be deleterious to post-harvest quality, and Nell et al. (1989) showed that terminating fertilizer use some weeks before marketing could be beneficial for pot chrysanthemums. High nitrogen (N) levels appear particularly damaging to subsequent longevity, and pot plant species reported to benefit from low N include begonia, campanula, cyclamen, New Guinea impatiens, poinsettia, rose and schefflera (Braswell *et al.*, 1982; Druege, 2001; Grantzau, 1988; Serek, 1990; ter Hell and Hendriks, 1995).

Post-harvest longevity has traditionally been determined by holding plants in a single post-harvest environment intended to simulate 'normal' home-life conditions. However, pot plants may be exposed to a wide range of 'normal' home-life conditions (watering, illumination, temperature etc), and robustness to withstand varied conditions is, potentially, a most important component defining pot plant quality. Product robustness against conditions of use has long been recognised as important in manufacturing industry and there is an extensive bibliography on research methodology aimed at making products robust against varied conditions of use. A wide range of options are discussed by Nair (1992), including methods for the combined analysis of means and variances across a range of environments. The choice of noise factors for simulated environments for industrial products has been studied by a number of authors including Gilmour (1991) and Tuck *et al.* (1993), building on the suggestions of Taguchi (1987). Tsai *et al* (1996) and Bisgaard and Steinberg (1997) discuss robust estimation for 2-level factorial experiments. Edmondson (1996) initiated the adaptation of robust product design to pot plants production in a study of biometric methods for investigating shelf and after-sales quality of pot plants.

UK-grown pot plants can be expected to have higher potential post-harvest quality and longevity than imported produce, as they should be fresher due to a much shorter marketing chain. However, the two products are likely to appear similar at the time of delivery to the retailer, and there is currently no objective method to determine the potential post-harvest quality and longevity of a pot-plant at the point of sale. A possible way of doing this may be to utilise chlorophyll fluorescence (CF). The effectiveness of CF to quantify the effects of imposed plant stress factors, such as low temperature, air pollution and drought on photosynthetic function has recently been reviewed by Bolhar-Nordenkampf and Oquist (1993) and by Harbinson (1995). van Kooten *et al* (1991) have studied the use of chlorophyll fluorescence for predicting the post-harvest longevity of pot plants and have concluded that modulated fluorescence is a fast and non-invasive technique to determine physiological status over time. More recently, van Kooten (personal communication, December 1996).has studied the applicability of chlorophyll fluorescence techniques to predict post-harvest longevity for the Flower Auctions in Holland. The results of these studies are confidential, but he has written, "The methodology is suitable for judging potential plant longevity, but the relationship is not simple and it takes a few precautions to make sure you are not measuring unwanted effects. The Dutch auctions have tried to measure this relationship themselves, but “have found it difficult to create the proper circumstances to measure reproducibly”.

This project has aimed to improve the competitiveness of UK pot plant producers and to benefit consumers (and retailers) by facilitating the production and delivery of more consistent and longer-lived pot plants to the point of sale. This has been done by:

- **Identifying and quantifying key production and supply chain factors influencing post-harvest quality and longevity in a wide range of home-life environments, and adapting robust product design procedures used in manufacturing industry to identify protocols giving enhanced post-harvest product robustness to withstand varied post-harvest environmental and handling treatments.**

- **Evaluating the usefulness of novel chlorophyll fluorescence techniques to provide objective criteria of potential post-harvest quality and longevity.**

The project has focused on poinsettia and begonia as 'model' plants that account for about 27% of the value of the pot plant sector. However, the principles established have application to all pot plant species and most other horticultural commodities.

3.3. Production and treatment regimes

Years 1 and 2 of the project used commercially grown poinsettia and begonia. These plants were subjected to varying levels of marketing stress, and were then tested for quality and longevity in a range of simulated home-life conditions at Efford. Years 3 and 4 of the project used poinsettia and begonia produced experimentally at Efford using a range of nutritional regimes. These plants were also tested for quality and longevity using the same home-life conditions that were used for the commercial plants. Physiological characters were measured weekly during home life and quality scores were simultaneously assigned by an 'expert' assessor. These measurements enabled quality in poinsettia and begonia to be modelled and defined objectively in terms of measured physiological variables **(Objective 1)**. The models were then used to assess the effects of marketing and home-life treatments on quality loss in home life (**Objective 3**) and the effects of nutritional regimes on home-life quality and longevity (**Objective 4**). In a parallel procedure, screening techniques were developed using chlorophyll fluorescence (CF) to monitor 'stress' in poinsettia and begonia **(Objective 2).** The utility of CF was then determined as a means of predicting potential home-life longevity at the point of despatch or sale **(Objective 5)**.

3.3.1. Transport and home-life treatments – Years 1 and 2

In Year 1, commercially-grown poinsettia (Sonora and Spotlight) and commercially-grown begonia (Balli and Batik) were transported from Double H Nurseries Ltd (New Milton, Hampshire) to HRI Efford at the end of the second week of December for poinsettia and at the end of the fourth week of April for begonia.

The plants were transported in three different ways, representing three levels of simulated supply chain stress:

- *Minimum stress*: plants were transported over the short distance from New Milton to Efford with the minimum possible stress, ensuring that minimum temperatures did not fall below 14-15°C. The plants were de-sleeved and held at 15°C with 1,000 lux lighting (14 hours/day) for 48 hours until the start of a simulated retail phase

- *Commercial marketing stress*: plants were transported from New Milton, via commercial depots, to retail outlets close to Efford using either the Sainsbury retail chain or the Safeway chain. The pots were collected from the local retail outlet, transported to Efford, and then subjected to a simulated retail phase at the same time as the minimum stress plants. The temperature trace in Fig. 1 (top), obtained using monitoring equipment accompanying the pots, shows that although 'heated' transport had supposedly been used, poinsettia transport temperature fell to 10°C during transit from New Milton to the depot in Swindon in Year 1.

- *Cold-transport stress*: Pots were rapidly transported to Efford and then cold-stored, while still sleeved and boxed, at 7°C for 2 days. They then entered the simulated retail phase, along with pots from the other marketing treatments. Fig. 1 (bottom) shows temperature traces from collection to the start of the simulated retail phase for poinsettia in Year 1.

The simulated retail phase was at 18°C and 1,000 lux lighting (14 hours/day). Pots from the minimum stress treatment were without sleeves but pots from the other two marketing treatments remained sleeved. After 48 hours, all pots were de-sleeved and home-life treatments were started.

Home-life treatments were imposed for 6 weeks and comprised all combinations of two contrasting temperatures (16°C or 21°C) given in separate controlled-temperature rooms, two lighting levels for 14 hours/day (600 lux or 300 lux) achieved by the use of 50% mesh shading and two watering regimes. For poinsettia, the watering regimes were standard or wet, with the wet treatments achieved by standing the pots in saucers holding approximately 1 cm of water. For begonia, the watering regimes were standard or fluctuating, with the fluctuating treatment achieved by allowing pots to dry out almost to wilting before re-watering.

In total, there were 48 treatments comprising 2 cultivars x 3 supply chains x 8 home-life environments for each of poinsettia and begonia, with three replicate plants per treatment combination (144 pots in total).

Physiological characteristics were measured weekly. For poinsettia, the characteristics included leaf loss, bract loss, bract colour, leaf colour, number and stage of development of cyathia and the incidence of *Botrytis*, BEN and other disorders. For begonia, the characteristics included counts of green buds and coloured buds, open single flowers and open double flowers, bud drop, flower drop and leaf drop, counts of damaged leaves, leaf colour, flower colour and the incidence of *Botrytis*. In addition, on each recording occasion, an expert assessor scored individual poinsettia plants for bract, leaf and cyathium quality (Mr Gary Shorland), and individual begonia plants for flower, foliar and overall plant quality (Mr Mike Holmes). Each of these quality attributes was scored using an absolute ten-point personal assessment scale: 1 to 2 = very poor; 3 to 4 = poor; 5 to 6 = good; 7 to 8 = very good; 9 to 10 = excellent.

Experimentation for poinsettia and begonia in Year 2 was essentially a repeat of that in Year 1. However, for poinsettia, the home-life phase was extended to 8 weeks to give more time for the decline in quality to approach a minimum. In addition to bract, leaf and cyathium quality scores, overall plant quality scores were also recorded. Safeway provided the commercial marketing chain for poinsettia but, unfortunately, the whole batch of plants was misdirected during transportation and had to be replaced by a consignment of plants supplied direct from the production nursery to Efford. For the purposes of data interpretation, this chain has to be regarded as closer to the minimum stress treatment than to the intended commercial supply treatment. Sainsbury provided the commercial marketing chain for begonia.

3.3.2. Production treatments – Years 3 and 4

Poinsettia

i) Year 3

Regimes. The production treatments for Year 3 poinsettia had two quantitative levels of feed nitrogen (N) combined with two quantitative levels of feed potassium (K). These nutritional treatments were started 2 weeks after potting when the plants were fully established and in active growth (establishment was at 125 ppm N and 0 ppm K), and levels of N (but not K) were proportionally reduced with stage of growth as shown in Table 1a. The combination of low N and low K was regarded as the commercial standard.

Table 1a. N and K nutritional treatments used in poinsettia production in Year 3

Nitrogen (N)	Potassium (K)	Growth Stage	ppm N	ppm K	N:K Ratio
Low	Low	1	225	175	1.3 : 1
		2	175	175	1 : 1
		3	150	175	0.9 : 1
High	Low	1	335	175	1.9 : 1
		2	260	175	1.5 : 1
		3	225	175	1.3 : 1
Low	High	1	225	350	0.6 : 1
		2	175	350	0.5 : 1
		3	150	350	0.4 : 1
High	High	1	335	350	1 : 1
		2	260	350	0.7 : 1
		3	225	350	0.6 : 1

Leaf and growth medium assays. Leaf tissue was collected on two occasions during production and Table 1b shows the average percentage N, K and Ca leaf tissue concentration averaged over the two occasions. Similarly, the growth medium mineral content was assayed on three occasions during production and Table 1c shows the average percentage N, K and Ca averaged over the three occasions, together with the average conductivity.

Table 1b. Leaf mineral content during production (sampled weeks 42 and 46) for N and K nutritional treatments used in poinsettia production in Year 3

Treatment	Level	Nitrogen (%)	Potassium (%)	Calcium (%)
Potassium	Low	4.25	2.66	1.35
	High	4.34	3.28	1.13
Nitrogen	Low	4.15	3.09	1.11
	High	4.45	2.84	1.37
Lsd (5%)		0.30	0.14	0.19

Table 1c. Growth medium mineral content during production (sampled weeks 40, 42 and 46) for N and K nutritional treatments used in poinsettia production in Year 3

Treatment	Level	Nitrogen (mg/l)	Potassium (mg/l)	Calcium (mg/l)	Conductivity (μS)
Potassium	Low	204	164	152	319
	High	198	390	98	411
Nitrogen	Low	123	280	71	323
	High	279	273	179	406
Lsd (5%)		98	78	60	160

ii) Year 4

Regimes

The poinsettia production treatments for Year 4 had four quantitative levels of nitrogen feed combined in all factorial combination with two quantitative levels of K (Table 2a). As in Year 3, nutritional treatments were first applied 2 weeks after potting, and levels of N (but not K) were gradually and proportionally reduced with stage of growth.

Table 2a. N and K nutritional treatments used in poinsettia production in Year 4

Nitrogen	Potassium	ppm N Growth Stage			Ppm K Growth Stage		
		1	2	3	1	2	3
N1	K-	150	117	100	175	175	175
N2	K-	195	150	130	175	175	175
N3	K-	225	200	170	175	175	175
N4	K-	335	260	225	175	175	175
N1	K+	150	117	100	350	350	350
N2	K+	195	150	130	350	350	350
N3	K+	225	200	170	350	350	350
N4	K+	335	260	225	350	350	350

Leaf and growth medium assays. Leaf tissue was collected on four occasions during production and Table 2b shows the average percentage N, K and Ca leaf tissue concentration averaged over occasions. Similarly, the growth medium mineral content was assayed on four occasions during production and Table 2c shows the average percentage N, K and Ca averaged over occasions, together with the average conductivity.

Table 2b. Leaf mineral content during production (sampled at weeks 39, 43, 45 and 48) for N and K nutritional treatments used in poinsettia production in Year 4

Treatment	Level	Nitrogen (%)	Potassium (%)	Calcium (%)
Potassium	K-	4.18	2.46	1.31
	K+	4.07	2.91	1.12
Lsd (5%)		0.14	0.12	0.12
Nitrogen	N1	3.92	2.86	1.07
	N2	4.14	2.77	1.15
	N3	4.24	2.66	1.22
	N4	4.20	2.46	1.42
Lsd (5%)		0.19	0.17	0.16

Table 2c. Growth medium mineral content during production (sampled at weeks 39, 43, 45 and 48) for N and K treatments used in poinsettia production in Year 4

Treatment	Level	Nitrogen (mg/l)	Potassium (mg/l)	Calcium (mg/l)	Conductivity (μS)
Potassium	K-	91	161	111	250
	K+	95	296	80	285
Lsd (5%)		15	29	11	28
Nitrogen	N1	54	219	62	233
	N2	66	230	70	245
	N3	99	238	96	279
	N4	153	226	154	313
Lsd (5%)		21	41	16	39

iii) Transport and home-life stress

Two varieties, Sonora and Spotlight, were tested and in each year plants were given either a minimum supply chain stress or a commercial stress, provided by the Sainsbury supply chain in Year 3 and the Safeway supply chain in Year 4. In addition, an extra sub-set of 16 plants, representing all nutritional treatments and both cultivars, was given a cold-transport stress treatment, as in Years 1 and 2. In Year 3, the home-life treatments comprised all factorial combinations of two levels of temperature (16ºC or 21ºC), two lighting levels (600 lux or 300 lux) and two levels of watering (control or wet) but in Year 4 the watering treatment comparison was dropped. Physiological scores and expert scores were recorded during home life essentially as in Years 1 and 2.

In summary, there were 128 factorial combinations in each year and these were tested using single plant plots in a non-replicated factorial trial. In addition, a batch of 16 cold-stressed plants was also included making 144 pots in total.

Begonia

i) Year 3

Regimes. The production treatments for Year 3 begonia were two quantitative levels of N combined with two quantitative levels of K, as shown in Table 3a. Treatments started two weeks after potting (a week after the pinch at the onset of short days). The low levels were slightly lower than the current commercial practice at the time of the experiment, whereas the high levels were higher than the current commercial practice. Feed levels were halved four weeks before the plants were marketed. For the Year 3 experiment, Balli was no longer available and Mariette was substituted.

Table 3a. N and K nutritional treatments used in begonia production in Year 3

Nitrogen	Potassium	Growth Stage	ppm N	ppm K	N:K Ratio
Low	Low	Initial	100	100	1:1
		Final	50	50	1:1
High	Low	Initial	300	100	3:1
		Final	150	50	3:1
Low	High	Initial	100	300	1:3
		Final	50	150	1:3
High	High	Initial	300	300	1:1
		Final	150	150	1:1

Leaf and growth medium assays. Leaf tissue was collected on four occasions during production and Table 3b shows the average percentage N, K and Ca leaf tissue concentration averaged over the four occasions. Similarly, the growth medium mineral content was assayed on four occasions during production and Table 3c shows the average percentage N, K and Ca averaged over occasions, together with the average conductivity.

Table 3b. Leaf mineral content during production (sampled at weeks 12, 14, 16 and 18) for N and K nutritional treatments used in begonia production in Year 3

Treatment	Level	Nitrogen (%)	Potassium (%)	Calcium (%)
Potassium	Low	2.70	1.67	1.29
	High	2.77	1.83	1.24
Nitrogen	Low	2.64	1.77	1.16
	High	2.83	1.73	1.37
Lsd (5%)		0.19	0.11	0.09

Table 3c. Growth medium mineral content during sampled at weeks 12, 14, 16 and 18) for N and K nutritional treatments used in begonia production in Year 3

Treatment	Level	Nitrogen (mg/l)	Potassium (mg/l)	Calcium (mg/l)	Conductivity (μS)
Potassium	Low	55	61	64	174
	High	53	129	51	196
Nitrogen	Low	16	90	30	146
	High	91	100	85	223
Lsd (5%)		24	29	22	24

ii) Year 4 The production treatments for Year 4 were four quantitative levels of nitrogen combined in all combinations with two quantitative levels of potassium. The nutritional treatments were applied when the plants were fully established and in active growth (one week after the pinch). The eight combinations of nutrient treatments are summarised in Table 4a. For the Year 4 experiment, Mariette was no longer available and Blitz was substituted.

The Year 4 begonia experiment was commenced in the spring/summer of 2002 but production and disease problems meant that results from the trial were unusable. However, DEFRA provided additional funding to allow the experiment to be repeated in 2003 and the results reported here are from the 2003 experiment. To avoid confusion, the results from the 2003 begonia trial will be referred to as the Year 4 begonia trial throughout this report.

Table 4a: N and K nutritional treatments used in begonia production in Year 4

Nitrogen (N)	Potassium (K)	ppm N	ppm K	N:K Ratio
N1	K-	100	100	1 : 1
N2	K-	166	100	1.7 : 1
N3	K-	233	100	2.3 : 1
N4	K-	300	100	3 : 1
N1	K+	100	300	0.33 : 1
N2	K+	166	300	0.55 : 1
N3	K+	233	300	0.78 : 1
N4	K+	300	300	1 : 1

Leaf and growth medium assays. Leaf tissue was collected on four occasions during production and Table 4b shows the average percentage N, K and Ca leaf tissue concentration averaged over the four occasions. Similarly, the growth medium mineral content was assayed on five occasions during production and Table 4c shows the average percentage N, K and Ca averaged over occasions, together with the average conductivity.

Table 4b. Leaf mineral content during production (sampled at weeks 16, 18, 20 and 22) for N and K nutritional treatments used in begonia production in Year 4

Treatment	Level	Nitrogen (%)	Potassium (%)	Calcium (%)
Potassium	K-	3.06	2.42	1.95
	K+	2.81	2.75	1.78
Lsd (5%)		0.19	0.24	0.12
Nitrogen	N1	2.91	2.66	1.71
	N2	2.85	2.61	1.79
	N3	3.06	2.60	2.02
	N4	2.91	2.46	1.95
Lsd (5%)		0.27	0.34	0.17

Table 4c. Growth medium mineral content during production (sampled at weeks 14, 16, 18, 20 and 22) for N and K nutritional treatments used in begonia production in Year 4

Treatment	Level	Nitrogen (mg/l)	Potassium (mg/l)	Calcium (mg/l)	Conductivity (μS)
Potassium	K-	165	93	194	266
	K+	130	218	111	245
Lsd (5%)		30	18	14	45
Nitrogen	N1	85	172	102	208
	N2	130	155	130	233
	N3	174	152	173	269
	N4	202	145	206	311
Lsd (5%)		43	26	19	64

iii) Transport and home-life stress

At the end of the production period in mid-May, plants were subjected either to minimum supply chain stress or commercial marketing stress using the Safeway marketing chain in Year 3 and the Sainsbury's chain in Year 4. The plants were then subjected to a standard simulated retail phase followed by a range of simulated home-life treatments. In Year 3, these comprised all factorial combinations of two levels of temperature (16 ºC or 21 ºC), two lighting levels (600 lux or 300 lux) and two levels of watering (control or fluctuating). In Year 4, the simulated home-life treatments consisted of all factorial arrangement of 2 levels of temperature (16ºC and 21ºC in the two home-life rooms) and 2 lighting levels (600 lux and nominally 300 lux, obtained by shading).

Physiological scores and expert scores were recorded during home life, as in Year 1 and 2. Chlorophyll fluorescence was measured on the same occasions, using a *pea* double-hit procedure (Plant Efficiency Analyser, Hansatech Instruments Ltd., King's Lynn, UK) on three leaves of each plant. The immediate effects of the different temperatures in the home-life rooms were equalised to 18 ºC and the shading removed prior to measurement following the same protocol used in previous years. All begonia expert quality scores were made by Mr Mike Holmes (Double H Nurseries) using his personal assessment criteria on a ten-point scale. In addition, plants were also assessed by five Efford staff members (Assessors 1-5) on 8 occasions throughout the trial.

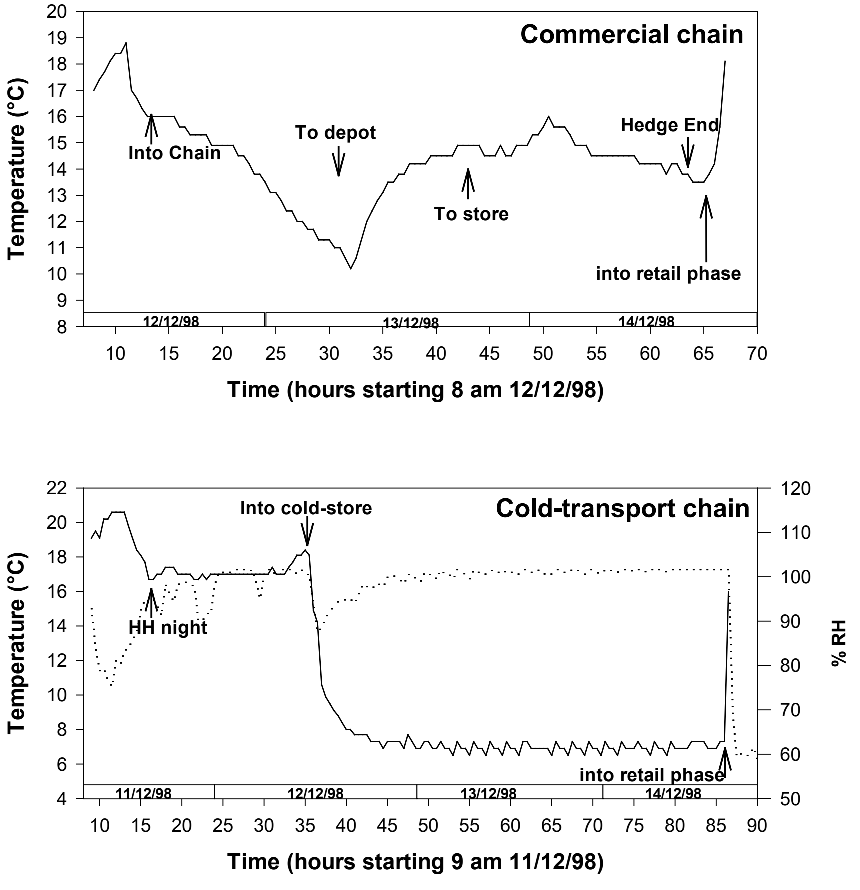


Fig. 1. Temperature and humidity logged during the poinsettia marketing phase in Year 1

3.4. Development of objective quality models (Objective 1)

3.4.1. Objective quality scores

Observed pot plant quality is based on numerous aspects of plant performance and appearance. In the case of poinsettia, these include observed leaf, bract and cyathia drop, leaf and bract paling, incidence of BEN etc. In the case of begonia, flowering characteristics are probably of key importance. Loss of plant quality over time can be related to deterioration in the physiological status of the plant. In this study, plant quality deterioration was defined objectively using a two-stage procedure. First, ordinal regression analysis methods were used to correlate the observed quality scores with a single integrated measure of plant quality based on the measured plant physiological characteristics. Second, the objective quality scores were modelled over time to assess the effects of transport stress treatments and home-life factors on home-life quality and longevity.

Model specification

In each experiment, N individual pot-plants were scored at each of T evenly-spaced time points, usually weekly, on an ordinal scale with K categories (an integer-valued scale from 1 to K where K represents the optimum score). The expert ordinal scores were related to the plant physiological data by fitting a regression model. Let Y_{it} represent the score of the i^{th} pot-plant at the t^{th} time and let $\boldsymbol{x}_{it}$ represent a multivariate vector of measured plant physiological variables observed for each unit at each time point $t = 1,\ldots,T$. Then, the probability that an ordinal quality score, Y_{it}, falls within a particular score category can be related to the measured variables, $\boldsymbol{x}_{it}$, by a proportional odds model based on cumulative logits (McCullagh and Nelder, 1989, Chapter 5)

$$\log\left(\frac{\mu_{itk}}{1-\mu_{itk}}\right) = \beta_{0k} + \boldsymbol{x}'_{it}\boldsymbol{\beta}. \qquad (3.1)$$

Here, $\mu_{itk} = P(Y_{it} \le k)$ is the cumulative probability for scores $Y_{it} \le k$, the β_{0k} for $k = 1,\ldots,K-1$ are cut-points to be determined from the data and $\boldsymbol{\beta}$ is a vector of model parameters. The cut-points $(-\infty < \beta_{01} < \ldots < \beta_{0(K-1)} < \infty)$ define the divisions between the ordinal score categories on the cumulative logit scale and the proportional odds model assumes that the regression parameters are the same for each category defined by the set of cut-points. For plant quality score data, this assumption translates into the necessary assumption of consistency of scoring on the part of the assessor throughout the assessment period, and independence of scores from experimental treatments. Thus we have assumed that the quality categories were equivalently spaced and represented the same levels of quality at each assessment occasion and within each experimental plot.

Equation (3.1) can be derived from an underlying but unobservable variable q_{it} on the set of real numbers $-\infty < q_{it} < \infty$. Thus, equation (3.1) transforms the ordinal scale to a continuous scale based on the linear predictor $\boldsymbol{x}'_{it}\hat{\boldsymbol{\beta}}$, where the cut points β_{0k} define the class boundaries on the continuous scale of measurement. The predicted response $\boldsymbol{x}'_{it}\hat{\boldsymbol{\beta}}$ is a linear function of the measured variates $\boldsymbol{x}_{it}$ and is a convenient model for relating the expert ordinal scores to the measured physiological variates.

To ensure that higher values of q_{it} indicate higher scores, we choose

$$q_{it} = -\boldsymbol{x}'_{it}\hat{\boldsymbol{\beta}} \qquad (3.2)$$

to represent the fitted objective quality score for the i^{th} unit at the t^{th} time point. The cut points β_{0k} give a partition of the objective score q_{it} into intervals $\beta_{0(k-1)} < q_{it} \le \beta_{0k}$ where the k^{th} interval covers the continuous objective score for the k^{th} ordinal score category $Y_{it} = k$. In summary, q_{it} is a measure of plant quality on a continuous scale and is a linear function of the physiological variates. For instance, in begonia, objective quality may be determined by, amongst many other characteristics, flower count and leaf drop

$$q_{it} = -(\hat{\beta}_1.(\text{flower count}) + \hat{\beta}_2(\text{leaf drop}) + \cdots),$$

where the model parameters $(\hat{\beta}_1, \hat{\beta}_2, \ldots)$ represent the contributions of each of the physiological measurements to overall plant quality.

A scale without fixed reference points is difficult to interpret, so it is desirable to re-scale the model. This can be achieved by simply adding a location parameter (r) to q_{it} to arbitrarily fix the location. For instance, by choosing r appropriately the mean objective quality of the experimental population can be set to be 100 at the start of home life. To give meaningful predictions on the original ordinal score scale, q_{it} is re-scaled by defining the mean score (over the K categories) and constraining the mean score to lie in the interval ½ … (K + ½). Then, the fitted expert score S_{it} can be written as

$$S_{it} = (K + \tfrac{1}{2}) - \frac{K}{(K-1)} \sum_{k=1}^{K-1} \exp(\beta_{0k} + \boldsymbol{x}'_{it}\boldsymbol{\beta}) / (1 + \exp(\beta_{0k} + \boldsymbol{x}'_{it}\boldsymbol{\beta})) \qquad (3.3)$$

Parameter estimation

As pot-plants were scored repeatedly at weekly intervals, the ordinal scores for an individual plant were likely to be correlated. The standard, likelihood based methods for estimating the model parameters $(\beta_{0k}, \boldsymbol{\beta})$ of equation (1), described in the literature (McCullagh and Nelder, 1989, Chapter 5), assume independence between data points and are thus unlikely to produce good estimates of the true model parameters. The approach adopted here for parameter estimation was to use a modification of the generalized estimating equation (GEE) methodology originally proposed by Liang and Zeger (1986). GEEs are non-likelihood based methods for fitting marginal models to repeated measures when the response has a distribution in the exponential family (see Hardin and Hilbe 2002 for a full discussion of GEEs). In the absence of a likelihood function, the model parameters are estimated by solving multivariate analogues of the quasi-likelihood functions (Wedderburn 1974). Resulting estimates are not maximum likelihood, but have asymptotic normality and consistency (Liang and Zeger 1986). Appendix 6.1 contains a full description of the algorithm used for parameter estimation.

The ten-point scale used for the quality assessments gave very few or zero counts in some categories and the scale was re-coded prior to modelling by pooling together certain of the categories. The re-coding varied between years depending on the distribution of scores within the original quality score categories. In principle, the ten-point expert score scale was re-coded to give the following five-point scale; 1 or 2 = 1 (very poor), 3 or 4 = 2 (poor), 5 or 6 = 3 (good), 7 or 8 = 4 (very good) and 9 or 10 = 5 (excellent). For some data sets there were very few scores in the lowest categories (1 and 2), so these were merged with the next lowest category (3 and 4) to give a four-point scale.

In Years 1 and 2 of the project a separate relationship was fitted for each cultivar, as there was no reason to believe that the relationship between quality scores and plant physiology would be the same for each cultivar. GEE were formulated and fitted to the data using a user-defined procedure `GEEORDINAL`, based on the procedure `GEE` in GenStat (2000). All pairwise interactions between physiological variates were initially included in the model but many were discarded because they were not significant and did not contribute to the model.

3.4.2. Model fitting for poinsettia

Year 1

In Year 1, bract, leaf and cyathium quality scores were recorded for each plant. Unfortunately, overall plant quality score records were not collected therefore model fitting had to be restricted to the bract, leaf and cyathium quality scores. Standard generalised linear model methodology showed that, in Sonora, the important physiological characters for leaf quality were: foliar colour, leaf loss, numbers of lost side-shoots, the presence or absence of *Botrytis*, and pale bracts. In Spotlight, the important plant characters were bract loss, leaf loss and foliar colour.

Year 2

Table 5 gives parameter estimates and standard errors for the best fitting model for poinsettia cultivars Sonora and Spotlight for Year 2. The coefficients β_{01}, β_{02}, β_{03} and β_{04} (cut-points) define the divisions between these five expert score categories on the cumulative logit scale. The model coefficient associated with a physiological variate represents the effect of a unit change in the corresponding plant quality variate, expressed on a cumulative logit scale. Positive coefficients show that as the corresponding physiological variate increased the perceived quality decreased and vice-versa for negative coefficients. Note that the relative size of parameter estimates is not an indication of the relative importance of the corresponding characters as the characters are not all measured on the same scale. The model coefficients in Table 5 for the two cultivars taken separately indicate that:

Sonora

i) Quality was markedly reduced by cyathia and bract drop, pale leaves and bracts, and by BEN.

ii) A significant interaction between the proportion of cyathia present and the presence of pale leaves showed that high quality required both a high cyathia count and dark leaves

iii) A significant interaction between pale bracts and BEN showed that high quality required both dark bracts and absence of BEN.

Spotlight

i) Quality was significantly reduced by cyathia, bract and leaf drop, pale leaves and BEN.

ii) A significant interaction between of pale leaves and BEN showed that high quality required both dark leaves and absence of BEN.

Table 5. Parameter estimates and standard errors for Year 2 poinsettia data

	Sonora		Spotlight	
Model term	Estimate	S.e.	Estimate	S.e.
β_{01}	-4.84	0.60	-3.99	0.50
β_{02}	-2.49	0.50	-1.89	0.44
β_{03}	0.01	0.51	-0.25	0.45
β_{04}	2.74	0.59	3.87	0.54
Cyathia Proportion†	-4.48	0.57	-5.03	0.54
Bract Drop	0.12	0.03	0.11	0.03
Bract Edge Necrosis‡	1.16	0.39	0.71	0.34
Pale Leaves‡	1.95	0.44	4.53	1.19
Pale Bracts‡	1.29	0.49	-	-
Leaf Drop	-	-	0.07	0.02
Cyathia Proportion x Pale Leaves	7.03	2.55	-	-
Bract Edge Necrosis x Pale Leaves	-	-	-3.08	1.16
Bract Edge Necrosis x Pale Bracts	-1.40	0.51	-	-

† Proportion of cyathia still present on the plant. ‡ Indicator variables representing absence or presence of indicated physiological characteristic.

Residual plots for the Year 2 data indicated that the model fit was consistent between experimental treatments at each occasion during home life. This showed that the same relative importance attached to each plant characteristic for each treatment and throughout home life.

Using the parameter estimates from Table 5, a mean objective quality score (*q*) of 100 was defined at the end of the first week of home life by using location parameters (*r*) of 96.3 and 95.9 for cultivars Sonora and Spotlight respectively. Table 6 gives the expected and observed numbers of plants in each of the five expert score categories for the data grouped by objective quality score. The expected counts appear to agree well with the observed counts across all categories.

Table 6. Test of goodness of fit of the Year 2 poinsettia objective quality model: comparison of observed (Obs.) and expected (Exp.) numbers of observations in defined plant quality categories

Expert score	1 (Very Poor)		2 (Poor)		3 (Good)		4 (Very Good)		5 (Excellent)		
	Obs.	Exp.	Obs.	Exp.	Obs.	Exp.	Obs.	Exp.	Obs.	Exp.	Total
Sonora											
<91.5	4	3.4	1	1.4	0	0.2	0	0.0	0	0.0	5
91.5-93.8	9	10.5	23	21.9	11	9.4	0	1.1	0	0.1	43
93.8-96.3	3	2.4	15	17.7	56	56.3	32	28.6	2	3.1	108
96.3-99.0	1	0.3	5	3.0	24	24.4	62	59.2	21	26.0	113
>99.0	0	0.0	1	0.3	4	3.2	26	32.0	96	91.6	127
Total	17	16.7	45	44.3	95	93.5	120	120.9	119	120.	396
Spotlight											
<91.9	8	8.5	3	3.6	1	0.7	1	0.2	0	0.0	13
91.9-94.0	15	15.0	34	32.7	16	17.6	7	6.5	0	0.1	72
94.0-95.7	2	4.0	17	18.8	31	28.5	26	24.1	0	0.6	76
95.7-99.8	3	1.1	9	7.1	25	23.3	115	113.8	22	28.7	174
>99.8	0	0.0	0	0.1	1	0.6	21	27.3	51	45.0	73
Total	28	28.6	63	62.4	74	70.8	170	171.9	73	74.4	408

Years 3 and 4

Unfortunately, the planned overall plant quality score records were not collected in Year 3 making it impossible to validate the relationship between quality and physiology observed in Year 2. However, validation was done in Year 4 and Table 7 gives the parameter estimates and standard errors for the best fitting model for poinsettia using the Year 4 data. Expert quality scores, recorded on a ten-point scale, were re-coded as follows: 10 or 9 = 4 (Excellent), 8 or 7 = 3 (Very Good), 6 or 5 = 2 (Good) and 4 or 3 or 2 or 1 = 1 (Poor or Very Poor). Only a very few scores were recorded in categories 1 and 2 on the original ten-point scale so it was necessary to pool these scores into the scores in categories 3 and 4 to obtain good estimates of the cut-points between score categories. The inclusion of a cultivar term in the model did not significantly improve the fit of the model showing that the observed differences in quality scores between the cultivars could be accounted for by differences in the measurable plant characteristics. The model coefficients associated with the physiological variates in Table 7 represent the effects of unit change of the respective variate on plant quality, expressed on a cumulative logit scale.

Table 7. Parameter estimates and standard errors for Year 4 poinsettia data

Model term	Estimate	S.e.
β_{01}	-4.270	0.393
β_{02}	-2.124	0.383
β_{03}	1.097	0.392
Bract Drop	0.087	0.019
Leaf Drop	-0.071	0.017
Cyathia Proportion	-3.953	0.285
Bract Colour	0.891	0.159
Pale leaves (upper)	0.857	0.174
Pale leaves (lower)	0.488	0.182
Botrytis	1.962	0.409
Pale Bracts	1.064	0.334
Cyathia Proportion x Botrytis	-5.355	1.738
Bract Drop x Botrytis	-0.104	0.032

As in Table 5, positive model coefficients show that when the corresponding physiological variate increases the quality decreases, and vice-versa for negative coefficients. The model coefficients in Table 7 therefore indicate that:

i) High bract drop reduced plant quality.
ii) High leaf drop increased plant quality
iii) Cyathia drop reduced plant quality.
iv) Poor bract colour reduced plant quality.
v) The presence of pale upper and lower leaves, *Botrytis* and pale bracts all reduced plant quality.
vi) There was a significant negative interaction between cyathia drop and botrytis showing that high quality required both low cyathia drop and low botrytis
vii) There was a significant negative interaction between bract drop and botrytis showing that high quality required both low bract drop and low botrytis

Residual plots showed that the model fit was consistent with the experimental treatments, indicating that there was no significant lack of fit between the treatments. Using the parameter estimates from Table 7, objective quality scores (*q*) were calculated for each plant at each occasion using equation (3.2) as $q_{it} = -\boldsymbol{x}'_{it}\hat{\boldsymbol{\beta}}$. Table 8 tests for goodness of fit by comparing the observed and predicted numbers of plants in each of the expert score categories (1,2,3,4), for the data grouped into the four intervals on the objective score scale produced from the cut-points. The predicted counts in the cut-point categories agree with the observed counts indicating that the model fit was reasonably good.

Table 8. Test of goodness of fit of Year 4 poinsettia objective quality model: comparison of observed (Obs.) and expected (Exp.) counts in cut-point categories

Quality Score	1 (Poor)		2 (Good)		3 (Very Good)		4 (Excellent)		
	Obs.	Exp.	Obs.	Exp.	Obs.	Exp.	Obs.	Exp.	Total
(-∞,-4.3]	26	23.0	10	11.5	1	2.4	0	0.1	37
(-4.3,-2.1]	95	96.4	181	178.	109	104.	0	6.6	385
(-2.1,1.1]	9	15.3	74	76.6	242	233.	74	73.7	399
(1.1,∞)	0	0.4	2	3.0	55	58.9	367	361.	424
Total	130	135.	267	269.	407	398.	441	442.	1245

In summary, model fitting has shown that overall plant quality can be described by readily measurable plant characteristics in poinsettia. An objective quality score, determined as a linear combination of plant physiological characteristics provides an appropriate model for integrated plant quality

3.4.3. Model fitting for begonia

Year 1

Model fitting for begonia in Year 1 used a standard generalised linear model methodology rather than the methodology described in section 3.4.1. This indicated (data not shown) that the important physiological characters for quality in Balli and Batik were flower count, flower drop, bud count, damaged flower count, bud drop and damaged leaf count. Objective quality scores were fitted and modelled for begonia in essentially the same way as for poinsettia in subsequent years.

Year 2

Table 9 gives estimates of key parameters determining plant quality in begonia cultivars Balli and Batik in Year 2, together with standard errors. No plants were scored as class 5, excellent, and for this reason there are only three cut-point coefficients on the cumulative logit scale. Residual plots showed that there were no significant deviations from the fitted model. Table 9 indicates that:

Balli

i) A high flower or bud count markedly improved plant quality.
ii) Quality was markedly reduced by flower or bud drop and by damaged leaves.
iii) There was a significant negative interaction between flower drop and bud drop showing that high quality required both low flower drop and low bud drop.
iv) There was a significant negative interaction between flower drop and damaged leaf count showing that high quality required both a low flower drop and a low damaged leaf count.

Batik

i) A high flower or bud count markedly improved plant quality.
ii) Quality was markedly reduced by flower or bud drop and by damaged leaves.
iii) There was a significant negative interaction between flower drop and bud drop showing that high quality required both low flower drop and low bud drop.

Table 9. Parameter estimates and standard errors for Year 2 begonia data

	Balli		Batik	
Model term	Estimate	S.e.	Estimate	S.e.
β_{01}	-3.031	1.042	-2.671	1.029
β_{02}	-0.503	1.037	1.610	0.992
β_{03}	2.284	1.030	4.846	1.082
Flower Count	-0.190	0.037	-0.155	0.024
Flower Drop	0.570	0.088	0.300	0.039
Bud Drop	0.494	0.093	0.713	0.131
Damaged Leaf Count	0.624	0.122	0.278	0.082
Bud Count	-0.053	0.011	-0.052	0.010
Flower drop x bud drop	-0.043	0.010	-0.056	0.016
Flower drop x damaged leaf count	-0.059	0.017	-	-

Using the parameter estimates from Table 9, a mean objective quality score (q) of 100 was constructed for the end of the first week of home life by choosing location parameters (r) of 94.6 and 93.1 for cultivars Balli and Batik respectively. Table 10 gives the expected and observed numbers of plants in each of the four expert score categories for the data grouped by objective quality score for each cultivar. The expected counts in each of the cut-point categories agreed well with the observed counts across all categories for both cultivars.

Table 10. Test of goodness of fit of Year 2 begonia objective quality model: comparison of observed (Obs.) and expected (Exp.) counts in cut-point categories

Expert score	1 (Very Poor)		2 (Poor)		3 (Good)		4 (Very Good)		
	Obs.	Exp.	Obs.	Exp.	Obs.	Exp.	Obs.	Exp.	Total
Balli									
<91.5	21	21.0	8	7.0	0	0.9	0	0.1	29
91.5 - 94.1	16	14.9	29	32.8	16	14.8	3	1.5	64
94.1 - 96.8	1	1.8	17	13.9	35	37.0	15	15.4	68
>96.8	0	0.2	0	1.8	24	21.6	171	171.4	195
Total	38	37.9	54	55.5	75	74.3	189	188.3	356
Batik									
<90.4	1	2.3	2	0.7	0	0.0	0	0.0	3
90.4 - 94.7	5	4.6	41	44.9	20	16.5	1	1.0	67
94.7 - 97.9	1	0.4	23	18.2	52	55.4	15	17.0	91
>97.9	0	0.0	1	1.7	29	30.2	168	166.1	198
Total	7	7.3	67	65.5	101	102.1	184	184.2	359

Year 3

Table 11 gives parameter estimates and standard errors for the best fitting model for begonia using Year 3 data. Quality scores, recorded on a ten-point scale, were re-coded to give the following scale; 10 or 9 = 4 (Excellent), 8 or 7 = 3 (Very Good), 6 or 5 = 2 (Good) and 4 or 3 or 2 or 1 = 1 (Poor or Very Poor). Only a handful of scores were recorded in categories 1 and 2 of the original ten-point scale so it was necessary to pool these together with the scores in categories 3 and 4 in order to give good estimates of the cut-points between score categories during model fitting. The model coefficients associated with the physiological variates in Table 11 represent, as before, the effects of unit change of the respective variate on plant quality, expressed on a cumulative logit scale.

The model coefficients in Table 11 indicate that:

i) High flower counts increased plant quality in cultivar Mariette but not in Batik where flower counts remained high throughout home life.
ii) High flower drop reduced quality.
iii) High bud drop reduced quality.
iv) High damaged leaf count reduced quality.
v) The presence of pale leaves reduced quality.
vi) Significant Flower drop x Bud drop, Flower drop x Damaged leaves and Cultivar x Flower interaction effects indicated that the effects of the corresponding variables were less than additive.

Table 11. Parameter estimates and standard errors for Year 3 begonia data

Model term	Estimate	S.e.
β_{01}	-7.363	0.445
β_{02}	-4.402	0.393
β_{03}	-0.377	0.327
Cultivar	0.023	0.348
Flower count	-0.028	0.015
Flower drop	0.385	0.041
Bud drop	0.417	0.035
Damaged leaves	0.498	0.101
Pale leaves	1.867	0.231
Flower drop x Bud drop	-0.038	0.004
Flower drop x Damaged leaves	-0.037	0.014
Cultivar x Flower count	0.042	0.018

Residual plots showed that the model fit was consistent with the experimental treatments, showing that there was no significant lack of model fit. Objective quality scores (*q*) were derived from the parameter estimates in Table 11, and Table 12 shows comparisons of observed against predicted numbers of plants in each of the expert score categories. The predicted counts in the cut-point categories agree with the observed counts indicating that the model fit was realistic

Table 12. Test of goodness of fit of Year 3 begonia objective quality model: comparison of observed (Obs.) and expected (Exp.) counts in cut-point categories

Quality Score	1 (Poor)		2 (Good)		3 (Very Good)		4 (Excellent)		
	Obs.	Exp.	Obs.	Exp.	Obs.	Exp.	Obs.	Exp.	Total
(-∞,-7.4]	42	46.1	19	14.6	1	1.2	0	0.0	62
(-7.4,-4.4]	80	71.8	206	195.	56	72.6	0	1.9	342
(-4.4,-0.4]	11	6.8	65	84.6	309	300.	66	59.4	451
(-0.4,∞)	0	0.0	0	0.5	21	17.0	20	23.5	41
Total	133	124.	290	295.	387	391.	86	84.8	896

Year 4

Expert quality scores, recorded on a ten-point scale, were re-coded to give the following scale; 10 or 9 = 4 (Excellent), 8 or 7 = 3 (Very Good), 6 or 5 = 2 (Good) and 4 or 3 or 2 or 1 = 1 (Poor or Very Poor). Only a handful of scores were recorded in categories 1 and 2 of the original ten-point scale so it was necessary to pool these together with the scores in categories 3 and 4 in order to give good estimates of the cut-points between score categories during model fitting. All pairwise interactions between explanatory variates were initially included in the model, but were discarded when not significant. Table 13 shows the parameter estimates and standard errors for the best fitting model.

Table 13: Parameters and standard errors for Year 4 begonia data

Model term	Estimate	S.e.
β_{01}	-8.326	0.519
β_{02}	-6.494	0.482
β_{03}	-2.313	0.434
Cultivar	-0.789	0.200
Flower Drop	0.229	0.023
Bud Drop	0.239	0.046
Foliar Colour	2.048	0.245
Flower Colour	2.462	0.349
Damaged Leaves	-0.257	0.082
Flower Drop x Bud Drop	-0.016	0.004

The model coefficients associated with the physiological variates in Table 13 represent the effects of unit change of the respective variate on plant quality, expressed on a cumulative logit scale. Positive coefficients indicate that as the corresponding physiological variate increased the quality decreased and vice-versa for negative coefficients. The model coefficients from Table 13 indicate:

i) Blitz had a lower quality score than Batik, irrespective of the recorded physiological characteristics
ii) High flower drop reduced plant quality
iii) High bud drop reduced plant quality

iv) The presence of pale upper canopy leaves reduced plant quality
v) The presence of pale flowers reduced plant quality
iv) There was a significant negative interaction between flower drop and bud drop showing that high quality required both low flower drop and low bud drop.

The parameter estimates are consistent with those observed for data collected during the previous trials. Residual plots showed that the model fit was consistent with the experimental treatments, showing that there was no significant lack of fit between the treatments. The parameter estimates from Table 13, can be used to produce an objective quality score (q) for each plant at each occasion and Table 14 compares the observed and predicted numbers of plants for data grouped into the four intervals on the objective score scale produced from the cut-points. The predicted counts in the cut-point categories agree with the observed counts indicating that the model fit was reasonably good.

Table 14: Observed (Obs.) and expected (Exp.) counts in cut-point categories

Quality Score	1 (Poor)		2 (Good)		3 (Very Good)		4 (Excellent)		
	Obs.	Exp.	Obs.	Exp.	Obs.	Exp.	Obs.	Exp.	Total
(-∞,-8.3]	0	4.2	2	1.4	4	0.4	0	0.0	6
(-8.3,-6.5]	29	34.2	61	56.7	50	47.9	0	1.2	140
(-6.5,-2.3]	22	16.5	60	60.8	364	363.	86	91.4	532
(-2.3,∞)	0	0.1	0	0.6	28	31.8	159	154.	187
Total	51	55.0	123	119.	446	443.	245	247.	865

In summary, the model fitting process has shown that overall plant quality in begonia can be well described by measured plant characteristics. Models fitted for begonia indicated that, in general, parameter estimates were consistent between years. Increased flower drop, bud drop and damaged leaf count reduced quality, as did the presence of pale leaves. Increased flower and bud count improved quality. As in the case of poinsettia, the parameter estimates agreed well with the scores of the expert assessor

3.4.4. Conclusions

Mathematical procedures were developed that enabled quality to be objectively quantified by summing weighted measures of several measured physiological characters. These procedures were based on the analysis of observed relationships between physiological scores and measures of perceived quality recorded simultaneously by a grower expert. The parameters of the model represented the contributions of each of the physiological variates to overall plant quality. The model fitting procedure was based on a general estimating equation (GEE) approach, which quantified the effects of particular physiological characters on perceived quality. Major factors determining quality in poinsettia included cyathia, leaf and bract drop, leaf and bract paling and BEN. Major factors determining quality in begonia included total bud and flower count, bud and flower drop, and numbers of damaged leaves.

3.5. Robust product design methodology: the effects of home-life and supply chain treatments on plant quality (Objective 3)

3.5.1. Modelling quality loss

The effects of marketing and home-life treatments on the individual physiological characteristics of plant quality are of great importance. In this report, the effects of the individual quality characteristics have been summarised by a single objective quality score, as defined in Section 3.4, and the loss of quality during home-life has been summarised by the decline in the objective quality score. The response of the objective quality scores during home-life has been modelled over time to assess the effects of transport stress treatments and home-life factors on home-life quality and longevity.

Model specification

Objective quality scores typically decline during home life due to plant maturation and the stress of sub-optimal, home–life environments, although the scores for individual plants sometimes increased due either to inconsistent scoring or to real improvement in quality due, for example, to flower development. The quality decline process for individual plants in home life is complex and will not necessarily have a consistent simple functional form. For this reason, we have chosen to fit a time-based polynomial curve to the objective quality scores for each plant, and to analyse the derived coefficients using standard analysis of variance (ANOVA) methods (Diggle *et al*, 1994, chapter 6). A simple linear polynomial model may not always be the best fitting function for a particular plant but it is sufficiently flexible to fit most types of response function and, for that reason, was chosen here to describe the rate of quality decline during home life.

We denote the serial observations of the objective quality score for plant i as $\boldsymbol{q}_i' = (q_{i1}, q_{i2}, \ldots, q_{iT})$, and the T x R design matrix of within-plant variables (polynomial time trends) as $\boldsymbol{G}$. Then the objective quality scores for plant i can be modelled as

$$\boldsymbol{q}_i = \boldsymbol{G}\boldsymbol{\theta}_i + \boldsymbol{\varepsilon}_i, \qquad (3.4)$$

where $\boldsymbol{\theta}_i$ is an R-dimensional vector of unknown model parameters to be estimated, and $\boldsymbol{\varepsilon}_i$ is a T-dimensional vector of random error terms. We assume that $\boldsymbol{\varepsilon}_i \sim N(\mathbf{0}, \sigma_i^2 \mathbf{I}_T)$, where $\mathbf{0}$ is a T-dimensional vector of zeroes and $\mathbf{I}_T$ is a T x T identity matrix. The parameters of equation (3.4) are estimated by least squares for each individual plant and then a conventional ANOVA of treatment effects is made for each individual coefficient from $(\theta_{i1}, \theta_{i2}, \ldots, \theta_{iR})$ taken over the full set of plants.

The linear model

For much of the objective quality score data collected during the project, quality decline during home life was monotonic and was well described by a simple linear regression model fitted to the time course of scores for each individual plant. For a simple linear regression model, the matrix $\boldsymbol{G}$ in equation 3.4 has a column of 1's and a column of mean corrected times, representing the assessment occasions during home life. Using this model, the observed decline in objective quality scores during home-life can be described by the equation:

$$\boldsymbol{q}_i = \theta_{i1} + \boldsymbol{\tau}\theta_{i2} + \boldsymbol{\varepsilon}_i, \qquad (3.5)$$

where $\boldsymbol{q}_i' = (q_{i1}, q_{i2}, \ldots, q_{iT})$ is the vector of serial observations of the objective quality score for plant *i* and $\boldsymbol{\tau}' = (1-\bar{t}, \ldots, T-\bar{t})$ is the vector of observation times, centred about the mean $(\bar{t})$. The model parameters θ_{i1} and θ_{i2} represent the mean quality during home life and the rate of quality decline respectively.

A derived quantity based on the simple linear model that proved useful in quantifying the effects of the experimental treatments on plant quality was *plant useful longevity.* The useful longevity of a plant during home life was defined to be the time in weeks from de-sleeving until the quality had declined to the division point between very good and good quality. The appropriate model cut-point parameter β_{0k} was used to estimate this division point and the useful longevity for plant *i* was estimated from the expression

$$\text{longevity}_i = \frac{(\beta_{0k} - \theta_{i1})}{\theta_{i2}} + \bar{t}. \qquad (3.6)$$

Figs 2a and 2b show the mean quality decline profiles (solid line with x symbols) for two, idealised treatments, each comprising four plants and labelled as A and B respectively. The mean useful longevity for treatment A is approximately 2½ weeks and for treatment B is approximately 4 weeks. Thus, treatment A would be preferred to treatment B, as it would give, approximately, an additional 1½ weeks of useful home-life longevity.

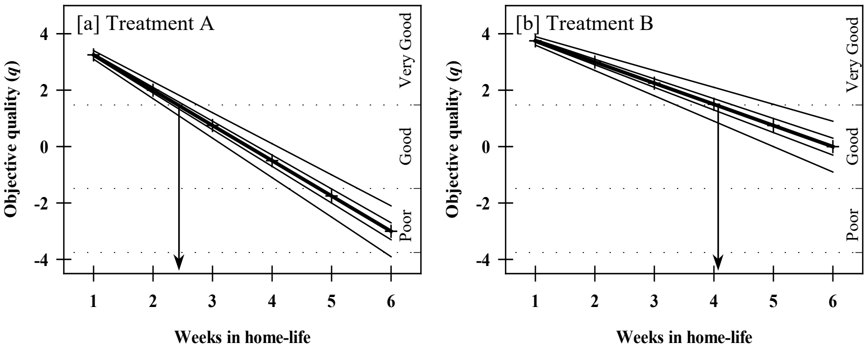


Fig. 2[a-b]. Mean quality decline profiles (solid line with x symbols) and individual plant quality decline profiles for two hypothetical treatments, A and B.

The longevity analysis would be adequate for detecting the main effects of experimental treatments on mean quality during home life but would not be adequate for certain other important features of the data. For example, Figs 3a and 3b show the mean quality decline profiles (solid line with x symbols) for two idealised treatments, each comprising four plants and labelled as C and D respectively. The mean longevity for treatment C and treatment D is approximately 2¼ weeks and thus an analysis based purely on mean longevity would rate treatments C and D as equal.

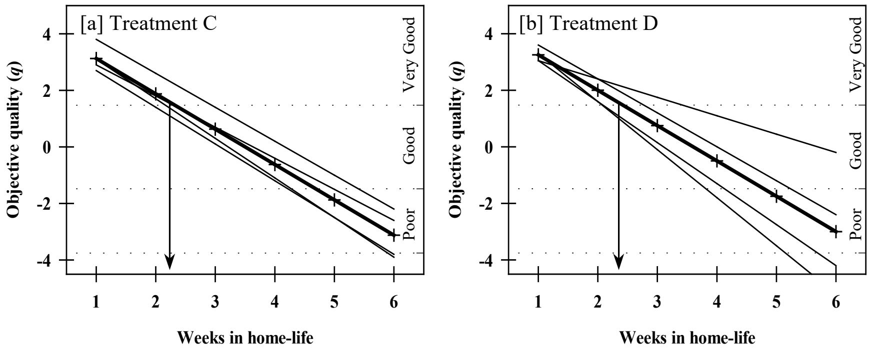


Fig. 3[a-b]. Mean quality decline profiles (solid line with x symbols) and individual plant quality decline profiles for two hypothetical treatments, C and D.

However, although mean longevity does not differ between treatments C and D, plants in treatment D have much more variable rates of quality decline and of mean quality than plants in treatment C. Treatments that give lower plant-to-plant variability during home life are said to give improved *robustness*. So, in addition to determining those treatments that improve mean longevity, it is also of interest to identify treatments that give reduced variability over a range of home-life environments and so give improved robustness during home life. The performance of a production treatment within different home-life environments can be investigated by identifying significant interactions between the production and home-life treatment factors in the analysis. This, together with the usual residual analysis, allows the identification of those production treatments that are *robust* across the range of experimental home-life environments.

3.5.2. Effects of home-life and supply chain factors

Poinsettia

Expert quality loss scores for poinsettia were available only for Years 2 and 4 therefore quality loss during home-life could be modelled only on the basis of objective quality scores from those two years. In Year 2, quality loss for each plant was modelled using equation (3.4) with fitted linear, quadratic and cubic terms. Figs 4a and 4b show the resulting quality loss functions during home life for each of the 24 treatments per cultivar for Sonora and Spotlight, respectively (Year 2 data). Each curve was averaged over the three plants comprising a specific treatment. The scatter of the curves reflects the treatment effects on quality for each cultivar taken separately. The main effects of any particular treatment can be shown by averaging over all the component curves at the fixed levels of that treatment. Figs 4c and 4d show the main effects of supply chain, Figs 4e and 4f show the main effects of home-life temperatures and Figs 4g and 4h show the main effects of home-life lighting.

In Year 4, a simple straight line model was found adequate to model the decline in quality during home life and the main treatments effects for Year 4 are shown in Figs. 5[a-d] for cultivar (5a), supply chain (5b), light (5c) and home-life temperature (5d).

Supply chain effects

Cold transport had adverse effects on quality in each of the poinsettia cultivars in both Years 1 and 2. Effects were clearly apparent after only one week in home life and, in the case of Sonora, the effects persisted for the following six weeks. Although there was an immediate severe deleterious effect of cold stress in both cultivars, the subsequent rate of decline was somewhat less for cold-stressed plants than for non cold-stressed plants. For Spotlight, the average cold-stressed plants actually appeared to be of higher quality than the non cold-stressed plants after 5 or 6 weeks of home life. Analysis of the effects of cold transport on individual physiological characters showed that cold transport promoted bract loss in the first two weeks after de-sleeve. Cold stress also exacerbated leaf loss, increased cyathia loss (Year 2 only) and gave paler bracts. Fig G3 (Grower Summary) illustrates the extreme effects of cold treatment during the marketing of poinsettia.

An additional extra sub-set of 16 plants was included in the Year 4 trial and these plants were subjected to simulated cold transport. Although they were not included in the formal analysis of objective quality scores, they nevertheless confirmed that the Year 4 cold-stress plants showed the same increased post-marketing bract and leaf loss, increased loss of bract and leaf colour, and overall reduced quality observed in the previous years of the trial.

In Year 1, the commercial chain plants were either intermediate in quality between the cold-transport plants and the minimum stress plants, or similar to the cold-transport plants. This was probably because temperatures had been allowed to fall to a low value during delivery to the supply depot (Fig. 1). Unfortunately, in Year 2, it was not possible to judge the effects of the commercial transport chain, as the transport chain plants were lost and replacement plants had to be obtained. As can be seen in Figs 4c and 4d, the replacement plants responded very similarly to the minimum stress plants. In Year 4, the minimum stress plants had a small, but significant, increase in longevity of 2.8 days relative to the commercial chain (Fig 5b).

Home-life effects
After two weeks in home-life, poinsettia plants maintained in cool (16°C) conditions were of markedly higher quality than plants maintained at 21°C (Figs 4e, 4f and 5d). In Year 2, the quality benefit of low home-life temperatures was maintained throughout home life in the case of Sonora, and to the fifth week of home life in the case of Spotlight. Higher temperature promoted cyathia loss (starting after 7 days of home life in Year 1 and after 14 days in Year 2), and reduced bract colour. High light (600 lux) in home life gave improved objective quality scores relative to those for low light (300 lux) in Year 4 (Fig. 5c) and for Spotlight in Year 2 (Fig. 4h) and this effect was continuous throughout home-life. The combination of cool temperature and high light was particularly beneficial (Fig. G.5, Grower Summary). There were no clear effects of watering treatments on plant quality.

Begonia

Figs 6a and 6b show the wide variation in individual begonia objective quality loss profiles due to treatment effects in Year 2 while Figs 6c-h show the corresponding main supply treatment effects in Year 2. Figs. 7a-d show the main effects of cultivar, supply chain, home-life light and home-life temperature treatment effects on begonia objective quality during Year 3 and Figs 8a-d show the corresponding information for Year 4.

Supply chain

Objective quality in Batik was greater throughout home life for the minimum supply chain treatment than for either of the two other supply treatments in Year 2 (Fig.6d). As the supply chain plants were not cold-stressed and as both the supply chain and the cold-stress plants showed a similar loss of quality, it seems likely that the loss of quality of these two treatments was a generalised stress response. The beneficial effects of minimum stress were less obvious for Balli but, as was also seen for Batik, the minimum stress plants had greater flower counts throughout home life. This effect was seen also in Year 1, with minimum stress plants having greater flower numbers throughout home life relative to either cold-transport plants or commercial chain plants. In Year 3, objective quality was greater throughout home life in the minimum supply chain treatment than in the commercial treatment (Fig. 7b). Year 4 again showed a clear average loss of quality due to marketing (Fig 8b). The cultivar effect in Year 4 showed better quality from Blitz compared with Batik but due to the change of variety in Year 4, this result could not be compared directly with previous years.

Home life factors

Home-life temperature had by far the most pronounced effect on objective quality scores (Figs 6e, 6f, 7d and 8d), and results in Years 2, 3 and 4 were broadly similar to those obtained in Year 1. The decline in quality was much greater in both cultivars at 21°C than at 16°C, due primarily to greater flower and bud drop, and this led to the observed differences in perceived quality during weeks 3-5 of home life. Other deleterious effects of the higher temperature treatment were paler flowers and leaves.

High light (600 lux) gave higher objective quality scores than low light (300 lux) in Year 2 (Figs 6g, h), with differences continuing to increase throughout home life. As with cool temperature, the high light treatment gave consistently higher total flower counts throughout home life in Year 2 compared with low light. There was no significant lighting effect on objective quality in Year 3 or Year 4 (Fig. 7c and 8c). A beneficial effect of high light was observed in Year 1 but only from the third week onwards. The lighting treatments had no significant effect on flower and bud drop in Years 1 or 2 therefore high lighting must have shown its effect simply by promoting bud opening. High light also retarded the decline in flower colour score in both Years 1 and 2, and in foliage colour score in Year 2 but not in Year 1. The home-life combination of cool temperature and high light was particularly beneficial to begonia longevity in both years (Fig. G.7, Grower Summary). There were no clear effects of watering treatments on plant quality.

3.5.3. Conclusions

Decline in quality was modelled by fitting objective quality scores to the observed expert quality scores. The fitted scores were then used to compare the effects of transport chain and home-life factors on post-harvest quality and longevity. Cold-temperature transport had large adverse effects on the subsequent home-life longevity of poinsettia. For begonia, both the cold treatment and the transport chain had an adverse effect on Batik but less effect on Balli. The combination of cool home-life temperature (16°C) and high light (600 lux) proved particularly beneficial to home-life quality in both pot plant species.

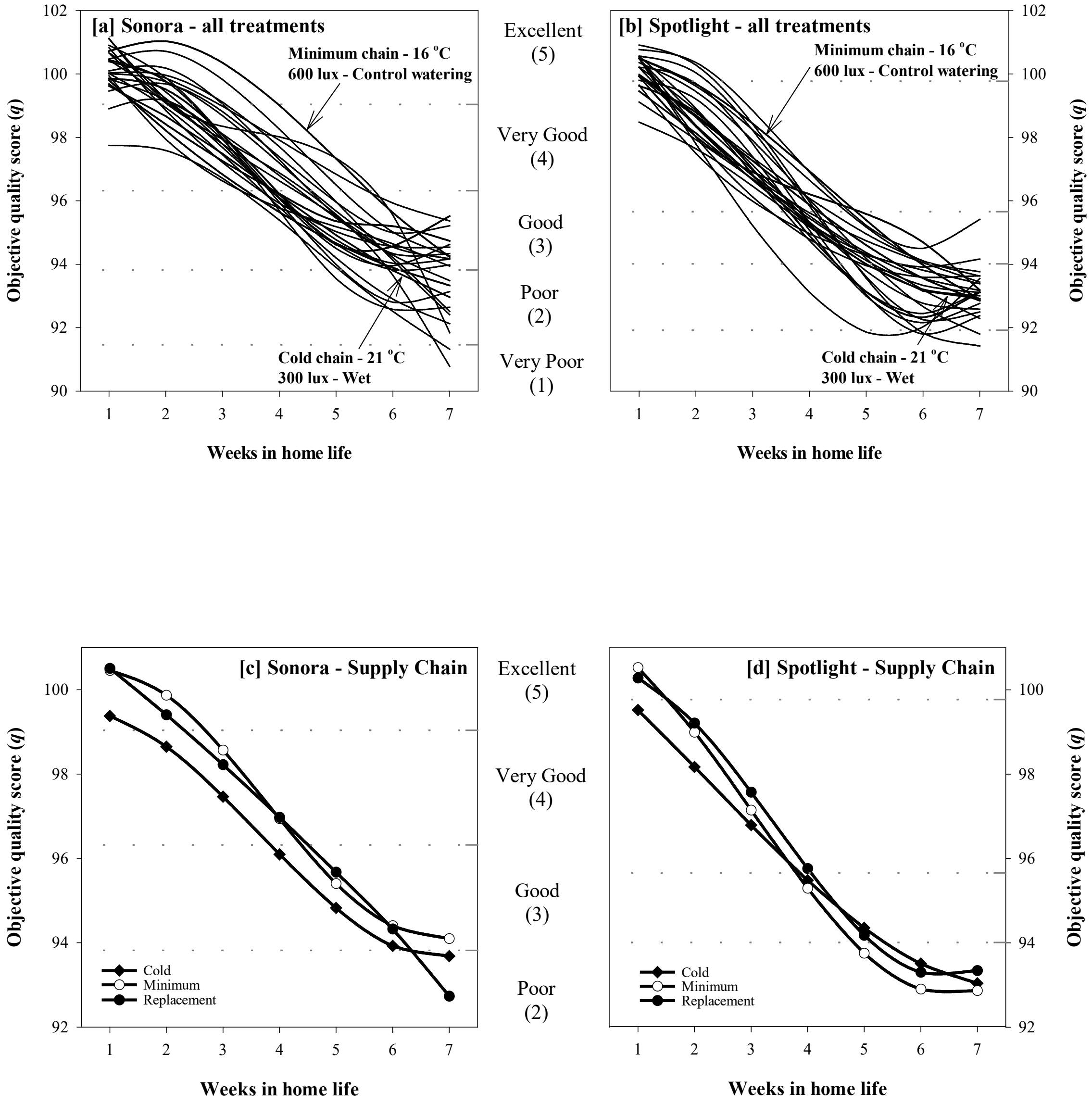


Fig. 4 [a-d]. Experimental treatment effects on poinsettia objective quality scores (q) during home life in Year 2.

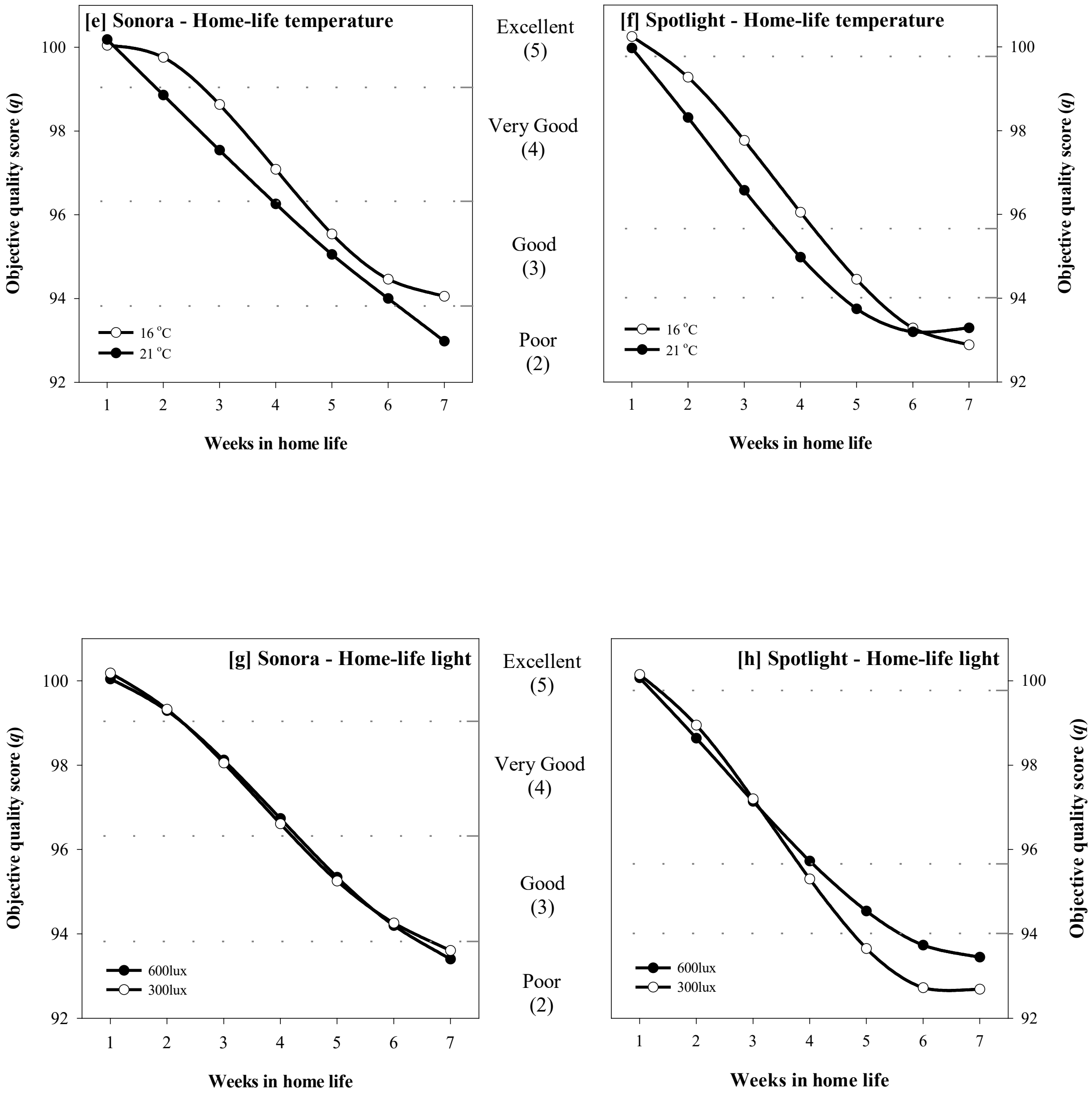


Fig. 4 [e-h]. Experimental treatment effects on poinsettia objective quality scores (q) during home life in Year 2.

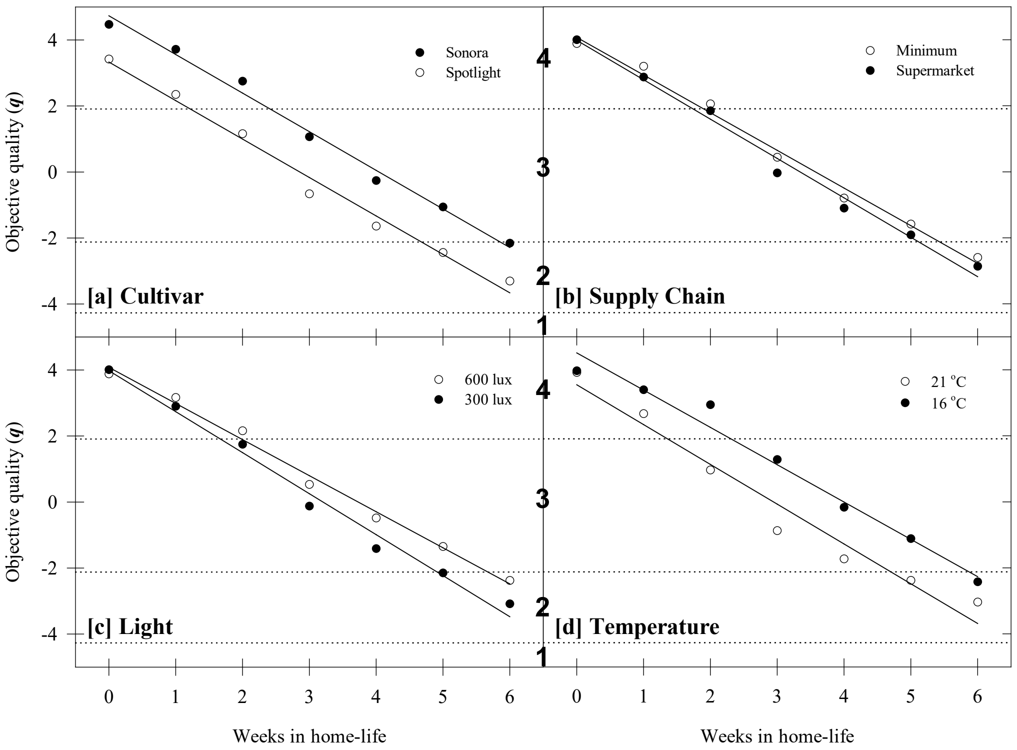


Fig. 5 [a-d]. Experimental treatment effects on poinsettia objective quality scores (q) during home life in Year 4 (4 = excellent, 3 = very good, 2 = good and 1= poor or very poor).

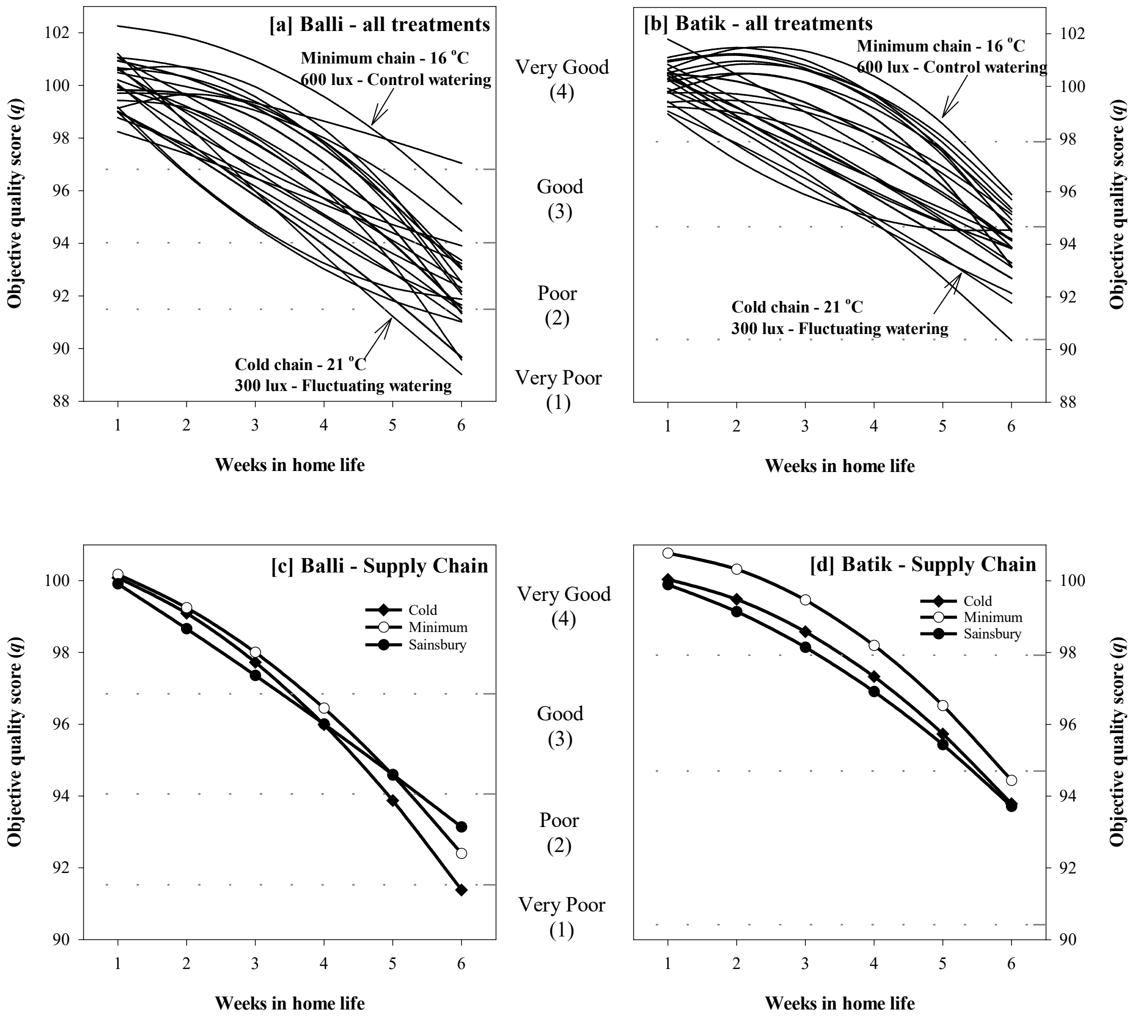


Fig. 6 [a-d]. Experimental treatment effects on begonia objective quality scores (q) during home life in Year 2.

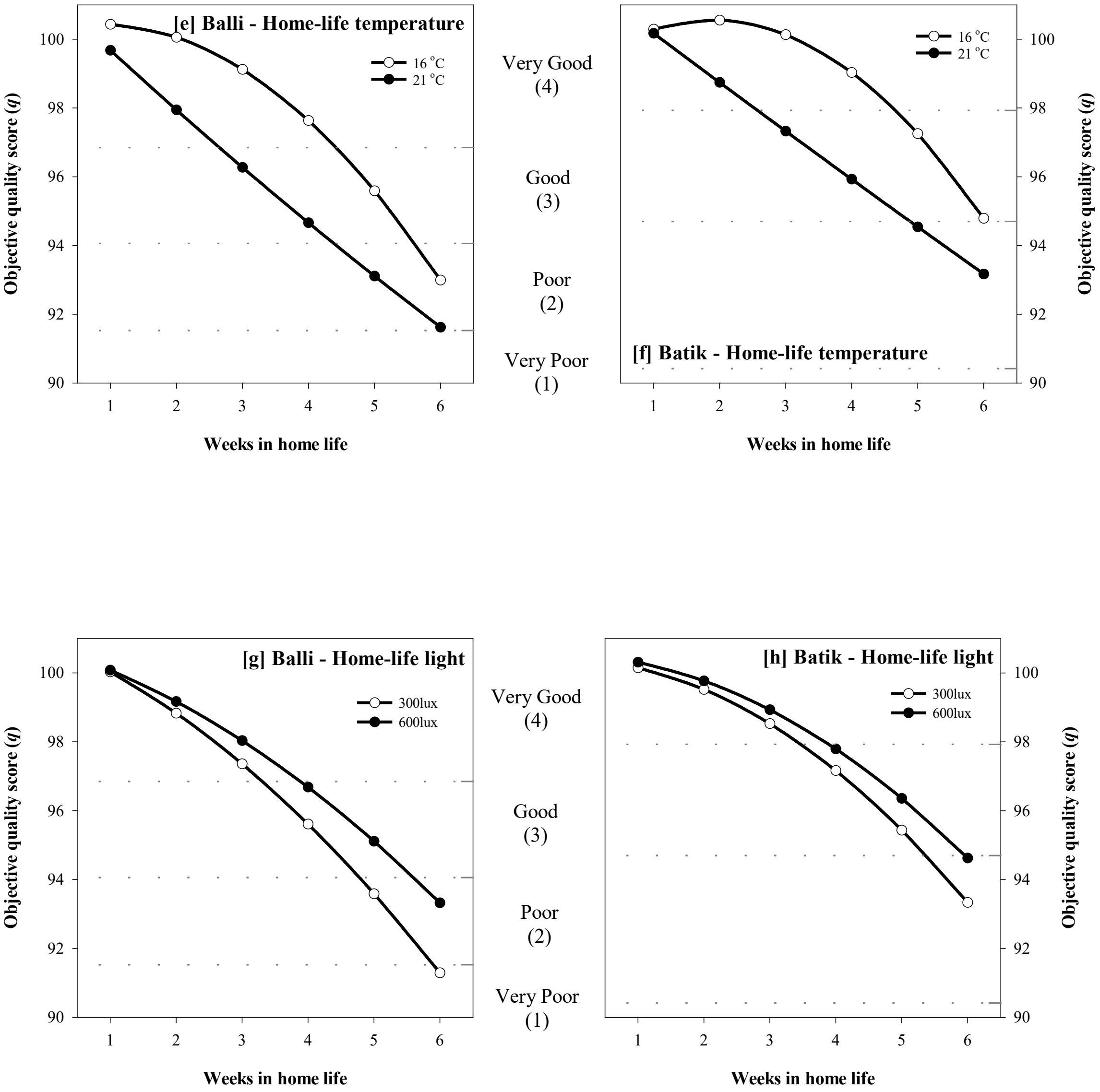


Fig. 6 [e-h]. Experimental treatment effects on begonia objective quality scores (q) during home life in Year 2.

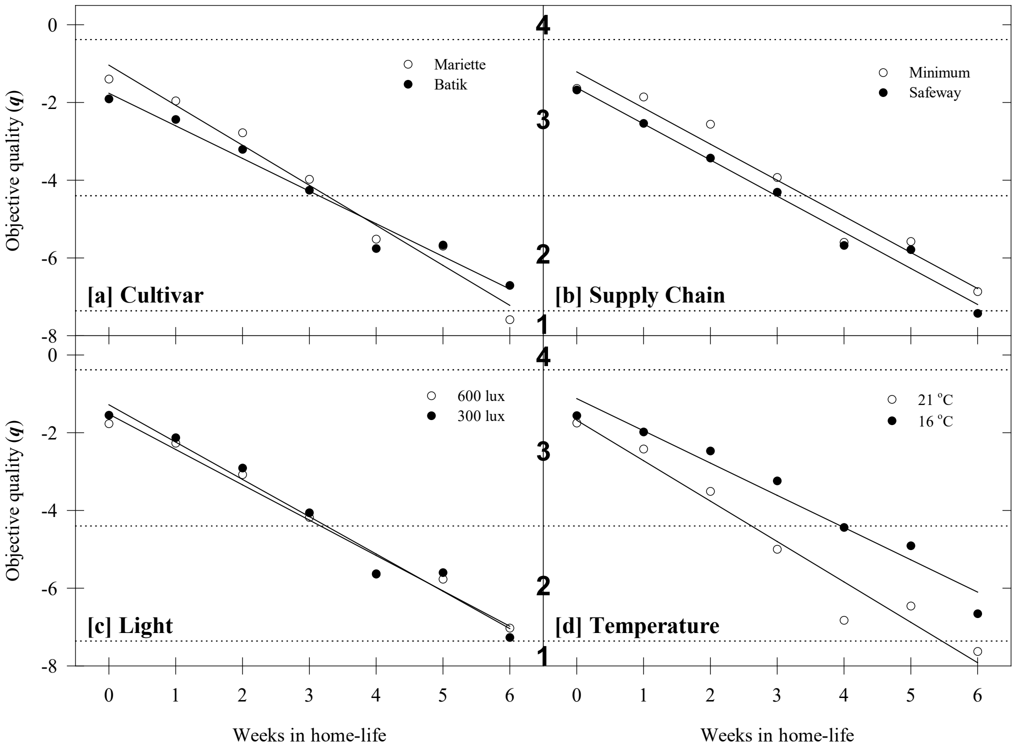


Fig. 7 [a-d]. Experimental treatment effects on begonia objective quality scores (q) during home life in Year 3 (4 = excellent, 3 = very good, 2 = good and 1= poor or very poor).

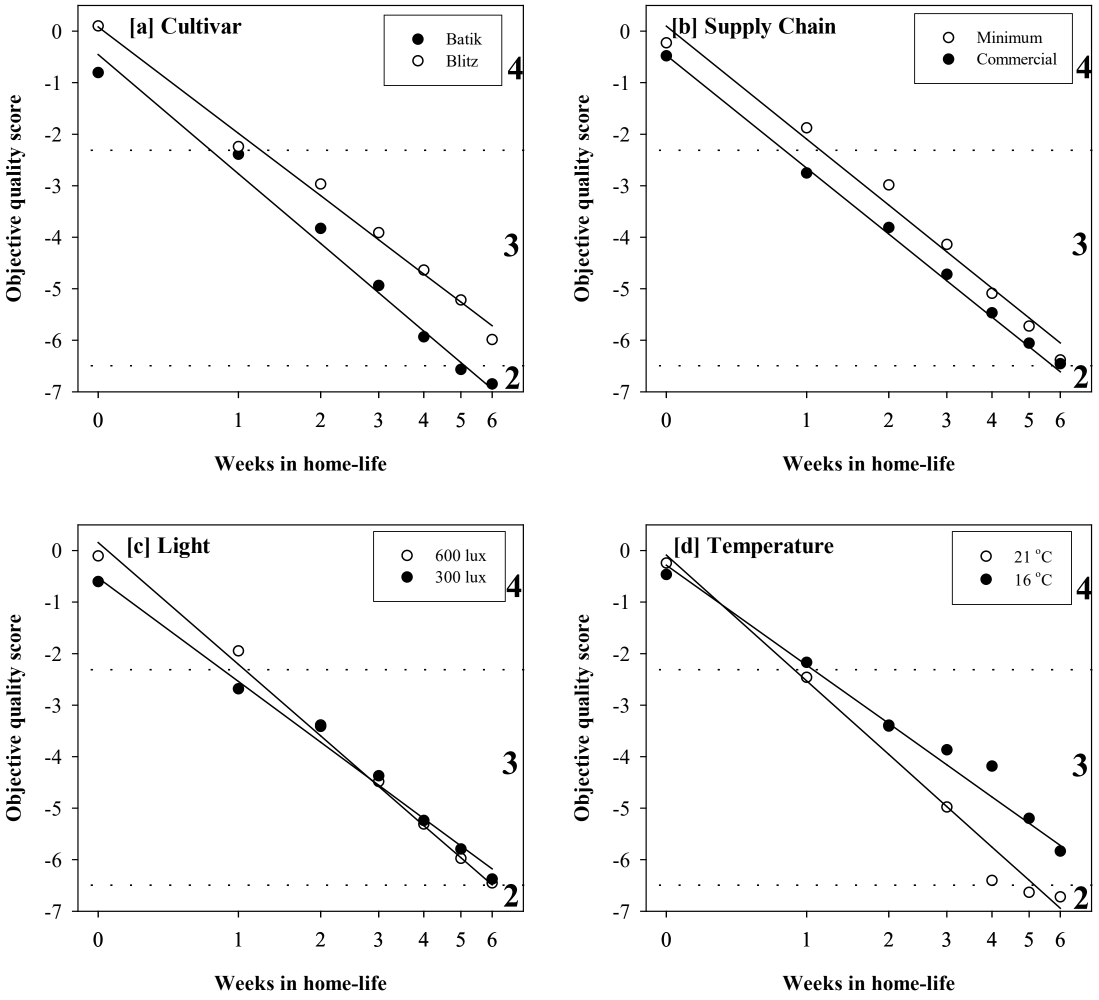


Fig. 8[a-d]. Experimental treatment effects on begonia objective quality scores during home-life in Year 4

3.6. Utility of robust product design methodology (Objective 4)

3.6.1. Robust Product Design Methodology

Theory of methodology

Robust product design is a collection of techniques used in manufacturing industry to improve the quality (performance) of products. Robust product design aims to identify production methods that minimise the variation of a product over a range of operating conditions without reducing the mean performance of the product. The methodology involves *control* factors and *noise* factors where control factors are those factors that can be easily manipulated (controlled) such as, for example, nutrient feeds during pot-plant production and noise factors are those that are difficult or impossible to control such as post-production environmental variables. Robust product design aims to identify the production control factor settings that reduce the variation due to the post-production noise factors.

The experimental design used for the pot-plant project examined the effects of a range of control factors (production and supply chains) on plant quality over a range of noise factors (home-life environments). For each combination of production treatment i in home-life environment j, the mean quality during home-life expressed on the objective quality score scale ($\theta_{i1}^{(j)}$), the rate of quality decline during home-life (objective quality score units per week) ($\theta_{i2}^{(j)}$), and the home-life longevity measured in weeks until plant quality was rated as 'good' on the expert assessor scale ($L_i^{(j)}$), were determined as performance measures of pot-plant quality during home life.

The first step in the analysis was to identify the levels of the production treatment factors (control factors) that increased the mean values of $\theta_{i1}^{(j)}$ and $L_i^{(j)}$ and reduced the magnitude of $\theta_{i2}^{(j)}$ across the range of home-life environments. This was done to assess whether production factors can be adjusted to give plants with higher average quality and longevity and a slower mean rate of quality decline over the full set of home-life environments tested in the project.

The second step was to test for significant interactions between control factors and noise factors. This analysis was intended to determine whether the production treatments gave significantly different mean levels of $\theta_{i1}^{(j)}$, $\theta_{i2}^{(j)}$ and $L_i^{(j)}$ in the different home-life environments. For example, two production treatments may give similar $\theta_{i2}^{(j)}$ when averaged across all home-life environments, but one treatment may give plants that perform equally across all home-life environments ($\theta_{i1}^{(1)} \approx \theta_{i1}^{(2)} \approx \theta_{i1}^{(3)} \approx \theta_{i1}^{(4)}$), whereas the other may give plants that perform well in environments $j = 1$ and 2 and badly in environments $j = 3$ and 4 ($\{\theta_{i1}^{(1)} \approx \theta_{i1}^{(2)}\} > \{\theta_{i1}^{(3)} \approx \theta_{i1}^{(4)}\}$). Production treatments giving plants that performed equally well across the range of home-life environments would be said to be *robust*. It is important that production systems produce plant that are of high mean quality during home life, but it is also important that the plants are robust across the range of potential home life environments. The final step in the analysis was to examine the residuals of the fitted model, including all of the important interactions, in order to identify any other treatment factors that may have affected plant-to-plant variability.

Example of methodology

An example of the model fitting procedure is shown in Table 15 using the data from the Year 4 home-life trial for poinsettia. The control factors tested were the factorial combination of 2 levels of cultivar, 2 levels of potassium, 2 levels of supply chain and 4 levels of nitrogen. Each of these 32 production treatments was tested in the 4 home-life environments given by the factorial combination of 2 levels of temperature and 2 levels of light. Estimates of $\theta_{i1}^{(j)}$, $\theta_{i2}^{(j)}$ and $L_i^{(j)}$ were analysed separately by ANOVA over the full set of experimental treatments.

Table 15. Estimates of mean quality, $\theta_{i1}^{(j)}$ (objective quality score), rate of quality decline, $\theta_{i2}^{(j)}$ (objective quality score units per week), and home-life longevity, $L_i^{(j)}$ (weeks) within each of the 32 combinations of control factors in each of the 4 home-life environments (noise factors) for the Year 4 poinsettia trial.

Control Factors †					**Noise Factors** ‡											
i	**C**	**N**	**K**	**S**	**T (+)**						**T (-)**					
					L (+)			**L (-)**			**L (+)**			**L (-)**		
					$\theta_{i1}^{(1)}$	$\theta_{i2}^{(1)}$	$L_i^{(1)}$	$\theta_{i1}^{(2)}$	$\theta_{i2}^{(2)}$	$L_i^{(2)}$	$\theta_{i1}^{(3)}$	$\theta_{i2}^{(3)}$	$L_i^{(3)}$	$\theta_{i1}^{(4)}$	$\theta_{i2}^{(4)}$	$L_i^{(4)}$
1	1	1	1	1	2.3	-0.9	9.0	0.4	-1.2	6.2	1.1	-0.9	7.4	-0.3	-1.2	5.5
2	1	2	1	1	1.9	-1.0	7.9	2.4	-1.5	6.9	0.6	-1.2	6.3	-0.6	-1.4	5.1
3	1	3	1	1	1.7	-1.2	7.3	0.3	-1.4	5.8	0.3	-1.2	6.0	-0.2	-1.6	5.2
4	1	4	1	1	1.0	-1.3	6.5	1.4	-1.0	7.4	2.0	-1.9	6.2	0.4	-2.0	5.2
5	1	1	2	1	2.5	-0.9	9.4	1.4	-1.4	6.6	0.7	-1.1	6.4	0.2	-1.3	5.9
6	1	2	2	1	6.1	-1.2	10.6	2.0	-1.1	7.8	0.6	-1.1	6.4	1.2	-0.9	7.7
7	1	3	2	1	2.6	-0.9	9.4	2.7	-1.4	7.5	0.1	-1.1	6.1	-0.1	-1.2	5.7
8	1	4	2	1	1.7	-1.3	7.0	0.1	-1.2	5.8	1.2	-1.6	6.0	0.2	-1.0	6.3
9	2	1	1	1	0.6	-1.2	6.2	0.0	-1.2	5.8	-1.2	-1.1	4.9	-1.1	-1.3	4.9
10	2	2	1	1	0.5	-1.1	6.3	-0.9	-1.5	4.9	-0.9	-0.9	5.3	-0.5	-1.3	5.2
11	2	3	1	1	-0.2	-0.6	7.0	-1.1	-1.5	4.7	-1.2	-1.2	4.8	-1.2	-0.9	5.0
12	2	4	1	1	0.7	-1.3	6.1	0.1	-1.2	5.9	-0.7	-1.2	5.2	-1.2	-1.1	4.8
13	2	1	2	1	-0.1	-1.3	5.6	0.1	-1.4	5.6	-0.8	-1.1	5.2	-0.6	-1.2	5.2
14	2	2	2	1	0.0	-1.1	5.9	0.5	-1.2	6.3	-0.6	-1.1	5.4	-0.2	-0.9	6.1
15	2	3	2	1	0.2	-1.1	6.2	0.3	-1.2	6.0	-0.2	-0.9	6.1	-1.0	-1.3	4.9
16	2	4	2	1	0.8	-1.0	6.9	-0.3	-1.3	5.4	-0.7	-1.1	5.3	-1.2	-1.2	4.8
17	1	1	1	2	2.5	-0.9	9.1	1.8	-1.1	7.6	1.5	-1.0	7.6	0.1	-1.3	5.8
18	1	2	1	2	2.5	-0.8	9.7	2.2	-1.0	8.4	1.2	-0.9	7.9	0.5	-1.2	6.1
19	1	3	1	2	2.2	-0.9	8.9	1.9	-1.2	7.3	1.4	-1.2	7.0	0.9	-1.9	5.6
20	1	4	1	2	2.6	-0.8	9.6	1.9	-0.8	9.2	0.4	-1.3	5.9	1.0	-1.0	7.2
21	1	1	2	2	1.6	-1.0	7.7	0.4	-1.2	6.1	0.9	-1.0	7.0	0.7	-1.2	6.3
22	1	2	2	2	1.8	-1.0	7.9	1.8	-1.1	7.4	1.6	-1.0	7.9	0.0	-1.1	5.9
23	1	3	2	2	1.6	-1.1	7.4	2.0	-1.1	7.9	0.6	-1.1	6.4	-1.0	-1.5	4.8
24	1	4	2	2	1.9	-0.9	8.3	1.5	-1.1	7.2	0.3	-1.3	5.9	-0.4	-1.3	5.3
25	2	1	1	2	1.0	-1.3	6.3	0.1	-1.1	6.0	-0.1	-1.1	5.9	-1.6	-1.4	4.4
26	2	2	1	2	0.2	-1.1	6.2	0.7	-1.2	6.4	-0.4	-1.1	5.5	-0.4	-1.1	5.5
27	2	3	1	2	0.4	-1.0	6.4	-0.3	-1.3	5.4	-0.3	-0.8	6.2	-1.4	-1.3	4.6
28	2	4	1	2	0.6	-1.2	6.3	1.4	-1.2	7.0	0.0	-1.1	6.0	-0.1	-1.3	5.6
29	2	1	2	2	1.1	-1.1	7.0	-0.3	-1.2	5.5	-0.8	-1.4	4.9	-0.3	-1.4	5.3
30	2	2	2	2	1.0	-1.1	6.8	-0.6	-1.4	5.1	-0.8	-1.3	5.0	-0.4	-1.2	5.5
31	2	3	2	2	1.4	-1.1	7.1	0.6	-1.0	6.7	0.0	-0.9	6.4	-0.9	-1.5	4.8
32	2	4	2	2	1.3	-1.0	7.4	1.3	-0.9	7.7	-0.6	-1.0	5.5	-0.4	-1.4	5.2

† **C** = Cultivars (1 = Spotlight & 2 = Sonora), **N** = Nitrogen (N1, N2,N3 & N4), **K** = Potassium (1 = K+ & 2 = K-) and **S** = Supply Chain (1 = Minimum & 2 = Supermarket)
‡ **T(+)** = High Temperature (21 ºC), **T(-)** = Low Temperature (16 ºC), **L(+)** = High Light (600 lux) and **L(-)** = Low Light (300 lux).

Additional Consumer Quality Score Data

The original project plan was based on the use of a single assessor to score plant quality. This was done to ensure consistency and comparability between years. However, from the results of the first two years of the project, it became apparent that there was a need for additional quality scores based on consumer assessors. As part of an additional DEFRA short-term project (HH1529SPC), a complete set of five extra 'consumer' scores was collected on the Year 4 poinsettia data by five members of the HRI-Efford staff on each of the occasions that the expert scores were collected. This was complemented by a similar set of consumer score data collected on the Year 4 begonia data using additional funding supplied by the HDC. The mathematical models developed in Years 1 and 2 of the project have been used below to describe the impact of the production treatment effects on the expert quality assessments as originally planned. In addition, the consumer preference scores and the comparisons between the consumer scores and the expert scores have also been analysed.

3.6.2. Production effects on poinsettia robust home-life quality

Analysis of expert quality score data

Year 3

Unfortunately, the planned poinsettia expert quality scores were not recorded in Year 3. This meant that the objective quality estimates had to be based on the model developed in Year 2 and the conclusions regarding the effects of N and K on $\theta_{i1}^{(j)}$ and $\theta_{i2}^{(j)}$ could not be fully validated by examining model lack of fit effects in Year 3.

Figs 9 [a-b] show the effects of the production treatments on the decline in quality during home life in Year 3 using the quality model developed in Year 2. Solid lines represent quality decline profiles, symbols represent mean objective quality scores determined weekly for each treatment, and the dashed lines represent the divisions between the expert score categories (excellent, very good, good and poor), which correspond to the estimated model parameters $\hat{\beta}_{01}$, $\hat{\beta}_{02}$, $\hat{\beta}_{03}$ and $\hat{\beta}_{04}$ (see Section 3.4.2). Given reliable quality scores in Year 3, the individual linear regression parameter estimates, $\hat{\theta}_{i1}$ and $\hat{\theta}_{i2}$, for each plant could have been used to test for the treatment effects on the model parameters. However, the unavailability of the quality score made formal significance testing inappropriate and it was not possible to estimate model lack of fit. Nevertheless, Fig 9a strongly suggests that low N gave slightly higher quality plants than high N throughout home life whereas Fig 9b shows no evidence of any effects of K on poinsettia quality.

Year 4

The effects of N and K (see Table 2) on the decline in poinsettia quality during home life in Year 4 are shown in Figs 10 [a-b]. These plots were constructed using models based on the Year 4 relationships between the expert quality scores and the plant physiology. The figures show that the effects of nutrition on subsequent plant quality were small. However, there was some evidence that N had different effects on quality in Sonora than in Spotlight. Figs 11 [a-b] show that for Sonora, the rate of quality decline during home life was greater in the high N treatments (N3 and N4, closed symbols) than in the low N treatments (N1 and N2, open symbols) whereas for Spotlight there was no evidence of any such effect. Figs 11 [c-d] show the K effects for each cultivar taken separately and show that there was little evidence of any average K effect for either cultivar.

Further analysis of the Year 4 data showed that mean home-life quality was significantly higher in the low K treatment than in the high K treatment for the supermarket chain plants, but that the effects of K in minimum stress plants were negligible (Figs 12 [a-b]). Low temperature during home life (16ºC) also slowed the rate of quality decline relative to high temperature (21ºC) and this effect was more pronounced in Sonora than in Spotlight (Figs 12 [c-d]).

Longevity estimates were used to quantify the treatment effects on added time spent in the "excellent" or "very good" classes during home life. Low N increased longevity by 3½ days in Sonora, but did not increase longevity in Spotlight. The largest increases in longevity were given by the home-life treatments where low temperature (16ºC) increased longevity by 8.5 days relative to high temperature (21ºC), and high light (600 lux) increased longevity by 5.4 days relative to low light (300 lux). These effects were additive, so the increase in longevity for a plant at low temperature and high light over a plant at high temperature and low light was approximately 2 weeks. Compared to these effects, the improved longevity given by low N during production was relatively small.

The variances of the residuals were tested for significance at each level of the significant treatment factors. There was no evidence of any significant differences in variability in mean quality for any of the levels of the main experimental treatments in Year 4 and this showed that there was no evidence that any of the nutritional treatments improved robustness in poinsettia by reducing variability over home-life environments.

Analysis of consumer quality score data

The five additional sets of quality assessment records collected on poinsettia in Year 4 for the DEFRA additional funding project were compared with the single set of expert quality assessment collected for the LINK project. Correlations were calculated using Kappa correlation statistics and Table 16 shows the Kappa correlation for every possible pairwise correlation between the six sets of assessments.

Table 16 Kappa correlations showing the degree of agreement for pairwise comparisons between the six assessors used for the Year 4 poinsettia trial.

	Assessor 1	Assessor 2	Assessor 3	Assessor 4	Assessor 5	Expert
Assessor 1	1.00	-	-	-	-	-
Assessor 2	0.66	1.00	-	-	-	-
Assessor 3	0.35	0.46	1.00	-	-	-
Assessor 4	0.27	0.29	0.27	1.00	-	-
Assessor 5	0.64	0.70	0.56	0.28	1.00	-
Expert	0.45	0.52	0.31	0.14	0.46	1.00

The correlations show that although there was some evidence that the five consumer assessors were not fully consistent, three of them (1, 2 and 5) were in good agreement with each other and these three differed from the expert more than they differed from each other. The results for assessors 3 and 4 were less clear but there was some evidence that these two assessors differed more from the expert than they did from the other three assessors. Overall, the analysis showed there was considerable variation between the five consumer assessors but, excluding assessor 4, there was some evidence that the remaining four assessors differed more from the expert than from each other.

The relationship between each pair of assessors was further explored in Fig 13. This graph shows bivariate plots of the scores awarded by each assessor plotted against the scores awarded by every other assessor. The Y-axis shows the mean and standard deviations of the scores of one assessor plotted against the corresponding scores of a second assessor along the X-axis. The diagonal line is the expected relationship between two assessors with identical scores therefore deviations from the diagonal axis show the differences in scoring between the pair of assessors.

The most important feature of Fig 13 is the relationship between the expert assessor and the five consumer assessors. Apart from assessor four, who gave relative rather than absolute scores, there was reasonable agreement between the consumer assessors and the expert assessor at the beginning of home life but, in every case, the consumer gave substantially higher scores at the end of simulated home-life than the expert. These comparisons show that the consumer perception of quality loss was substantially different from the expert perception of quality loss. If the consumer scores had been used to assess plant quality instead of expert scores, it is possible that much more importance would have been attached to quality variability later in home-life. In particular, the consumers rated plants from even the most extreme home-life environment as of some value at the end of home-life whereas the expert scored them as valueless.

It is also apparent that, apart from the scores from assessor four, most of the consumer assessors were in good agreement with each other. This is important, as a consensus on quality must be defined before techniques can be developed to improve quality. These results show that there is good reason to assume a common definition of quality for pot-plants.

The graphical plots in Fig 14[a-f] show the mean quality profiles over time for the four nitrogen feed treatments in the Year 4 poinsettia trial assessed by the five consumer assessors and the expert assessor. The most important feature of these plots is that all five consumers were consistent and ranked the low N treatment (N1) best throughout home-life. Even assessor 4, who used relative rather absolute scores, gave the same high relative ranking for the low N treatment. However, the expert assessor gave the highest ranking to the N2 treatment regime. These results show that the expert scores were not fully consistent with consumer preferences and suggest that the choice of a nitrogen feed production regime for optimising post-harvest quality needs be conditioned on the preferences of the consumer rather than on the assumptions of the grower. Further work on this important aspect of ornamental plant quality could be of great importance to the horticulture industry.

3.6.3. Production effects on begonia robust home-life quality

Analysis of expert quality score data

Year 3

The individual linear regression parameter estimates of $\theta_{i1}^{(j)}$, $\theta_{i2}^{(j)}$ and $L_i^{(j)}$ were analysed separately to test for the effects of N and K treatments (see Table 3) on the model parameters for the Year 3 begonia data. Fig. 15a shows that, after averaging over all other factor effects, the mean quality at the start of home life was greater for high N than for low N plants, but the rate of quality decline was less for low N plants than for high N plants. In contrast, the effect of K averaged over all other factors, appeared small (Fig 15b).

Further analysis showed that the effect of N on the rate of quality decline differed between cultivars (Fig. 16 [a-b]). Thus, in Batik, the rate of quality decline due to low N was only marginally less than that for high N, whereas in Mariette the rate of quality decline due to low N was considerably less than for high N. The average rate of quality decline averaged over the two temperatures showed a slightly reduced rate of decline for the high K feed treatment (Fig. 16 [c-d]). The effects of the two N treatments on the rate of quality decline in the two home-life temperature environments appeared very similar (Fig 16 [e-f].

Longevity estimates were used to quantify the treatment effects on added time spent in the "excellent" or "very good" classes during home life. High N increased longevity by 3½ days in Batik, but had no effect on increasing longevity for Mariette. However, this was because the low N plants were much paler at marketing and were judged to be of much lower quality at the start of home life. If the low N plants had been of comparable quality to the high N plants at the start of home life, and had retained their lower rate of quality loss, then the low N plants would have had markedly greater longevity than the high N plants. If the manipulation of N during production can be regulated to give plants that are of high quality at marketing and also retain their reduced rate of quality loss during home-life, then quality and longevity during home-life could be substantially enhanced.

Year 4

The individual linear regression parameter estimates of $\theta_{i1}^{(j)}$, $\theta_{i2}^{(j)}$ and $L_i^{(j)}$ were analysed separately to test for the effects of N and K treatments (see Table 4) on the model parameters for the Year 4 begonia data. The individual linear regression parameter estimates, $\hat{\theta}_{i1}$ and $\hat{\theta}_{i2}$, for each plant were analysed separately with the usual assumptions of normality and constant variance, to test for treatment effects on the model parameters. Fig 17a-d shows the fitted models for the expert score data showing the main effects of the nitrogen, potassium, cultivar and supply chain factors on home-life quality and longevity. It should be noted that the Year 4 plants were of a higher overall quality than the Year 3 plants and the rate of decline of the plants during home-life better fitted an exponential decay curve rather than a linear decay curve. For this reason, the time axis of the graphs in Fig 17a-d have been transformed to a logarithmic scale to give a linear fit for the objective quality scores over time.

An important aim of the Year 4 begonia experiment was to test whether reduced nitrogen feed could be used to improve longevity during home-life. In the Year 3 begonia trial, plants produced using reduced nitrogen feed showed a slower rate of decline than plants produced using a standard feed (Fig 15). However, the low nitrogen plants from Year 3 were of relatively low initial quality and would have been non-saleable in normal commercial practice. A less extreme range of N feed treatments was used in Year 4 to avoid the problem of initial quality loss and Fig 17a (objective quality) and Fig 18a (foliar colour) do indeed show that the initial quality of all treatments was very similar at the point of sale. However, although all the plants were of high initial quality, there was very little difference in the rate of quality decline of the various nitrogen treatments during home-life. Therefore there was no evidence from the Year 4 data that the nitrogen feed treatments affected the home-life quality of the plants.

The potassium treatments had a small but measurable effect on quality at the point of sale with a slight loss in the initial quality score (Fig 17b) due to the high K treatment. The foliar colour (Fig 18b) was slightly affected by the potassium feed with the high K plants showing a slight reduction in foliar colour relatively to the low K plants. However, Fig 17b shows that the high K treatment plants had a small but significant reduction in the rate of quality decline during home-life after averaging over all other experimental factors. This shows that the high K treatment had some benefit in improving the longevity of begonia quality during home-life.

The effects of the supply chain and shelf-life treatments were consistent with the effects seen in previous trials except that Blitz was not directly comparable with Mariette used in Year 3. Mean quality during home-life was significantly greater for Blitz than for Batik, and the rate of quality decline was slightly greater for Batik than for Blitz (Fig 17c). Mean quality during home-life was greater for the minimum stress chain than for the commercial chain although the rates of quality decline did not differ from each other (Fig 17d). Also, the low temperature treatment (16ºC) increased mean quality and slowed the rate of quality decline relative to the high temperature treatment (21ºC) and the low light treatment slightly reduced the rate of quality decline relative to the high light treatment (not shown).

Analysis of potassium feed effects on percentage flower drop

The effects of potassium on the rate of quality decline in begonia (Fig 17b) were examined in detail by examining the effects of potassium on the individual determinants of plant quality. Fig19a-d shows the effects of potassium feed levels on percentage cumulative flower drop for the two varieties in each of Year 3 and 4 of the experiment. There was a very strong contrast between the high temperature plants and the low temperature plants with the high temperature plants showing accelerated flower drop relative to the low temperature plants.

Fig 19a-d shows good evidence that the high K feed treatment gave increased resistance to flower drop relative to the low K feed treatment in the high temperature home-life environment, especially during early home-life. The strongest response was seen in Year 4 (Figs 19c-d) where there was good evidence that high K reduced flower drop at high temperature, especially for Batik but also for Blitz. For Blitz, there was no evidence of any effect of any K effect in the low temperature regime whereas for Batik there was some reduction in flower drop due to high K in the low temperature regime but only after two or three weeks of home life. The evidence from Year 3 (Figs 19a-b) was less strong but there was some evidence that high K reduced flower drop in Mariette in the high temperature regime, especially in the critical first two weeks of home life.

In both years, flower drop was a significant determinant of plant quality (Tables 11 and 13) although in both years there were also other important determinants of plant quality. Fig 15b shows no <u>average</u> quality improvement due to potassium in Year 3 while Fig 17b shows only a modest reduction in the rate of quality decline due to high K in Year 4. However, Fig 19 show clear evidence that high K had a significant effect in reducing flower drop at the high home-life temperature and especially during the early weeks of home-life. This shows that high K can increase robustness to flower drop caused by high home-life temperature and therefore shows that increased K during production can improve the robustness of begonia against early flower drop in high temperature home-life environments.

Analysis of consumer quality score data

The five additional sets of quality assessment records collected on begonia in the Year 4 HDC additional funding project were compared with the single set of expert quality assessment collected for the LINK project. Correlations were calculated using Kappa correlation statistics and Table 17 shows the kappa correlation for every possible pairwise correlation between the six sets of assessments.

Table 17 Kappa correlations showing the degree of agreement for pairwise comparisons between the six assessors used for the Year 4 begonia trial.

Assessor 1	1.00					
Assessor 2	0.05	1.00				
Assessor 3	0.03	0.33	1.00			
Assessor 4	0.00	0.38	0.44	1.00		
Assessor 5	0.03	0.36	0.37	0.51	1.00	
Expert	-0.10	0.22	0.23	0.36	0.29	1.00
	Assessor 1	Assessor 2	Assessor 3	Assessor 4	Assessor 5	Expert

Apart from Assessor 1, whose scores were essentially uncorrelated with the other five assessors, the scores of the remaining five assessors were all positively correlated. Although the correlations were not strong, it is notable that Assessors 2-5 generally had higher correlations with each other than they had with the expert assessor. As with poinsettia, this suggests that the expert assessments of quality did not fully accord with the consumer assessments of quality.

Figs 20[a-f] show the quality scores of the individual assessors plotted against time for each of the four nitrogen treatments. Assessor I appears to have scored the plants on a relative scale at each time point rather than on an absolute scale and this probably explains the lack of correlation between Assessor 1 and the other five assessors. Of the remaining five assessors, it is very apparent that whereas the expert assessor was very consistent and saw no evidence of any differences between the nitrogen treatments the consumer assessors did show preferences between the nitrogen treatments. All the consumer assessors (including Assessor 1) appear to have shown a preference for the low rate nitrogen treatments and especially for N2 compared with the high rate nitrogen feed treatments. However, this preference occurred throughout home-life and therefore seems to have been based on the general average appearance of the plant rather than on any evidence of extended home-life. Nevertheless, these results do suggest that manipulating the nitrogen feed regime can have an impact on consumer preference and may be an important consideration when defining the plant quality required by the consumer.

3.6.4. Conclusions

Mean quality during home life, the rate of decline during home life and the home-life longevity were all used to provide appropriate performance measures of pot-plant quality during home life. These measures were used to compare the effects of production factors on post harvest quality and to test for important interactions between production and home-life factors.

In the Year 3 begonia trial, the low N feed substantially reduced the rate of quality loss but because the initial quality at marketing was severely reduced, the reduced rate of quality loss in home life did not result in improved longevity. In the Year 4 begonia trial, a higher minimum level of N feed was used to try to improve initial quality. The Year 4 plants were, indeed, of the required commercial standard but, unfortunately, there was no evidence of any reduction in the rate of quality decline.

In poinsettia there was some weak evidence that reduced N during production increased longevity but the effect was very small (about 3.5 days for cv Sonora) and was probably of no commercial significance.

There was good evidence that high K feed during production reduced begonia flower drop in high-temperature home-life environments, especially during early home-life. Hence high K increases begonia robustness against high temperature home-life environments.

The mathematical models of nitrogen effects were all based on the quality scores made by the expert assessor. However, the non-expert assessors all showed a preference for the low N treatments and modelling consumer preferences might have shown a different model for nitrogen feed effects. If the consumer is indeed tolerant of low nitrogen effects in begonia, it might be possible to reduce nitrogen feed and produce more robust plants without necessarily reducing consumer acceptability at the point of sale.

The extra information from the DEFRA study showed that the quality scores assigned by the expert did not fully agree with the quality scores assigned by the consumers. The consumer scores seemed reasonably consistent with each other but the sample size was too small to test for separate groups or clusters within the set of consumers. Overall, the results suggest that preference variability between assessors may generate an additional level of variability that needs to be accounted for in the design of robust quality in ornamental plants

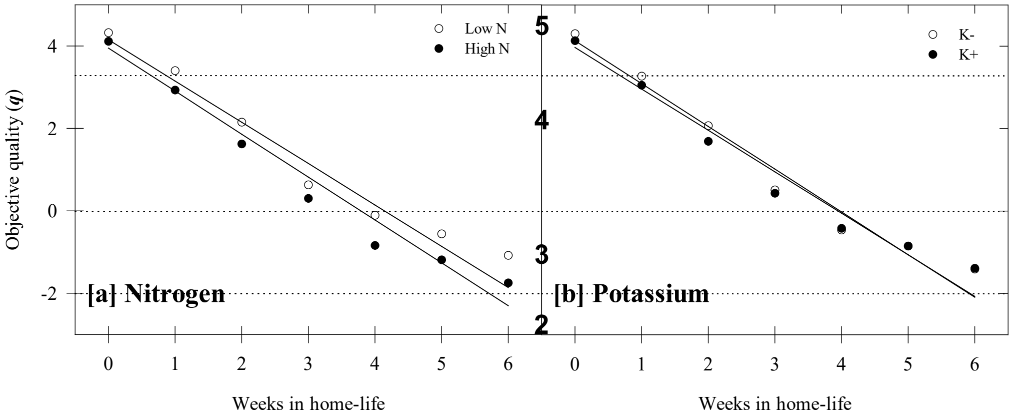


Fig. 9. Main effects of N and K on objective plant quality of poinsettia during home life in Year 3 (5 = Excellent, 4 = Very Good, 3 = Good and 2 = Poor)

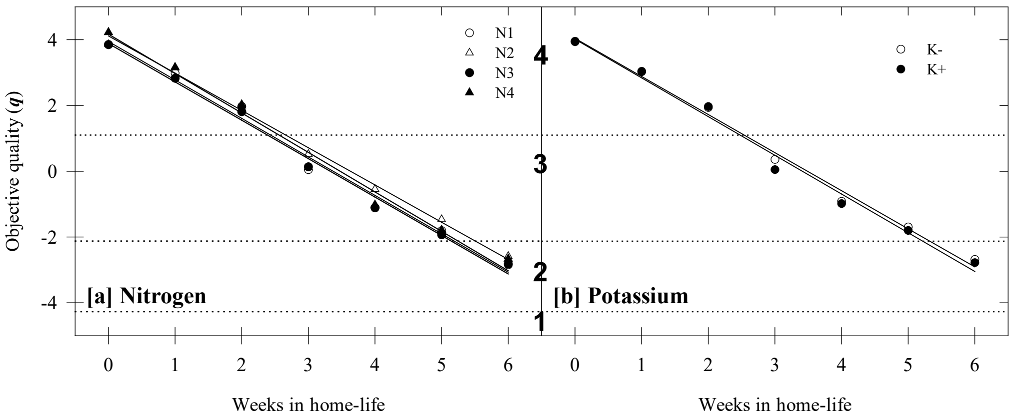


Fig. 10. Main effects of N and K on objective plant quality of poinsettia during home life in Year 4 (4 = Excellent, 3 = Very Good, 2 = Good and 1 = Poor or Very Poor)

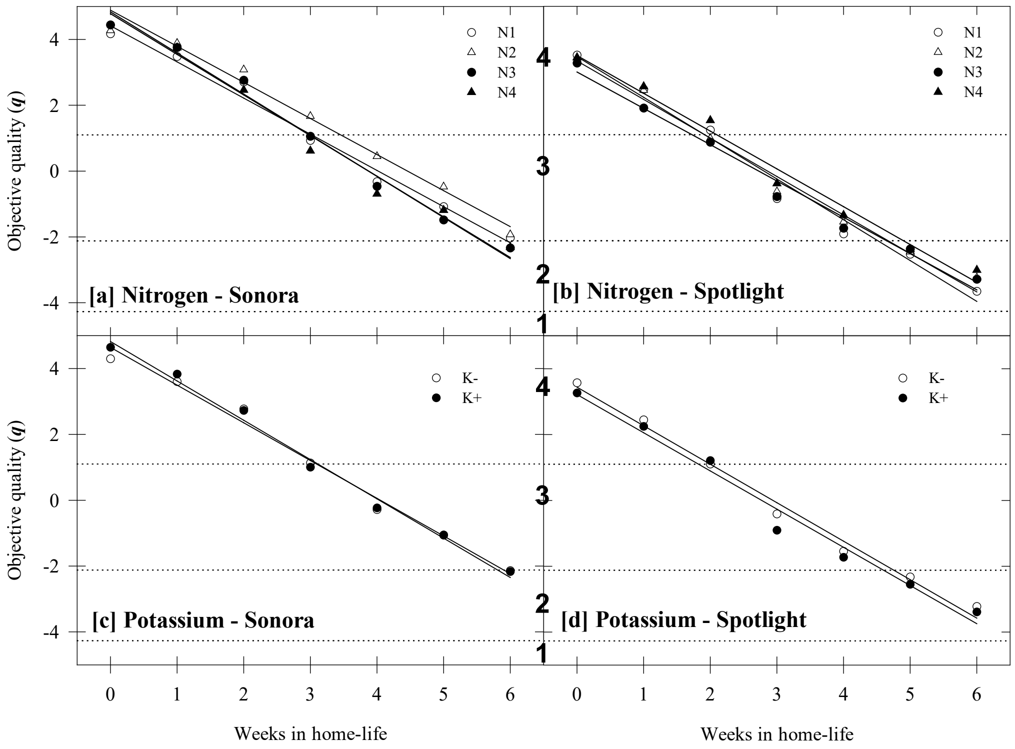


Fig. 11. Interactions between level of N and cultivar, and between level of K and cultivar for poinsettia objective plant quality in Year 4 (4 = Excellent, 3 = Very Good, 2 = Good and 1 = Poor or Very Poor)

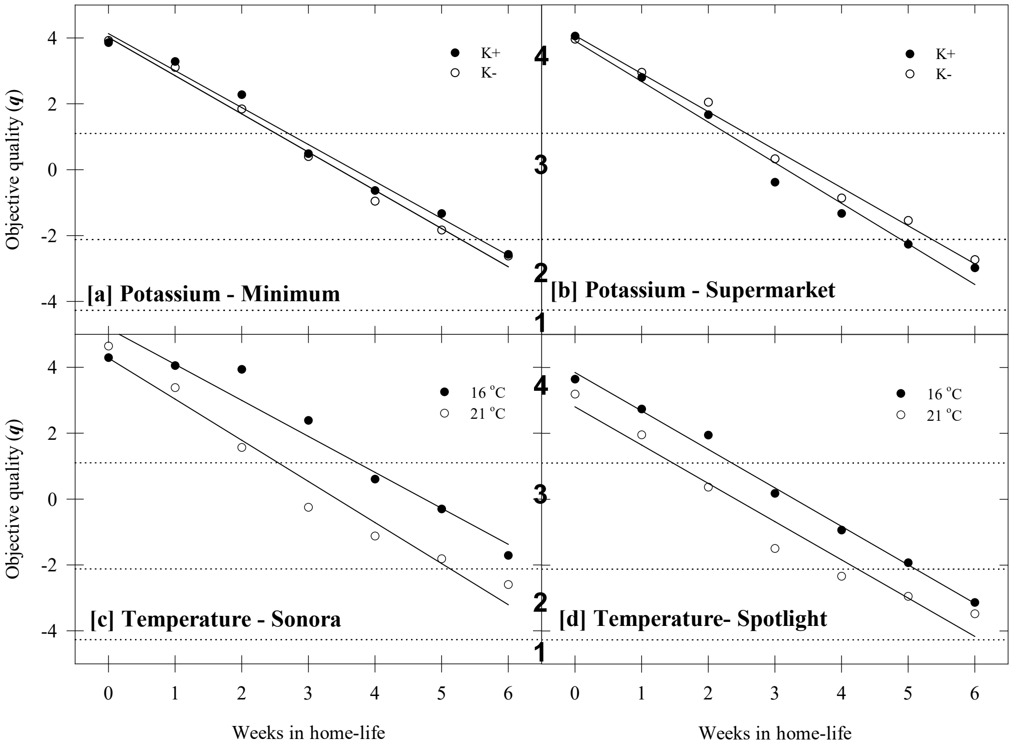
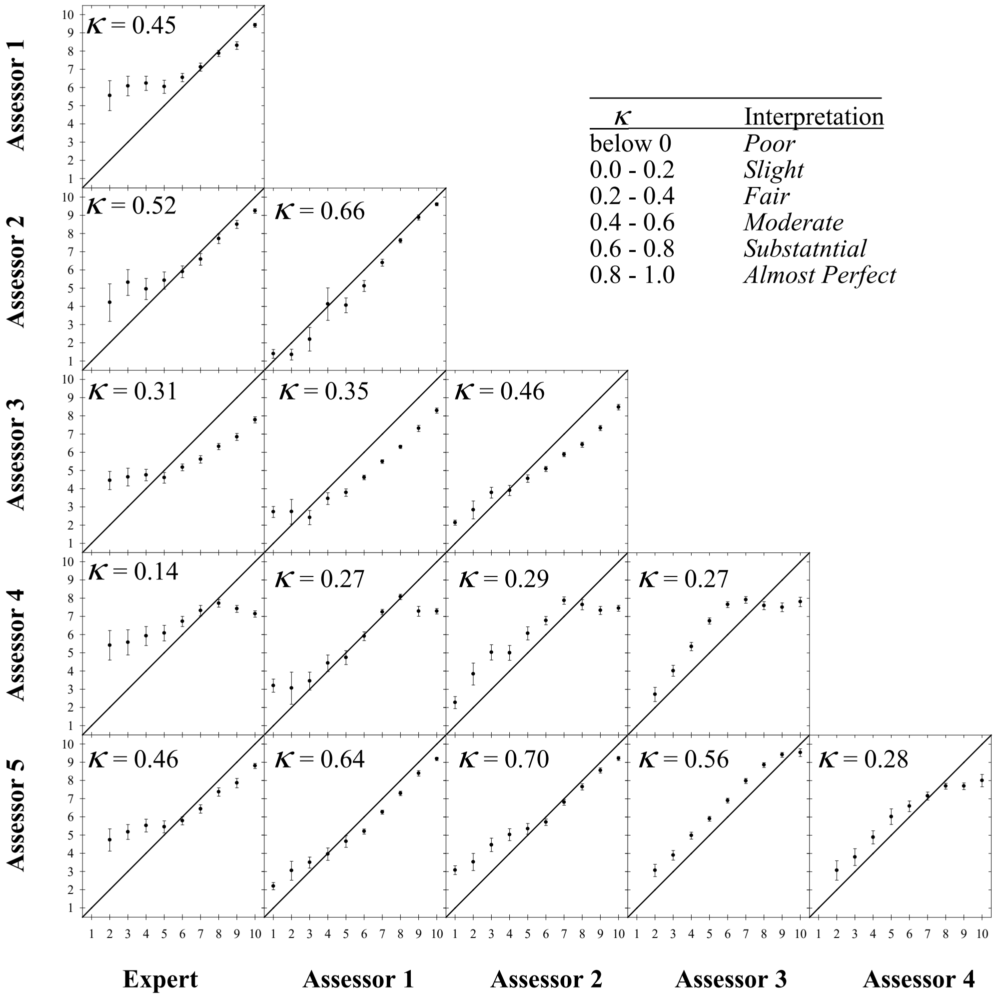


Fig. 12. Interactions between level of K and supply chain, and between home-life temperature and cultivar for poinsettia objective plant quality in Year 4 (4 = Excellent, 3 = Very Good, 2 = Good and 1 = Poor or Very Poor)



κ	Interpretation
below 0	*Poor*
0.0 - 0.2	*Slight*
0.2 - 0.4	*Fair*
0.4 - 0.6	*Moderate*
0.6 - 0.8	*Substatntial*
0.8 - 1.0	*Almost Perfect*

Fig. 13. Pairwise graphical plots showing the relationship between the quality scores for the Year 4 Poinsettia data for each pair of assessors. The bivariate plots show the scores assigned to each individual pot-plant by each pair of assessors and the error bar shows the variability of the Y-axis observations relative to the X-axis observations. The diagonal lines represent the lines of perfect agreement.

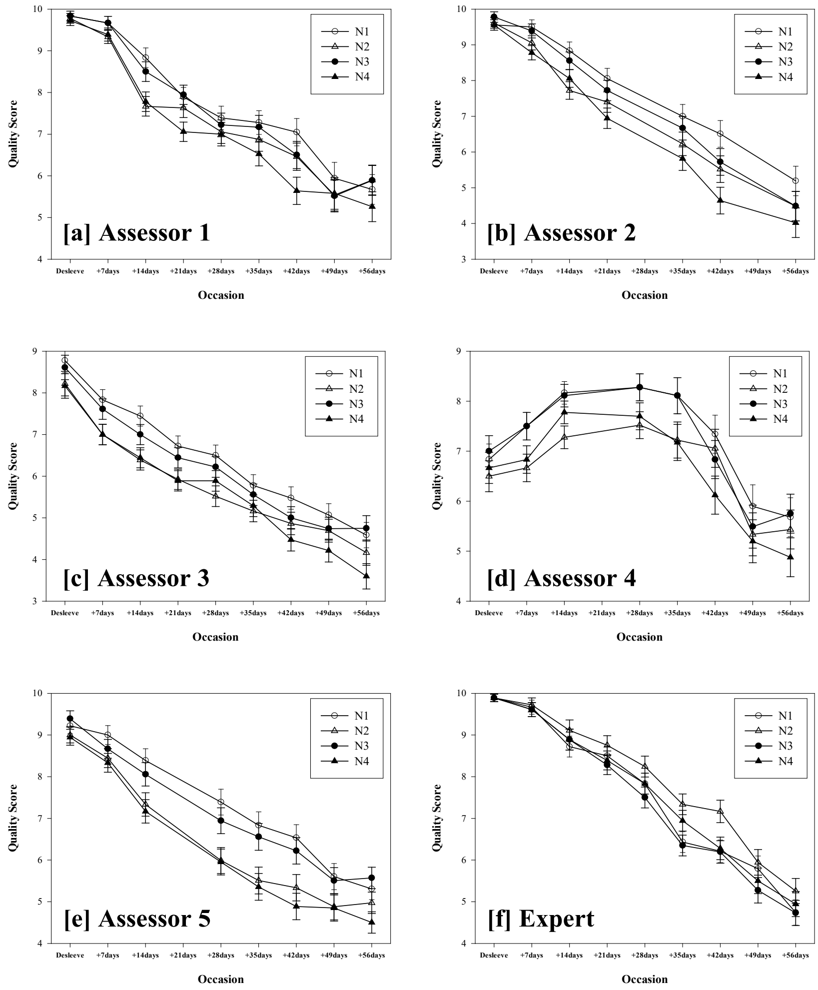


Fig. 14. Pairwise graphical plots showing the mean quality scores for four nitrogen feed treatments in the Year 4 poinsettia trial as assigned by five consumer assessors

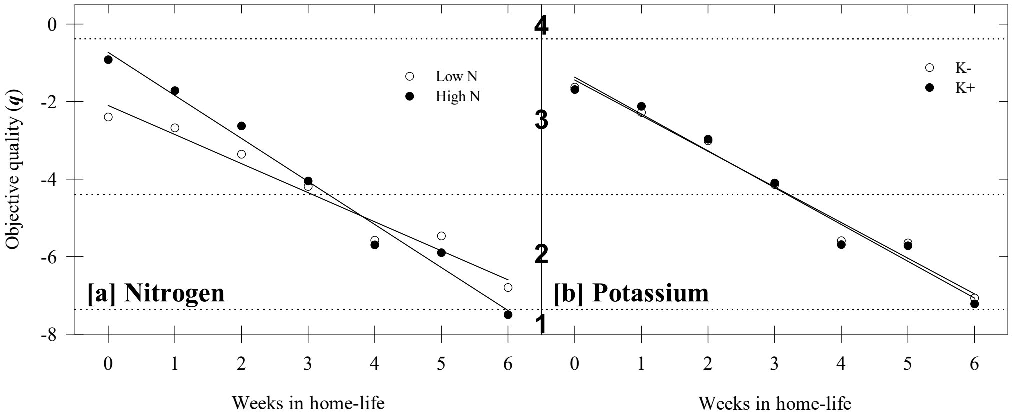


Fig. 15. Main effects of N and K on objective plant quality of begonia during home life in Year 3 (4 = Excellent, 3 = Very Good, 2 = Good and 1 = Poor or Very Poor)

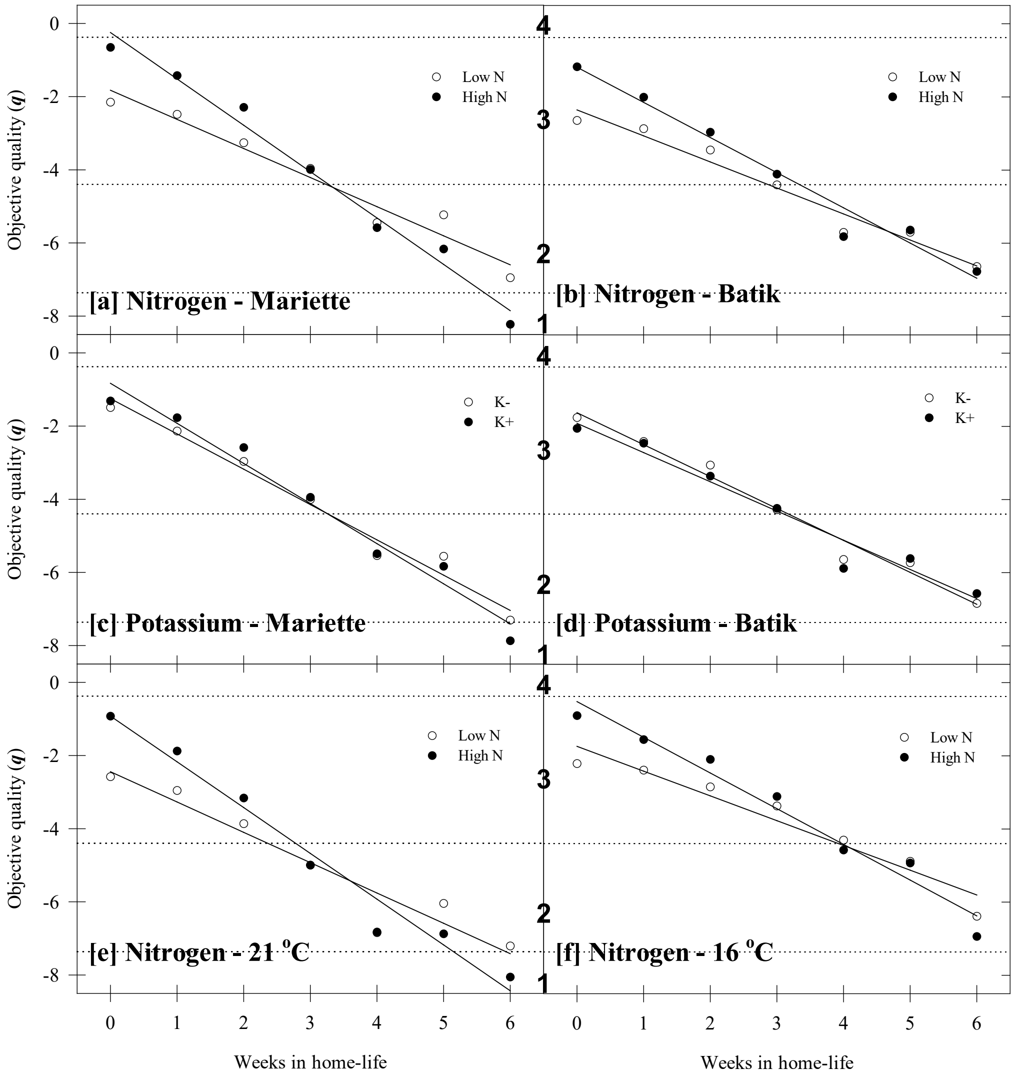


Fig. 16a-f. Interactions between level of N and cultivar, level of K cultivar, and between home-life temperature and N for begonia objective plant quality in Year 3 (4 = Excellent, 3 = Very Good, 2 = Good and 1 = Poor or Very Poor)

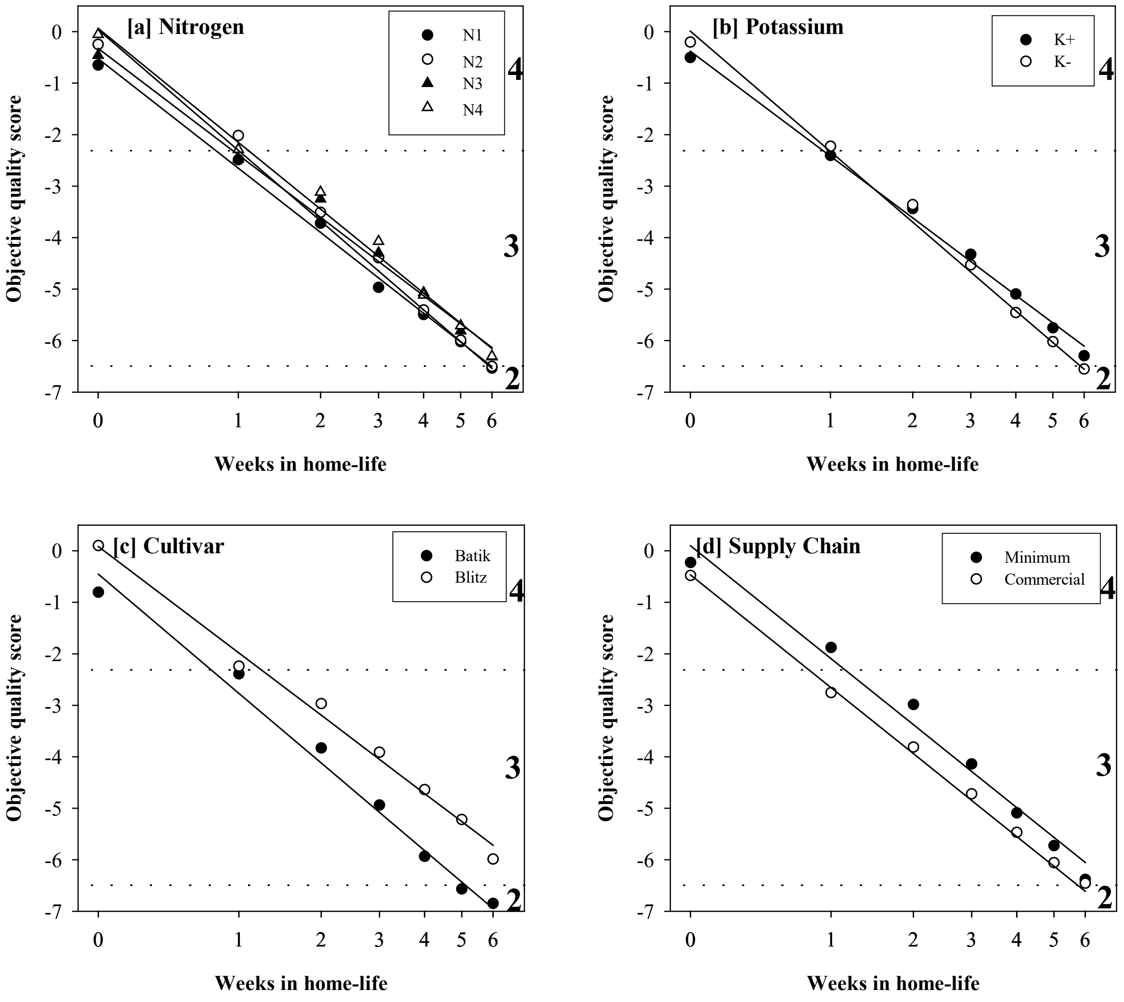


Fig 17[a-d]: Effects of nitrogen treatments [a], potassium treatments [b], cultivars [c] and supply chain treatments [d] on objective plant quality of begonia during home life in Year 4

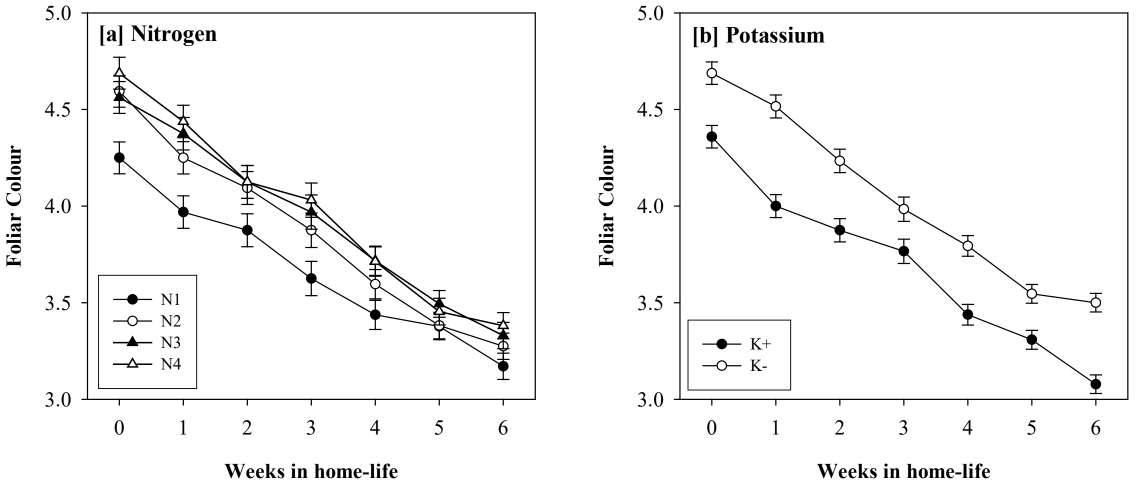


Fig 18[a-b]: Foliar colour score (1-5; 5 = dark green & 1 = severe paling) for begonia plants in summer 2003, for nitrogen [a] and potassium treatments [b] during production

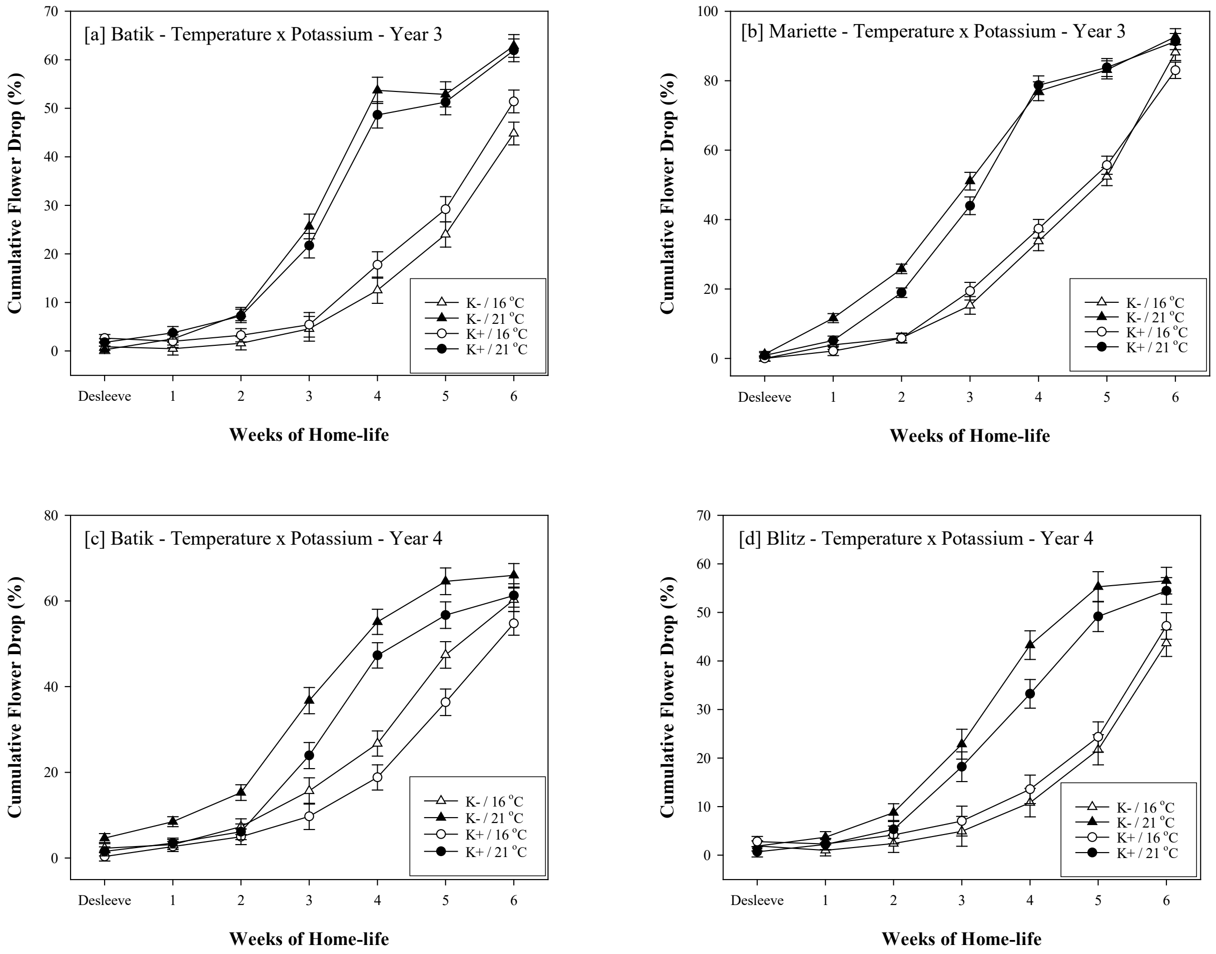


Fig 19[a-d]: Effects of potassium treatments on percentage cumulative flower drop in begonia at two home-life temperatures for [a] Batik Year 3, [b] Mariette Year 3, [c] Batik Year 4, [d] Blitz Year 4.

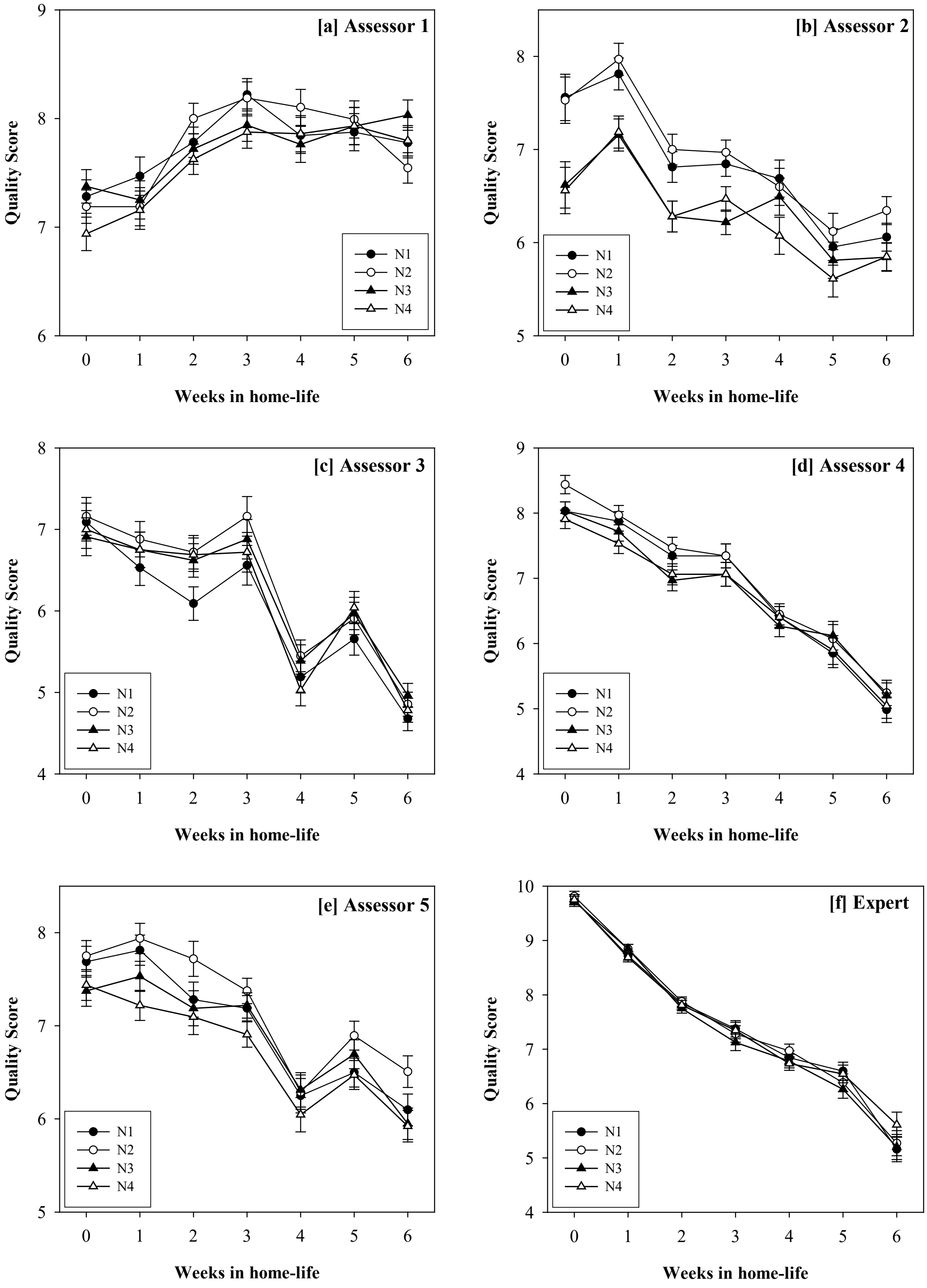


Fig. 20[a-f]. Mean quality scores for four nitrogen feed treatments in the Year 4 begonia trial as assigned by the five consumer assessors and the expert assessor

3.7. Development of chlorophyll fluorescence (CF) screening techniques (Objective 2)

3.7.1. Theory and experimental details

Light energy is absorbed by chlorophyll molecules and used in photosynthesis. However, some of the absorbed light is always lost as heat or re-emitted as chlorophyll fluorescence (CF). If a dark-adapted leaf is exposed to a flash of light, the resulting CF emission can be used as a highly sensitive indicator of photosynthetic function (Bolhàr-Nordenkampf and Öquist, 1993). Stress conditions, such as chilling, high temperature, drought etc can reduce the photosynthetic quantum conversion efficiency of a plant and this loss of efficiency can be detected non-invasively and non-destructively by using an appropriate fluorimeter to measure CF. In this project, the CF efficiency of stressed pot-plants was studied to develop appropriate screening techniques for determining the predictive value of CF as an indicator of potential pot plant longevity.

The characteristics of CF emission from illuminated dark-adapted leaves were first described by Kautsky and Hirsch (1934). There is a rapid rise from an initial level, Fo, to a maximal intensity, Fp, where Fp depends upon the intensity of illumination, and an overall maximal value Fm is achieved under light saturation conditions. There then follows a decline phase that finally reaches a steady state (Ft). One of the most useful fluorescence parameters (Bolhàr-Nordenkampf and Öquist, 1993) has proved to be Fv/Fm where Fv (variable fluorescence) is calculated as Fm-Fo. This ratio is highly correlated with the net photochemical efficiency of photosystem II and can be expected to decline under stress conditions. In this project, Fv/Fm has been monitored and recorded for each trial throughout the project.

Recently, Professor Reto Strasser (consultant to the project) and others have proposed alternative measures to characterise photosynthetic efficiency. These alternatives have become feasible due to improvements in the resolution of fluorescence induction kinetics. Additionally, intermediate staging points such as Fj and Fi can now be identified in the rise between Fo and Fm, and fluorescence values at these points can be integrated into a single measure in the so-called, JIP tests (Strasser and Strasser, 1995). The particular derived parameter that we have used here to contrast with Fv/Fm, was derived by Prof. Strasser and is referred to as the CF 'Performance Index' (PI).

Two types of fluorimeter have been used in the project, both manufactured and made available by Hansatech Ltd (consortium member). They were the Plant Efficiency Analyser (PEA) and the Modulated Fluorescence Measuring System (MFMS). Of these, the PEA proved the most useful and the results presented here relate exclusively to this instrument. Starting in Year 2, the use of the PEA was extended to compare 'second hit' CF values with 'first hit' CF values. This was done to test if the recovery period of plants subjected to a first hit stress was compromised by the way the plants were produced, marketed or maintained in the home environment.

CF parameters were recorded in parallel with the collection of the plant quality data and plant physiological data (see section 3.4.1.). Measurements were taken from each plant at the production site (HH Nurseries or Efford) immediately prior to marketing, again at the start of home life (at de-sleeve for all but minimum stress plants), and weekly thereafter.

Three (single hit) measures of CF were taken from each plant on each sampling occasion. This was done by repeatedly cycling through all the plants, taking one measurement per plant each time. In the case of begonia, the three measurements per plant reflected leaf position in the canopy, upper canopy, middle canopy and lower canopy. From Year 2 onwards, all leaves were side shoot leaves, but in Year 1 lower canopy leaves were taken to be basal leaves on the main stem. Second hit recordings were done only on one plant per treatment rather than on all three and, in the case of begonia, only on upper canopy leaves.

Leaf clips were attached to appropriate leaves at least 1 hour prior to measurement to give sufficient time for dark adaptation (see Fig. 21). Equipment settings for poinsettia in Year 1 were based on the findings of an initial pilot study. However, improvements in treatment discrimination were given in all subsequent poinsettia trials (and in all trials with begonia) by increasing the light intensity to 100% and adding additional filters to attenuate the fluorescence signal and to prevent scaling errors.

Fig. 21. Leaf clips attached to lower canopy (basal) leaves of begonia plants prior to measurement of CF in Year 1

It became apparent early in the study that the environmental conditions under which the CF monitoring was carried out and particularly the temperature and the lighting prior to dark adaptation, had significant effects on the measured CF parameter values. In an attempt to control this effect in the Year 1 poinsettia trial, the two home-life rooms at 16°C and 21°C were both brought to 18°C at least 2 hours prior to the start of recording. This was not adequate, however, and in all subsequent trials (both poinsettia and begonia), rooms were brought to 18°C the evening before measurements were due to be taken. Shading was removed on the morning of assessment, and all plants were watered immediately prior to measurement.

3.7.2. Monitoring CF activity

Fig. 22 shows comparative first hit CF measurements, expressed both as Fv/Fm and as PI, for poinsettia plants in Year 2. Trends were similar to those in Year 1 (not shown), but changes in the instrumentation greatly improved treatment discrimination in Year 2. Fv/Fm and PI showed very similar trends, but on the basis of standard errors of observations, PI was judged to give the better treatment discrimination.

Fig 22 shows a clear increase in the CF scores between those made at the production nursery prior to transport and those made in the home-life facility (at de-sleeve) at Efford. It is unlikely that the photosynthetic efficiency improved as a consequence of transport and it seems more likely that the increase reflected differences in the environmental conditions in which the measurements were made. Following de-sleeve, CF scores declined progressively with time in home life, effectively mirroring the progressive reductions seen in the quality of the individual pot plants.

The detrimental effects of cold-transport on photosynthetic efficiency were clearly indicated by significantly reduced values of CF (particularly as PI) at de-sleeving (Fig. 22a). Reduced CF was apparent for a further week when CF was monitored as Fv/Fm whereas when CF was monitored using PI, the effect was observed for most of home life. The effects of transport treatment on the decline in PI during home life closely resembled effects shown up by objective quality scores and PI clearly showed good potential as an indicator of batches of plants that have been cold-stressed during the marketing phase.

CF measured both by F_v/F_m and by PI declined faster when the home life temperature was 21°C than when it was 16°C, particularly during the first three weeks of home life (Fig. 22b). The effect was very similar to that shown by objective quality scores. High light during home-life gave an increase in CF (Fig 22c) and was correlated with the beneficial effects of high light on the objective quality scores. High light reduced bract and leaf loss and benefited expert quality scores after 3 weeks in home life. As was found for the objective quality, there were no effects of the watering treatment on CF (not shown).

As shown in Fig. 22d, CF scores were consistently much higher for Sonora than for Spotlight throughout home life, and this was also the case in Year 1. This means that CF is cultivar specific and that, in practice, CF predictions would need to be calibrated against specific cultivars.

Figs 23a-b show comparative first hit and second hit PI scores for the effects of supply chain and home-life temperature on poinsettias in Year 2. For clarity, trends for the replacement chain plants have been omitted. Trends for the cold and minimum chain treatments differ slightly from those shown in Fig 22[a-d] (and have larger associated error bars) as they are based on the reduced number of plants used for the recording of second hit CF. Also, they did not start until 7 days after the start of home life, when improvements were made in the second hit instrument set-up. Overall, the second hit PI values were much smaller than first hit values, and there was no evidence of increased sensitivity to the effects of the treatments. This was also the case when CF was monitored as Fv/Fm and when CF scores were expressed as the ratio of first hit values to second hit values (data not shown). The Year 3 data gave essentially similar conclusions.

Fig. 24 shows comparative first hit CF measurements, expressed both as Fv/Fm and as PI, for begonia plants in Year 2. The plots show that PI gave greater treatment discrimination than Fv/Fm, as was found for poinsettia. The CF values declined with time in home life in a similar manner to the objective quality scores. The commercial marketing chain gave significantly lower PI than either of the other chains during the first three weeks of home life (Fig. 24a), and PI was lower at 21ºC than at 16ºC throughout home life (Fig. 24b). These results generally show that PI agrees well with the quality scores on begonia and poinsettia recorded at the same time as the PI reading.

However, although values of PI were lower at 300 lux than at 600 lux in Year 1 (not shown), there was little effect of lighting treatments on PI in Year 2 (Fig. 24c), and this contrasted with the marked effects of light on objective quality score. As for poinsettia, there was a marked cultivar effect on PI, with values for Batik being significantly higher than those for Balli (Fig 24d).

Fig. 25a-b show comparative first hit and second hit PI scores for the effects of supply chain and home-life temperature on begonia in Year 2. As for poinsettia, the second hit PI scores gave no greater treatment discrimination in begonia than did the first hit scores. This was found also in Year 3. Ratios of first hit to second hit scores also failed to improve treatment discrimination.

Of particular importance in the begonia trials, values of PI differed with leaf position. Much higher PI scores were obtained in Year 2 from the middle canopy leaves than from either the upper or the lower canopy leaves. Comparisons with Year 1 were difficult since changes had been made between Years 1 and 2 in the criteria used to select leaves for monitoring therefore the effects of leaf position were re-examined in Year 3. Again, there were marked differences in first hit PI scores due to leaf position and, as shown in Table 18, highest scores were again given by the middle canopy leaves. Lower canopy leaves gave higher PI scores than upper canopy leaves from week 2 onwards. These results show that leaf selection is an important factor that must be taken into account when developing CF as an objective measure of quality in begonia.

Table 18. First hit PI scores for upper, middle and lower canopy begonia leaves at each assessment occasion in Year 3

	Assessment Occasion (Week number in home life)							
Leaf Type	In g'house	At de-sleeve	1	2	3	4	5	6
Upper	15.6	24.7	17.4	10.6	9.7	8.4	7.8	7.3
Middle	18.6	30.6	26.2	24.0	22.6	19.3	16.7	16.8
Lower	17.8	24.2	19.5	19.3	17.9	15.9	15.0	14.1
LSD 5%	2.3	2.3	2.0	1.7	1.6	1.3	1.3	1.1

3.7.3. Leaf greenness effects

The large variability in the CF data obtained from the first three years of the trial suggested that additional information would be needed to improve the CF predictions if the methodology was to have practical application. One possibility was to use chlorophyll content as a covariate for the CF measurements and in the final begonia experiment of the project Hansatech Instruments provided a chlorophyll content meter to investigate the relationship between PI and leaf greenness. This instrument was then used to measure leaf greenness in the Year 4 begonia trial using the same leaves at the same time that were used for the CF readings.

Table 19 shows the observed correlations between the CF parameters, leaf greenness and PI for the Year 4 begonia plants at de-sleeving and the objective quality and flower drop after two weeks in home-life.

Table 19: Correlations between the CF parameters, PI and leaf greenness (Green) at de-sleeving and objective quality (Q_{week2}) and cumulative flower drop (Fdrop$_{week2}$) at week 2 of home-life for the Year 4 begonia trial

PI	1.00													
Green	0.36	1.00												
Q_{week2}	0.02	0.15	1.00											
Fdrop$_{wee}$	0.17	0.03	-0.39	1.00										
F_0	-0.05	-0.11	-0.27	0.10	1.00									
F_m	0.11	-0.04	-0.18	0.06	0.75	1.00								
F_v/F_m	0.28	0.10	0.15	-0.08	-0.48	0.15	1.00							
T_{Fm}	0.10	-0.01	-0.16	0.20	0.27	0.00	-0.36	1.00						
Area	0.58	0.15	-0.12	0.12	0.48	0.58	0.06	0.34	1.00					
F_1	-0.29	-0.18	-0.23	0.05	0.95	0.80	-0.38	0.16	0.33	1.00				
F_2	-0.42	-0.22	-0.21	0.02	0.88	0.76	-0.34	0.10	0.23	0.99	1.00			
F_3	-0.59	-0.26	-0.14	-0.03	0.70	0.69	-0.20	-0.03	0.05	0.89	0.95	1.00		
F_4	-0.48	-0.21	-0.14	-0.02	0.70	0.78	-0.09	-0.04	0.12	0.88	0.92	0.96	1.00	
F_5	-0.03	-0.09	-0.17	0.04	0.74	0.98	0.14	-0.04	0.41	0.82	0.81	0.77	0.85	1.00
	PI	Green	Q_{week2}	Fdrop$_{wee}$	F_0	F_m	F_v/F_m	T_{Fm}	Area	F_1	F_2	F_3	F_4	F_5

Figures 26[a-g] show plots of Greenness against PI for begonia data at desleeving and at weeks one to six during home-life. The Figures show that there was a clear trend during home-life for PI and leaf greenness to become more highly correlated throughout home-life. However, at de-sleeving the correlation between greenness and PI was very weak, as shown in Table 19.

Figures 27[a-b] and 28[a-b] compare the PI and the chlorophyll content, respectively, for both the nitrogen and the potassium treatments over the full period of home-life. The figures show the four main nitrogen treatments and the two potassium treatments where the error bars indicate ± one standard error about the plotted values. The two sets of figures show clear nitrogen treatment effects and these are clarified in Figs 29[a-b] which show trends for low (N1 & N2) versus high (N3 & N4) nitrogen treatments. The high nitrogen treatments had higher chlorophyll content than the low nitrogen treatments at all assessments but the PI effect showed a much smaller difference due to the N treatment effects. It is important to note that at the beginning of home-life there was a large difference in leaf greenness but no difference in PI.

The low potassium treatment had higher chlorophyll content than the high potassium treatment (Fig 28b) from week 2 of home-life but there was relatively little difference in PI between the K treatments (Fig 27b).

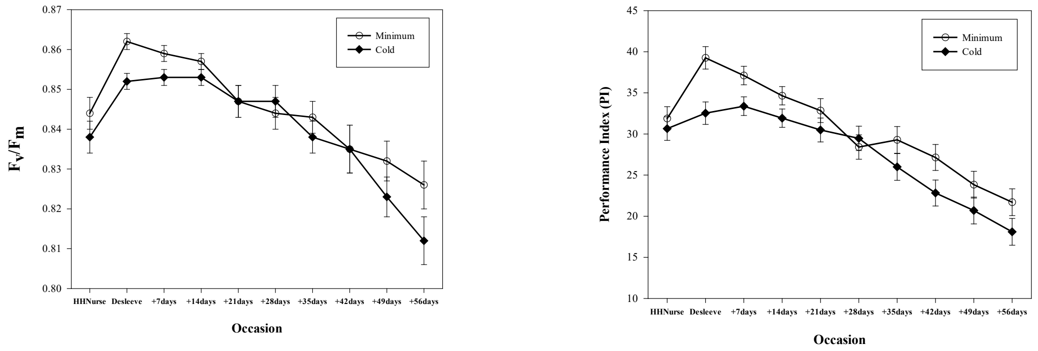
As leaf greenness was measured on only the final begonia trial, it was not possible to reach firm conclusions about the possible role of greenness as a covariate for the CF measurements. However, the low correlation between greenness at de-sleeving and quality and flower drop after two weeks (Table 19) suggests that greenness is unlikely to provide a useful covariate for PI for begonia quality prediction.

3.7.4. Conclusions

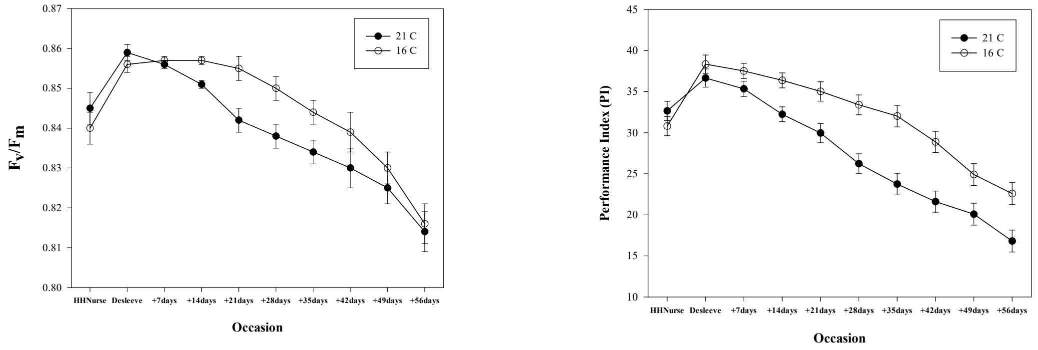
Of the several procedures and measures of CF tested, the 'first hit' Performance Index (PI), devised by Professor Strasser of the University of Geneva, was shown to be the most effective, with scores essentially mirroring objective plant quality assessments made at the same time as the CF readings. CF was strongly correlated with the objective measure of plant quality observed at the same time as the CF readings. However, a number of factors needed to be standardized for comparative CF scores to be meaningful. These included the environment itself, since factors such as temperature and light at the time of measurement were shown to have large effects on CF. Another important factor that affected CF scores in begonia was leaf position (age). In both poinsettia and begonia, CF score was markedly influenced by cultivar.

Greenness was examined as a covariate for PI in the final begonia trial of the project but there was insufficient data available from this trial to reach any definite conclusion about the relationship between leaf greenness and PI. However, the data that was collected showed no convincing evidence that greenness was usefully correlated with subsequent plant quality therefore it is unlikely that greenness would have any value as a covariate for PI for individual begonia plants.

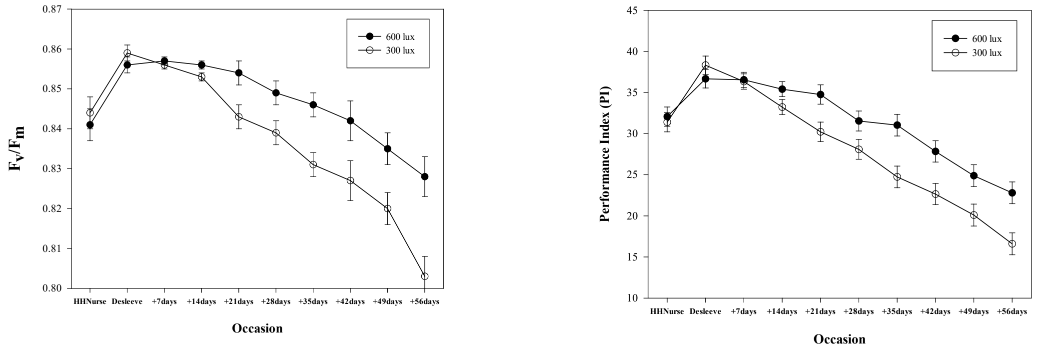
[a] Supply Chain



[b] Home-life temperature



[c] Home-life light



[d] Cultivar

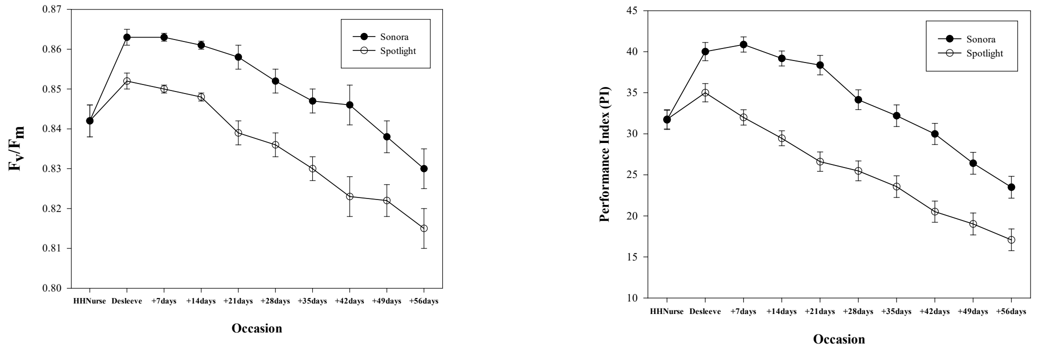
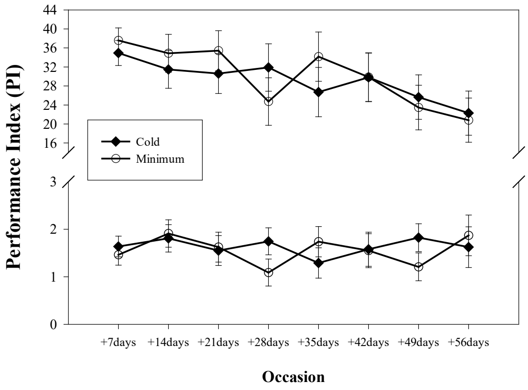


Fig. 22 [a-d]. Effects of a) supply chain, b) home-life temperature, c) light and d) cultivar on CF of poinsettia during home life in Year 2: left, Fv/Fm; right, PI.

[a] Supply Chain



[b] Home-life temperature

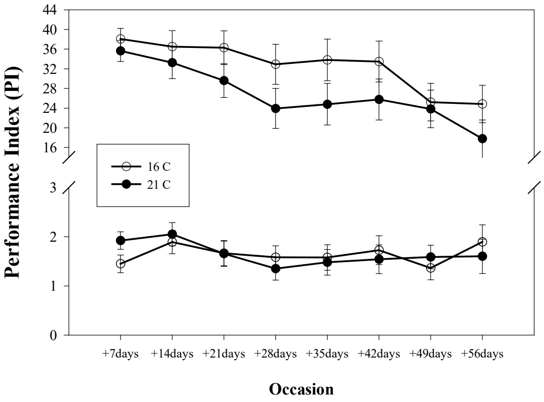
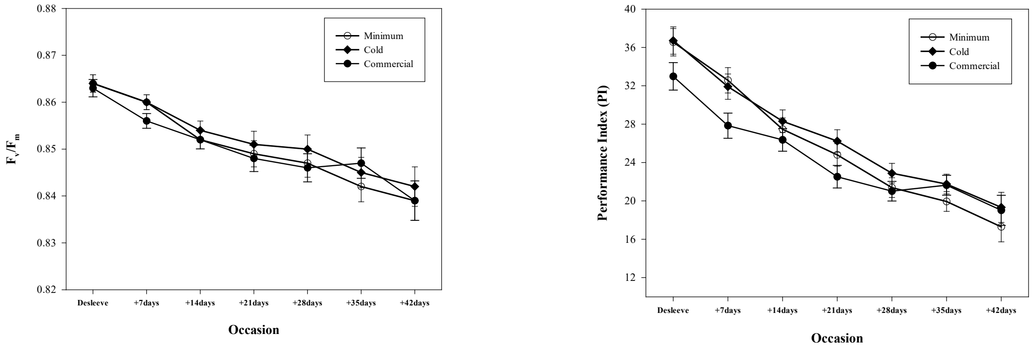
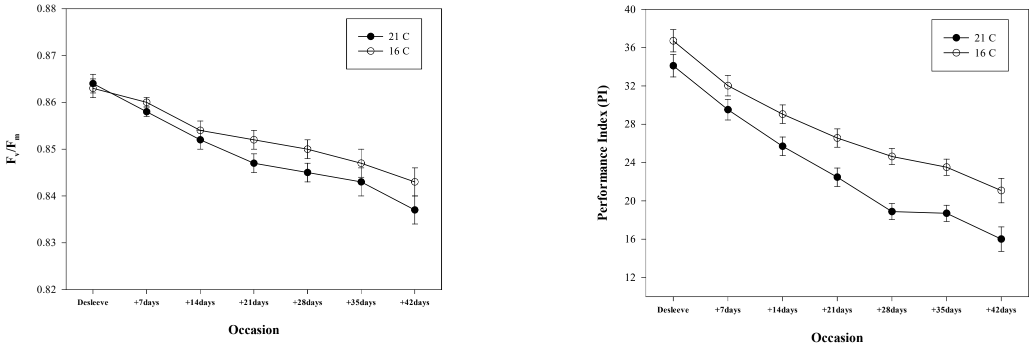


Fig. 23. Effects of [a] supply chain and [b] home-life temperature on CF performance index (PI) of poinsettia during home life in Year 2: upper pair of lines in each case, first hit values; lower pair of lines, second hit values.

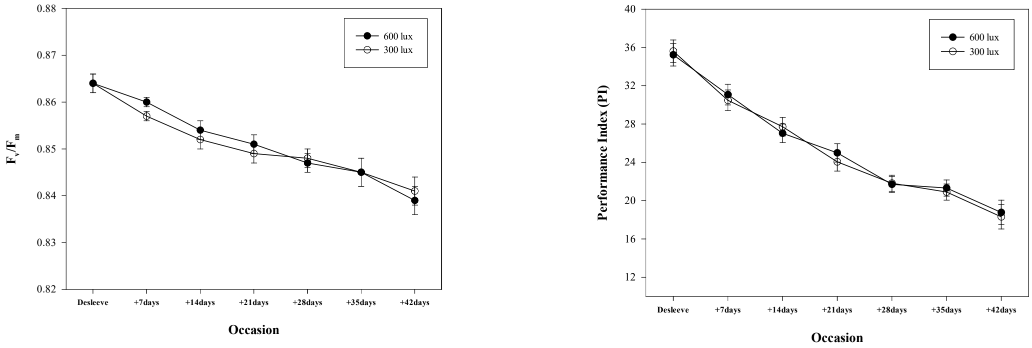
[a] Supply Chain



[b] Home-life temperature



[c] Home-life light



[d] Cultivar

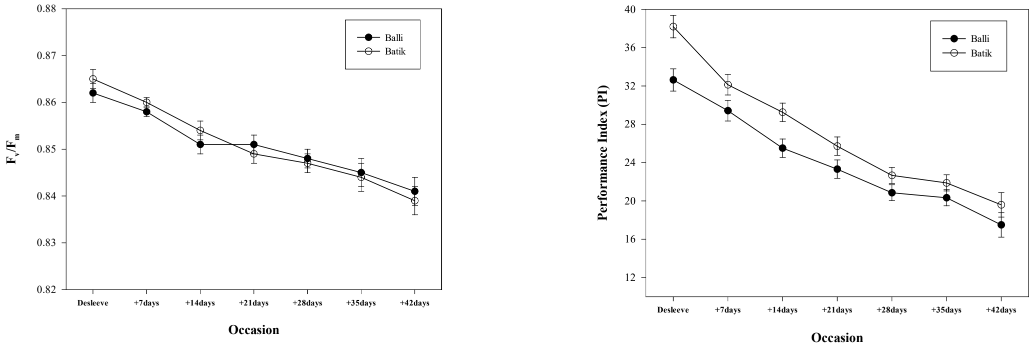
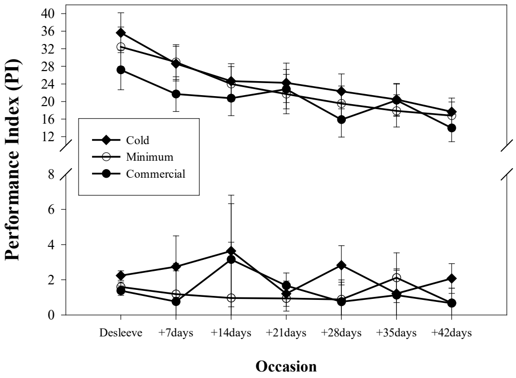


Fig. 24 [a-d]. Effects of a) supply chain, b) home-life temperature, c) light and d) cultivar on CF of begonia during home life in Year 2: left, Fv/Fm; right, PI.

[a] Supply Chain



[b] Home-life temperature

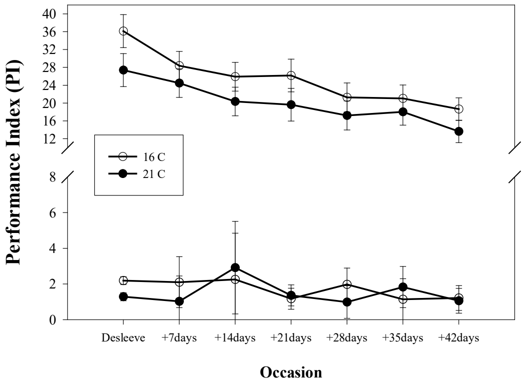


Fig. 25. Effects of [a] supply chain and [b] home-life temperature on CF performance index (PI) of begonia during home life in Year 2: upper pair of lines in each case, first hit values; lower pair of lines, second hit values.

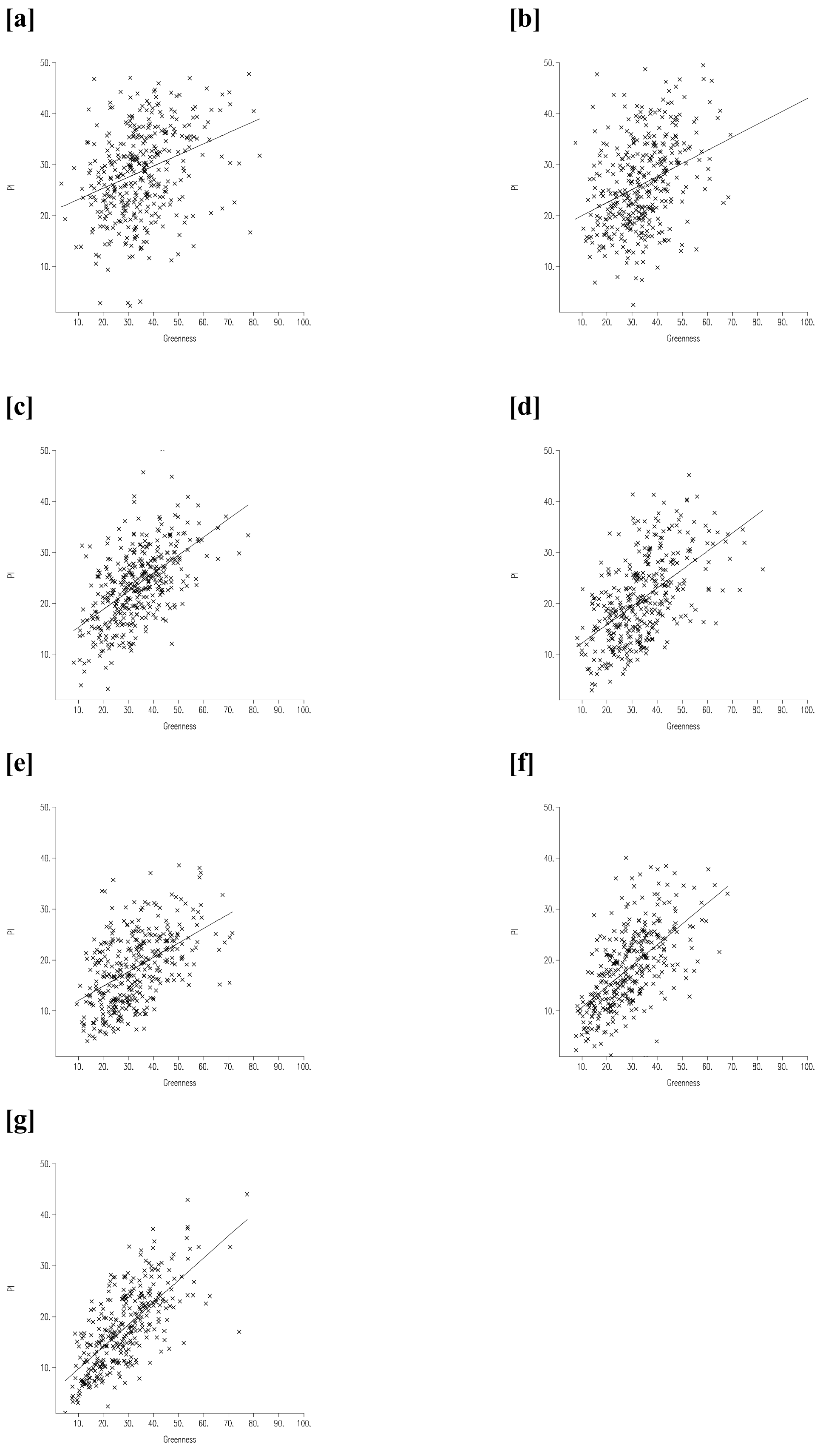


Fig 26[a-g]: Relationship between Greenness and PI for begonia data at desleeving [a], week 1 [b], week 2 [c], week 3 [d], week 4 [e], week 5 [f] and week 6 [g] for Year 4 begonia plants in home-life rooms.

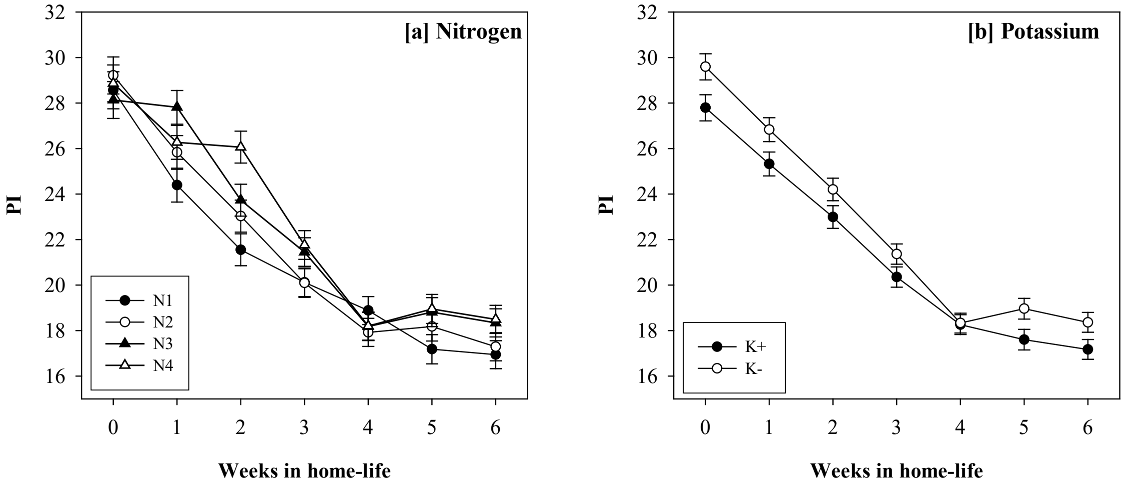


Fig 27[a-b]: Chlorophyll fluorescence PI for begonia plants in home-life rooms at Efford, summer 2003, for nitrogen treatments [a] and potassium treatments [b] during production

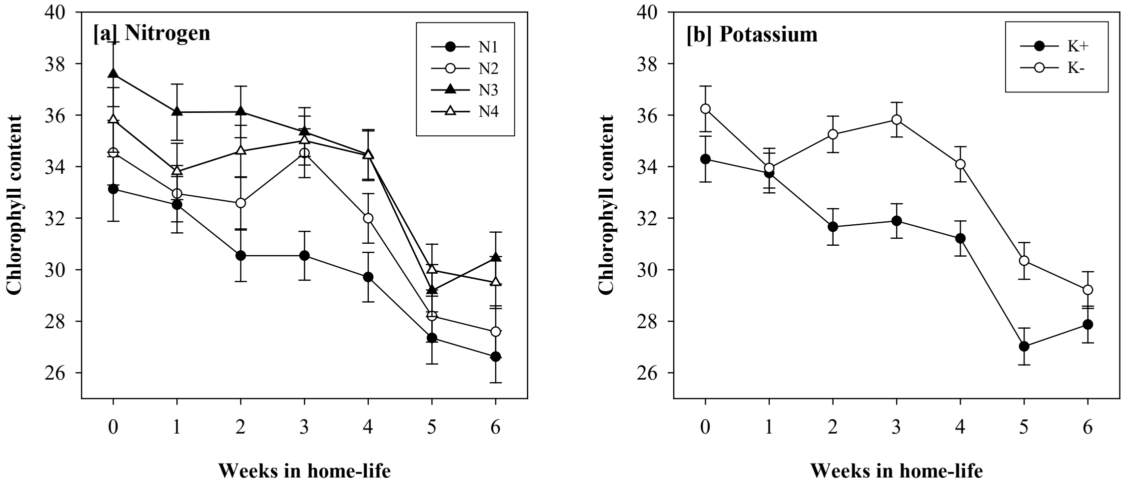


Fig 28[a-b]: Chlorophyll content for begonia plants in home-life rooms at Efford, summer 2003, for nitrogen treatments [a] and potassium treatments [b] during production

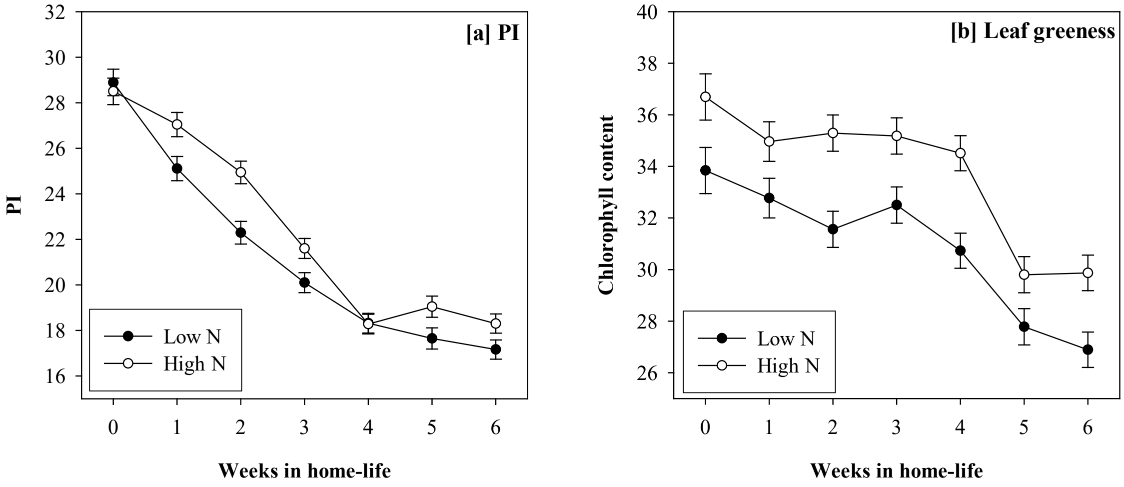


Fig 29[a-b]: Low nitrogen (N1 & N2) and high nitrogen (n3 & N4) treatment effects on PI [a] and chlorophyll content [b] for begonia plants in home-life rooms at Efford, summer 2003

3.8. Predictive utility of chlorophyll fluorescence (Objective 5)

3.8.1. Predictions of plant death in poinsettia

Although CF was routinely shown throughout the project to be strongly correlated with pot plant quality at the time of measurement, additional analyses were required to test whether CF scores could be predictive of future quality and longevity. An unplanned opportunity to test predictive potential occurred in Year 3 when a large number of poinsettia plants deteriorated so rapidly in home life that they had to be removed before the end of the trial, showing almost total leaf wilt. The cause of the decline was not identified but could have been caused by a pathogenic agent affecting individual plants. CF measurements were recorded as originally intended therefore the unplanned plant deaths gave the opportunity to test the effectiveness of CF as a predictor of plant mortality.

Methodology

The probability of plant death in any particular week was modelled by fitting a logistic binomial regression of individual plant death based on the observed CF predictor variables (first and second hit values for PI and Fv/Fm) of the individual plants in the previous week. Let π_{it} be the probability of plant death at week t for plant i, and let $x_{i(t-1)}$ be a measured predictor variable for plant i at week t-1. Let β determine the effect of the predictor variable on the probability of subsequent removal of a plant from the trial. Then a suitable logistic regression model relating $x_{i(t-1)}$ to π_{it} is:

$$\log\left(\frac{\pi_{it}}{1-\pi_{it}}\right)=\alpha+\beta x_{i(t-1)}, \qquad (3.7)$$

Separate models were fitted for each CF predictor variable and, in addition, the effect of including the observed predictor variable from two weeks prior to plant death in addition to the predictor variable from the previous week was also tested. Removal of a plant from the trial was regarded as equivalent to plant death.

Results

Fig. 30 [a, b] show the fitted model based on first hit PI (referred to as $PI^{(1)}$) for Sonora and Spotlight respectively, and Table 20 gives the estimates of model parameters and standard errors. The x's in the Figs represent the recorded PI data, and the solid lines are the fitted curves for the probabilities of the subsequent removal of a plant from the trial given the observed value of $PI^{(1)}$. The open symbols (o) represent the proportions of plants subsequently removed from the trial averaged over a range of values of $PI^{(1)}$ and plotted at the mean value of the $PI^{(1)}$ for that range. The plotted proportions were not used to fit the model but are presented as a summary of the data to show the goodness of fit of the model to the data. The error bars represent ±2 standard errors for the summary proportions.

From Fig. 30, and from the significant negative regression values for β for Sonora and Spotlight in Table 20, it is apparent that a lower value of PI$^{(1)}$ at time t-1 significantly increased the probability of removal of a plant from the trial at time t. The regression coefficients shown in Table 20 for PI$^{(1)}$ at time t-1 are statistically significant, but the very high variability apparent in Figs 30 [a, b] for individual plant observations shows that the method gave only weak predictions of individual plant deaths.

Table 20. Estimated model parameters for poinsettia death data, 2000/1

CF measure	Cultivar	Parameter	Estimate	s.e.	t-value
PI$^{(1)}$	Sonora	α	0.902	0.795	1.13
		β	-0.1152	0.0249	-4.63
	Spotlight	α	-0.112	0.520	-0.22
		β	-0.0871	0.0233	-3.74
$F_v/F_m^{(2)}$	Sonora	α	23.00	5.79	3.97
		β	-35.21	7.90	-4.46
	Spotlight	α	14.31	3.41	4.20
		β	-23.49	4.93	-4.76

The relationship observed for $F_v/F_m^{(2)}$ was similar to that observed for PI$^{(1)}$ as indicated by the significant negative regression values for β for Sonora and Spotlight in Table 20. The relationship observed for $F_v/F_m^{(2)}$ was stronger than that observed for $F_v/F_m^{(1)}$, indicating that the second hit ratio, $F_v/F_m^{(2)}$, was a more sensitive indicator of subsequent removal from the trial than was the first hit ratio. Nevertheless, the relationship remained too weak to enable prediction on the basis of individual plant measurements.

The simple logistic binomial regression model was further extended to incorporate CF data recorded at week t-2 in addition to that recorded at t-1 and this was done for $F_v/F_m^{(2)}$ and PI$^{(1)}$. Results of an analysis of deviance showed that the inclusion of additional CF data at t-2 after fitting CF data at t-1 gave no significant additional increase in predictive power.

A similar predictive model for plant death was fitted using the leaf and bract quality scores. Neither bract quality or leaf quality was predictive of plant death.

Summary

CF did appear to be a potentially useful predictor of plant death based on this analysis, but only when recorded one week prior to plant death. The most useful CF predictors were the first-hit performance index, PI$^{(1)}$, and the second-hit ratio $F_v/F_m^{(2)}$. There was no evidence that the predictive relationship changed with time.

The scatter of the plotted data values and the magnitude of the standard errors shown, for example, in Fig 30(a-b) indicate that even the most useful CF predictors gave only very weak predictions of subsequent plant death. The CF data collected from this trial would not have been useful for reliable prediction of individual plant deaths but nevertheless, it does appear that CF could have value for screening damage or disease to whole batches of plants.

3.8.2. Predictive power of CF for batch effects in poinsettia

Imposed marketing stress had a significant impact on plant longevity in home life and plants subjected to different degrees of marketing stress were used in each year of the project to test the predictive power of CF for home-life quality and longevity. By the end of Year 3, it had been clearly shown that, due to the high variability of individual plant predictions, CF was not useful for the prediction of the future home-life performance of individual plants. However, CF did appear to have some potential as a means of identifying batches of plants damaged during marketing.

Fig. 31 shows the batch mean square root of cumulative bract drop one week after marketing for each transport chain in Years 2, 3 and 4, plotted against the corresponding batch mean PI score measured at de-sleeve. The error bars for each symbol represent a 95% confidence interval for the batch mean PI. The Year 1 results have been excluded because the chlorophyll fluorescence machine set-up in Year 1 was very different from that used in subsequent years therefore the PI scores were not comparable.

The clear association in Year 2 between high bract drop by week 2 of home life and low PI at de-sleeve in the cold-stress treatment can be clearly seen. However, although effects due to cold stress on bract drop were at least as great in Year 4 as in Year 2, there was no evidence in Year 4 of any associated drop in the PI score at de-sleeve. The PI batch score for the cold treatment in Year 4 was at least as high as for the commercial batches in Years 2, 3 and 4, and for the minimum stress batch in Year 2. There was weak evidence that minimum stress marketing in Year 3 and 4 was associated with higher PI scores than other marketing treatments. Nevertheless, from these results, it appears that CF does not have the predictive power necessary to act as a reliable indicator of potential quality for poinsettia at the point of sale.

Another possible predictive measure of batch quality that was tested was the plant-to-plant variability in PI within batches. A batch that contains some diseased or damaged plants might be expected to show greater plant-to-plant variability than a batch containing uniformly high quality plants. However, when plant-to-plant variability was compared between batches in each year, there was no consistent evidence that variability differed significantly between batches.

3.8.3. Predictive power of CF for batch effects in begonia

Fig. 32 shows the batch mean square root flower drop data for begonia, one week after marketing for each of the transport chains used in Years 1 to 4, plotted against the batch mean PI score measured at de-sleeve. The 5% LSD shown on the plot was calculated from a pooled estimate of the within-batch variability, and is appropriate for comparing batch means. Overall, the data does indeed, indicate that PI at de-sleeve can be predictive of subsequent quality measured by flower drop after one week of home life. A linear regression line fitted to the complete set of 10 batch means explained 62.2% of the variability between batch means and gave the following parameter estimates:

Parameter	Estimate	Standard error
Mean	2.740	0.508
Slope	-0.066	0.017

It should be noted that in Year 1 the PI readings included two canopy leaves and a basal leaf whereas in future years the recording was changed to three canopy leaves, upper, middle and lower. The PI readings for the Year 1 data were based on only two canopy leaves and it is possible that if three canopy leaves had been used as was done in subsequent years, the Year 1 data might have been closer to the fitted regression line.

The regression model shows that for each unit drop in the PI there was an increase of about 0.066 in the square root cumulative flower drop count. Over the extreme range of PI values shown in Fig 32, this is equivalent to an average cumulative increase of about one additional lost flower per plant per week. Although apparently modest, this is an average batch effect whereas, in practice, there could be a range of severity effects with some plants in a batch much more severely affected than others in the same batch. By setting a suitably high PI batch rejection value, batch screening could be used to screen out defective batches and thus to ensure that all plants in a batch are of marketable quality.

3.8.4. Statistics required for PI batch data sampling plans

Tables 21 and 22 provide a complete summary of the PI batch data information collected over the four years of the project for poinsettia and begonia at desleeving. They show the batch means and standard errors of the means together with the number of measurements (n), the batch variance, the residual degrees of freedom for the variance estimates and the percentage coefficients of variation (CV's). Note that the CV's were much larger for begonia than for poinsettia. Begonia plants continued to grow and develop during marketing and home-life and thus had a wider range of leaf types (ages) than poinsettia, which does not develop new leaves after marketing. Batch means differed between cultivars in both species and batch variances were generally higher for batches with higher mean responses. However, CV's appeared broadly similar for cultivars of the same species. Generally, there were no consistent treatment effects on batch variances.

Tables 21 and 22 provide all the information that would be required to develop batch sampling plans based on standard statistical methodology if CF was to be developed commercially for batch screening using the cultivars tested within this project.

Table 21. Mean, standard error of mean (se), variance and coefficient of variation (CV) of PI for supply chain and production treatment batch means for poinsettia in Years 2, 3 and 4 at desleeving.

Treatment	Year	Chain	Cultivar	n†	Mean	se†	Variance	Res. df†	CV(%)†
Supply Chain	4	Supermarket	Sonora	96	44.29	0.82	64.6	414	18.2
		Minimum	Sonora	96	48.81	0.85	69.2	414	17.0
		Cold	Sonora	24	44.21	1.57	59.4	414	17.4
		Supermarket	Spotlight	96	35.27	0.61	36.1	414	17.0
		Minimum	Spotlight	96	38.51	0.63	38.1	414	16.0
		Cold	Spotlight	24	34.92	0.93	20.7	414	13.0
	3	Supermarket	Sonora	96	46.49	0.64	39.6	372	13.5
		Minimum	Sonora	96	51.26	0.76	55.6	372	14.6
		Cold	Sonora	-	-	-	-	-	-
		Supermarket	Spotlight	96	31.77	0.68	44.3	372	21.0
		Minimum	Spotlight	96	34.70	0.68	44.3	372	19.2
		Cold	Spotlight	-	-	-	-	-	-
	2	Supermarket	Sonora	72	44.69	1.21	106.1	418	23.1
		Minimum	Sonora	72	40.75	0.94	63.6	418	19.6
		Cold	Sonora	72	32.69	0.75	40.9	418	19.6
		Supermarket	Spotlight	72	35.71	0.61	26.4	418	14.4
		Minimum	Spotlight	72	37.76	0.64	29.8	418	14.5
		Cold	Spotlight	72	32.38	0.83	49.4	418	21.7
Production	4	N1	Sonora	54	44.40	1.13	68.4	414	18.6
		N2	Sonora	54	45.97	1.14	70.2	414	18.2
		N3	Sonora	54	47.25	1.06	60.5	414	16.5
		N4	Sonora	54	47.54	1.11	66.5	414	17.2
		N1	Spotlight	54	36.85	0.87	41.1	414	17.4
		N2	Spotlight	54	36.49	0.76	31.5	414	15.4
		N3	Spotlight	54	36.30	0.80	34.6	414	16.2
		N4	Spotlight	54	37.01	0.80	34.7	414	15.9
	3	Low N	Sonora	96	48.88	0.68	44.3	372	13.6
		High N	Sonora	96	48.87	0.73	50.9	372	14.6
		Low N	Spotlight	96	32.92	0.72	49.3	372	21.3
		High N	Spotlight	96	33.56	0.64	39.3	372	18.7

† n = number of measurements, se = standard error of mean, Res. Df = residual degrees of freedom for variance estimates and CV(%) = coefficient of variation expressed as a percentage

Table 22. Mean, standard error of mean (se), variance and coefficient of variation (CV) of PI for supply chain and production treatment batch means for begonia in Year 2, 3 and 4 at desleeving.

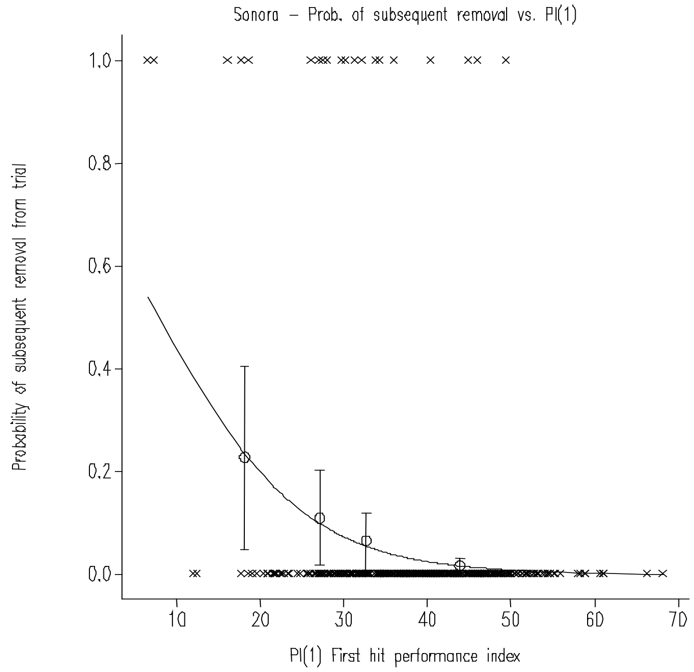
Treatment	Year	Chain	Cultivar	n†	Mean	se†	Variance	Res. df†	CV(%)†
Supply Chain	4	Supermarket	Batik	-	-	-	-	-	-
		Minimum	Batik	-	-	-	-	-	-
		Cold	Batik	-	-	-	-	-	-
		Supermarket	Mariette	-	-	-	-	-	-
		Minimum	Mariette	-	-	-	-	-	-
		Cold	Mariette	-	-	-	-	-	-
	3	Supermarket	Batik	96	27.0	0.91	80.2	372	33.1
		Minimum	Batik	96	31.4	0.96	88.6	372	30.0
		Cold	Batik	-	-	-	-	-	-
		Supermarket	Mariette	96	23.0	0.67	43.1	372	28.6
		Minimum	Mariette	96	24.5	0.70	47.6	372	28.2
		Cold	Mariette	-	-	-	-	-	-
	2	Supermarket	Batik	72	36.2	1.05	79.7	420	24.7
		Minimum	Batik	72	39.9	1.24	110.4	420	26.4
		Cold	Batik	72	38.7	1.28	118.6	420	28.2
		Supermarket	Balli	72	29.8	0.82	48.3	420	23.3
		Minimum	Balli	72	33.3	1.03	76.5	420	26.3
		Cold	Balli	72	34.8	0.92	60.9	420	22.4
Production	4	N1	Batik	-	-	-	-	-	-
		N2	Batik	-	-	-	-	-	-
		N3	Batik	-	-	-	-	-	-
		N4	Batik	-	-	-	-	-	-
		N1	Mariette	-	-	-	-	-	-
		N2	Mariette	-	-	-	-	-	-
		N3	Mariette	-	-	-	-	-	-
		N4	Mariette	-	-	-	-	-	-
	3	Low N	Batik	96	25.3	0.89	76.3	372	34.5
		High N	Batik	96	33.2	0.98	92.5	372	29.0
		Low N	Mariette	96	21.3	0.65	41.1	372	30.0
		High N	Mariette	96	26.1	0.72	49.5	372	27.0

† n = number of samples, se = standard error of mean, Res. df = residual degrees of freedom for variance estimates and CV(%) = coefficient of variation expressed as a percentage

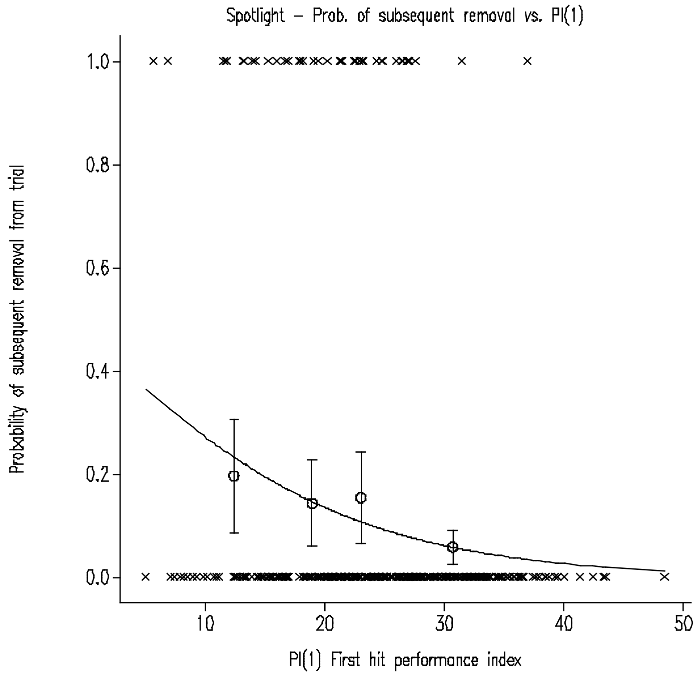
3.8.5. Conclusions

CF did appear to be a potentially useful predictor of plant death in the Year 3 poinsettia trial, but only when CF was recorded one week prior to plant death. The most useful CF predictors were the first-hit performance index, PI, and the second-hit ratio F_v/F_m. However, the scatter of the data values and the magnitude of the standard errors indicated that CF was likely to have greater value for batch screening in poinsettia than for the screening of individual plants. The potential value for batch screening appeared to be confirmed in Year 2 by a clear association between high bract drop during home life and low PI score at de-sleeve for cold-stressed plants. However, this relationship was not confirmed in Year 4, and it was concluded that CF was not sufficiently stable to provide a reliable indicator of potential quality at the point of sale for poinsettia.

For begonia, regression analyses involving individual plants in Year 3, indicated that CF (as PI) could be predictive of the effects of supply chain treatment on subsequent plant quality. However, as with poinsettia, there was substantial variability in the quality predictions and it seems probable that the potential for batch screening is greater than that for individual plants. Combining batch data for transport treatments over years did, indeed, appear to indicate a role for CF as a predictor of subsequent quality as a simple linear relationship was found between batch PI at de-sleeve and subsequent flower drop, over the first week of home life. Although the increase in flower drop due to marketing stress appeared modest, the effect was statistically highly significant and appeared to be well defined over the four years of the project. The Year 1 data appeared to have a slightly lower PI than expected relative to flower drop but this may have been due to the different choice of leaf types used in Year 1 resulting in only two rather than three canopy leaves being measured. Nevertheless, the negative regression relationship between batch means shown in Fig 32 was very highly significant and appeared to be well defined for at least three of the four begonia experiments.



[a] cv. Sonora



[b] cv. Spotlight

Fig. 30 [a, b]. Fitted probability of subsequent poinsettia plant death in week *t* based on chlorophyll fluorescence PI$^{(1)}$ scored at week *t*-1

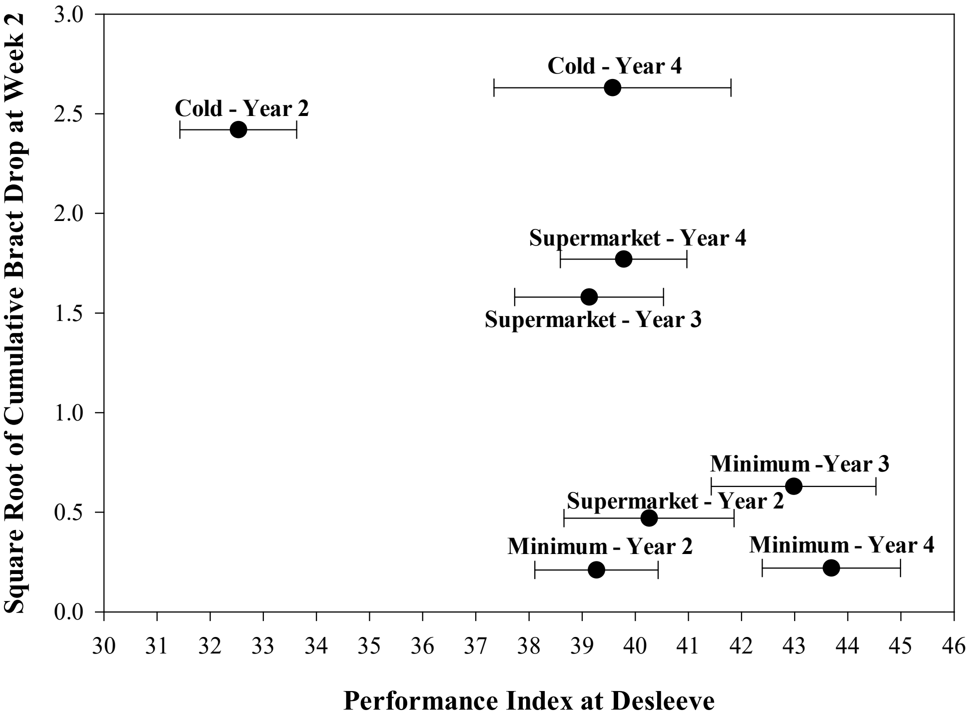


Fig. 31. Mean performance index (PI) at de-sleeve and square root of cumulative bract drop two weeks after de-sleeve for poinsettia supply chain treatments in Year 2, 3 and 4

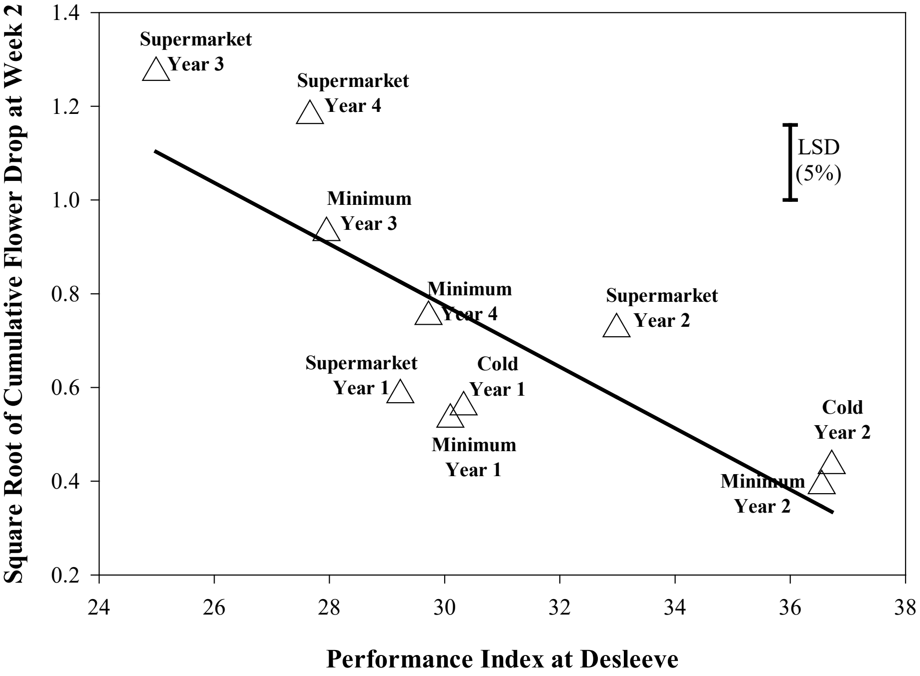


Fig. 32. Mean performance index (PI) at de-sleeve and square root of cumulative flower drop two weeks after de-sleeve for begonia supply chain treatments in Years 1, 2, 3 and 4

4. TECHNOLOGY TRANSFER

General outputs
The project has provided the following general outputs for the horticulture industry:

1. A mathematical methodology for the objective determination of quality in any ornamental plant species.
2. Unambiguous evidence that low-temperature (below 14°C) transport promotes subsequent quality loss in poinsettia and markedly reduces home-life longevity.
3. Generalized recommendations on ideal home-life environments for poinsettia and begonia for incorporation into customer care information.
4. Recommendations for nutritional treatments to be used during the production of poinsettia and begonia to maximize home-life robustness and quality.
5. Recommendations regarding test conditions needing to be met for effective CF assessment of quality.
6. A regression model for begonia batch flower drop prediction during home-life based on CF measurements during marketing
7. The principles developed in this project have equal applicability to other horticultural commodities and can be expected to contribute significantly to grower/retailer/consumer benefit in the future

Exploitation by consortium members
Specific example of project exploitation by consortium members include:

i) HDC
HDC has exploited the methodology by developing a programme of research on bedding plants using the methods developed in this project.
HDC will Produce a Wall Chart showing Transport and Customer Care Information by May 2004
HDC News Article on Nutritional Treatments and Post-Harvest Longevity will be published by March 2004
A feature in HDC News on the potential use of chlorophyll fluorescence in horticulture: HDC in association with Hansatech Ltd and Debbie Rees of NRI will be produced by 30 June 2004

ii) Retail
Safeway Stores plc have exploited the findings of the project as part of their quality evaluation procedures. This includes sourcing pot plants to minimise transport chains. Understanding customer expectations regarding 'quality' requires further investigation and this will be undertaken 'in house' by both retail partners.

iii) Hansatech
Hansatech Instruments Ltd already produces a new generation of chlorophyll fluorimeter (Handy-PEA) which automatically calculates the performance index (PI).
Hansatech Instruments Ltd will continue to pursue in-house development of the technique over the next 2 years with a view to producing a screening device suitable for use in the nursery and retail environments.
Hansatech Instruments Limited will attempt to set up collaboration with a supplier of poinsettia and begonia to further develop the technique to meet the needs of the industry.

iv) Scientific
Professor Strasser will collaborate with Hansatech to further exploit the scientific data for modelling CF response data including the additional chlorophyll content measurements.

v) Double H Nurseries Ltd
Now have preferred use of data logging for transport chain records during marketing
Will use the knowledge of the main quality variates for poinsettia and begonia to target home-life tests for quality assurance for customers.
Will use the knowledge of the main quality variates for poinsettia and begonia to assess the quality characteristics needed in new varieties
Will use the nutrient feed information from the project to achieve the best nutrient balance for crop quality and good shelf and home-life.

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6. APPENDICES

6.1. Generalised Estimating Equations (GEE)

Model specification

In a marginal regression model, only the first two moments of the responses for each experimental unit are required, whereas for a full likelihood model strong additional assumptions about the distribution and the dependence structure of the repeated measures are needed. The generalized estimating equations (GEE) method of Liang and Zeger (1986) was devised to avoid the strong assumptions of the full likelihood model. Generalized estimating equations provide a method of fitting marginal models to repeated measures whenever the response has a distribution in the exponential family. In the absence of a known likelihood function, model parameters can be estimated by solving multivariate analogues of the quasi-likelihood functions (Wedderburn 1974). Although the resulting estimates are not maximum likelihood, they do have asymptotic normality and consistency (Liang and Zeger 1986).

Generalized estimating equations are formed by re-defining the set of K ordinal responses as K-1 binary responses and then fitting a proportional odds model for the marginal probabilities assuming a suitable choice of working correlation matrix for the dependencies between the binary responses (Kenward, Lesaffre and Molenberghs, 1994). At each time point, each experimental unit has an ordinal score between 1 and K and these scores are transformed into K-1 new binary variables by the relationship $Z_{itk} = 1$ if $Y_{it} \le k$ or $Z_{itk} = 0$ if $Y_{it} > k$ for $k = 1, \ldots, K-1$ to give a data vector $\boldsymbol{Z}'_{it} = (Z_{it1}, \ldots, Z_{it(K-1)})$ of K-1 binary variables for each experimental unit at each time point $t = 1, \ldots, T$. There are S explanatory variates, $\boldsymbol{x}_{it}$, at each of the T time points giving a complete T x S matrix $\boldsymbol{X}_{0i} = [\boldsymbol{x}_{i1}, \boldsymbol{x}_{i2}, \ldots, \boldsymbol{x}_{iT}]'$ of explanatory variates for each experimental unit. The complete data matrix including the cut-points and the explanatory variables for each experimental unit is $\boldsymbol{X}_i = [\mathbf{1}_T \otimes \mathbf{I}_{K\text{-}1}, \boldsymbol{X}_{0i} \otimes \mathbf{1}_{K\text{-}1}]$. Here, $\mathbf{1}_T$ and $\mathbf{1}_{K\text{-}1}$ are T dimensional and K-1 dimensional vectors of unit elements respectively and $\mathbf{I}_{K\text{-}1}$ is the K-1 dimensional identity matrix. Let the data vector of binary measurements for the i^{th} experimental unit be $\boldsymbol{Z}'_i = (\boldsymbol{Z}'_{i1}, \ldots, \boldsymbol{Z}'_{iT})$ and let $\mathbf{E}[\boldsymbol{Z}_i] = \boldsymbol{\mu}_i$ where $\boldsymbol{\mu}'_i = (\boldsymbol{\mu}'_{i1}, \ldots, \boldsymbol{\mu}'_{iT})$, $\boldsymbol{\mu}'_{it} = (\mu_{it1}, \ldots, \mu_{it(K\text{-}1)})$ and $\mu_{itk} = P(Y_{it} \le k)$. Let $\boldsymbol{\beta}'_0 = (\beta_{01}, \ldots, \beta_{0(K-1)})$ be a K-1 dimensional vector of cut-point parameters and let $\boldsymbol{\beta}'_1 = (\beta_1, \beta_2, \ldots, \beta_S)$ be an S dimensional vector of regression parameters and let $\boldsymbol{\beta}' = (\boldsymbol{\beta}'_0, \boldsymbol{\beta}'_1)$. Let $g(.)$ represent the logit function. Then equation (3.1) can then be re-expressed as

$$\mathbf{E}[\boldsymbol{Z}_i] = g^{-1}(\boldsymbol{X}_i \boldsymbol{\beta}). \quad (6.1)$$

Let $\boldsymbol{D}_i = \partial[\boldsymbol{\mu}_i(\boldsymbol{\beta})]/\partial\boldsymbol{\beta}$ and $\boldsymbol{W}_i = \boldsymbol{V}_i^{1/2} \boldsymbol{R}_i \boldsymbol{V}_i^{1/2}$ where $\boldsymbol{V}_i^{1/2}$ is a matrix containing the square roots of the variances of the elements of $\boldsymbol{Z}_i$ along the leading diagonal, $\text{var}(Z_{itk}) = \mu_{itk}(1 - \mu_{itk})$ and $\boldsymbol{R}_i$ is the correlation matrix of the elements of $\boldsymbol{Z}_i$. For any given set of explanatory variables, and assuming that the model equations are fully identified, the model parameters of equation (6.1) can be estimated by iterative re-weighted least squares to give a convergent solution of the generalized estimating equations

$$\sum_{i=1}^{N} \boldsymbol{D}_i' \boldsymbol{W}_i^{-1}\left(\boldsymbol{Z}_i - \boldsymbol{\mu}_i\right) = \mathbf{0}. \qquad (6.2)$$

Equation (#.2) will produce consistent estimates of the model parameters as $N \to \infty$, even if the covariance structure of $\boldsymbol{Z}_i$ is not correctly specified (Liang and Zeger 1986). However, the efficiency of estimation of the parameter vector $\boldsymbol{\beta}$ and the reliability of inference will be improved if $\boldsymbol{R}_i$ is chosen to be close to the true correlation matrix of $\boldsymbol{Z}_i$.

A consistent estimator of the covariance matrix of the parameter estimates is given by the robust estimator:

$$\boldsymbol{V}_\beta = \left[\sum_{i=1}^{N} \boldsymbol{D}_i' \boldsymbol{W}_i^{-1} \boldsymbol{D}_i\right]^{-1} \left[\sum_{i=1}^{N} \boldsymbol{D}_i' \boldsymbol{W}_i^{-1} \operatorname{cov}(\boldsymbol{Z}_i) \boldsymbol{W}_i^{-1} \boldsymbol{D}_i\right] \left[\sum_{i=1}^{N} \boldsymbol{D}_i' \boldsymbol{W}_i^{-1} \boldsymbol{D}_i\right]^{-1}. \qquad (6.3)$$

An estimate of $\boldsymbol{V}_\beta$ can be found by replacing $\boldsymbol{\beta}$ by its current estimate and cov($\boldsymbol{Z}_i$) by $\left(\boldsymbol{Z}_i - \boldsymbol{\mu}_i\right)\left(\boldsymbol{Z}_i - \boldsymbol{\mu}_i\right)'$ on the off-diagonal blocks (Liang and Zeger, 1986), and $\boldsymbol{V}_i^{1/2}\left(\mathbf{I}_T \otimes \boldsymbol{S}\right)\boldsymbol{V}_i^{1/2}$ on the diagonal blocks. Equation (6.3) is a robust estimator of variance, as it is consistent regardless of the specification of $\boldsymbol{W}_i$. Hypotheses of the form H_0: $\boldsymbol{B\beta} = \mathbf{0}$ can be tested using the Wald statistic $(\boldsymbol{B}\hat{\boldsymbol{\beta}})'[\boldsymbol{B}\hat{\boldsymbol{V}}_\beta \boldsymbol{B}']^{-1}(\boldsymbol{B}\hat{\boldsymbol{\beta}})$, which, under a null hypothesis, has an asymptotic chi-square distribution with degrees of freedom equal to the rank of $\boldsymbol{B}$.

Specification of $\boldsymbol{R}_i$

The first stage in estimating $\boldsymbol{R}_i$ is to take account of the correlations between the derived binary responses, $\boldsymbol{Z}_i$, within each time point. It can be shown (see Kenward, Lesaffre and Molenberghs, 1994) that the expected correlation between binary variables Z_{itj} and Z_{itk} is given by $\rho_{jk} = \rho_{kj} = \{\exp(\beta_{0j} - \beta_{0k})\}^{1/2}$ where $j < k$. By assumption, the same correlation matrix applies to every set of binary variables at every time point $t = 1,\ldots,T$ and can be written as the (K-1) x (K-1) dimensional matrix

$$\boldsymbol{S} = \begin{bmatrix} \rho_{11} & \cdots & \rho_{1(K-1)} \\ \vdots & \ddots & \vdots \\ \rho_{(K-1)1} & \cdots & \rho_{(K-1)(K-1)} \end{bmatrix}.$$

Assuming the longitudinal model is smooth and continuous, the matrix of correlations between observations on consecutive pairs of vectors of binary observations on the same unit must approach $\boldsymbol{S}$ as the interval between the observations approaches zero. Therefore a natural limiting model for the correlation matrix between consecutive vectors of observations is $\alpha\boldsymbol{S}$ where $0 < \alpha < 1$ is a weighting coefficient that approaches unity as the interval between the observations decreases. Further, if we assume a first-order autoregressive model for α, the correlation matrix between vectors of observations separated by t evenly-spaced intervals becomes $\alpha^t\boldsymbol{S}$. Under these assumptions, a natural model for the working correlation matrix for the set of vectors of observations on the same individual is $\boldsymbol{R}_\alpha = \boldsymbol{C}_\alpha \otimes \boldsymbol{S}$, where $\boldsymbol{C}_\alpha$ is the T x T dimensional autoregressive matrix

$$\boldsymbol{C}_\alpha = \begin{bmatrix} 1 & \alpha & \cdots & \alpha^{T-1} \\ \alpha & 1 & \ddots & \vdots \\ \vdots & \ddots & \ddots & \alpha \\ \alpha^{T-1} & \cdots & \alpha & 1 \end{bmatrix}.$$

The correlation matrix $\boldsymbol{R}_\alpha$ is a function of a single unknown scalar parameter α and, under this model, the problem of estimating the weighting matrix $\boldsymbol{W}_i$ in equation (6.3) reduces to the problem of estimating α in $\boldsymbol{R}_\alpha$.

Estimation of α

The variance matrix $\boldsymbol{V}_\beta$ is a function of the unknown parameter α, therefore α can be estimated by minimising $\boldsymbol{V}_\beta$ or, equivalently, by minimising $\log|\boldsymbol{V}_\beta|$. To ensure that the correlation matrix $\boldsymbol{R}_\alpha$ remains positive definite during model fitting, α can be constrained to lie on the interval (-1, 1) by working with the transformed correlation parameter ϕ, where $\phi = \log(1+\alpha) - \log(1-\alpha)$ for $-1 < \alpha < 1$. Using the current estimates of the model parameters $\hat{\boldsymbol{\beta}}_m$ and covariance matrix $\boldsymbol{V}_{\beta_m}$ after m iterations of a fitting algorithm, a new estimate of ϕ is given, using Newton's method, by

$$\phi_{m+1} = \phi_m - \frac{\dfrac{\partial}{\partial\phi}\log|\boldsymbol{V}_{\beta_m}|}{\dfrac{\partial^2}{\partial\phi^2}\log|\boldsymbol{V}_{\beta_m}|}. \qquad (6.4)$$

Expressions for the first and second derivatives of $\log|\boldsymbol{V}_\beta|$ can be calculated relatively simply. Fitting proceeds by using an initial estimate of α to estimate $\hat{\boldsymbol{\beta}}$ and $\boldsymbol{V}_\beta$, using equations (6.2) and (6.3), and an updated estimate of α follows from equation (6.4). The fitting procedure is iterated until convergence is achieved. An approximate standard error for the transformed correlation parameter $\hat{\phi}$ can be estimated by using an approximation to maximum likelihood estimation (Lindsey 1996, Chapter 3). Let $\log|\boldsymbol{V}_\beta(\phi)|$ be a continuous function on the interval $(-\infty, \infty)$ and let $\log|\boldsymbol{V}_\beta(\phi)|$ have a minimum on this interval, then a second-order approximation for $\log|\boldsymbol{V}_\beta(\phi)|$ at $\hat{\phi}$ is given by $\log|\boldsymbol{V}_\beta(\hat{\phi})| + \tfrac{1}{2}(\phi-\hat{\phi})^2 \dfrac{\partial^2}{\partial\phi^2}\log|\boldsymbol{V}_\beta(\hat{\phi})|$. Thus the function $|\boldsymbol{M}(\phi)|$, where $\boldsymbol{M}(\phi) = \boldsymbol{V}_\beta^{-1}(\phi)$ and $|\boldsymbol{V}_\beta(\phi)|^{-1} = |\boldsymbol{V}_\beta^{-1}(\phi)|$, can be approximated by a Normal function with mean $\hat{\phi}$ and standard deviation $s^2 = 1\big/\left(\partial^2 \log|\boldsymbol{V}_\beta(\hat{\phi})| \big/ \partial\phi^2\right)$ giving

$$|\boldsymbol{M}(\phi)| \approx k\exp\left\{-(\phi-\hat{\phi})^2 \big/ 2s^2\right\}.$$

An approximate standard error for the parameter $\hat{\phi}$ is given by $s = \left(\sqrt{\dfrac{\partial^2}{\partial\phi^2}\log|\boldsymbol{V}_\beta(\hat{\phi})|}\right)^{-1}$ and approximate confidence intervals for $\hat{\phi}$ can be calculated using the Normal error distribution. Confidence intervals for $\hat{\alpha}$ can be obtained by back-transforming the interval boundaries for $\hat{\phi}$ to the original scale of α using the transformation $\hat{\alpha} = (\exp\hat{\phi} - 1)/(\exp\hat{\phi} + 1)$.