

Project title: Narcissus: Chlorine dioxide – assessing crop safety in daffodils treated in hot-water treatment

HDC project number: BOF 70a

Project leader(s): Gordon Hanks
Consultant
2 Malvern Close
Spalding
Lincolnshire
PE11 2DQ

T: 01775 723916
M: 07789 336325
E: gordon.hanks@talktalk.net

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Key worker(s): Gordon R Hanks BSc, MPhil, FIHort, MSB, CBiol

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Project co-ordinator(s): Adrian Jansen (Lingarden Bulbs Ltd)

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The results and conclusions in this report are based on an investigation conducted over a two-year period. The conditions under which the experiments were carried out and the results have been reported in detail and with accuracy. However, because of the biological nature of the work it must be borne in mind that different circumstances and conditions could produce different results. Therefore, care must be taken with interpretation of the results, especially if they are used as the basis for commercial product recommendations.

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GROWER SUMMARY

Headline

As a biocide in the hot-water treatment of daffodil bulbs, chlorine dioxide appeared to have no adverse effects over a two-year growth period, compared with using 'FAM 30' + 'Bravo 500', the current recommendation. For one of the two varieties tested, 'Mando', treatment with chlorine dioxide had a positive effect on the number of stems, the rate of shoot development, stem length and flower count. A negative impact on bulb yield was observed at the end of the two-year period which, though possibly an artefact of the procedures used, warrants further investigation.

Background

To manage stem nematode (*Ditylenchus dipsaci*) and base rot (basal or *Fusarium* rot caused by *Fusarium oxysporum f.sp. narcissi*) in daffodils, hot-water treatment (HWT) of the bulbs is essential. For decades, the HWT dip used for treating daffodil bulbs has invariably contained formalin (formaldehyde), a biocide that provides general disinfection of the bulbs, water and equipment, as well as apparently augmenting the kill of stem nematodes by the hot water itself. At the end of 2008 clearance to use formalin in the agriculture/horticulture industry in the EU was withdrawn without any alternative being available. Effective alternative biocides for use in HWT have therefore been urgently sought. As a result of HDC-funded projects (BOF 61, 61a, 61b and 61c) an iodophore biocide, 'FAM 30', had been suggested as an alternative to formalin, where appropriate in tank-mix with a chlorothalonil fungicide ('Bravo 500'). However, the practical use of 'FAM 30' is hampered by the rapid dissipation of its active ingredient (iodine) in bulb-dipping tanks and difficulties in assessing iodine concentrations on-farm using a 'dip-stick' test. Another biocide, chlorine dioxide (ClO₂), was investigated in this project following successful research in the USA; it has the advantage that its concentration can be automatically monitored and maintained in bulb-dip tanks. The main aim of the present project was to determine whether chlorine dioxide treatment resulted in any adverse or phytotoxic effects on the crop.

Summary of the project and main conclusions

As a feasibility study, two daffodil stocks ('Mando' and 'Quirinus') were treated in 2010 with chlorine dioxide (4 to 5ppm) using HWT facilities at a commercial farm (project BOF 70). The technique was judged to have potential for practical on-farm use, and the treated bulbs were planted in the field along with comparable batches treated in HWT with 'FAM 30' + 'Bravo 500', the dip additives currently recommended as a result of the earlier projects. Growth and development of the four batches of bulbs was assessed during two-year-down growing.

After shoot emergence in the first year (2011) 'Mando' plants previously treated with chlorine dioxide were both more numerous and more advanced than those treated with 'FAM 30' + 'Bravo 500'. The number of shoots or stems per plot, and stem length, were greater in the chlorine dioxide-treated plants than in the standard 'FAM 30' + 'Bravo 500' treatment (on average by 42% for numbers and 12% for length). The plots of 'Quirinus' showed no such difference. Crop development, measured as the passage through standard growth stages (GS), was similar for both treatments, and, apart from minor flower damage typical of that caused by HWT, flower quality was normal in all four batches of plants.

In 2012 there was a strong trend for variety 'Mando' previously treated with chlorine dioxide to produce more stems than those treated with 'FAM 30' + 'Bravo 500'. This was not the case for 'Quirinus'. Stem length, crop development rate and the incidence of disease symptoms were unaffected by treatment in either variety. Flower yields, scaled-up from counts on sample areas, were higher for chlorine dioxide-treated plants than for bulbs treated with 'FAM 30' + 'Bravo 500', by 28% for 'Mando' and 5% for 'Quirinus'. No appreciable defects in bud or flower quality were observed in either 'Mando' or 'Quirinus' or in either treatment.

The approximate bulb yields for the four blocks were recorded by the grower. The yields were poor, with percentage bulb weight increases between 33 and 121%, almost certainly a result of a very dry growing season in 2011 followed, in the case of the earlier 'Mando', by serious foliar damage from 2012's late frosts. Of serious concern, however, was the apparently reduced yield of both cultivars following HWT with chlorine dioxide, compared with the blocks treated with 'FAM 30' + 'Bravo 500'. The percentage increase for 'Mando' was 33% for chlorine dioxide and 94% for 'FAM 30' + 'Bravo 500', and for 'Quirinus' the figures were 42 and 121%, respectively. However, since the data were from an unreplicated observation and the bulbs in the two 'treatments' may have received different handling and husbandry at some stages, despite the efforts made to avoid this, the result should be treated with caution, especially as in all other respects the growth and development of bulbs from the two 'treatments' were either equal or better when chlorine dioxide was used. Following the other results just described, this conclusion was surprising and may have been an artefact of the procedures used, but it cannot at this point be confirmed or repudiated.

This means that UK growers should continue to rely for HWT on 'FAM 30' (or similar) biocides plus ensuring that the appropriate temperature-time combination is used. From the results of project BOF 61b, 61c and 63b, this means using 'FAM 30' at a rate between 4 and 8L product/1000L water (the higher rate being used for stocks in which there are base rot problems, despite the slightly reduced first-year crop vigour this may produce) and a regular HWT of 3¼ hours at 44.4°C. It would not be wise to continue to rely entirely on 'FAM 30' as the continuing availability of a product or active substance cannot be assumed, and because of the rapid dissipation of iodine that can occur in HWT tanks.

Financial benefits

This project provided useful information about chlorine dioxide, a potential replacement for formalin in daffodil bulb HWT. However, while the effects of chlorine dioxide on bulb growth and development were mainly neutral or positive in comparison with HWT using 'FAM 30' + 'Bravo 500', using chlorine dioxide appeared to result in a reduction of bulb yields; although this was considered very likely an artefact of procedures used, until this result can be clarified no financial benefits can be given.

Action points for growers

Bulb growers should continue to rely for daffodil bulb HWT on using 'FAM 30' (or similar) biocides and ensuring that the appropriate temperature-time combination is used. From the results of project BOF 61b, 61c and 63b, this means using 'FAM 30' at a rate between 4 and 8L product/1000L water (the higher rate being used for stocks in which there are base rot problems, despite the slightly reduced first-year crop vigour this may produce) and a regular HWT of 3¼ hours at 44.4°C. When using 'FAM 30' in bulb-dipping tanks care should be taken to ensure the biocide is topped-up as necessary.

SCIENCE SECTION

Introduction

In daffodil growing, hot-water treatment (HWT) is generally considered essential for the management of stem nematode (*Ditylenchus dipsaci*) and (with the addition of a suitable biocide or fungicide) base rot (basal or *Fusarium* rot caused by *Fusarium oxysporum f.sp. narcissi*). Until its use in agriculture and horticulture was banned in the EU in 2008, it was also considered essential to add formalin (formaldehyde) to the dip to augment the effect of the high temperature on stem nematodes, since nematodes that left the bulbs and entered the circulating dip had been shown to be more resilient to high temperatures than those remaining in the bulbs. It now appears likely that the addition of formalin to HWT tanks also acted as a general biocide dealing with the (sometimes considerable) bioload (biological contamination) within HWT tanks and their associated pipe-work.

Following the withdrawal of formalin no practical alternative biocide was available as a replacement. Although other biocides had been tested from time to time, the industry preference was for formalin because it was cheap, effective and growers had long experience of using it. Therefore the HDC funded a series of projects which considered other treatment options and screened some candidate biocides against stem nematode (project BOF 61), and, having identified an iodophore biocide ('FAM 30') as a possible replacement for formalin, carried out field trials to compare its effects with formalin (BOF 61a), evaluate its crop-safety and on-farm usage (BOF 61b and 61c) and further investigate its action on base rot spores (BOF 71 and 71a).

The results showed that 'FAM 30':

- ✓ Controlled the wool stage of stem nematode *in vitro* as a 3-hour cold (18°C) dip (at HWT temperatures the high temperature alone appeared sufficient for control) (BOF 61)
- ✓ Controlled chlamydospores (thick-walled resting spores) of the base rot fungus *in vitro* as a 3-hour cold dip or HWT (BOF 61)
- ✓ Appeared to be compatible in HWT with the chlorothalonil fungicide 'Bravo 500' (added to give further control of base rot) (BOF 61a and 61b)
- ✓ Compared favourably with formalin when used in HWT, with no phytotoxicity or adverse effects on the growth and development of the daffodil plant and crop other than a slight depression in growth in the year following HWT (BOF 61b)
- ✓ Controlled bioload in bulb-dipping facilities (BOF 61c)
- ✓ Appeared safe and convenient to use on-farm, with the normal precautions (BOF 61b).

However, there were some negative aspects of using 'FAM 30' as a bulb dip:

- x Iodine, the active ingredient of the biocide, dissipated rapidly (BOF 61c)
- x 'FAM 30' is strongly acidic, with implications for the corrosion of dip tanks and possible incompatibility with some pesticides
- x Whereas formalin was also effective as a short (15-25 minute) *cold* dip against spores of the base rot pathogen, 'FAM 30' may be ineffective when used in this way if the spores are not readily accessible (e.g. if there is much soil present) (projects BOF 71 and 71a).

Hence, despite the promising results obtained using 'FAM 30', it seemed unwise to depend upon it as the *only* biocide available for bulb dip use.

Amongst the many biocides available, chlorine dioxide (ClO₂) stands out for potentially greater use in production horticulture. It is a wide-spectrum biocide extensively used in fruit, vegetable and meat processing and in the treatment of potable water. It has the advantages that it can be readily generated on-site (by mixing sodium chlorite and hydrochloric acid) and accurately monitored and dosed. It does not generate any harmful by-products and is relatively unaffected by organic matter in dip tanks. In laboratory experiments in BOF 61 chlorine dioxide (as 'Harvest Wash') was found to be ineffective at killing stem nematodes at 18°C or base rot spores at HWT temperatures. However, in research at Washington State University (WSU), chlorine dioxide had been clearly demonstrated in a series of laboratory experiments to be an effective fungicide against a number of pathogens, including daffodil base rot, when used in HWT conditions, and the treatment was also shown to control the spread of base rot in daffodil bulbs without any phytotoxic effects (for references see the review of chlorine dioxide and its use in narcissus dipping, Appendix 2, 2011 Annual Report for BOF 70a). Chlorine dioxide has been used successfully by at least one major bulb grower in the Pacific North-West of the USA for several years without obvious adverse side-effects (G. Chastagner, personal communication).

Although the reason for the apparent discrepancy between the HDC and WSU studies is not clear, the more extensive body of evidence from the USA is persuasive. Consequently, the first stage of a feasibility study on chlorine dioxide in HWT was funded by the HDC in summer 2010 (project BOF 70). The outcome was:

- ✓ A major supplier of chlorine dioxide technology to industry, Tristel plc,¹ set up a farm-scale test run on a bulb farm
- ✓ Test runs with stocks of daffodil cultivars 'Mando' and 'Quirinus' showed no evident practical or operational reasons that might deter such use
- ✓ Chlorine dioxide was shown to be capable of dealing with a heavy bioload in the HWT tanks at the start of the test run, and thereafter it was possible to monitor and adjust the concentration of chloride dioxide easily

¹ <http://www.tristel.com/>

This encouraging start with chlorine dioxide suggested that extending BOF 70 would be worthwhile. Although there are other issues which will need to be addressed, such as its suitability for use in cold-dipping and its effects on stem nematode, the most pressing need was to assess any adverse effects that chlorine dioxide treatment might have on the growth and development of treated bulbs. In project BOF 70a the chlorine dioxide-treated bulbs from project BOF 70 were assessed under commercial conditions over the usual two-year-down growing period, and compared with equivalent batches of bulbs that had been treated with the currently recommended HWT dip, 'FAM 30' + 'Bravo 500'. The full results from both crop years are presented in this report.

Materials and methods

Hot-water treatment

The HWT facility used was at TH Charlton & Son Ltd, Spalding, Lincolnshire, and comprised two front-loading treatment tanks and a common, overhead holding tank (Secker Welding Ltd, Holbeach, UK). As used for the trial, the facility had a capacity of ca 21,000L, sufficient to use one treatment tank at a time with some reserve dip left in the holding tank. Each tank-load comprised six wooden bulk-bins each containing ca 0.6t of bulbs, a total of ca 3.6t of bulbs per load.

Full details of the HWT were given in the Final Report for project BOF 70. Briefly, the tanks were emptied and refilled with fresh mains water. On 5 August 2010 the dip was brought to a temperature of ca 49°C and the target concentration of chlorine dioxide, 4 to 5ppm (4 to 5mg/L), was achieved by the step-wise addition of mixed sodium chlorite and hydrochloric acid, using a spectrophotometer ('ChlordioXense'; Palintest Ltd, Gateshead, UK) to measure chlorine dioxide levels. No other chemicals - pesticides, adjuvants or anti-foam preparations - were added.

The three-hour HWT was started by pumping the solution to the tank into which the daffodil bulbs (ca 3.6t of cv 'Mando', mixed grades 'as lifted') had been loaded. The dip temperature was then just below the target treatment temperature of 44.4°C, which was then regained and maintained throughout the 3h-treatment. Sampling the dip and addition of further mixed sodium chlorite and hydrochloric acid continued throughout the HWT period, following which the dip was pumped to the alternate tank for overnight storage and the treated bulbs unloaded and ventilated immediately on a drying wall overnight. On 6 August this process was repeated with bulbs of cv 'Quirinus' (ca 3.6t of mixed small and large grades). All bulbs were kindly provided by TH Charlton & Son Ltd from their own stocks.

For comparative purposes, batches of the same two stocks received HWT with tank-mix iodophore biocide + chlorothalonil fungicide (as 8L 'FAM 30' + 1kg 'Bravo 500' per 1000L water), a recommended treatment that appears to be without significant adverse effects on daffodils (see project BOF 61b). These HWTs were carried out on 7 August 2011 for 'Mando' (treating a total of ca 12.4t over three separate HWT runs) and on 2 August 2011 for 'Quirinus' (treating ca 3.4t bulbs).

As far as practical, for both the 'Mando' and 'Quirinus' stocks, those allocated to the two 'treatments' were treated in an identical manner throughout regarding HWT and general bulb-handling procedures. This reduced the likelihood – but could not completely guarantee – that any differences between 'treatments' were due to factors other than the different chemicals added to the HWT dips.

Crop husbandry

'Mando' bulbs from both treatments were planted at site 1 ('Hallgate') on 10 August 2010 in two adjacent, roughly rectangular blocks surrounded by substantial areas of other daffodil stocks. 'Quirinus' bulbs from both treatments were planted in a similar fashion at site 2 ('Hog's Gate') on 7 August 2010. The bulb planting rate was 9t/acre (ca 22t/ha) with ridges at 0.90m-centres. As with the pre-planting operations, care was taken to use the same procedures (planting rate, use of planting machine, crop husbandry, etc.) for the four sets of bulbs so that differences due to bulb handling and husbandry were minimised. Crop husbandry followed the grower's usual techniques and was typical of daffodil growing in the south Lincolnshire region, except that flowers were picked from the first-year crop of 'Quirinus'. Flowers from both varieties were cropped in the second year, after centrally located areas (3m long x two ridges wide) in each of the four blocks had been marked with posts and tape and designated as 'no picking' areas, allowing additional assessments of flower quality to be made. The weather in both years was atypical: in 2011 conditions were unusually dry until late in the growing season, while in 2011-2012 a relatively mild winter encouraged early growth but was followed by severe cold when many cultivars approaching the flower-picking stage suffered low-temperature damage. The crops were grown for 2 years and lifted in mid-July 2012.

Crop assessment

Crops were checked at appropriate intervals, usually about weekly around the flowering period. 'Guard areas' at the sides and ends of each block were excluded from the assessed areas in order to avoid 'edge-effects' (Figure 1). In each of these four areas the crop was assessed in 10, 36cm-long sample areas arranged in an 'X' pattern (also shown in Figure 1). The following were recorded.

- The 'most usual' and maximum growth stages (GS; see Table 1). There were only minor differences between these two records, so only the 'most usual' GS are presented.
- The number and maximum length of shoots (at the earlier stages) and (or) stems (at the later stages), taking measurements from soil level to the tallest leaf-tip or the top-most part of the bud. Once any unmarketable stems were evident they were excluded from the regular stem counts.
- The number of poor quality stems, i.e. those unlikely to be marketable (those having dead flower buds, flowers with obvious petal or corona faults such as splits or notches in the petals and coronas with splits, or flowers that were small and 'starry'). These attributes are typical of flowers produced in the year after HWT. Flower quality was checked in fully opened blooms along a distance of 1m on either side of each sample area and in the 'no picking areas'.
- The number of fungal or other significant leaf lesions.
- The overall presence of pests, diseases and disorders was noted.

- In addition the grower provided information on the approximate flower and bulb yields he obtained from each of the four blocks.

Data interpretation

The initial study (project BOF 70) was designed as a farm demonstration, not a replicated, randomised experiment. However, it proved possible to grow the two batches of treated bulbs alongside comparable batches that had been treated with 'FAM 30' + 'Bravo 500' at around the same time. Apart from the dip additives, as far as practical the bulbs for both 'treatments' were handled in a comparable way throughout (storage conditions, planting rate, and so on), so it was considered not unreasonable to make direct comparisons between them. Nevertheless, any conclusions should be interpreted with caution.

The differences between pairs of treatment means (i.e. between chlorine dioxide and 'FAM 30' + 'Bravo 500' treatments) were tested for their statistical significance using the two-sample 'student's t-test' within 'Microsoft Excel'. Statistically significant differences between treatment means were noted at the 5, 1 or 0.1% level of probability, meaning that an observed difference could be expected to have occurred by chance once in 20, once in 100 or once in 1000 times, respectively. In some cases a lack of any difference between treatment means was self-evident and in this case no statistical tests were carried out (e.g. for GS, see Figures 4 and 10).

Figure 1

How the sampling areas were arranged

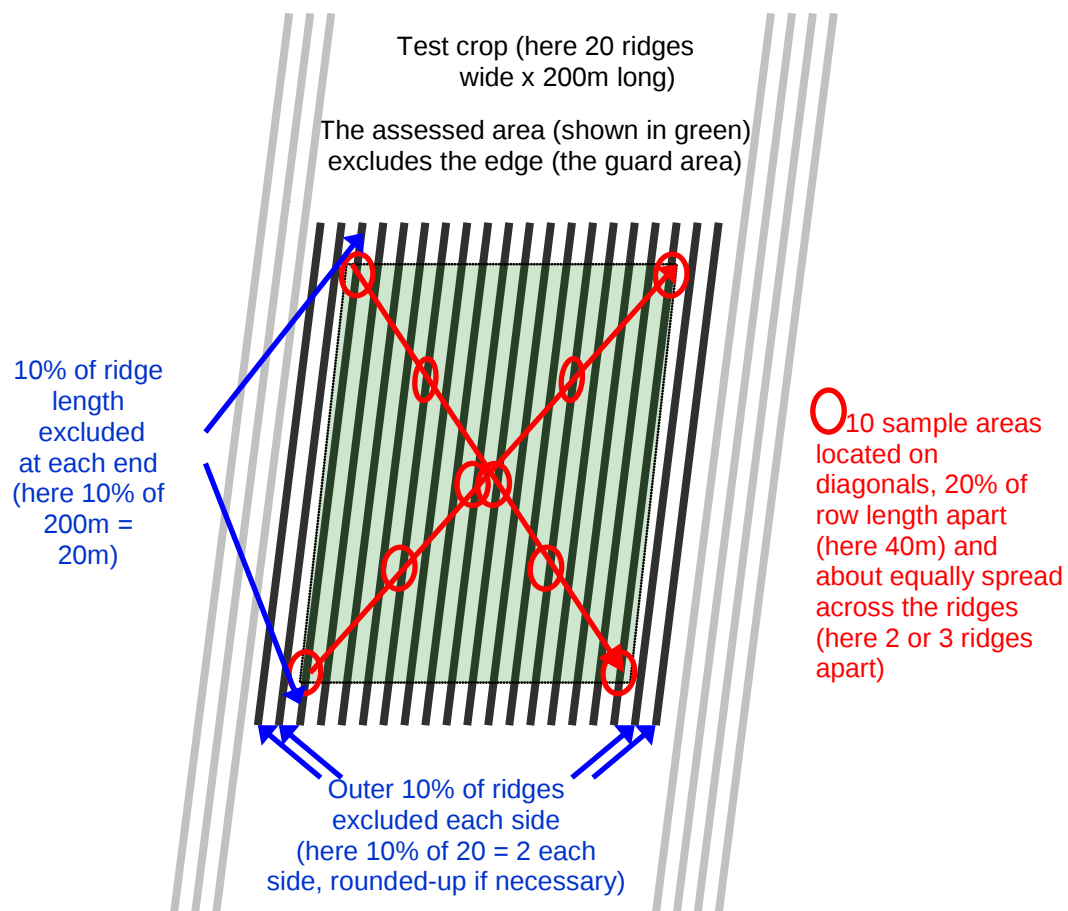


Table 1. A scale of Growth Stages (GS) for daffodils¹

Period	GS	Description	Notes
Unplanted bulb (GS 0)	0.1	'Dormant' bulb in storage	Bulbs would normally be planted at GS 0.1 or 0.2
	0.2	Root initial development evident close to the surface of the bulb	
	0.3	Shoot and/or roots emerging from stored bulb	Apply only to stored bulbs
	0.4	Bulb becoming desiccated with loss of skin, emerging roots or shoots becoming moribund	
	0.5	Bulb shrivelled, light in weight, or rotted	
Planted bulb (GS 1)	1.1	No clear emergence of shoot and/or roots	
	1.2	Roots and/or shoot emerging, <1cm in length	
	1.3	Roots and shoot elongating	
	1.4	Shoot tip close to soil surface	
Emergence (GS 2)	2.1	First shoots starting to emerge	Foliage height nominally 0
	2.2	Shoots elongating, but no buds obviously visible	Record maximum foliage height (and stem height for 2.3 and 2.4) ²
	2.3	Shoots elongating, tips of flower buds visible without pulling shoots apart	
Anthesis (GS 3)³	2.4	Full length of buds visible ('upright pencils')	Record maximum foliage and stem heights
	3.1	Flower buds still 'upright pencils' with no colour showing, but becoming clear of the foliage; flower cropping could have begun if a very tight stage is required and stem length is adequate	
	3.2	Flower buds are up to 'fat pencils' with no colour showing, flower cropping should have begun	
	3.3	Pedicels bending and/or spathes splitting, colour may be showing; a very late picking stage	Record maximum stem height
	3.4	Pedicels fully 'goose-necked' but flowers not open	This stage may pass quickly and variably
	3.5	Flowers (or florets) starting to open	
	3.6	Flowers fully open	For multi-headed types, 50% of florets open, senescing or senescent
	3.7	Flowers starting to senesce (petal tips dying)	
	3.8	Flowers (or florets) fully senescent, leaves still fully green and upright	
Post-flowering (GS 4)	4.1	Leaves still fully green, but at least some leaves starting to bend to ground	
	4.2	As 4.1, but some leaves bending conspicuously and at least some leaves with senescent (yellowing and dying) tips	
	4.3	Most leaves almost flat, with general incidence of senescence at the leaf ends	
	4.4	50% or more of leaf area senescent	
	4.5	Less than 10% leaf area remaining green	
	4.6	None (or a trace) of leaf area remaining green	
	5.1	Small amounts of green foliage remaining attached to bulbs	
'Summer dormancy' (GS 5)	5.2	Any foliage attached to the bulbs now dead	
	5.3	Dead foliage lost or removed	
	6.1	Bulb surface damp and/or not cleaned	
Lifted bulb (GS 6)	6.2	'First stage' drying (surface drying) complete	
	6.3	'Second stage' drying complete	
	6.4	Bulbs cleaned (and graded if appropriate)	

¹ Avoid the following when recording: plot or row ends; obvious rogues, off-types and atypically damaged/diseased plants; late flowers from lateral bulbs; and the most advanced plants if these are about 1% or less of the total

² Record shoot height from the point of emergence from the soil to the uppermost tip of foliage, and stem height from the point of emergence from the soil to the topmost tip if the bud, spathe or flower

³ If flowers cropped and no remnants left to estimate exact GS, record as '4.C' (cropped)

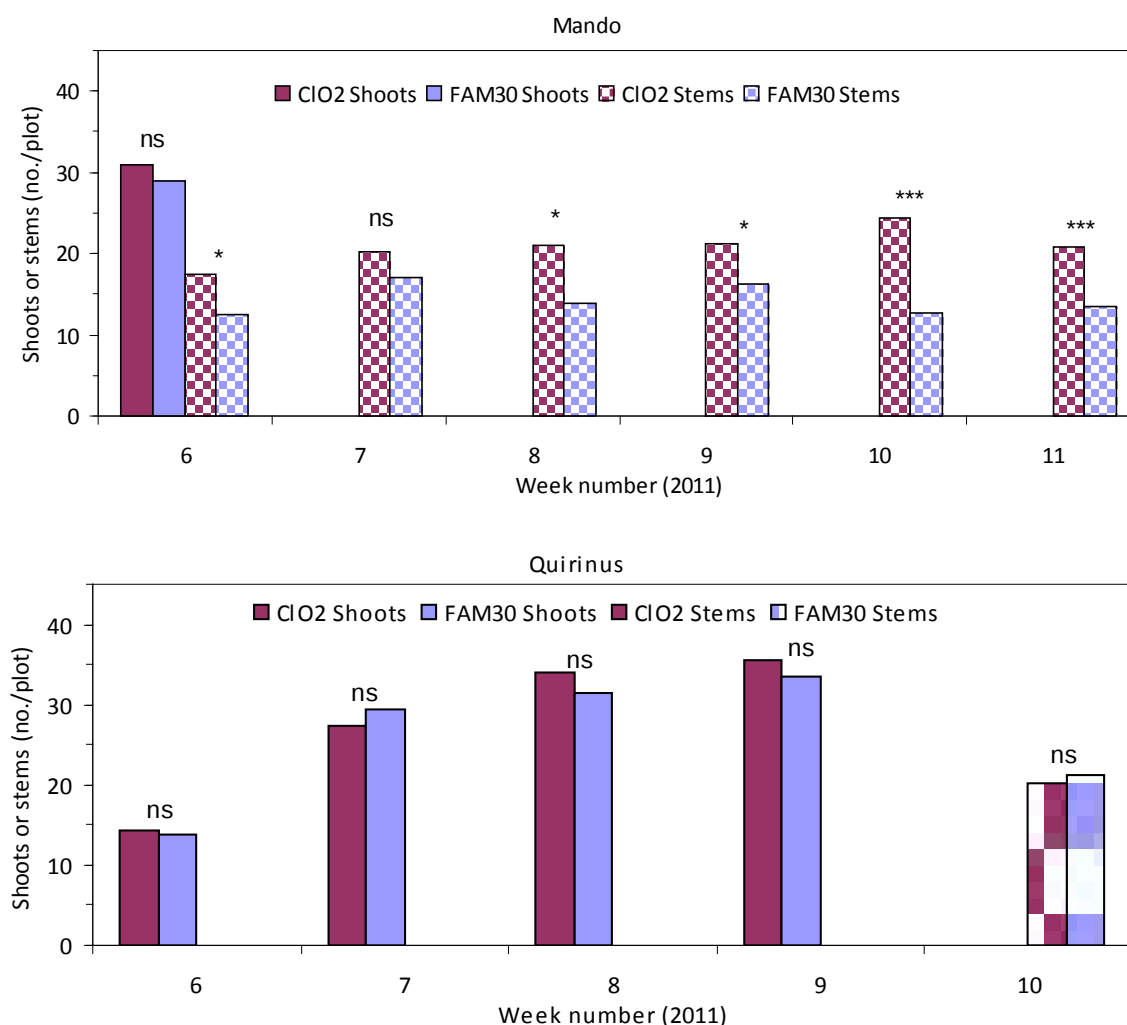
Results

Year 1: Shoot and stem counts

By week 5 of 2011 plants of 'Mando' had shoots up to 7.5cm high and there was an agreed visual impression that those previously treated with chlorine dioxide were both more numerous (per unit area) and more advanced than those treated with 'FAM 30' + 'Bravo 500'. At this date shoots of 'Quirinus' were still sparse, with shoots up to 2.5cm high, and there were no obvious differences between the two treatments.

Subsequent shoot and stem counts up to flowering are shown in Figure 2. 'Mando' was fully emerged by week 6, while 'Quirinus' continued to emerge until week 8. In 'Mando' the number of shoots or stems per plot was consistently greater, by 42% on average, in the chlorine dioxide-treated plants than in those treated with 'FAM 30' + 'Bravo 500' (see photographs in Figure 5). 'Quirinus' showed no such difference (see Figure 6).

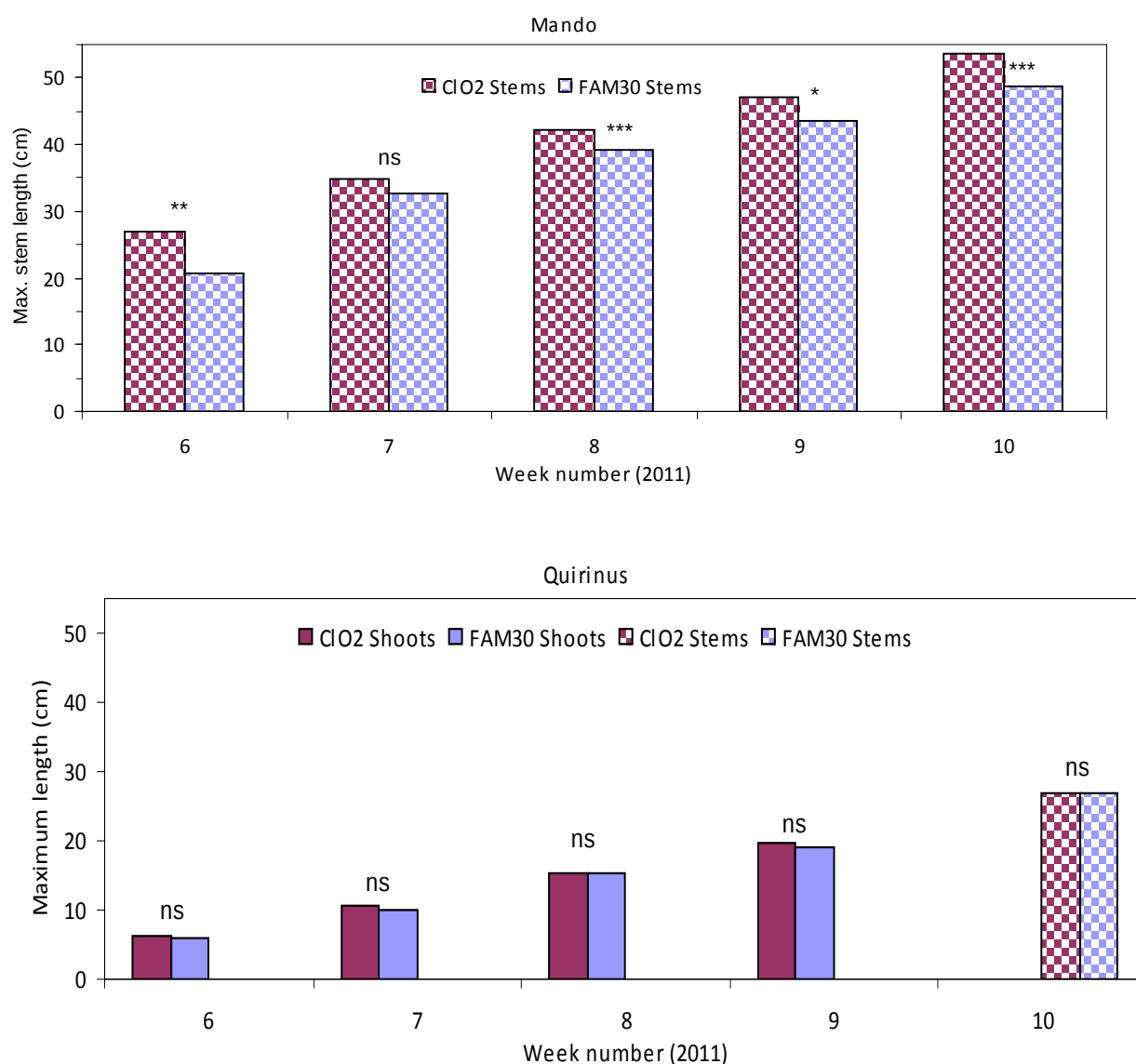
Figure 2. Shoot counts (during emergence) and stem counts (approaching flowering, stems with damaged flowers excluded) in daffodil 'Mando' (top) and 'Quirinus' (bottom) in year 1, following HWT with either chlorine dioxide (labelled ClO₂) or 'FAM 30' + 'Bravo 500' (labelled FAM30). Figures are means of 10 plots per treatment, and ns, *, ** and *** indicate that differences between pairs of values were not significant or were significant at the 5, 1 or 0.1% levels of probability, respectively.



Year 1: Shoot and stem length

Figure 3 shows maximum shoot or stem lengths for 'Mando' and 'Quirinus'. In 'Mando' the stems in the chlorine dioxide-treated plants were consistently taller (or earlier) than those which had been treated with 'FAM 30' + 'Bravo 500', by 12% on average, though this was not very obvious visually (see photographs in Figure 5). As for shoot and stem numbers, there was no such distinction for 'Quirinus' (see Figure 6).

Figure 3. Maximum shoot length in daffodil 'Mando' (top) and maximum shoot and stem lengths in 'Quirinus' (bottom) in year 1, following HWT with either chlorine dioxide (labelled ClO₂) or 'FAM 30' + 'Bravo 500' (labelled FAM30). Figures are means of 10 plots per treatment, and ns, *, ** and *** indicate that differences between pairs of values were not significant or were significant at the 5, 1 or 0.1% levels of probability, respectively.



Year 1: Crop development

Figure 4 shows the most usual Growth Stage (GS, see Table 1) in the period up to flowering in year 1: there were clearly no differences between chlorine dioxide- and 'FAM 30' + 'Bravo 500'-treated plants of either cultivar (see also photographs in Figures 5 and 6). (Statistical testing was not considered necessary.)

Figure 4. Most usual GS in daffodil 'Mando' (top) and 'Quirinus' (bottom) in 2011, following HWT with either chlorine dioxide (labelled ClO₂) or 'FAM 30' + 'Bravo 500' (labelled FAM30) in 2010. Figures are means of 10 plots per treatment.

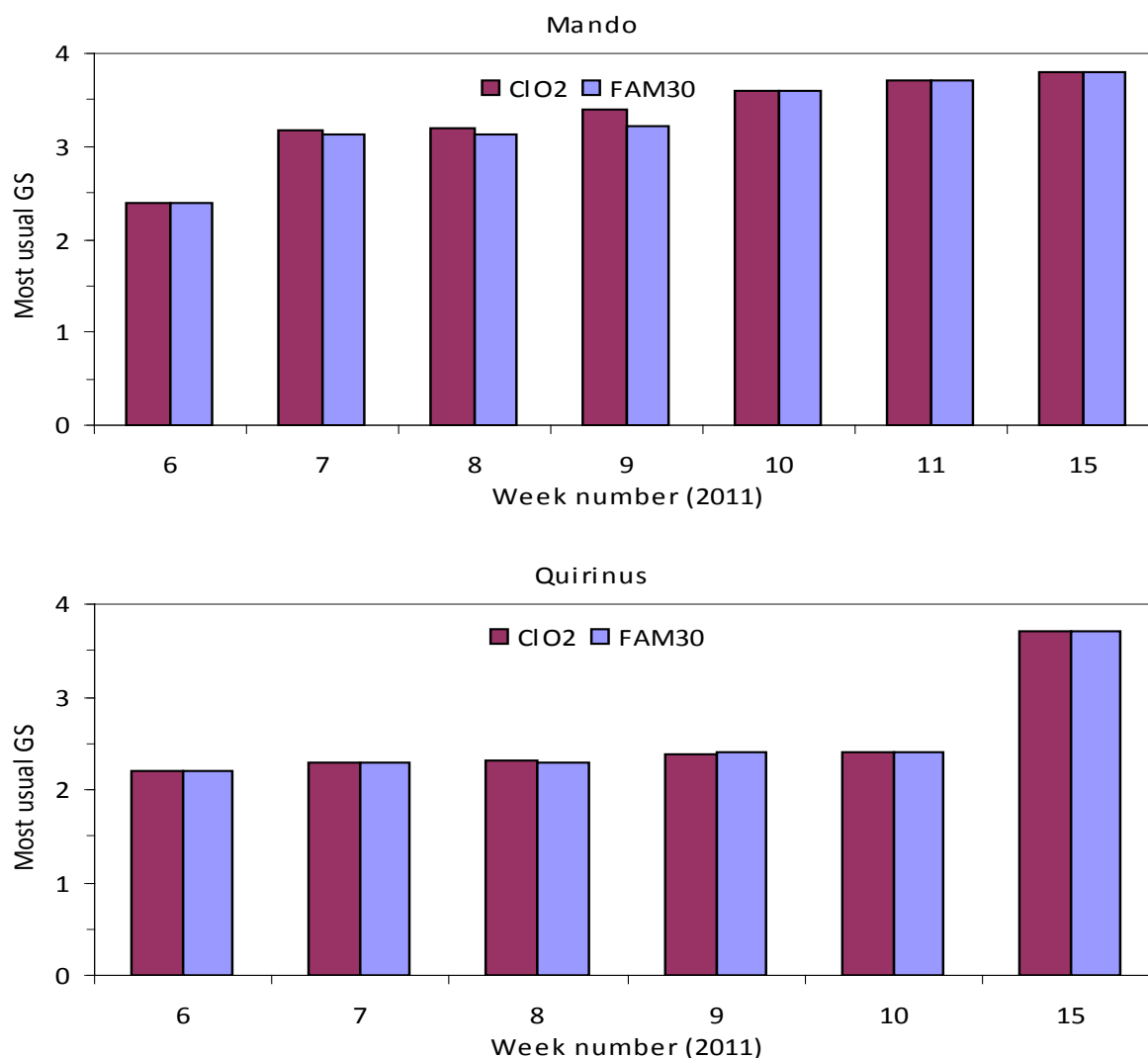


Figure 5. Daffodil 'Mando' in year 1: bulbs previously treated with chlorine dioxide or 'FAM 30' + 'Bravo 500'



(a)
Left:
18 February 2011
Left side (of centre line): chlorine dioxide-treated
Right side (of centre line): 'FAM 30' + 'Bravo 500'-treated

Below left (b) and right (c):
4 March 2011
Left side of each image: chlorine dioxide-treated
Right side of each image: 'FAM 30' + 'Bravo 500'-treated



(d)
Left:
12 March 2011
Left side: chlorine dioxide-treated
Right side: 'FAM 30' + 'Bravo 500'-treated

Below:
18 March 2011
(e) Left: chlorine dioxide-treated
(f) Right: 'FAM 30' + 'Bravo 500'-treated



Figure 6. Daffodil 'Quirinus' in year 1: bulbs previously treated with chlorine dioxide or 'FAM 30' + 'Bravo 500'



(a)
Left:
18 February 2011
Left side (of centre line): chlorine dioxide-treated
Right side (of centre line): 'FAM 30' + 'Bravo 500'-
treated



(b)
Left:
12 March 2011
Left side: chlorine dioxide-treated
Right side: 'FAM 30' + 'Bravo 500'-treated

Below: close-ups of (b)
(c) Left: chlorine dioxide-treated
(d) Right: 'FAM 30' + 'Bravo 500'-treated



(e)
Left:
11 April 2011
Left side: chlorine dioxide-treated
Right side: 'FAM 30' + 'Bravo 500'-treated

Year 1: Crop health

All areas of the trial showed occasional, mild foliar symptoms of HWT damage as mottled, roughened leaf tips (Figure 7a). Yellow leaf-tips, typical of damage caused by periods of low temperatures or frost, were frequent in all crops in the pre-flowering period. Mild symptoms of virus infection (yellow streaking on leaves) were seen occasionally throughout the stocks. At one end of the 'Mando' plantings leaf-tip damage characteristic of smoulder was prominent, possibly a 'row end' effect connected with spraying fungicides (this was in the 'guard area' so was not used in part of the regular assessments). Typical smoulder lesions were seen on the leaf-tips throughout the 'Mando' crop and in both treatments, an average of 0.5 plants per plot being affected; lesions were not seen on 'Quirinus'.

Year 1: Flower quality

In weeks 10 and 11 an average of 1.75 stems per plot (about 12% of the stems) carried damaged flowers in cv 'Mando' that had been treated with 'FAM 30' + 'Bravo 500', whereas in plants treated with chlorine dioxide less than 0.5% of the flowers were affected (Figure 7d-f). Damage took the form of minor splits in the petals or corona or, rarely, of empty spathes (due to the earlier death of the flower bud), all typical of mild HWT damage, though the difference between the two treatments was notable (suggesting some unintended difference in the parameters of the two HWT). Examined later, when the flowers were fully open (week 15), more flowers in both treatments showed notches in the petals, splits in the corona, or were small, 'starry' flowers, again, typical of HWT damage in the first year (Figure 7c).

Flowers of 'Quirinus' were cropped in their first year. Bud development appeared normal during the period up to cropping (Figure 6), though some small, starry flower were seen generally across the cultivar in both treatments (Figure 7b). In a late sample of the remaining flowers after cropping, in week 15, most if not all flowers had petal and (or) corona damage (notched petals, split coronas) or were small and 'starry', all typical of HWT damage.

Figure 7. Daffodil 'Mando' and 'Quirinus' showing typical HWT damage (HWTd) to leaves and flowers in year 1

(a) 'Quirinus' with HWTd to leaf-tips



(b) 'Quirinus' with HWTd to petals (notched petals)



(c) 'Mando' showing HWTd with small, 'starry' flowers



(d) 'Mando' showing HWTd to petals (notched petals) following 'FAM 30' + 'Bravo 500' treatment



(e) 'Mando' showing HWTd with split corona following 'FAM 30' + 'Bravo 500' treatment



(f) 'Mando' showing largely undamaged flowers following ClO₂ treatment

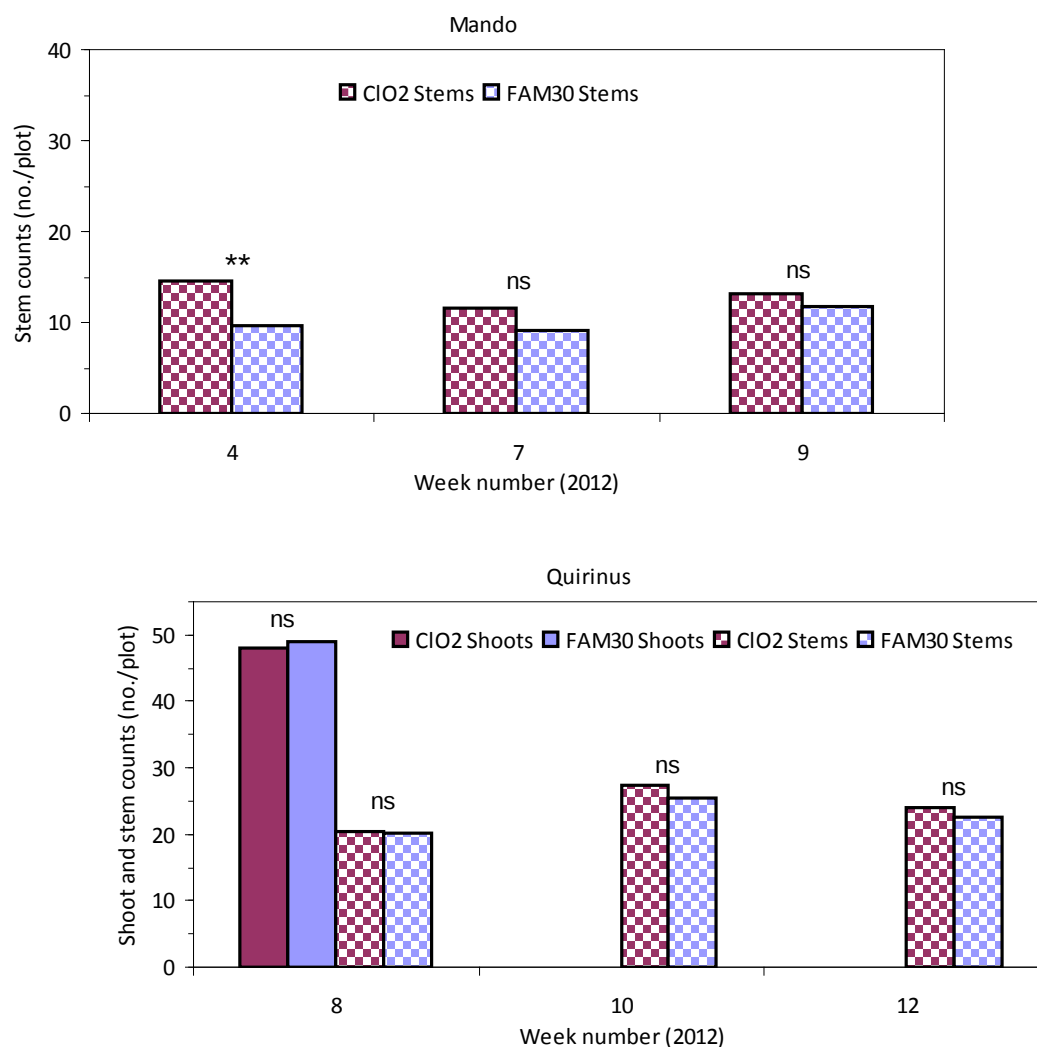


Year 2: Shoot and stem counts

In 2012 'Mando' were well advanced by week 4, with shoots and stems around 30cm-long (Figure 8). As in the previous year there was a visual impression that those previously treated with chlorine dioxide were more numerous than those treated with 'FAM 30' + 'Bravo 500'. This difference was confirmed by statistical analysis at week 4 (at the 1% level of probability) but not subsequently, although Figure 8 shows that this trend persisted (also see Year 2: Flower yield, below).

In 'Quirinus' shoot and stem counts over weeks 8 to 12 did not differ significantly between treatments (Figure 8), again confirming the previous year's results.

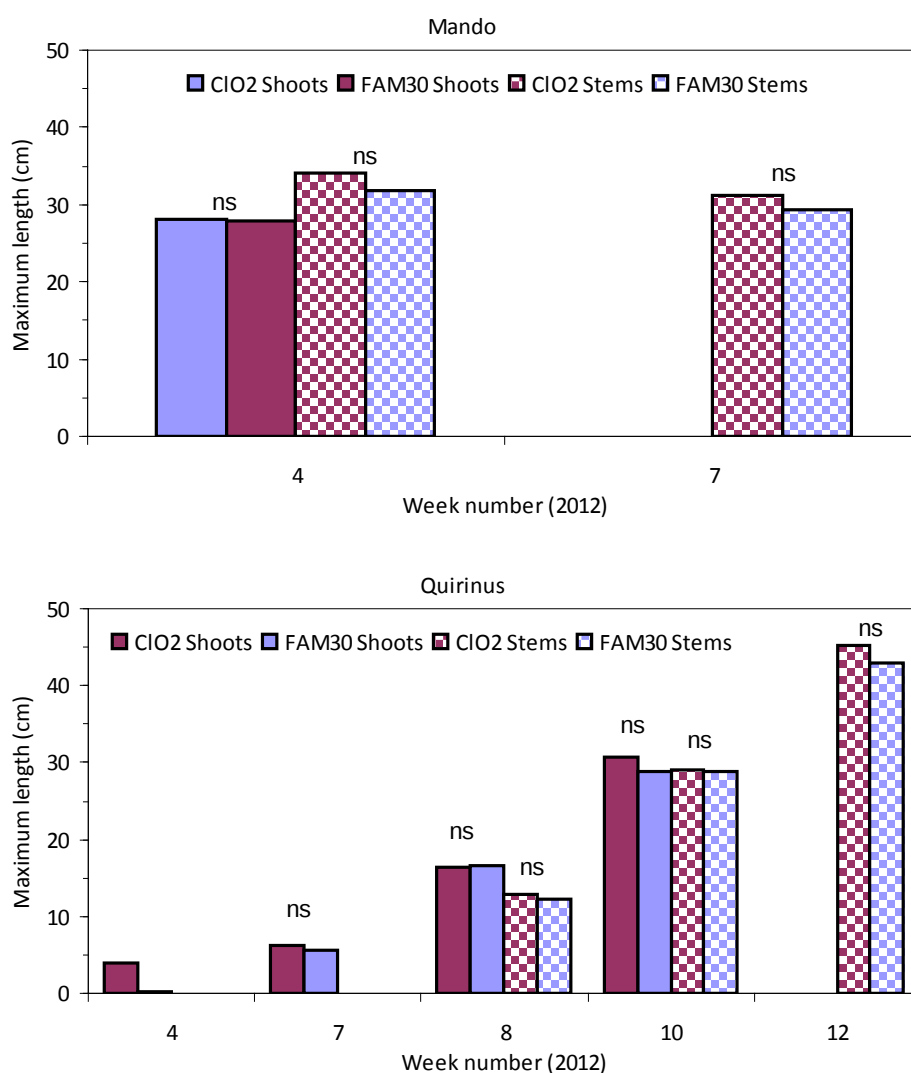
Figure 8. Stem counts (approaching flowering, stems with damaged flowers excluded) in daffodil 'Mando' (top) and shoot counts (during emergence) and stem counts in 'Quirinus' (bottom) in year 2, following HWT with either chlorine dioxide (labelled ClO₂) or 'FAM 30' + 'Bravo 500' (labelled FAM30). Figures are means of 10 plots per treatment, and ns, *, ** and *** indicate the differences between pairs of values were not significant or were significant at the 5, 1 or 0.1% levels of probability, respectively.



Year 2: Shoot and stem length

By week 4 'Quirinus' shoots from the chlorine dioxide treatment were about 4cm tall, while those from the 'FAM 30' + 'Bravo 500' treatment had not yet started to emerge. However, at subsequent assessments any between-treatment differences in mean shoot and stem lengths were not statistically significant (Figure 9). Differences in length between the 'treatments' of 'Mando' were also not significantly different.

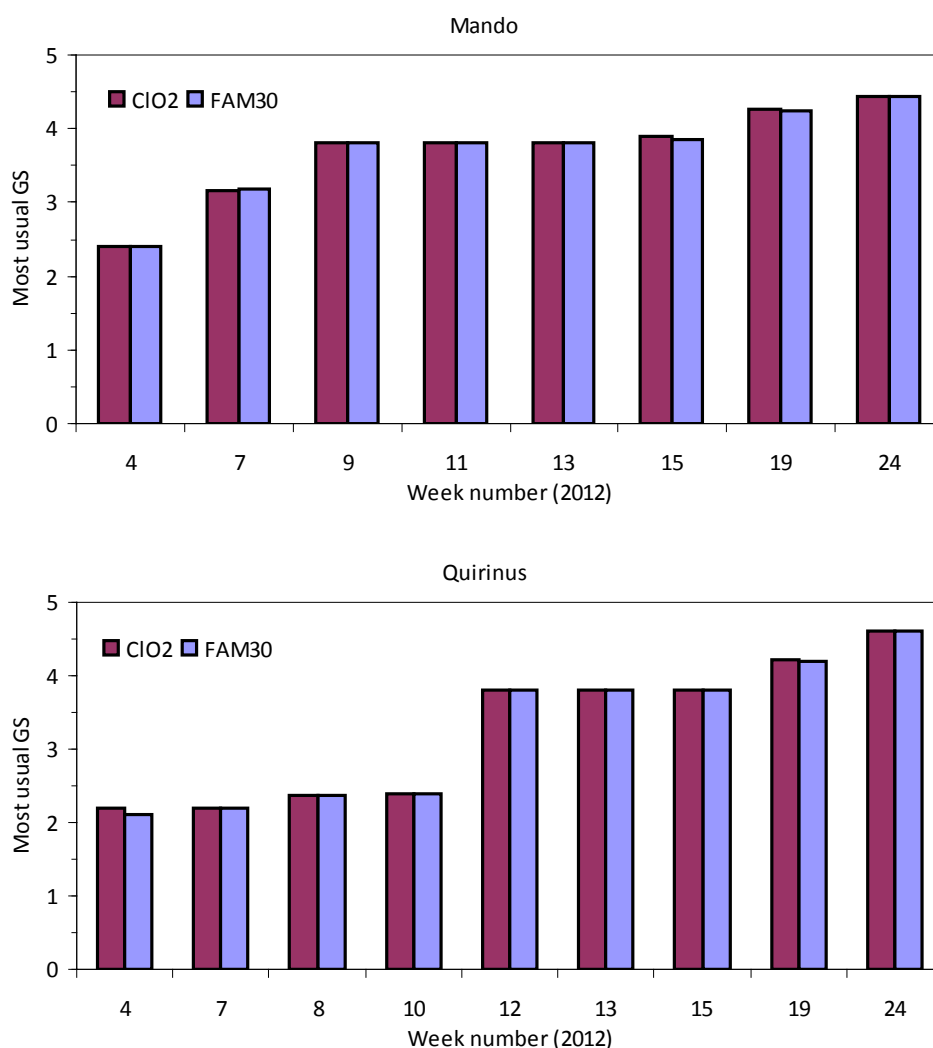
Figure 9. Maximum shoot and stem lengths in daffodil 'Mando' (top) and 'Quirinus' (bottom) in year 2, following HWT with either chlorine dioxide (labelled ClO₂) or 'FAM 30' + 'Bravo 500' (labelled FAM30). Figures are means of 10 plots per treatment, and ns, *, ** and *** indicate that the differences between pairs of values were not significant or were significant at 5, 1 or 0.1% levels of probability, respectively.



Year 2: Crop development

Figure 10 shows the most usual GS through the main growing period in year 2. There was clearly no difference in the rate of crop development between chlorine dioxide- and 'FAM 30' + 'Bravo 500'-treated plants of either cultivar.

Figure 10. Most usual GS in daffodil 'Mando' (top) and 'Quirinus' (bottom) in 2012, following HWT with either chlorine dioxide (labelled ClO₂) or 'FAM 30' + 'Bravo 500' (labelled FAM30). Figures are means of 10 plots per treatment.



Year 2: Crop health

Effective HWT would be expected to have some control of base rot and fungal foliar diseases (e.g. smoulder) as well as bulb-scale mites and other pests. In this project, however, relatively late, severe frosts caused significant crop damage in the form of yellow (later necrotic) leaf-tips, possibly exacerbating smoulder development, but perhaps also masking a proportion of the expected leaf-tip lesions. 'Mando', the earlier cultivar, was the more seriously affected by smoulder-like lesions developing at leaf-tips and leaf-breaks, with grey mould commonly occurring on the cut-ends of its flower stalks. Smoulder primaries, however, were infrequent, with a mean number of primaries per plot across all recording dates varying only between 0.13 and 0.23 and the mean number of 'classic' smoulder

lesions between 0 and 0.07, and, at such low frequencies, no effects due to cultivar or treatment could be inferred. Similar considerations applied to the very low frequencies of plants affected by base rot (plants yellowing and dying-down prematurely) or showing symptoms of bulb-scale mite. Virus symptoms (yellow foliar streaking) were seen very infrequently and only on 'Mando'. Later in the growing season trivial amounts of mild 'physiological rust' damage were seen on 'Mando' but not 'Quirinus'.

Year 2: Flower yield

The estimates of flower yields, obtained by recording in the sample areas, could be compared with the grower's predicted yields (based on previous crop performance) and the yield he actually achieved (Table 2).

The grower's yield prediction, somewhat over 510,000 stems/ha, far exceeded the yields achieved for 'Mando' and slightly underestimated it for 'Quirinus'; the actual yields were 58 and 116% of those predicted for the two cultivars, respectively. The discrepancy can largely be explained by the weather: in 2012 the earlier flowering 'Mando' came into flower during a period of late, very cold weather, while 'Quirinus' flowered after this extreme weather. This followed a very dry growing season in 2011 which would, in any case, have been expected to result in low flower numbers.

The estimated flower numbers derived from the sample area assessments for each HWT regime exceeded the yield actually achieved by the grower. This is unsurprising since, while commercial picking depended on the numbers of stems ready on the cropping days, a 'more scientific' stem count in the sample areas allowed for the inclusion of all stems present, whether approaching or at a cropping stage or even if already picked. For both cultivars the yield assessments for chlorine dioxide-treated plants exceeded those in the 'FAM 30' + 'Bravo 500' treatment, by 28% for 'Mando' and 5% for 'Quirinus', following the trend seen for both cultivars in Figure 8.

Table 2. Predicted and actual flower yields in 2012 for daffodils 'Mando' and 'Quirinus' treated in 2010 with chlorine dioxide (ClO₂) or 'FAM 30' + 'Bravo 500' in HWT. The figures are based on the bulb weights given in the text and planting at 9t/acre in ridges at 0.90m-centres.

<i>Cultivar</i>	<i>Flower yield (thousand stems/ha)</i>			
	<i>Grower's prediction¹</i>	<i>Grower's achieved²</i>	<i>Extrapolated from sample areas³</i>	
			<i>ClO₂</i>	<i>'FAM 30' + 'Bravo 500'</i>
'Mando'	513	296	397	311
'Quirinus'	516	598	730	693

¹ Based on previous years' experience with these stocks

² Bulked across both treatments, not picked separately

³ For each of the four blocks the flower counts from its ten sample areas were averaged and scaled-up to give an estimate of counts for the whole block

Year 2: Flower quality

No defects in bud or flower quality were observed in either 'Mando' or 'Quirinus' or in either treatment, apart from an occasional 'Mando' stem in which the spathe failed to split normally.

Bulb yield after 2 years

The approximate bulb yields for the four blocks are shown in Table 3. Since a percentage bulb weight increase² of <150% would generally be considered poor, the yields obtained here, between 33 and 121%, would be considered very disappointing - *under average conditions*. However, the overall low yields were almost certainly largely a result of a very dry growing season in 2011 followed, in the case of the earlier cultivar 'Mando', by serious foliar damage due to 2012's late frosts. Of more serious concern for the project, however, was the lower yield of both cultivars following HWT with chlorine dioxide, compared with those treated with 'FAM 30' + 'Bravo 500'. Since the data were from an unreplicated observation and the bulbs in the two 'treatments' may have unintentionally received different handling and husbandry at some points despite the efforts made to avoid it, this result should be treated with caution, the more so as in all other respects the growth and development of bulbs from the two 'treatments' were either equal or better where chlorine dioxide had been used; this point is considered further in the Discussion.

Table 3. Planted and lifted bulb weights after two years' growth for daffodils 'Mando' and 'Quirinus' previously treated with chlorine dioxide (ClO₂) or 'FAM 30' + 'Bravo 500' in HWT. Figures based on the bulb weights given in the text and planting at 9t/acre in ridges at 0.90m-centres.

Cultivar	HWT	Planted weight (t)	Lifted weight (t) ¹	% weight increase
'Mando'	ClO ₂	3.6	4.8	33
	'FAM 30' + 'Bravo 500'	12.4	24.0	94
'Quirinus'	ClO ₂	3.6	5.1	42
	'FAM 30' + 'Bravo 500'	3.4	7.5	121

¹ Based on recovering 0.6t per bin

² ((Weight lifted – weight planted) x 100) / weight planted

Discussion

Chlorine dioxide has many advantages as a biocide in production horticulture. Experiments in the USA (see Introduction) showed it was effective in killing the daffodil base rot pathogen under HWT conditions, while in the UK the practicality of using chlorine dioxide on-farm in HWT was confirmed in HDC-funded project BOF 70. The current project, BOF 70a, was set up to determine whether using chlorine dioxide in HWT resulted in any adverse effects on the bulbs, such as reduced growth or unmarketable flowers, in comparison with using 'FAM 30' + 'Bravo 500', a treatment developed in BOF 61 and subsequent projects as an alternative to formalin. In 2010 bulbs of an early and a mid-season daffodil cultivar, 'Mando' and 'Quirinus' respectively, were given HWT on-farm using either chlorine dioxide or 'FAM 30' + 'Bravo 500'; a proposal for a replicated, randomised trial was rejected and instead the four batches of treated bulbs were planted and observed over a two-year-down growing cycle.

At the start of the first growing season 'Mando' bulbs treated with chlorine dioxide had more shoots and shoots were at a more advanced stage than bulbs treated with 'FAM 30' + 'Bravo 500', and subsequently stem (flower) counts for 'Mando' remained consistently and significantly higher following chlorine dioxide treatment. The stems of chlorine dioxide-treated plants were also consistently taller. In contrast, 'Quirinus' showed no such difference between the two HWT additives. There were no significant differences in growth and development between crops from the two treatments in either variety, as measured by their progress through the growth stages. As expected in daffodils in the year after receiving HWT, the usual symptoms of HWT-induced damage - roughened leaf tips, splits in petals and corona, some small 'starry' flowers - appeared in all four blocks of plants and, despite the overall numbers of affected flowers being very low, there were more damaged flowers in 'Mando' plants from the 'FAM 30' + 'Bravo 500' treatment than in those treated with chlorine dioxide; there was no such effect in 'Quirinus'.

In 2012, the second growing season, there was again an impression early in the season that shoots in chlorine dioxide-treated 'Mando' were more numerous than in those treated with 'FAM 30' + 'Bravo 500', and this was confirmed for stem (flower) numbers by statistical analysis. Later in the season stem numbers failed to show a statistically significant difference between the two treatments, and the same applying throughout to the results for 'Quirinus'. Despite this, as Figure 8 shows, there did appear to be a trend towards higher flower numbers in chlorine dioxide-treated plants than in the alternative treatment, and this was in agreement with the actual flower counts obtained by the grower from the four areas. Overall, for 'Mando' the flower yield was poor, supplying only some 58% of the grower's expectation, and this could be explained by the weather in 2012, when the early-flowering 'Mando' was coming into flower during a period of late, very cold weather. This followed a very dry growing season in 2011, which would be expected, in any case, to result in low flower

numbers. In 2012 'Quirinus' flowered after the extreme weather, its actual flower yield exceeded the grower's estimates. There were no significant differences in shoot length, stem length or the rate of passage through GS between the treatments in either variety, and in this year no defects of commercial significance were observed in bud or flower quality in either cultivar or either treatment.

Effective HWT would be expected to have an effect on subsequent levels of base rot and fungal foliar diseases (e.g. smoulder) as well as bulb-scale mites and other pests. Here, the number of smoulder primaries, classic smoulder lesions and plants obviously affected by base rot were low throughout, and at such low incidence it was not feasible to infer any differences due to cultivar or treatment.

The percentage bulb weight increases obtained were poor, the low overall yields almost certainly a result of a very dry growing season in 2011 exacerbated, in the case of the earlier 'Mando', by serious leaf damage as a result of late frosts in 2012. The grower ensured that all four blocks of bulbs were treated in a comparable fashion throughout bulb handling, planting, husbandry and lifting, and the bulb yield was recorded for each block separately. It was therefore of serious concern that the bulb yield of both cultivars was much reduced following HWT with chlorine dioxide *vis-à-vis* the blocks treated with 'FAM 30' + 'Bravo 500'. This was a surprising and disappointing finding, given the prior lack of adverse effects of chlorine dioxide on either cultivar through the two-year growing period. This result is difficult to interpret: first, the apparent adverse effect of chlorine dioxide on bulb yield ran contrary to the other, apparently beneficial, effects of chlorine dioxide seen; and secondly, the effect on bulb yield was a *substantial* one and, therefore, difficult to 'explain away'. Despite the efforts made to ensure comparable handling of the four batches of bulbs, it is possible that some difficult-to-control factor(s) nevertheless *did* affect the results. Possible contributory factors could include within-field differences in drainage or nutrition, unavoidable spray-overlaps at some points in the field, or minor differences in HWT conditions or pre- or post-HWT storage. To some extent, a field factor (such as drainage) would seem to be too much of a coincidence, as the same apparent effect was seen in both cultivars despite their being sited in different fields some 1km apart. Such an effect might also have been expected to have shown up in other measurements, such as stem count or stem length. However, the smaller extent of HWT-damage observed in chlorine dioxide-treated 'Mando' is suggestive of a slightly lower (or shorter) HWT temperature having been applied in comparison with that in the 'FAM 30' + 'Bravo 500' treatment. A more optimal HWT timing and duration in the 'FAM 30' + 'Bravo 500' treatment would have given a better control of pathogens and therefore higher bulb yields, despite the occurrence of HWT damage. This may account for the unexpected result, since only a small range of temperatures separates those that are too low and therefore ineffective in the control of pathogens, and those that are too high and therefore cause heat injury to the daffodil tissues. Another likely contributory factor could be different profiles of bulb grades across the bulb bins. Bringing harvested bulbs from the field,

the grade-out at any one instant might be affected by local variations in soil conditions, or by the particular grade of bulbs that was planted in that spot two or three years earlier; taking bulbs from the yard to the field for planting, the distribution of grades in individual bins could be very much influenced by activity on the grading line at a specific moment, or by how the bulbs have been sorted or handled at some earlier stage of processing. In this project the 'Mando' bulbs treated with chlorine dioxide consistently showed a higher flower number than those from the 'FAM 30' + 'Bravo 500' treatment, and this could simply be a consequence of having – by chance – larger bulbs in the bins used for the chlorine dioxide treatment. For the 'Quirinus' bulbs the likelihood of variations in bulb grade from one bin to another were greater, since the stock consisted of mixed small and large grades and there would be no assurance that the ratio of grades would be the same in all bins. The effects of HWT regimes and bulb grade on bulb vigour and flower yield are well documented in the literature.

Although opportunities to make observations on what are essentially demonstrations, rather than replicated, randomised experiments, may seem convenient and economic, they can sometimes lead to confusion, as in this case. Despite the apparently neutral (or even beneficial) effects of chlorine dioxide treatment on stem length, plant development and flower yield and quality, the use of chlorine dioxide in daffodil HWT cannot be recommended until further checks have been made on its effect on bulb yield.

This conclusion means that UK growers should continue to rely on 'FAM 30' (or similar) biocides for daffodil HWT, along with ensuring that an appropriate temperature-time combination is used. From the results of project BOF 61b, 61c and 63b, this equates to using 'FAM 30' at a rate between 4 and 8L product/1000L water (the higher rate being used for stocks in which there are base rot problems, despite the slightly reduced first-year crop vigour this may produce) and a regular HWT of 3¼ hours at 44.4°C. As an alternative to 'FAM 30' cannot be proposed at this time, the continued reliance on that single product for use in HWT represents a significant risk to the daffodil industry.

Following these findings there are some obvious needs for further applied (near-market) research on daffodil HWT:

- Clarify the effect of HWT with chlorine dioxide on bulb yields
- Test chlorine dioxide for use in cold-dipping
- Test chlorine dioxide specifically against stem nematode (which does not appear to have been done)
- Develop protocols for maximising the effectiveness of 'FAM 30' and similar biocides in HWT
- Integrate the finding of these projects with those of BOF 74 and 74a (testing new fungicides for control of base rot in HWT).

Acknowledgements

The HDC and the author are indebted to Mr Tom Charlton for allowing his bulb stocks, HWT facilities and land to be used in this study. Thanks are due to Mr Adrian Jansen, Industry Representative for the project, for his encouragement and helpful comments on the text.

Technology transfer and other outputs

Talks on HDC-funded projects including BOF 70a were (or will be) given at the following meetings:

- HDC Narcissus Technical Seminar, Spalding, 14 April 2011
- HDC Narcissus Technical Seminar, Camborne, 5 May 2011
- HDC/BDGA Narcissus Technical Seminar, Spalding, 15 November 2012
- HDC/BDGA Narcissus Technical Seminar, Spalding, 22 May 2013

Information on BOF 70a was (or will be) included in the following articles:

- Hot new treatments for daffodils, HDC News no. 172 (April 2011), pp.28-30
- Hot-water treatment of daffodil bulbs, HDC Fact Sheet (due 2013)