FINAL REPORT

To:
Horticultural Development Council
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NARCISSUS: The cause of 'physiological rust' disorder

BOF 62

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Commercial - In Confidence

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CONTENTS PAGE

Page

Grower Summary

BOF 62

NARCISSUS: The cause of 'physiological rust' disorder

Final Report 2007

BOF 62 - NARCISSUS: The causes of 'physiological rust' disorder

Grower Summary

Headline

The hypothesis that environmental conditions linked to temperature and water availability result in the rust-like symptoms of narcissus physiological rust (NPR) was not confirmed.

Background and expected deliverables

In the early 1990s UK daffodil growers highlighted concerns about rust-like lesions being seen increasingly on flower stems and leaves. The severity of this 'rust' varied from mildly disfiguring to severely marking, and in the worst instances lesions were prominent along the length of the stem, the stem became brittle, and customers sometimes rejected such batches. The initially sporadic concerns of a few growers increased to a much wider concern that large quantities of daffodil flowers might become unmarketable.

The cause of rust was unknown, but it did not appear to be due to a disease or pest, and the condition became known as 'narcissus physiological rust' (NPR). NPR has been reported from crops in Cornwall and eastern England, on many cultivars, and on crops in their first, second and subsequent years of growth. The HDC undertook a survey of rust amongst its members in 2002 and 2003, which revealed:

- 75% of respondents had seen NPR in the previous four or five years
- Of those seeing NPR, 8% of their area had been affected, and turnover was reduced by an average of 2%

- Many cultivars were affected by NPR in the second crop year, and several in the first crop year as well

These findings did nothing to explain the recent increase in the occurrence of NPR. Considering the possible causes of NPR and of other physiological disorders in daffodils, it appeared likely that it resulted from unfavourable combinations of temperature and soil water availability. This project was set up to test this theory.

- The prime deliverable expected from the project was a clear confirmation or rejection of the proposed cause of NPR, with perhaps some additional information about the incidence of other daffodil physiological disorders.

Summary of the project and main conclusions

- When six daffodil cultivars were grown under a range of temperature and soil moisture levels there were no instances of NPR symptoms. Therefore, the hypothesis that environmental conditions linked to temperature and water availability cause the rust-like symptoms through disturbance of normal water relations, whilst not being definitively ruled out, was not confirmed.

- Symptoms of 'chocolate spot', a less serious but related disorder of daffodils, occurred in five of the six cultivars in the experiment, especially in cultivar 'Mando'. The symptoms of chocolate spot were distinct from those of NPR, with no suggestion that the two symptoms are aspects of a single disorder. Chocolate spot symptoms were more common in cool and intermediate temperatures than in warm temperatures, but did not appear to be related to soil moisture levels. Chocolate spot may therefore be a result of low temperatures at some key stage of the growing cycle.

Financial benefits

- The project showed that adverse combinations of temperature and soil water availability were unlikely to be the cause of NPR, therefore this cause

of the disorder may be eliminated, enabling future efforts to be concentrated on nutritional or other causes. As such, there are no direct financial benefits from the project.

Action points for growers

- Since unfavourable soil conditions are the likely cause of other disorders of daffodils, including 'chocolate spot' and various flowering disorders, it is important for bulb growers to maintain a high standard of soil structure management to prevent compaction and water-logging.

Science Section

Introduction

In the early-1990s UK narcissus (daffodil) growers highlighted concerns about rust-like lesions which were seen increasingly on the flower stems and leaves. In the UK field-grown narcissus cut-flowers have an annual farm-gate value of about £10m, largely dependent on the multiple-retail and export sectors, and it was feared that 'rust' (as the condition was soon christened) could significantly compromise sales. The cause of rust was unknown. The severity of the rust symptoms varied from mildly disfiguring to severely marking; in the worst instances, lesions were prominent along much of the length of the stem, the stem became brittle, and customers rejected such batches. From then onwards, the minutes of the HDC Bulb and Outdoor Flower (BOF) Panel recorded that the initially sporadic concerns of a few growers had increased to a much wider concern that large quantities of cut-flowers might become unmarketable. Not surprisingly, the initial reluctance of Panel members to commission research on the aetiology and management of rust – here referred to as 'narcissus physiological rust' (NPR) - declined.

NPR is distinct from the true rust symptoms found on narcissus in the Netherlands as a result of infection with *Aecidium narcissi* from the reed-grass *Phalaris arundinacea* used there as a covering material (van Aartrijk *et al*., 1995).

NPR has been reported from narcissus crops in Cornwall and eastern England, on many cultivars, and on crops in their first, second and subsequent years of growth. While there is the expected anecdotal information amongst the industry on the occurrence and causes of the disorder, no coherent theories had been proposed; consequently the HDC undertook a survey of NPR amongst its members in 2002 and 2003. This information was made available to the authors and has been summarised previously at HDC meetings. The main findings were as follows.

- 75% of respondents had seen NPR in the previous four or five years, of which 50% had seen flowers down-graded and 33% had experienced totally unmarketable flowers.
- Of those growers seeing NPR, 8% of their area had been affected, 4% of the area was down-graded, and 1% of the area was unmarketable. Turnover was reduced by an estimated 0 to 15%, with an average of 2%.
- Many cultivars were affected by NPR in the second crop year, and several in the first crop year as well. Cultivars reported as having a high incidence and severity of rust were 'Golden Ducat', 'Carlton', 'St Keverne', 'Standard Value' and 'White Lion'. Of these, 'Golden Ducat' and 'Mando' were mentioned a disproportionately large number of times when compared with the area of these cultivars grown.

These findings did nothing to explain the recent increase in the occurrence of NPR. Some of the cultivars mentioned above were also the most widely grown cultivars at the time, so little could be inferred from the survey results as to varietal susceptibility. Naturally, there was a tendency for speculation in the comments section of the questionnaire: in particular, it was said that the occurrence of NPR was weather-related: the "crop grows too fast after a cold frosty spell", and NPR was "… seen mainly in waterlogged areas…" However, the survey confirmed that NPR was a significant problem for UK growers, and strengthened the case for investigation.

NPR does not appear to have been described in current UK (e.g., Moore *et al*., 1979) or Dutch (e.g., van Aartrijk *et al*., 1995) advisory literature. The authors conducted a search of international research using the CABI database from 1973 to date, without locating any relevant information. The authors are not aware of other research on the topic.

There is no definite knowledge of the cause of NPR, and a number of possibilities had been considered. A pathogenic cause has been more-or-less ruled out. Unsuccessful attempts have been made to isolate pathogens from NPR lesions (various growers and advisors, personal communications). These

diagnostic attempts appear to have been conducted on an '*ad hoc*' basis; there is no record of a systematic approach.

By analogy with disorders in other horticultural crops, a nutritional cause of NPR had been suggested. Such deficiencies might be caused directly or by a pH-related effect (e.g., calcium, boron, molybdenum) or indirectly (e.g., of calcium by potassium, ammonium nitrate, magnesium or boron). Similarities with other disorders (e.g., corky pit of apples and pears, oedema or water-soaking of beet, or corky petiole in celery) suggested that NPR might result from boron deficiency (A A Tompsett, personal communication). However, no conclusions could be drawn from simple boron-spraying trials in Cornwall (A A Tompsett, personal communication). Several physiological disorders are known to be the result of calcium deficiency (e.g., bitter pit of apples, blossom end rot of tomatoes, or internal browning of Brussels sprouts). In spring 2004, therefore, soil and plant samples were taken from examples of non-'rusted' and 'rusted' narcissus crops in Lincolnshire (HDC BOF Panel, personal communication). Tissue nutrient concentrations were determined, but showed no clear trends which might indicate a link between nutrient levels and the severity of NPR. Other causes suggested include adverse water relations, an environmental cause (such as a combinations of unfavourable temperatures), or a genetic or pollutant-related cause.

There are some similarities of NPR to narcissus chocolate spot (NCS), a narcissus disorder that has been described in the advisory literature for many years (e.g., Moore *et al*., 1979), and which does not appear ever to have been serious enough to concern growers or warrant research. (NCS is not connected with the disease of the same name in beans, which is caused by *Botrytis fabae*.) NCS symptoms consist of elongated, chocolate-coloured spots that appear on the leaves in variable numbers – a symptomatology similar to, but distinct from, that of NPR. Like NPR, NCS does not appear to be pathological – no fungal, bacterial or viral agents have been isolated from the lesions (various growers and advisors, personal communications). NCS lesions result from the death of groups of epidermal cells, though the overlying cuticle remained intact, as shown by scanning electron-microscopy,

therefore excluding the entry of a causal agent (Moore *et al*., 1979). In variety trials conducted in 1967 and 1968 at Rosewarne Experimental Horticulture Station, Cornwall, most cultivars found to be affected by NCS were yellow trumpet and large-cup varieties, though not all showed symptoms in both years of the study; however, these types of cultivars also comprised the majority of cultivars in the study, so no inference of a genetic basis can be made. On the other hand, Moore *et al*. (1979) stated that "Casual observations suggest that the appearance of the [chocolate] spots is associated with increasing ambient temperatures".

While it is a distinct condition, NPR appears to have some parallels with NCS. Thus, no pathogen has been detected in either case, while a temperature-related cause has been suggested for each. A few other narcissus disorders are described in the literature as being occasionally encountered, some possibly related to environmental conditions (Table 1). Similarities between NPR, NCS and some disorders in Table 1 implicate temperature and water status as possibly important. Further, descriptions of oedemas in various horticultural crops suggest similarities to NPR.

The present report describes an experiment designed to test the hypothesis that NPR results from a sub-optimal combination of temperature and soil moisture levels.

Table 1. Some physiological disorders of narcissus (other than NPR and NCS).

Disorder	*Description and occurrence*	*Suggested predisposing factors*	*Factors found **not** remedial*	*Reference*
Bud death	Dead buds ('drumsticks') in *Narcissus poeticus* 'Flore Pleno'	• Warm slopes • Extreme temperatures • Hot, dry growing seasons • Wet autumns • Adverse water relations and a poor root system	• Irrigation • Mulch • Shading	Moore *et al*. (1979); Rees (1972); Tompsett (1972)
Bud death	Dead buds ('drumsticks') in forced double cultivars (especially 'Golden Ducat')	• Rapid growth • Dense bud tissues • Inability to translocate sufficient water	• Misting • Calcium sprays • Growth retardant sprays • Fungicides	Hanks (1992)
Bullhead	Abnormal flowers and flower emergence in 'Cheerfulness'	• Transmissible (genetic or viral) component • High temperatures	• Heat treatment (*re* viral component)	Moore *et al*. (1979); Tompsett (1979)
Wilting	Severe but temporary wilting in protected crops	• Bright morning sunshine following cool night	• None known	A R Rees (personal communication)

Materials and methods

Plant material

Bulbs of grade 12-14cm (circumference) of narcissus cultivars 'Carlton', 'Golden Ducat', 'Mando', 'Standard Value', 'St Keverne' and 'White Lion' were obtained from commercial suppliers in the Spalding area in September 2006. On 16 September five bulbs were planted *ca.* 10cm-deep (measured to the base of the bulb) in 20cm-diameter, 4L-capacity plastic plant-pots. The growing medium consisted of a blended peat/sand mix and John Innes no.1 compost (1:1, v/v), the peat/sand mix consisting of sphagnum peat and horticultural sand (3:1, v/v) amended with (all concentrations in $kg.m^{-3}$) ammonium nitrate (0.40), potassium nitrate (0.75), single super-phosphate (1.50), ground chalk (2.25), ground magnesian limestone (2.25) and fritted trace elements WM 255 (0.40) (ADAS, 1984). The plant-pots were placed in 19cm-diameter plant-pot saucers and were bottom-watered as required via drip irrigation to individual saucers, with three drippers per saucer. Valves controlled a separate supply of water for each row of pots.

Environmental treatments

Plant-pots were placed in a polyethylene and mesh 'thermogradient tunnel' (TGT; Wurr *et al*., 1996) at Warwick HRI, Wellesbourne. In the TGT a hot-air heating distribution system maintained a temperature gradient from *ca*. 4°C above outside temperature at the warm end, to slightly above outside temperature at the cool end.

For each of the six cultivars, environmental treatments consisted of high, medium and low temperatures (plants placed at the warm or cool end of the TGT or at the mid-point), combined with four soil moisture levels. In each temperature zone, the soil was maintained at four moisture levels from near-dry to very wet, with target levels of 42, 56, 69 and 82% of maximum capacity, respectively (subsequently referred to as dry, damp, wet and soggy treatments). Changes in sample pot weights in each temperature zone were used to monitor moisture loss at a minimum of weekly intervals, and during periods of rapid plant development and (or) warm and sunny weather,

monitoring was carried out more frequently. For each of the four soil moisture levels two pots, one from the South and one from the North side of the TGT were weighed to measure water loss which was taken as an average of the two pot weights. Once the critical percentage of maximum capacity was exceeded the saucers were filled with water through drippers as described above.

The plant-pots were arranged in a Latin square design with three replicate pots of each combination of temperature zone and moisture level for each of the six cultivars. This design ensured that, within each sub-block of pots, the same cultivar appeared only once within each row or column of the block layout. Each sub-block of pots was protected by a row of two guard pots. Appendix Figure A shows the layout of the pots within the TGT. Plate 1 illustrates the practical arrangements of the experiment.

A fungicide programme was applied to all plants at fortnightly intervals starting shortly after shoot emergence, alternating high-volume sprays of chlorothalonil (as 'Bravo 500', four sprays in total) and dichlofluanid (as 'Rovral Flo', three sprays in total), both used at recommended rates. No other chemical treatments were applied.

Records and data

Air temperatures within each zone were recorded hourly using thermistors inside radiation screens. Soil temperatures within pots were measured using thermistors buried at bulb depth. In addition to weighing sample pots to determine water loss (see above), soil moisture content was measured at four-hour intervals using theta probes (Delta-T). Representative digital photographs were taken regularly throughout the experiment.

At the start of the growing season the progress of shoot emergence was assessed three times per week. At the end of the growing season the extent of foliage senescence was assessed weekly. Flowers from all pots were individually picked as they reached anthesis, counting flower numbers and assessing the flower stems and flowers for damage. Following shoot

emergence, weekly assessments of plants for visible defects either due to disease, damage or disorder were made. Pots were scored for the incidence and severity of NCS on a scale from 0 (no incidence) to 6 (severe incidence).

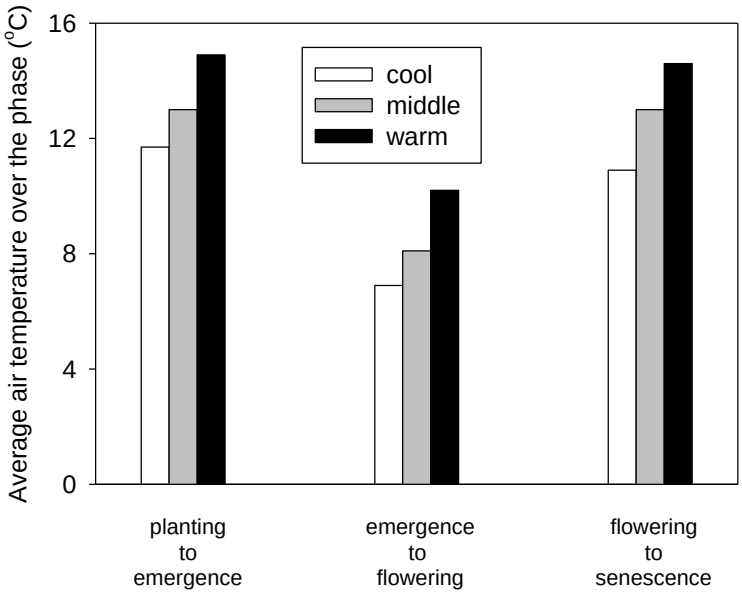
Data were subjected to analysis using the method of restricted maximum likelihood (REML) within the GenStat computer package (GenStat Committee, 2000).

Results

Temperatures and moisture levels

In this section temperatures and soil moisture levels are summarised in order to validate and quantify the treatment effects used. These confirmed that appropriate conditions were obtained throughout the study. The air temperature lift from the cool to the warm zone within the TGT was, on average, 3.2, 3.3 and 3.7°C for the phases planting to emergence, emergence to flowering, and flowering to foliage senescence, respectively (Figure 1).

Figure 1. Average air temperature for the zones within the TGT for three phases of development, defined by dates for cultivar 'Carlton'.



There was very little difference between the four compost moisture levels in soil temperature at bulb depth within each temperature zone, as can be seen in Figure 2. The average soil temperature lift from the cool to the warm zone was 2.4, 2.8 and 2.9°C for the phases planting to emergence, emergence to flowering, and flowering to foliage senescence, respectively. These data confirmed that operating with different water moisture levels would not have invalidated the requisite temperature effects.

The output from the theta probes is shown in Figure 3 for the south and north sides of the TGT. In general, distinct differences between the dry and soggy

treatments were maintained fairly well throughout the growth of the crop. However, Figure 3 also shows that the damp and wet treatments were less distinct, and that, in the warm end of the tunnel on the South side, it was more difficult to maintain the differences in hydration, even with the dry treatment. However, the overall moisture levels achieved ensured that the plants were maintained in a testing range of conditions: Figure 4 summarises these data over the three phases.

Figure 2. Soil temperatures at bulb depth averaged over three phases of development, defined by dates for cv 'Carlton'.

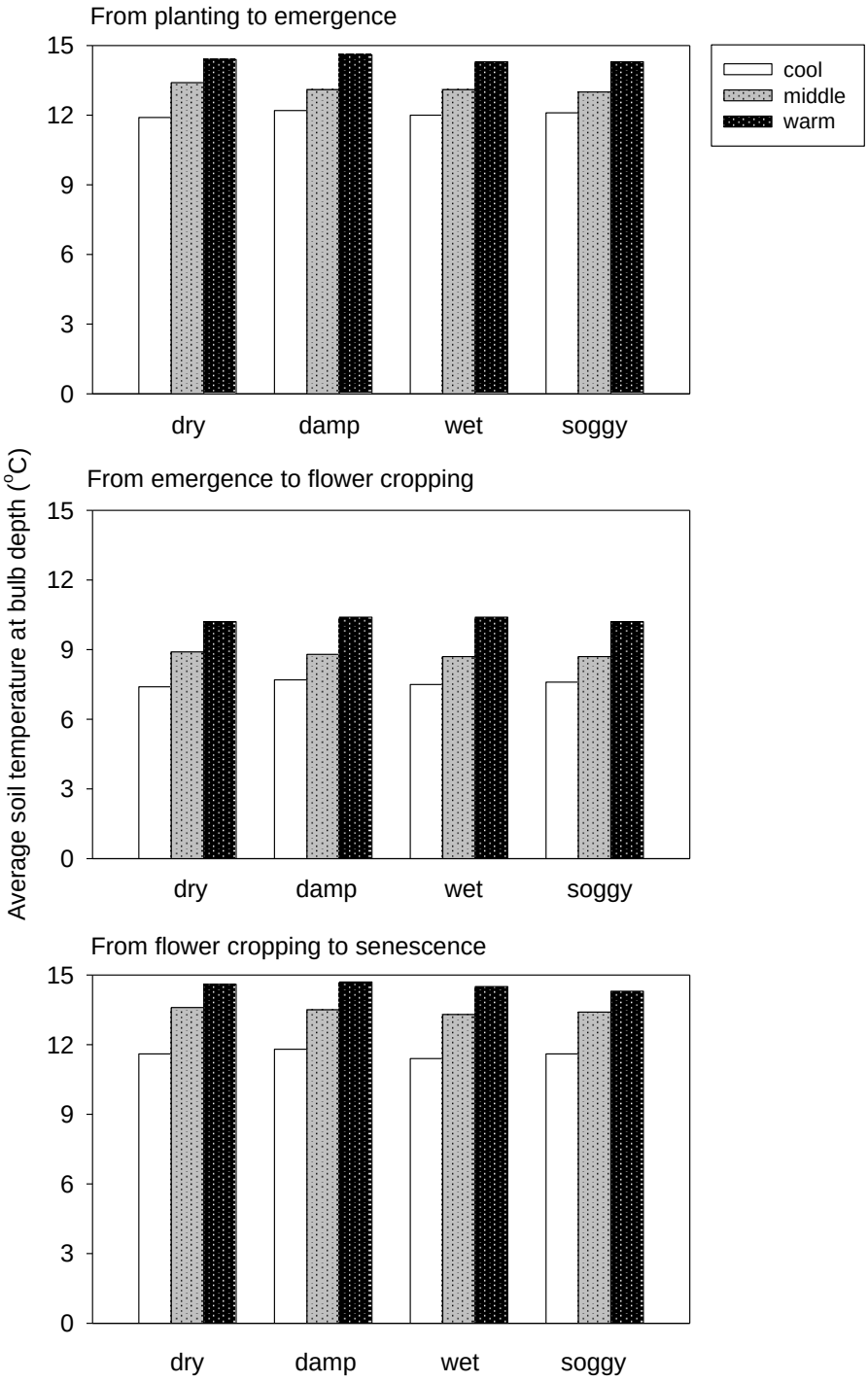


Figure 3. Output from the theta probes: increasing moisture content is indicated by higher mV readings.

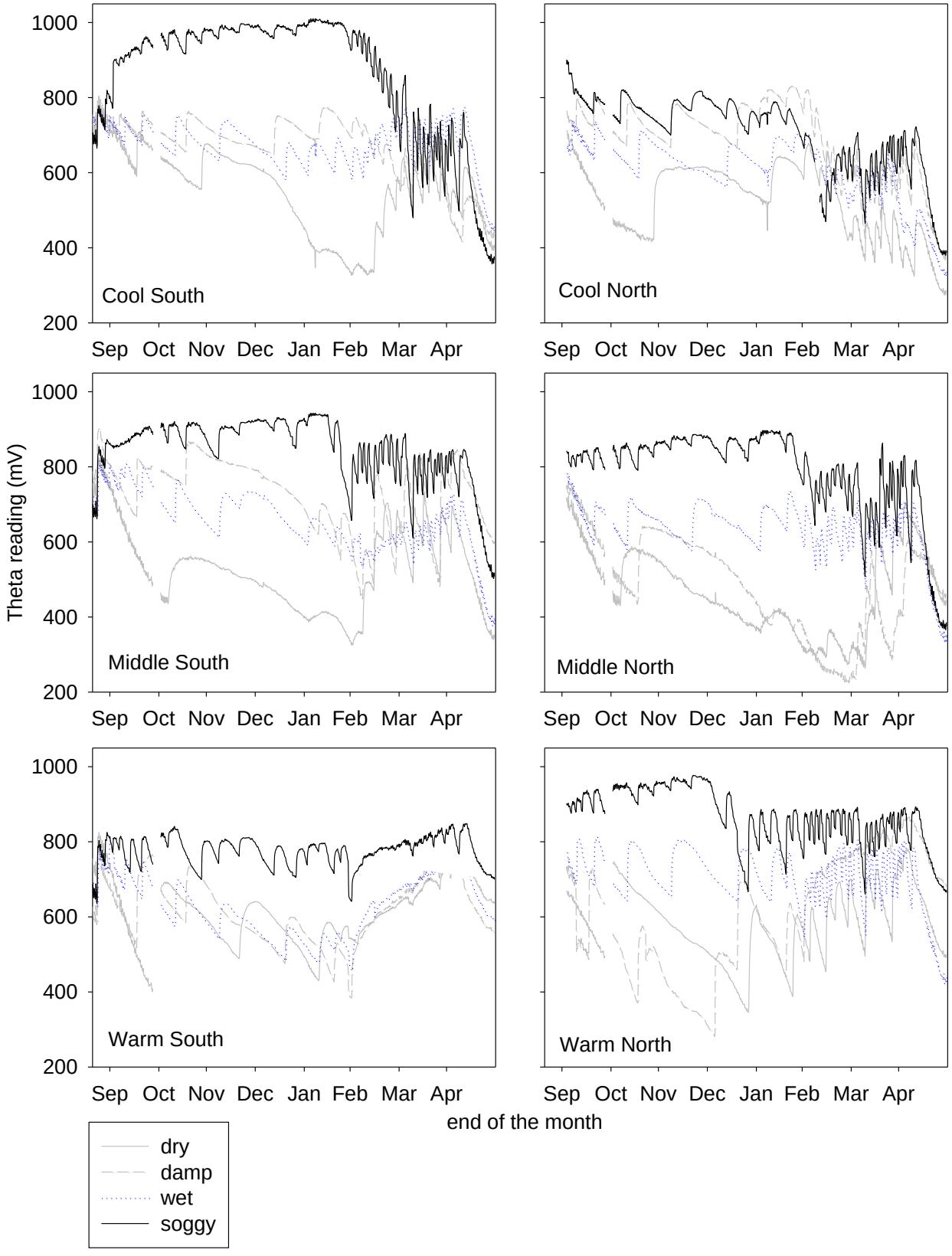
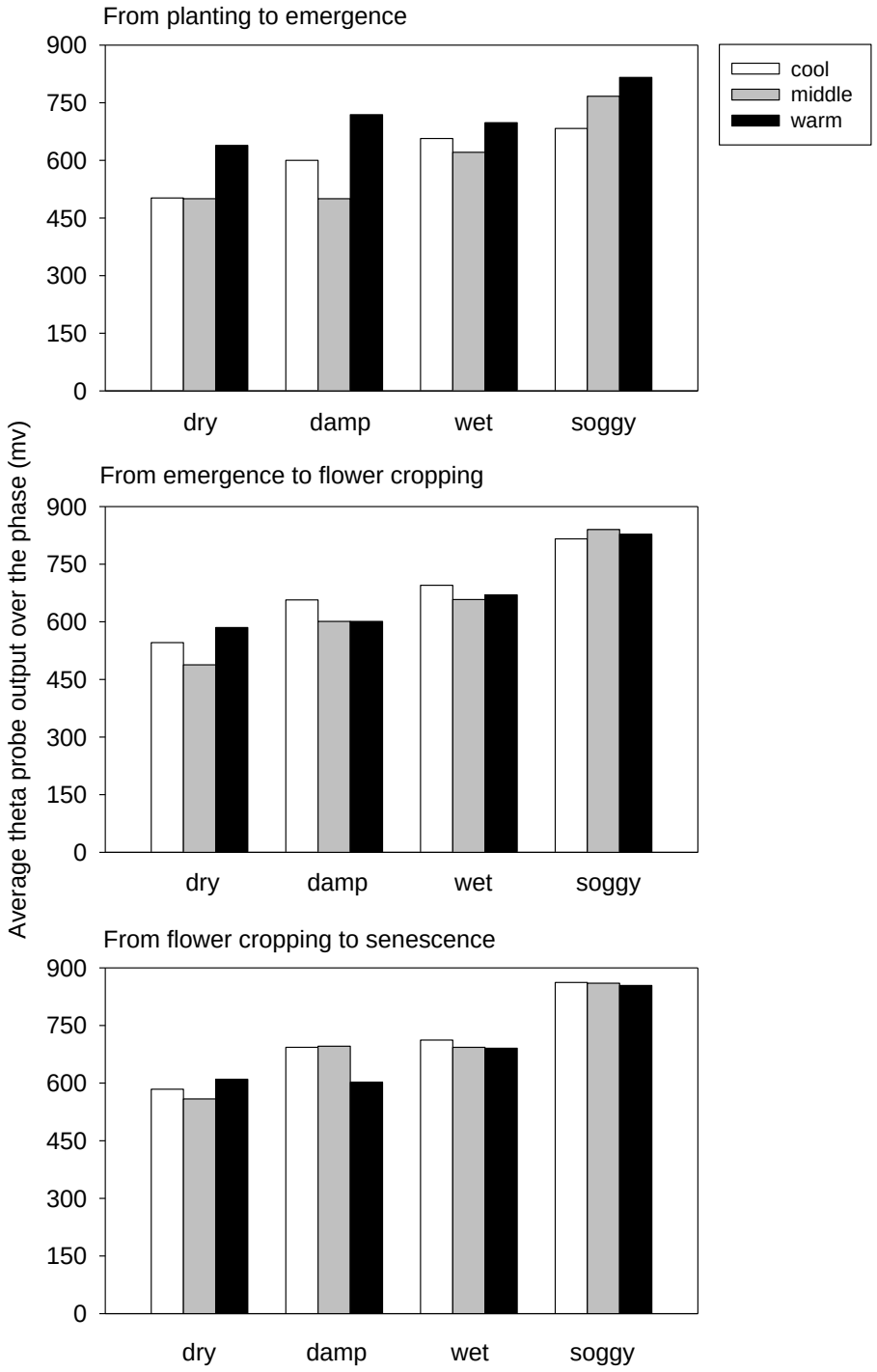


Figure 4. Summarised output from the theta probes averaged over three phases of development, defined by dates for cv 'Carlton'.



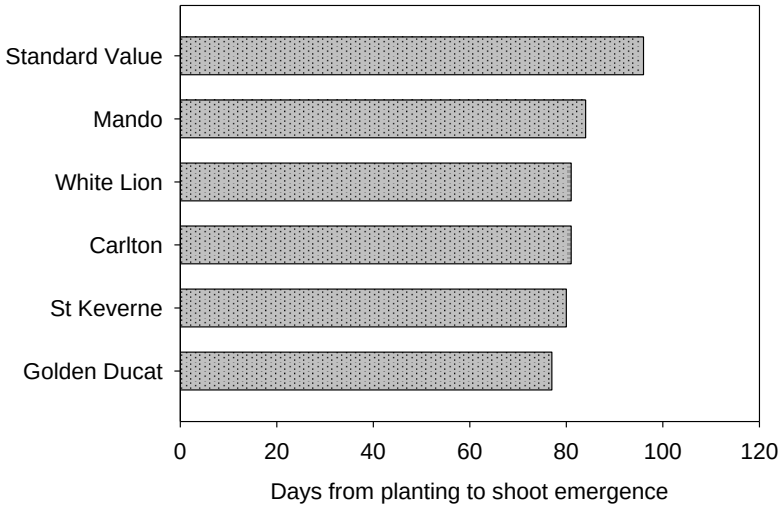
Time of shoot emergence

There were significant differences, at the 10%, 1% and 1% levels respectively, in the time of shoot emergence for the different zones, moisture levels and cultivars, but there were no significant interactions between the three factors and the differences were small. On average shoots emerged 84, 81 and 85 days after planting in the cool, middle and warm zones, respectively (SED 1.7). Narcissus shoots have a lower temperature optimum (*ca*. 5°C) for satisfaction of the cold requirement and a higher temperature optimum (*ca*. 13°C) for rapid growth once the cold period has been supplied (Rees, 1972); the observed earlier emergence at the intermediate temperature would be expected as a result of avoiding too high a temperature initially and too low a temperature later.

In the soggy moisture level shoots emerged on average earliest, 80 days after planting, followed by damp, wet and dry 83, 84 and 86 days after planting, respectively (SED 1.4). The importance of adequate irrigation for obtaining good growth of narcissus was demonstrated by Wurr *et al.* (2002), which this result may reflect, showing the tolerance of narcissus for wet conditions.

Figure 5 shows shoot emergence for the six cultivars, with 'Golden Ducat' emerging earliest and 'Standard Value' on average 19 days later.

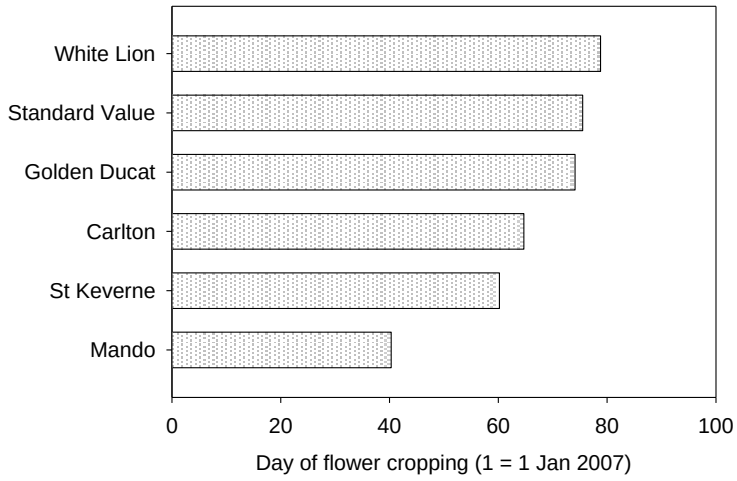
Figure 5 Time of shoot emergence for the six cultivars.



Time of flower cropping and the number of flowers

There were significant effects of temperature zone and cultivar, both at the 1% level, on the time of flower cropping, but no other significant main effect or interactions. On average flowers were cropped 10 days earlier in the warm zone than in the cool zone. This represents the normal response of narcissus to higher growing temperatures, once the cold requirement has been satisfied, and it is interesting to note that moisture level did not affect this parameter. As expected, there were differences between cultivars in the time of flower cropping (Figure 6): 'Mando' was the earliest to crop and was picked, on average, 39 days before the latest cultivar, 'White Lion' (SED 1.4).

Figure 6. Time of flower cropping for the six cultivars.



There were significant main effects and an interaction between temperature zone and cultivar, all at the 1% level, on the number of flowers produced (Figure 7). The total number of flowers picked from each pot varied from, on average, 1.8 for 'Golden Ducat' to a maximum of 9.4 for 'St Keverne'. It is known that 'Golden Ducat' is prone to flower loss at higher growing temperatures (Hanks, 1992).

Time of foliage senescence

There were significant effects of moisture, temperature and cultivar, all at the 1% level, on the time of senescence (Figure 8). On average senescence occurred 16 days later in the cool zone than in the warm zone, and there was a significant interaction between moisture level and temperature (at the 1% level), such that differences due to temperature were most pronounced in dry treatments and least pronounced in the wettest treatments. These observations were as expected: the senescence of narcissus can be considerably delayed by cool and wet weather. There was a range of 12 days in the date of 50% foliar senescence over the six cultivars.

Figure 7. The total number of flowers picked and the number of those flowers showing bulb-scale mite damage for the six cultivars.

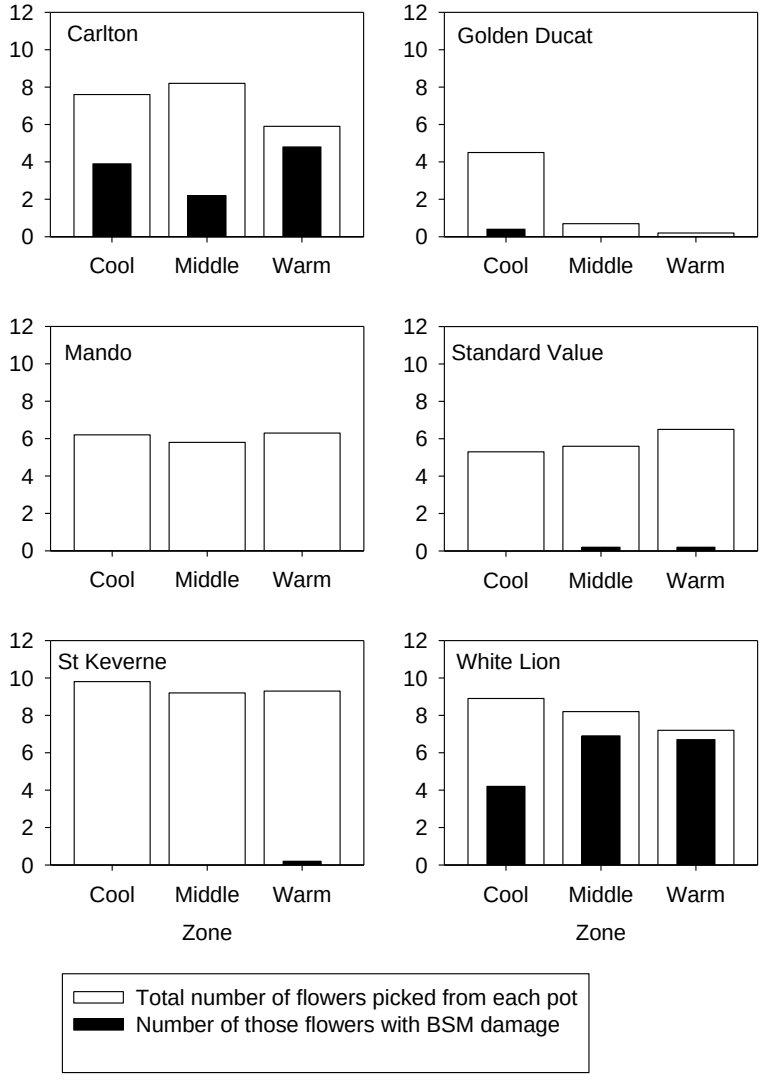
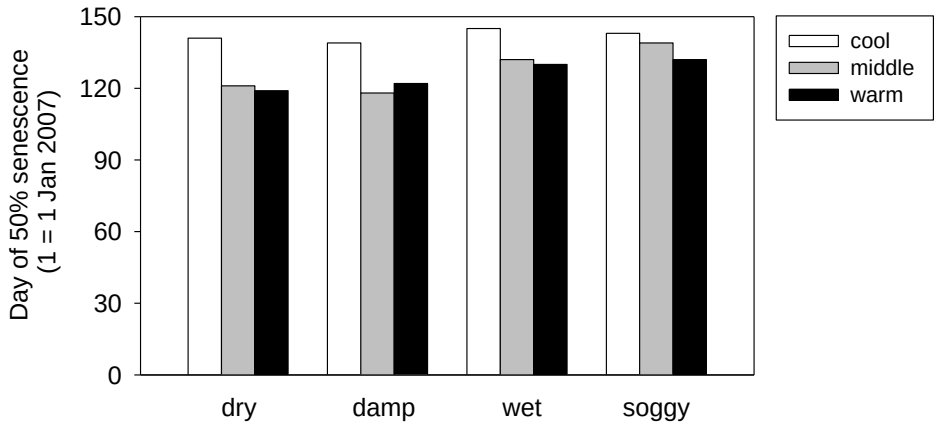


Figure 8. Day of 50% foliage senescence averaged over the six cultivars.



Visible defects

Flowers and flower stems on some plants were damaged by bulb-scale mites (BSM), which appeared to be due to some bulb stocks being infested from receipt. This was the most obvious defect found in the experiment. There was BSM damage (e.g. 'saw-tooth' edges to stems and leaves) on the flowers and flower stems of some cultivars, especially 'White Lion' (average 5.9 flowers and flower stems per pot with damage) and 'Carlton' (average of 3.6), and these figures were represented by the black bars in Figure 7. There was no BSM damage on flowers and flower stems of 'Mando'.

Careful weekly assessments of all plants failed to detect any symptoms of NPR. However, NCS was seen in all cultivars except 'St Keverne', and to the greatest extent in 'Mando'. Figure 9 shows the percentage of bulbs showing incidence of NCS over all the sample dates for all six cultivars in the three temperature zones. NCS symptoms were more marked in plants in the cool and middle zones than in the warm zone. Table 2 shows the scores for NCS on 'Mando' on 18 April 2007. There was a significant effect of temperature within the TGT, but no effect of the moisture level.

Figure 10 shows the percentage of bulbs showing incidence of NCS over all the sample dates for all the cultivars for the four moisture levels. There was no evidence of any effects of moisture levels, except possibly for Golden Ducat, where there may be a trend of increasing NCS incidence with increasing

moisture level, but the data are so few that it is impossible to draw any conclusions from this.

NCS lesions were carefully observed over the whole growing season: they maintained their chocolate-like appearance, with no suggestion that 'rust' and 'chocolate' symptoms were interchangeable, or that the nature of the lesions changed over time. Plate 2 illustrates the progress of NCS symptoms over the whole growing season.

Table 2. Mean NCS scores and numbers of bulbs affected by NCS in cv 'Mando' on 18 April 2007. Scores on a 0 to 6 basis, 0 = none to 6 = severe.

NSC score or number of bulbs affected	*Zone in the TGT*			*SED*
	Cool	*Middle*	*Warm*	
Score on stem	5.7	5.6	1.5	0.30
Score on leaf	4.7	5.1	2.1	0.51
Number of bulbs affected	3.5	4.2	2.5	0.42

Figure 9. The percentage of bulbs showing incidence of NCS for all cultivars over all observation times for the temperature zones.

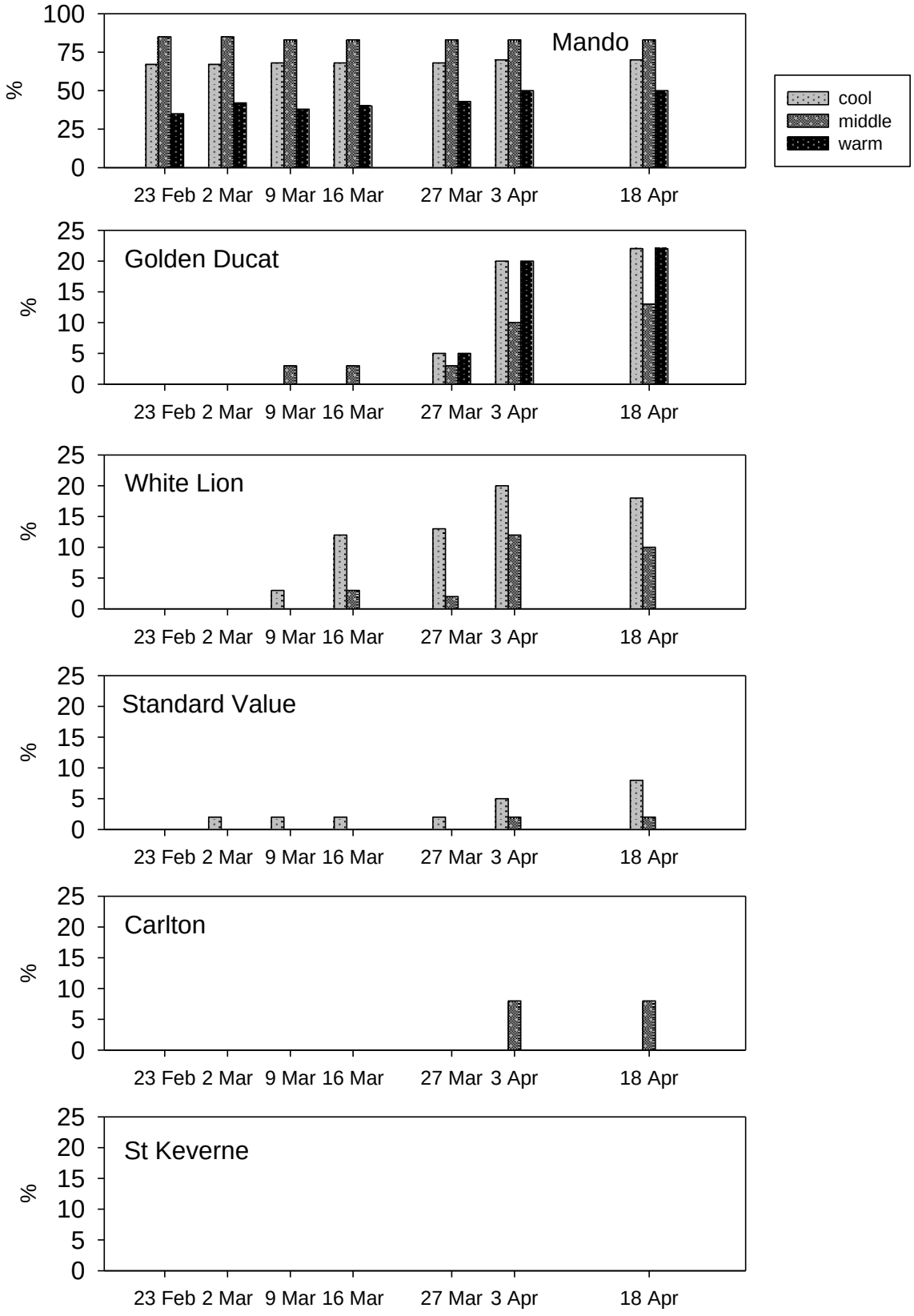
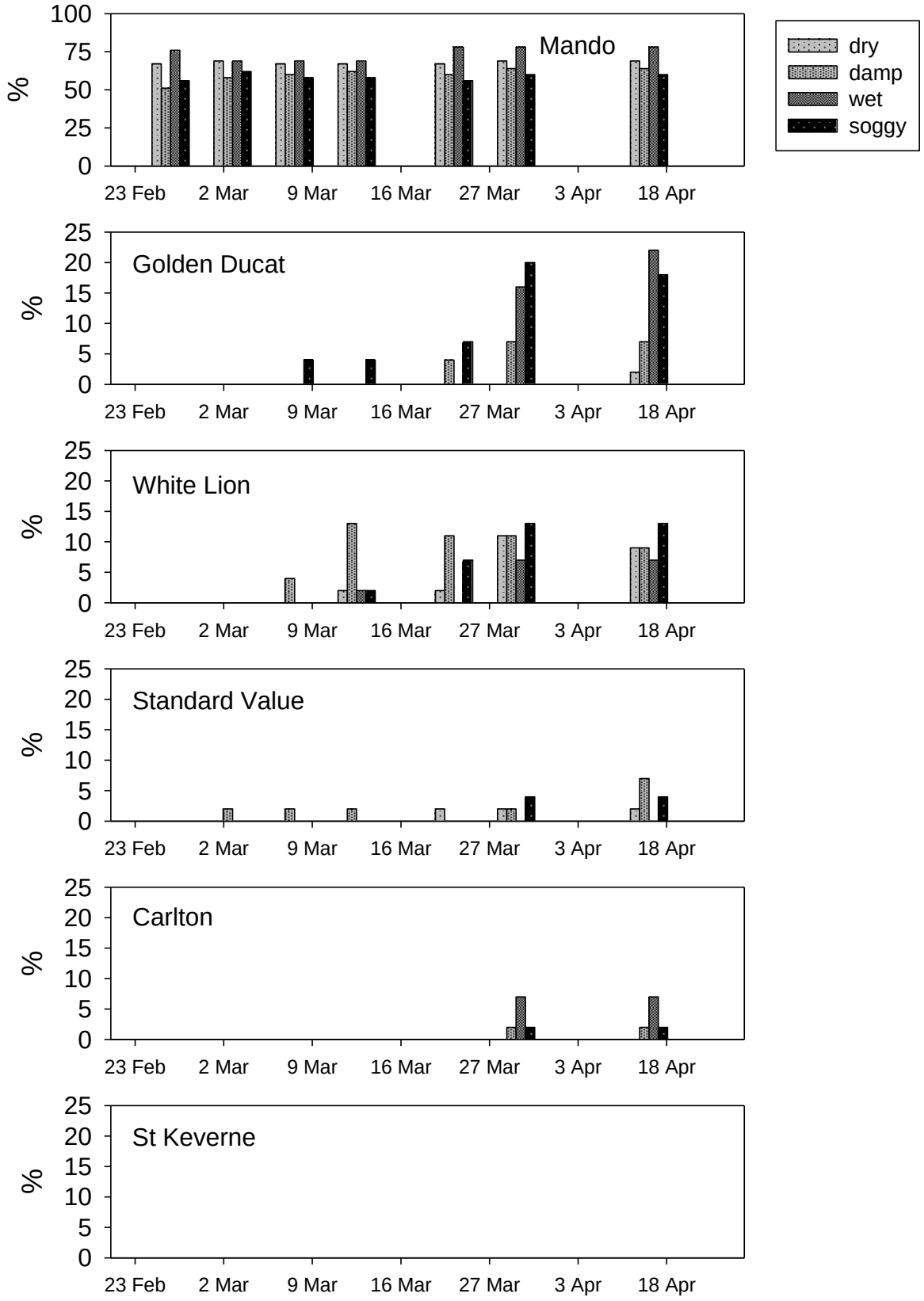


Figure 10. The percentage of bulbs showing incidence of NCS for all cultivars over all observation times for all moisture levels.



Discussion

This project was an investigation of the hypothesis that narcissus 'rust' (NPR) is a physiological disorder resulting from growth under adverse combinations of temperature and soil moisture levels. Previous observations led the authors to discount a pathological or nutritional cause of NPR. On the other hand, grower observations on NPR, and previous reports on the similar condition called narcissus chocolate spot (NCS), suggested that environmental conditions linked to temperature and water availability might cause the rust-like symptoms through disturbance of normal water relations. To test the hypothesis, narcissus plants of six cultivars were grown over one growing cycle under a range of soil moisture levels at temperatures from significantly below to significantly above natural ambient temperatures. In no instance, however, were the typical NPR symptoms seen on the experimental plants, and so this hypothesis, whilst not being definitively ruled out, could not be confirmed. In the present circumstances it is unlikely that additional industry funding will be available to further this investigation, and the HDC BOF Panel should consider whether it should press for research at a more strategic level, which could investigate the causes of NRP at a cellular or sub-cellular, rather than whole-plant, level.

NCS, however, occurred in five of the six cultivars, and severely so in 'Mando'. The symptoms were confirmed as being distinct from those of NPR; the appearance of the 'chocolate' lesions did not change over time, so there is no suggestion that the two symptoms are aspects of a single disorder. NCS symptoms were more common in cool and intermediate temperatures than in warm temperatures, but there was little suggestion that it was related to soil moisture levels. NCS may therefore be a result of low temperatures at a key stage of the growing cycle.

Because of these 'negative' findings, little advice can be given to growers on the way to prevent NPR. Despite the perceived robustness of narcissus plants for growing under a wide range of soil conditions, there is still good reason to emphasise the need for the careful management of soil structure in narcissus growing. Land preparations and all subsequent workings should always take

into account the need to reduce compaction and water-logging to a minimum, since these factors are also likely to be involved with various flowering disorders of narcissus (Table 1; Rees, 1972). In the absence of definitive knowledge on the effects of trace element deficiency on NPR, NCS and other narcissus disorders, growers should consider applying trace elements as on a routine basis.

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Plate 1 General over view of the experimental layout and methods for recording of the environmental data

Water is delivered to each pot via 3 drippers

Thermocouples measuring soil temperature at bulb depth within pots

A Delta-T theta probe measuring soil moisture

View at the start of flowering showing the tubes distributing heat along the gradient

At start-up, a general view from the middle zone

A closer view of the cool end

Plate 2 Typical Narcissus Chocolate Spot symptoms photographed over time

23 February 2007

2 March 2007

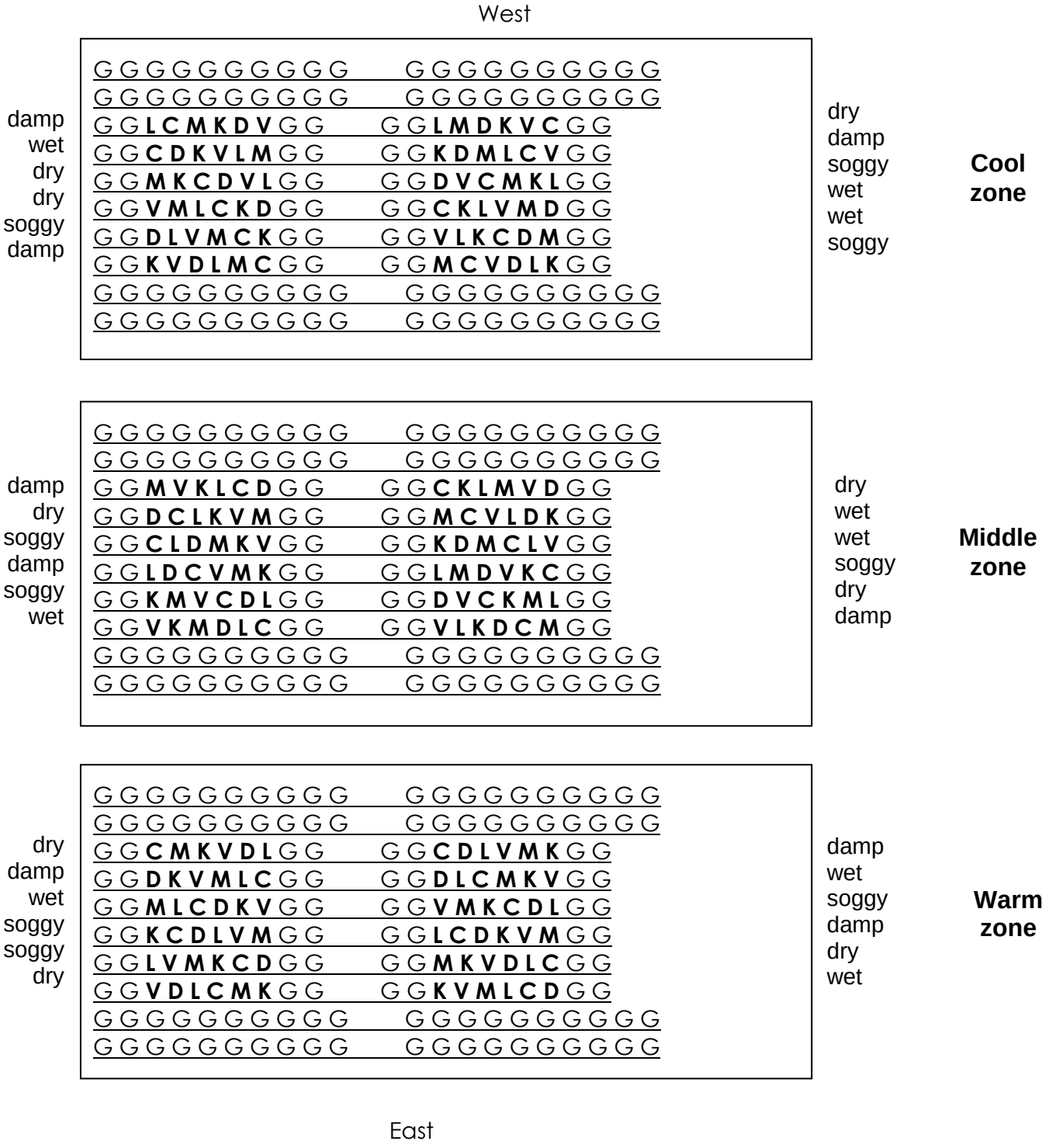
9 March 2007

16 March 2007

27 March 2007	3 April 2007	18 April 2007

Appendix

Figure A. Detailed plan of the pot layout in the TGT.



Key to cultivars: 'Carlton' **C**, 'Golden Ducat' **D**, 'Mando' **M**, 'Standard Value' **V**, 'St Keverne' **K**, 'White Lion' **L**; G indicates guard pots.