HORTICULTURE RESEARCH INTERNATIONAL
WELLESBOURNE

Report to: Horticultural Development Council,
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This project was carried out at Horticulture Research International, Littlehampton as an HDC-funded PhD studentship by Mr Mark Maddock, registered as a post-graduate student at the University of Nottingham. The full report of the investigation is currently being written up as a thesis to be submitted to the University of Nottingham in fulfilment of the requirements of the award of PhD. Copies of the thesis will, in due course, be available from the HDC Office in Petersfield. The project summary below has been written to provide growers with the main findings of the investigation by the joint project supervisors, Dr Allen Langton of Horticulture Research International, Wellesbourne, Dr Jeff Atherton of the Department of Agriculture and Horticulture, University of Nottingham, Sutton Bonington and Dr Nigel Dungey of Van Heyningen Brothers, Littlehampton.

CONTRACT REPORT

The effects of day and night temperatures and interactions with other environmental factors on plant stem elongation

HDC PC41c

PC 41c: The effects of day and night temperatures and interactions with other environmental factors on plant stem elongation

Final Report Summary

Background

Control of plant stem elongation is vital to the production of high quality protected ornamentals and is currently achieved by a heavy reliance on the use of plant growth regulator chemicals. However, growers are coming under increasing pressure, both environmental and financial, to reduce chemical application, and alternative height control strategies are sought. One alternative is the manipulation of day and night temperatures; plants of almost all species grown at high day temperature and low night temperature tend to be tall whilst those grown at low day temperature and high night temperature tend to be short. It is generally believed that it is the difference (DIF) between the day and night temperatures which is important in determining response, with +DIF (day temperature greater than night temperature) giving tall plants and -DIF (day temperature lower than night temperature) giving short plants. Temperature regimens based on this principle are already used by many growers but little is known of the underlying mechanisms involved and of the limits to control imposed by interaction with other factors such as light.

Specific objectives

1. To investigate the effects of contrasting DIF regimens on stem elongation and to determine, in particular, whether responses are mediated via the influence of day and night temperatures on plant photosynthesis and respiration.

2. To determine whether the effects of DIF temperature treatments are modified by light level.

3. To develop procedures for the continuous monitoring of stem elongation and to use these to determine the influences of DIF temperature regimens on elongation during the day and during the night and to determine whether DIF temperature responses are mediated via effects on internal water stress.

Results

Objective 1.

DIF temperature responses were studied in young plants of the tomato cultivars, Moneymaker and Ailsa Craig, and the chrysanthemum cultivars, Delta and White Fresco. The regimens used all gave the same 24 hour temperature average of 20°C (day length = night length = 12 hours) and were 16°C day / 24°C night (-8°C DIF), 20°C day / 20°C night (0°C DIF) and 24°C day / 16°C night (+8°C DIF). These

temperatures were achieved to within +/- 1°C under standardized lighting conditions (37 W/m^2 PAR) given by fluorescent lighting in plant growth rooms without CO_2 enrichment and without control of vapour pressure deficit. Vapour pressure deficit was generally within the range 1.0 - 0.2 kPa which is usually regarded as having little effect on plant physiology and development, but typically reached 1.3 kPa during the day in the +DIF regimen. Growth in replicate experiments was monitored at regular intervals over a period of up to 20 days following initial exposure to treatment.

Essentially similar results were obtained for the two tomato cultivars. Effects on stem elongation rapidly became apparent and total plant height was greatly reduced in the -DIF treatment compared with that in the +DIF treatment. Taking 'Moneymaker' in one of the experiments as an example, a reduction of *c.* 38% was shown at the final sampling. Height in the 0DIF treatment was intermediate between that in -DIF and that in +DIF. Reduced plant height in the -DIF treatment reflected a large reduction in internode length (*c.* 32% shorter in -DIF) accompanied by a small reduction in leaf number (*c.* 8.5% fewer macroscopically visible leaves in -DIF). It was expected that leaf number differences would be small since leaf production is generally determined by the average 24-hour temperature and this was the same in the three treatments. Reductions in internode length were not accompanied by changes in stem diameter and free-hand stem sectioning and microscopic examination showed that the basis for reduced internode length in -DIF was reduced cell length. Fresh and dry weights were not significantly affected by treatment in one experiment and were increased in the 0DIF treatment over those in both fluctuating temperature treatments in a second experiment. There were no significant effects on average leaf area but leaves were noticeably paler in colour following -DIF treatment and a reduction in chlorophyll content per unit leaf area was shown following chemical extraction.

Responses in chrysanthemum were similar to those in tomato but were less marked. In the case of 'Delta', for example, the reduction in plant height in -DIF was *c.* 23.5% with all of this being accounted for by reductions in internode length. Fresh and dry weights were greatest in +DIF but differences were not significant. There were no obvious effects on leaf area but leaves in -DIF were noticeably paler in colour. Given this similarity of response between tomato and chrysanthemum and restrictions on space and time, it was decided to confine further investigation to just one of the species. Tomato cultivar Moneymaker was chosen since this showed the greatest responsiveness to treatment and plants could be raised quickly and easily for experimentation, either in the greenhouse or in the growth rooms themselves.

Photosynthetic activity and respiration were studied using tomato plants which had been placed in -DIF and +DIF temperature regimens *c.* 10 days before. A portable Ciras 1 Combined Infra-Red Gas Analysis System was used for this purpose with measurements being made both in the day (2 - 3 hours after lights on) and in the night (10 hours after lights off). Final calculations remain to be done but visual inspection of the data suggests that there were no consistent differences in either assimilation rate during the day or in dark respiration. That there were no obvious

differences in carbon fixation appears somewhat surprising since reductions in chlorophyll content in -DIF would have been expected 10 days after the start of treatment. Estimates were also made of stomatal conductance and transpiration rate using the Ciras 1 equipment but differences due to treatment, if any, were small.

The effects of longer term exposure to DIF temperature treatments were examined by germinating seed and raising plants of 'Moneymaker' tomato in the same three DIF temperature regimens used in the short term exposure experiments reported above. By 35 days after sowing, -DIF plants were 51.6% shorter than +DIF plants, with average internode length reduced by 47.0% and number of visible leaves reduced by 9.7%. In contrast to the findings for short term exposure, however, fresh weight in the -DIF plants was also reduced by 43.0%, dry weight by 41.5% and average leaf area by 31.0%. Interestingly, plants raised in 0DIF, whilst intermediate between -DIF and +DIF plants in stem length, had the greatest fresh and dry weights and the greatest average leaf area. There were no differences due to treatment in the relative concentrations (expressed as µg/g dry weight of leaf tissue) of starch, glucose, fructose, hexoses or galactose.

It was concluded that whilst short term exposure to -DIF gives height reductions with no obvious growth penalty, longer term exposure does reduce carbon fixation, presumably as a consequence of reductions in chlorophyll content per unit leaf area. It further appears clear that reductions in stem elongation are not a consequence of reduced assimilation rate, but that reduced assimilation can be an accompanying longer term consequence of growing in -DIF temperature regimens.

Objective 2

The effects of the three standard DIF temperature regimens were assessed under three levels of lighting by adjusting the number of fluorescent lamps over the plants to give irradiances during the 12-hour day of 12.4, 35.5 and 72.0 W/m^2 PAR. These lighting levels approximate to light integrals outside a greenhouse (with 60% light transmission) of 1.79, 5.11 and 10.37 $MJ/m^2/day$ (equivalent to average days on the South Coast of England in late December, mid February and early April). There were large differences in visible leaf number after 17 days of treatment as was to be expected and comparisons of stem extension were made on a per internode basis.

Light itself had a very marked effect on extension growth and, averaging over the three DIF regimens, length was reduced by 45% at the highest level compared with that at the lowest level. The difference between the intermediate and the highest level was much less, just 13.5%. Internode length in -DIF was less than that in +DIF at all three light levels, but the difference was greatest under the lowest light conditions where the potential for stem elongation was greatest. Thus, the reduction in internode length given by -DIF compared to +DIF at low light was 49.5%, whilst the difference at high light was 18.4%. It can be expected, therefore, that the effects of -DIF in commercial practice in summer will be less pronounced than in winter.

A series of greenhouse experiments was carried out with photoperiodic daylength extension to determine the effects of DIF on tomato under conditions where the daylength exceeded 12 hours. These were done in the early Spring when temperature in the greenhouse could be controlled with reasonable accuracy. All plants received 9 hours of natural light supplemented with either 3, 9 or 15 hours of low irradiance light (0.4 W/m^2 PAR) given by the use of fluorescent lamps. Thus, plants received photoperiods of either 12, 18 or 24 hours.

In a first experiment where the temperature was maintained continuously at 20°C there were no observed effects of photoperiod on stem length. It was concluded, therefore, that either low irradiance light was seen by the plants as 'night' from the standpoint of stem extension responses, or that low irradiance light was seen as 'day' but that the rate of stem extension at 20°C was rather similar in the day and in the night.

In a second experiment, the day extension lighting treatments were retained but a 12 hour day / night -DIF temperature regimen was imposed (16°C day / 24°C night) with 'day' comprising the 9 hours of natural light plus 3 hours of extension lighting. Again, there were no obvious effects of photoperiod on plant height suggesting that low irradiance lighting is seen by the plant as 'night'. Some confirmation of this was given in a third experiment where day extension lighting to 24 hours at a higher irradiance, 10 W/m^2, gave significant height increases. It seemed, therefore, that an irradiance of somewhere between 0.4 and 10 W/m^2 was required to signal day with regard to stem extension responses.

Objective 3

Linear voltage displacement transducers (LVDTs) were used in attempts to monitor stem extension growth continuously. In essence, cotton thread was attached to the growing tip of a plant, run over a pulley system and secured to a magnetic iron core which dropped down through an assembly of electric coils as the plant grew, generating electrical signals which could be related to the rate of movement of the core and, hence, the growth of the plant. This is a system which has been widely used by other researchers but which was found to be unsatisfactory in this study, mainly because the imposed environmental conditions directly affected the equipment itself as well as the plants, and because there was too much air movement in the controlled environment rooms. Instead, a procedure was developed based on the periodic photography of plant stems on which white paint dots had been marked at the start of experimentation. Increases in internode length could be determined by computer-based measurement of the distance between adjacent dots on the stem and calibration against a scale placed against the plant at the time it was photographed. In general, plants were photographed every 4 hours over an experimental period of *c.* 100 hours.

Considerable variation was shown between replicate plants in a given temperature treatment, and both negative and positive growth increases were shown between consecutive measurements. Nevertheless, basic trends were clear. Internodes of plants growing in the standard -DIF temperature regimen showed less increase

in length compared to those in 0DIF or +DIF regimens. Increases for three representative internodes over 100 hours in the three treatments in one particular experiment were 0.31, 0.36 and 0.54 mm respectively. Of particular note, however, was the observation that most of the extension growth occured during the night. Thus, even though day comprised a greater proportion of the 100 hours than did night, growth during the night accounted for 87.5, 81.8 and 96.3% of total growth made in the three respective DIF regimens.

A possible reason why plants show greatest extension growth during the night is that transpiration is greatly reduced or absent so increasing turgor pressure within the plant, movement of water into stem cells and increase in cell size. An experiment was carried out, therefore, to determine growth responses during the day and the night under conditions where internal water pressures during these times were similar. It was reasoned that if DIF temperature responses were still shown, it would indicate that extension growth was not mediated via the direct effects of temperature (or associated vapour pressure deficit) on turgor pressure within the plant.

Detached tomato stems were 'plumbed' into a pressurized water supply carried in rubber tubing attached to a pressure pump. Water pressure was gradually increased until the plants just started to show guttation (droplets of water visible on the surface of leaves and stems) and extension growth was monitored over 100 hours in the three standard DIF temperature regimens using the photographic technique described above. Growth increases for three internodes over this period were very small, 0.08 mm (-DIF), 0.15 mm (0DIF) and 0.04 mm (+DIF), and clearly far less than in plants growing on their own roots. It was tentatively concluded, therefore, that a growth substance is normally produced in the roots and translocated to the stems where growth occurs. In the absence of this, growth is severely restricted and DIF responses are not shown. Growth was too limited to gain any clear indication as to whether differences in growth between day and night were still shown in the absence of internal water stress. The importance of substances produced in the roots for stem growth has been suggested by others following experiments, for example, where gibberellin deficient mutant tomato plants were grafted on to wildtype rootstocks and where growth of the scion was promoted, presumably by the translocation upwards of gibberellins from the rootstock. It has not been previously suggested, however, that growth substances produced in the roots may play a part in determining DIF temperature responses.

Conclusions

1. Short term exposure of tomato and chrysanthemum plants to contrasting DIF regimens, 16°C day / 24°C night (-8°C DIF), 20°C day / 20°C night (0°C DIF) and 24°C day / 16°C night (+8°C DIF), resulted in large differences in stem length. -DIF plants were shorter than 0DIF plants and 0DIF plants were shorter than +DIF plants. These differences in stem length were largely or wholly accounted for by differences in internode length and these, in turn, were given by effects of DIF on cell size. Reductions in stem length in -DIF plants were not accompanied by differences in stem diameter.

2. Plant fresh weight, dry weight and average leaf area were little affected by short term exposure to DIF, but leaves were noticeably paler in colour in -DIF due to reductions in chlorophyll content. There appeared to be no consistent differences in photosynthetic assimilation rate during the day or in dark respiration after ten days of treatment.

3. Longer term exposure of tomato plants (35 days from sowing) to -DIF did result in reduced fresh weight, dry weight and leaf area compared to +DIF plants. However, 0DIF plants, whilst being intermediate in height, had a greater fresh weight, dry weight and average leaf area than plants in either of the fluctuating temperature treatments.

4. It was concluded that whilst short term exposure to -DIF gives height reductions with no obvious growth penalty, longer term exposure does reduce carbon fixation, presumably as a consequence of reductions in chlorophyll content per unit leaf area. It further appears clear that reductions in stem elongation are not a consequence of reduced assimilation rate, but that reduced assimilation can be an accompanying longer term consequence of growing in -DIF temperature regimens.

5. Light level had a very marked effect on extension growth, with average internode length being shortest at the highest irradiance. Internode length in -DIF was less than that in +DIF at all three light levels tested, but the difference was greatest under the lowest light conditions where the potential for stem elongation was greatest. It can be expected, therefore, that the effects of -DIF in commercial practice in summer will be less pronounced than in winter.

6. Low irradiance (0.4 W/m^2 PAR), day extension lighting to give photoperiods of 12, 18 or 24 hours appeared to have no effect on the stem length of tomato under 0DIF conditions or when a conventional 12 hour day/night -DIF regimen (16°C day / 24°C night) was superimposed. Increased height was recorded, however, when extension lighting to 24 hours was given at 10 W/m^2 PAR. It was concluded that an irradiance of somewhere between 0.4 and 10 W/m^2 was required to signal day with regard to stem extension responses.

7. Procedures were developed to monitor tomato plant growth at regular intervals during the day and the night. Internodes of plants growing in the standard -DIF temperature regimen showed less increase in length compared to those in 0DIF or +DIF regimens. Of particular note, however, was the observation that most of the extension growth occured during the night. Thus, growth during the night accounted for 87.5, 81.8 and 96.3% of total growth made in the three respective DIF regimens.

8. Detached tomato stems were grown in the standard DIF regimens under positive water pressure such that guttation was shown on the leaves and stems. It was expected that this would enable differences in extension growth to be measured under conditions where internal water pressures within the plants would not be affected by treatment. However, growth increases were

very small and far less than those shown by plants growing on their own roots. It was tentatively concluded, therefore, that a growth substance is normally produced in the roots and translocated to the stems where growth occurs. In the absence of this substance, growth is severely restricted and DIF responses are not shown.