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Project leader:	Rob Lillywhite, University of Warwick
Report:	Third annual report, December 2018
Previous report:	Second annual report, December 2017
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AUTHENTICATION

We declare that this work was done under our supervision according to the procedures described herein and that the report represents a true and accurate record of the results obtained.

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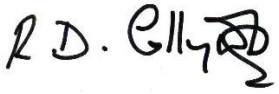
Signature  Date 10th March 2019

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GROWER SUMMARY

Headline

At the end of the third year of the project, growers should be aware of the following:

- First year results of the filtration and UV sterilization experiments have demonstrated that there was no negative treatment effects on flower production
- Thermal treatment of bulbs at 60°C for up to five minutes did not have a negative effect on plant growth or flower production but longer and hotter treatments did impose significant reductions in both
- Commercial testing of a chlorine dioxide dosing system has demonstrated that it can be successfully retrofitted to hot water treatment (HWT) tanks and that it appears to be an effective biocide
- In-tank fungicide testing confirms previous HDC guidelines that active ingredients are lost in HWT

Background

Hot water treatment (HWT) of narcissus bulbs is used to control pests and diseases, notably stem nematodes, bulb scale mites and Fusarium basal rot. This has been the standard approach for at least 70 years. For most of that time, formalin was added to HWT tanks as a general biocide i.e. to reduce inoculum in the tank water, however approval for formalin was withdrawn in 2008. Work in AHDB funded project BOF 061a (Lole, 2010) identified FAM 30 as a viable alternative and this has since become standard practice in the UK. However, FAM 30 is expensive in comparison to formalin and the result has been that growers do not always use it at the required rate and this issue is exacerbated since FAM 30 rapidly depletes in tanks under a high bioload.

Other biocide alternatives have been considered, notably chlorine dioxide which was demonstrated to be effective against spread of Fusarium (Chastagner and Riley, 2002) and is believed to be currently used by American Narcissus growers. However, in AHDB Horticulture project BOF 061a (Lole, 2010), chlorine dioxide was assessed alongside a number of alternative biocides, but was not considered further as FAM 30 was found to be more effective. The use of chlorine dioxide was further reviewed in BOF 070 (Hanks, 2010) which suggested that additional investigations were required before it could be recommended to growers.

Other biocides previously examined include peroxyacetic acid (Hanks and Linfield, 1999), hydrogen peroxide and UV (Stewart-Wade, 2011) but tank bioload was again found to reduce their efficacy so further commercial scale evaluation is required before they can be recommended. Non-chemical biocidal approaches, e.g. UV and thermal treatment, have been used in other water-based treatment systems and appear to offer a viable alternative to chemical approaches but their efficacy is known to be very dependent of water clarity, which is a problem with high bioload HWT (Pettitt, 2016). The issue of high HWT tank bioload was reported in BOF 070 (Hanks, 2010) and generating a solution to this

issue is probably key in improving the efficacy of all biocides and biocidal approaches (and probably fungicides as well).

Project aim

The aim of this project is to examine a range of candidate biocides (chlorine dioxide, hydrogen peroxide and didecyl dimethyl ammonium chloride) and physical approaches (thermal and UV treatment) for their efficacy and ease of use against stem nematode and Fusarium basal rot.

The project has been divided into eight objectives:

1. Review of the literature
2. *In vitro* laboratory tests
3. Assess the feasibility and cost of retrofitting biocide delivery systems to existing HWT tanks
4. Assess impact of different treatments on infrastructure
5. Small-scale tank tests
6. Commercial scale testing
7. Field trials
8. Health and safety considerations

Summary

This report covers the period January 2018 to December 2018 which is the third year of the four year project to investigate new or improved biocidal approaches in the hot water treatment of daffodil bulbs. This period saw the completion of on-farm testing and continuing monitoring of field crops.

Filtration and UV trials

On farm trials to examine both filtration and UV sterilization were carried out in September 2017 at Carwin Farm in Cornwall. Trial methodology was described in the 2017 Annual report. The bulbs were replanted in autumn 2017 and assessed in spring 2018. Stem length was measured on the 26th March 2018 and the number of flowers was assessment on 17th April 2018. Flower production will be assessed again in Spring 2019.

These initial first-year results show that neither filtration nor UV sterilization appear to have any negative effect on flowering. It is interesting, if not unexpected, that dipping using clean water gave better results. A fuller analysis will be carried out on flowering in spring 2019 when the crop will have been down for 18 months.

Thermal treatment of bulbs

Thermal treatment of bulbs was not included within the project remit but a number of growers expressed an interest in this approach. The decision was therefore made to trial it and include the results in this project. Small-scale laboratory tests were conducted at the University of Warwick and on-farm testing

was carried out in Cornwall. Temperatures between 60 and 70°C degrees, and durations between 3 and 12 minutes were tested. Assessment was a mixture of observation and disease incidence

Although there was some inconsistency in the approach and results, taken together the trials do provide some positive results. Short dips in the range 60-65°C of around five minutes did reduce the incidence of rots with few negative effects. The temperature is likely hot enough to provide control of surface pathogens and short enough to avoid physiological damage, however dips at 70°C or at 60-65°C for more than five minutes had a negative impact on bulbs. Given the logistical difficulty of handling large volumes of bulbs on farm it is unclear at this stage whether, with such small tolerances and the potential for significant losses, this method of sterilisation is practical.

Chlorine dioxide testing

Chlorine dioxide was demonstrated to be effective against spread of *Fusarium* (Chastagner and Riley, 2002) and is believed to be currently used by American Narcissus growers. However, in AHDB Horticulture projects BOF 061 and 061a, ClO_2 was assessed alongside a number of alternative biocides, but was not considered further as FAM 30 was more effective. The use of ClO_2 was further reviewed in BOF 070 with the recommendation that further investigations were required before it could be recommended to growers. This project continues that work, and in 2018 undertook full on-farm testing of a commercial chlorine dioxide dosing system.

Scotmas Ltd undertook trials on Jack Buck Farms in Lincolnshire in July and August 2018. An automated dosing system, using precursor chemicals, was installed on one of the HWT tanks (pictured opposite). This uses a concentration monitoring system to achieve a set residual chlorine dioxide value which indicates that all pathogens have been destroyed. Trials were conducted over a period of two weeks until a steady residual level of ClO_2 could be maintained (in this case 1.6ppm).

Laboratory analysis of tank water confirmed that the system controlled 99% of all pathogens which is considered a successful outcome.

Analysis of fungicide concentrations in HWT

In 2018, AHDB commissioned some additional work to examine the degradation of fungicides within HWT of bulbs. That work is reported here for convenience. In spring 2018, five growers agreed to take part in fungicide concentration monitoring trials. Gowers were sent pre-numbered collection tubes and a protocol for sampling. Sampling began on the first day of dipping and continued at regular intervals for the next week. The aim was to track how fungicides concentrations varied over time using the grower's normal starting and refill quantities of fungicides. The samples were returned to Warwick Crop Centre and levels of chlorothalonil (Bravo 500) and thiabendazole (Storite or Tezate) were analyzed, using HPLC with UV detection. Standards of known concentration were run alongside the samples to allow standardization of the results. The recommended rate for chlorothalonil is 500ppm (equivalent to 1l Bravo 500 per 1000l water) although half-rate is sometimes used. Thiabendazole is normally used (in the presence of an acidifier) at 275ppm (1.25l Storite per 1000l water). Following collation of the samples and data, two sets of observations were excluded as not being sufficiently robust or complete.

The three growers all used chlorothalonil as their main fungicide although one also used thiabendazole as well. The key outcome was that no grower, after two days, had a measurable level of chlorothalonil that was greater than 35% of the dosed rate. At the end of the second day of dipping, growers 1, 2 and 3 had 68ppm (27%), 176ppm (35%) and 97ppm (19%) of their target values, respectively. These results support the findings of the HDC Factsheet 10/13 that states that a stable concentration of about 25% of the target concentration is achieved after two days.

Concentrations for Grower 3 did vary over the course of dipping which might be due to the use of thiabendazole as well or another the use of top-loading tanks which might result in accumulation of more sediment than front loading tanks.

Grower 2 had the highest levels measured and these were maintained well into the season and it may be relevant that the samples from Grower 2 were also consistently the clearest in terms of visual appearance/sediment level. Earlier work in BOF 061c also showed relatively low levels of chlorothalonil compared to dosed rate and it was suggested that this was in part due to the sedimentation of the chemical and this is certainly backed up by the very high levels of chlorothalonil detected in the end of year tank sediment.

Thiabendazole (used only by Grower 3) showed a similar pattern to chlorothalonil with an initial value of approximately 50% of dosed rate which then stabilized at a lower level after the first two days of dipping.

Overall, the results support the findings published in the HDC Factsheet 10/13 that active ingredients are 'lost' from the circulating dip during HWT. To some extent this is as expected, as fungicides will only provide protective control of pathogens if they are adsorbed by the bulb or adhere to the bulb surface.

However, loss of active ingredients also occurs through heat and chemical degradation and through sequestration into tank sediments. The ratio across these different losses is unknown although it may be possible to reduce any negative impact through improved understanding of the chemical interactions between different fungicides, biocides and acidifiers. However, this is difficult as the continuing loss of active ingredients, and the different rates used, make this a never-ending task. Minimising tank sediments and bioload through improved bulb cleaning is easier to achieve.

Financial Benefits

At this stage of the project, it is possible to provide an initial assessment of the financial benefits arising from the research. Based on the approach and costs incurred during the chloride dioxide trials undertaken in Lincolnshire, we estimate that the cost of using FAM 30 and chloride dioxide are £4.45 and £7.00 per treated tonne of bulbs, respectively. The use of FAM 30 requires no set up costs while that for the chloride dioxide system were between £5 and £10k based on the system used.

Action Points

Growers should continue to ensure that any bulbs destined for HWT are as clean as possible. This will reduced both tank bioload and sediment and improve the efficacy of all chemical ingredients.

Growers should consider the use of automated chlorine dioxide dosing systems as on-farm trials have shown them to be successful. Early indications suggest no downsides although two years of monitoring will be undertaken before a final recommendation is provided.

Growers should be cautious of using manual chlorine dioxide dosing during HWT of daffodils as the on-farm trials have demonstrated that automated dosing is require to achieve control of pathogens.

SCIENCE SECTION

Filtration Trials – bulb assessments

On farm trials to examine both filtration and UV sterilization were carried out in September 2017 at Carwin Farm in Cornwall. These trials were described in the 2017 Annual report. The bulbs were replanted in autumn 2017 with the intention to assess then in spring 2018 and 2019. The first assessment was carried out on the 26th March 2018 when stem length was measured (five stems were measured from each of five rows for each treatment). A second assessment on 17th April 2018 counted flower number (flower counts were taken in a randomly selected 1.5m length of each of five rows for each treatment) (Table 1).

Figure 1. Trial plots March 2018, Carwin Farm

The treatments were:

- Batch 1 was dipped in fresh water with additional filtration.
- Batch 2 was run after batch 1 using both filtration and UV sterilization.
- Batch 3 was run using end of season water with the remnants of the season's standard chemicals, with additional filtration.
- Batch 5 was run with end of season water using both filtration and UV sterilization.

Table 1. Stem heights and flower counts for trial bulbs spring 2018

Batch	Mean stem height (cm)	Mean flower count
1. Clean water. Filtration only	22.2	24.6
2. Clean water. Filtration + UV	21.8	10.2
3. Dirty water. Filtration	18.2	4.0
5. Dirty water. Filtration + UV	18.7	7.4

These initial first-year results show that neither filtration nor UV sterilization appear to have any negative effect on flowering. It is interesting, if not unexpected, that dipping using clean water gave better results. A fuller analysis will be carried out on flowering in spring 2019 when the crop will have been down for 18 months.

Thermal treatments - bulb assessments

Work in year one of the project demonstrated that thermal treatment was a very effective biocidal approach with complete control of Fusarium spores being achieved at temperatures above 60°C. In practice, this means sterilizing the tank water overnight, in the absence of bulbs, by increasing the temperature to 60°C. This ensures that the water is sterile at the start of every day. This was tested at both Carwin and Bosahan Farms in Cornwall and was successfully achieved without any technical issues. The effectiveness of this approach is unknown as it is difficult to separate the benefits of the thermal treatment from on-going chemical treatments, both biocides and fungicides. There is an obvious financial penalty to additional heating of water but it isn't possible to estimate if this is cost is offset, or good value, due to lower incidence of disease while the bulbs are in the ground. In-field observation could not detect any difference between untreated and treated bulbs.

During the Narcissus Growers Workshops in May 2017 there was interest in using thermal treatments on bulbs themselves. Although bulbs are normally dipped at 44°C to avoid tissue damage, while at the same time controlling stem nematodes and bulb scale mites, the effect of short-term dipping at 60°C or more was unknown. Whilst thermal treatment of bulbs was not part of the project remit, it was decided to examine it as a student project. This was funded by the University of Warwick but the results are reported here as a matter of convenience.

During the summer of 2017, laboratory and on-farm trials were undertaken at Warwick Crop Centre and Carwin Farm, respectively. Bulbs were dipped at either 60°C, 65°C or 70°C for either 3, 5 or 10 minutes.

Laboratory trials at Warwick Crop Centre

Commercially obtained bulbs were subjected to 11 treatments which comprised a control and ten further treatments using a combination of time and temperature (Table 2). The trials used the existing small-scale testing facility at Warwick Crop Centre. This comprises a series of temperature controlled water baths. Each treatments used a batch of ten bulbs. Post-dipping, five bulbs were incubated at 25°C for 30 days while the other five were planted into individual pots. This dual approach allowed the development of rots to be assessed very quickly and also allowed any heat-induced physiological damage to be assessed in spring 2018.

Table 2 Thermal treatment at Warwick Crop Centre

Treatment number	Duration (minutes)	Temperature (°C)
T0	-	-
T1	3	60
T2	5	60
T3	8	60
T4	10	60
T5	3	65
T6	4	65
T7	5	65
T8	10	65
T9	3	70
T10	5	70
T11	10	70

The incidence of basal rot following the 30 day incubation period in shown in Figure 2. It is clear from the results that temperatures of 65°C and above increased the incidence of rots observed in bulbs. Given the contrast between the bulbs incubated at 60°C and those at 65°C and above, we believe that it is rots from heat-induced damage rather than basal rot that is the main problem. In contrast, bulbs treated at 60°C are no worse, and possibly better, than the control. This suggests that treatment at 60°C for up to 5 minutes may be effective in controlling surface pathogens.

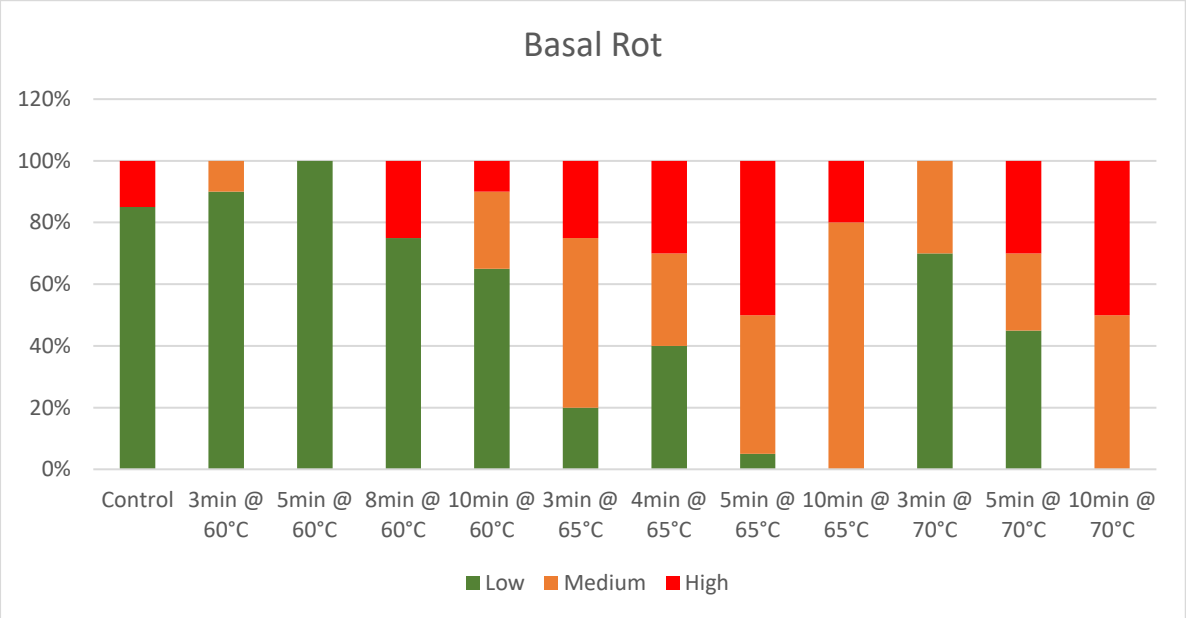

Figure 2 Incidence of basal rot following 30 day incubation (Warwick thermal trial)

Bulb assessments 2018

In spring 2018, the individually planted bulbs (20 per treatment) were assessed for flower numbers and subsequently the bulbs were dug up, dissected and assessed for rots (Table 3).

Table 3 Flower counts across 20 pots for bulbs dipped at 60-70°C for 3-10 minutes

Treatment	Duration (minutes)	Temperature (°C)	Flower Count
T0	-	-	4
T1	3	60	11
T2	5	60	3
T3	8	60	9
T4	10	60	6
T5	3	65	13
T6	4	65	10
T7	5	65	20
T8	10	65	5
T9	3	70	2
T10	5	70	3
T11	10	70	0

In contrast to the incubation study, the results were a little counter-intuitive. In terms of temperature, treatment at 65°C (Treatments 5-8) provided highest flower counts compared to 60°C and 70°C. The effect of duration was also inconsistent but somewhere between three and five minutes seems to be ideal. The low number of flowers for the control treatment was unexpected, however the 70°C treatments had a clearly negative effect on the bulbs. It should also be remembered that these were pot-grown first-year flowers so may not be representative of commercial practice.

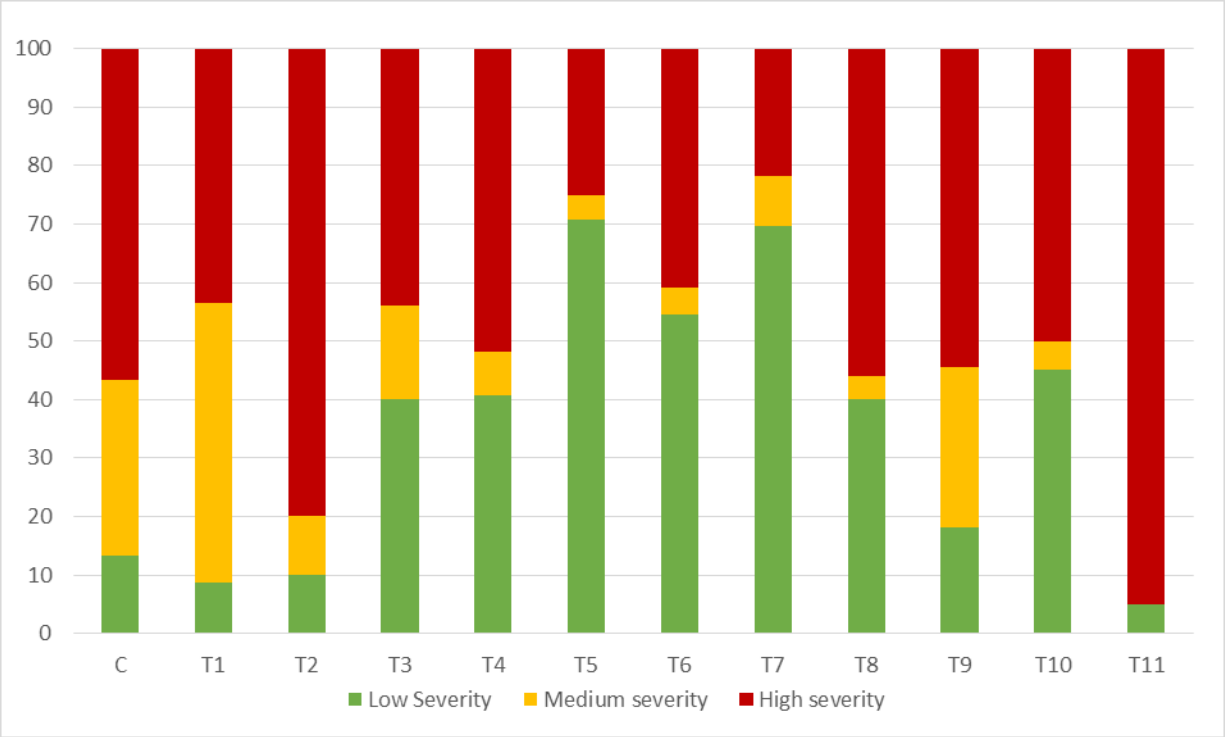

Figure 3 Base rot scores for bulbs dipped at 60-70°C for 3-10 minutes, spring 2018

When the same bulbs were assessed for rots there appeared to be a correlation with flower number. In the case of the control treatment it would appear that the low flower number was due to a high level of rot in the bulbs. In the case of the 65°C treatments the relatively low level of rot correlates well with the high flower number also observed. This suggests that 65°C was the optimum temperature with a duration somewhere between three and five minutes. The fact that 65°C was optimum for flower production but worse than 60°C for initial rot development is not helpful but does illustrate the difficulty in obtaining consistent results in this type of research. The major finding is that a short dip between 60°C and 65°C does not appear to cause major physiological damage to bulbs although the effects on first year flowers is variable.

Carwin Farm

Heat treatments were also trialled at Carwin Farm in Cornwall. Two varieties (Finland and St Patrick's Day) were used as they had significant base rot and so were of little commercial value. The treatments were (1) a control which wasn't dipped, (2) dipped for 5 minutes at 60 °C and (3) dipped for 11.5 minutes at 60°C. Core temperature was recorded during the dipping process. Dipping for 5 and 11.5 minutes raised the core temperature to 51 and 56°C respectively (ambient was about 20°C).

The bulk of the bulbs were planted as normal but two subsamples of bulbs were cut open to assess the level of rot. The first, straight after dipping, and the second after being incubated for 28 days at 25°C in moist conditions (to encourage rots to develop). Post-dipping, the levels of rot across both varieties were fairly consistent with 20-25% of Finland and 25-35% of St Patrick's Day bulbs showing evidence

of rots (Figure 4). After incubation the 5 minute treated Finland bulbs showed slightly lower levels ofbase rot than the untreated, however the 11.5 minute treated Finland bulbs and all of the St Patrick's Day showed very high levels of damage (65-92.5%) (Figure 5). Following dipping, the remaining bulbs were replanted for assessment in spring 2018 and 2019.

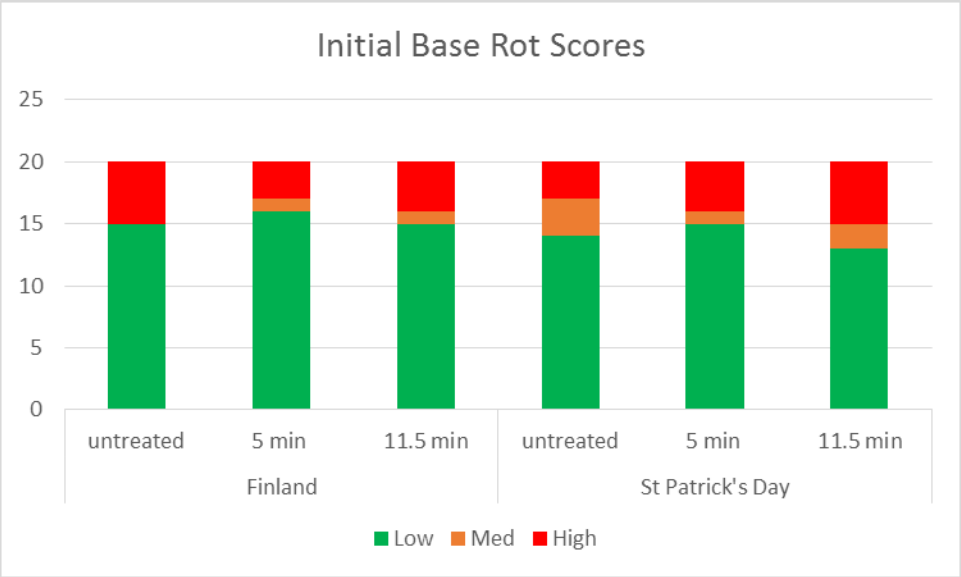

Figure 4 Initial base rot scores straight after dipping

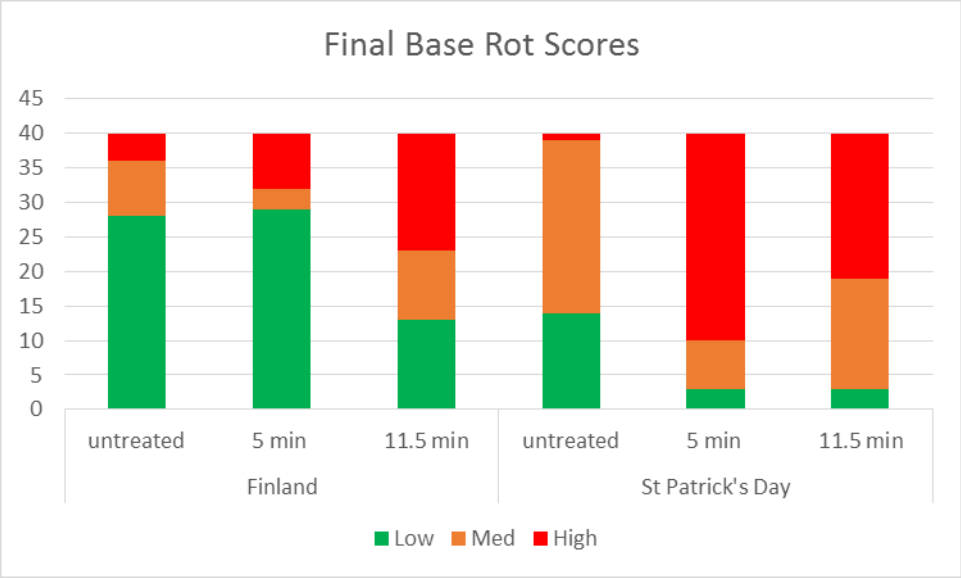

Figure 5 Base rot scores straight after incubation

Flower assessments

The trial plots were assessed on 26th March 2018. The number of flowers were counted in a representative 1.5m length of each of the two rows for each treatment (Table 4 and Figure 6). The results show that dipping bulbs at 60°C for five minutes had a slightly negative effect in comparison to not dipping the bulbs but the difference was not significant. However, dipping for 11 minutes did have a significant negative effect, suggesting that a dip of this length is damaging to the bulbs.

Table 4 Flower numbers along a 1.5m length for bulbs dipped for 0, 5 and 11 minutes.

Variety	Time at 60°C (min)	Mean flower count
Finland	0	29.5
Finland	5	24.0
Finland	11	2.0
St Patrick	0	24.5
St Patrick	5	20.0
St Patrick	11	0.5

Work in year 1 of this project demonstrated that dipping for three minutes at 60°C provide effective control of (surface) Fusarium, so while it is too early to recommend this approach, in situations where pest control is not a priority, short dipping is likely to be an effective approach to surface sterilisation of bulbs without affecting bulb physiology or flower yield. However, it is recognised that the logistical difficulties of short dipping are considerable, especially with drive-in front-loading HWT systems. The trial will be assessed again in spring 2019.

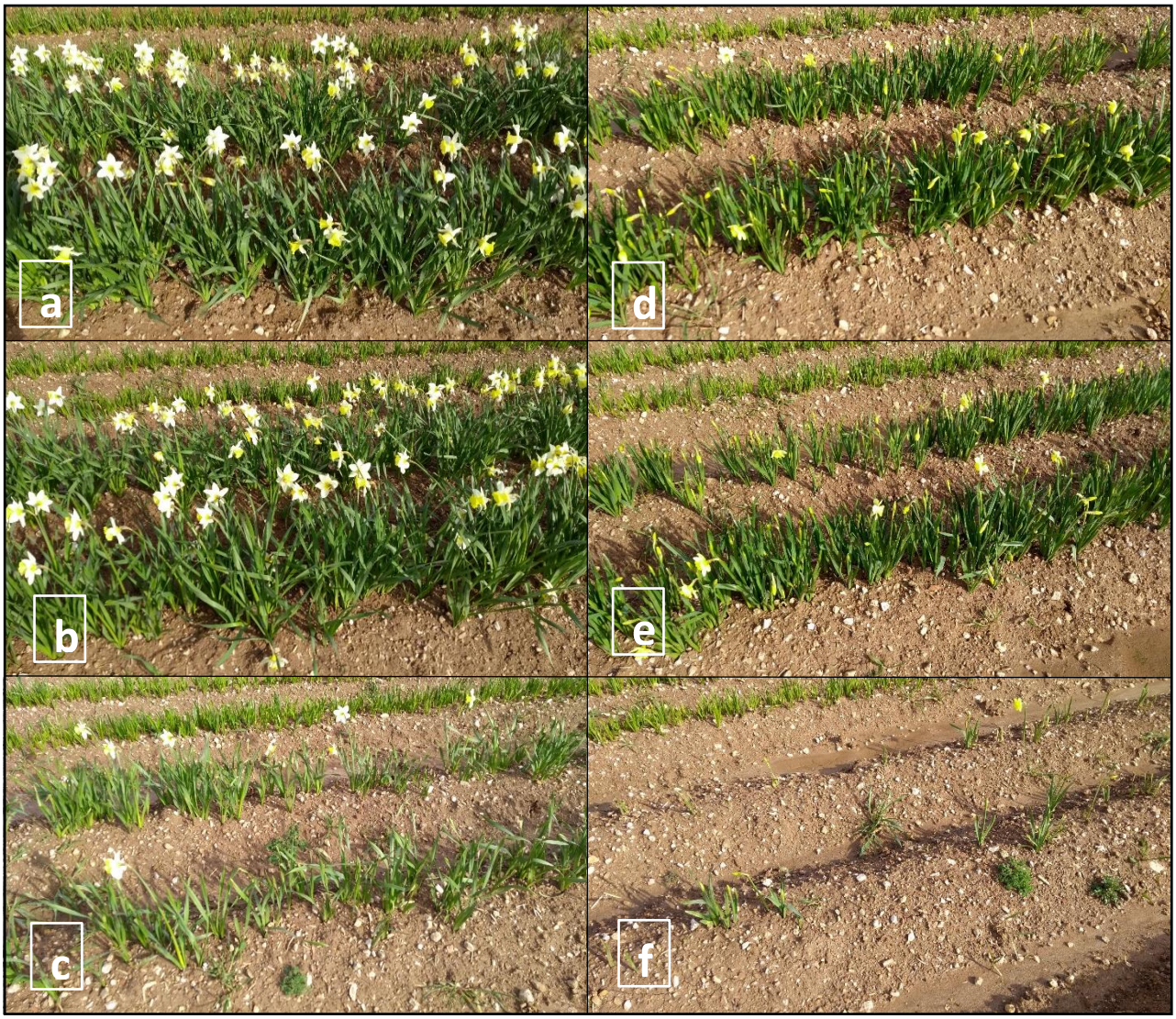

Figure 6 Year 1 flowers post thermal treatment (a = Finland, control; b = Finland, five minutes; c = Finland, eleven minutes; d = St Patrick's Day, control; e = St Patrick's Day, five minutes; and f = St Patrick's Day, eleven minutes).

Thermal treatments conclusion

Although there was some inconsistency in the approach and results, taken together the trials do provide some interesting results. Short dips in the range 60-65°C of around five minutes can reduce the incidence of rots with few negative effects. The temperature is likely hot enough to provide control of surface pathogens and short enough to avoid physiological damage, however dips at 70°C or at 60-65°C for more than five minutes can have a negative impact on bulbs. Given the logistical difficulty of handling large volumes of bulbs on farm it is unclear at this stage whether, with such small tolerances and the potential for significant losses, this method of sterilisation is practical.

Water clarity in HWT tanks

One of the major issues in HWT is preventing bioload, principally soil, from entering the tanks. Although all growers clean their bulb stocks prior to dipping, some soil still adheres to bulbs which is subsequently released into the tank upon dipping. It is this bioload which reduces the efficacy of chemical treatments.

During the course of our trials in 2018, water samples were collected from a variety of growers throughout the dipping season. It was notable that these samples, looked at together, exhibited an obvious variation in appearance depending on the site of collection (Figure 7). We attribute this to the volume of soil entering the tanks.

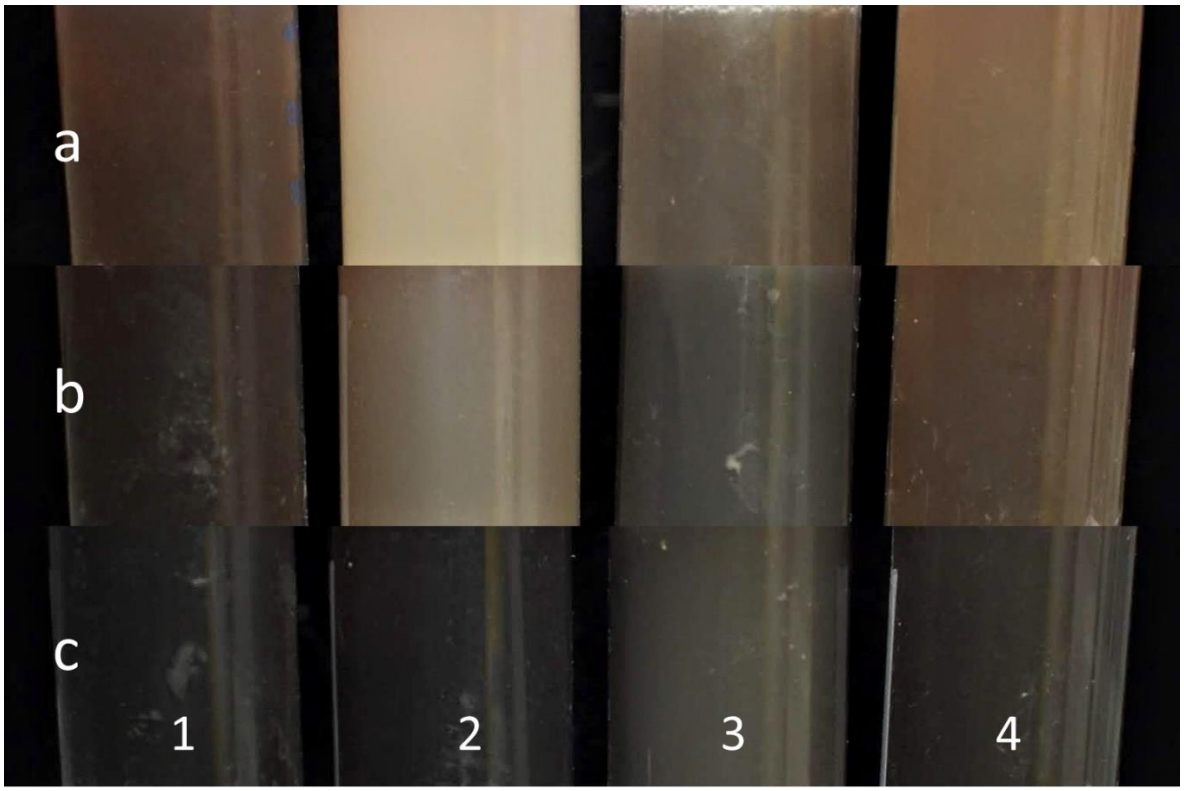

Figure 7 Water samples collected from the dipping tanks of 4 growers (1-4). Samples 1a-4a were taken after the first run of the season, samples 1b-4b were taken on the second day of dipping and samples 1c-4c were taken late in the dipping season.

This comparison of HWT water clarity shows that there is significant variation in the amount of soil particles entering the HWTs across different growers, despite the efforts made by all growers to clean their bulbs effectively before treatment. Given the negative impact that increased bioload has on the efficacy of all currently used chemical treatments it may be beneficial for growers to try and achieve the levels of bioload observed in Grower 2's HWT. This means employing all possible methods of removing soil from bulbs prior to dipping, taking steps to remove the collected sludge from the bottom of tanks between bulb loads and potentially changing the water more frequently.

Chlorine dioxide

Chlorine dioxide (ClO_2) is increasingly being used by the potable water treatment industry as an alternative disinfectant to conventional chlorine. Dissolved ClO_2 is an oxidiser and as such chemically reacts with microbiological pathogens by destroying their cell wall. The use of dissolved ClO_2 for disinfection has tended to be in applications where the treated water is reasonably clear, however, its use in sewage treatment is increasing, giving encouragement for its application in systems demanding high concentrations, such as agricultural disinfection and sterilisation.

Chlorine dioxide was demonstrated to be effective against spread of *Fusarium* (Chastagner and Riley, 2002) and is believed to be currently used by American Narcissus growers. However, in AHDB Horticulture projects BOF 61 and 61a, ClO_2 was assessed alongside a number of alternative biocides, but was not considered further as FAM 30 was more effective. The use of ClO_2 was further reviewed in BOF 70 which recommended that further investigations were required before it could be recommended to growers.

Chlorine Dioxide can be dosed in two ways. A solution with pre-dissolved ClO_2 can be added to the system; although, the transportation of high concentrations of highly reactive chemicals presents additional challenges. This approach has been trialled in the past by some commercial growers but was found to be logistically difficult and ineffective. Alternatively, ClO_2 can be dissolved into water by performing on-site chemical reactions and dosing via an automated dosing system. This method yields more accurate concentrations and reduces the potential for dangerous chemical reactions during transportation. In addition, ClO_2 concentration can be continuously recorded using chemical probes or real-time chemical analysis.

A number of uncertainties currently limit the employment of ClO_2 into current HWT facilities, most importantly, the required dosing rates need to be adjustable for varying biological demand; which subsequently varies between bulb batches and turbidity levels. However, a major benefit of ClO_2 is the elimination of chemical by-product and need for controlled chemical disposal; apparent when employing current disinfectants and fungicides. This may have significant benefits for operators who can adjust their dosing concentrations in accordance with biological load and turbidity.

Chlorine Dioxide disinfection

Chlorine dioxide can be produced by mixing solutions of sodium chlorate and hydrogen chloride to produce chlorine dioxide, sodium chloride and water. The gaseous chlorine dioxide is then dissolved into the water using a solution method. Gaseous ClO_2 is extremely flammable, becoming explosive at high concentrations, and is potential dangerous to handle. Solute chlorine dioxide can be achieved using an alternative chemical process which eliminates the need for a gaseous stage and is safer as a result. It has been shown to be an effective biocide and disinfectant even at low concentrations and is increasingly employed in the potable and waste water treatment industries. One benefit is the minimal corrosion caused to infrastructure rendering it applicable to distribution networks and metal piping.

Chastagner and Riley (2002) showed ClO_2 at 5 ppm was effective at controlling the spread of *Fusarium inoculum* during HWT; however, 5 ppm may be excessively high and therefore a minimum of 2.5ppm should be considered. Additionally, dissolved ClO_2 remains highly affective between the pH ranges of 4 to 10. Cayanan *et al* (2009) compare the treatment efficacy for different plant pathogens using dissolved ClO_2.

A number of considerations need to be taken into account when assessing the feasibility of ClO_2 disinfection. Firstly, a high concentration may be required to overcome the initial bioload of the water, thus setting the maximum dosage. Secondly, ClO_2 must be synthesised on-site given the dangers of transportation, requiring capital investment on storage and chemical equipment. Thirdly, the dosing systems are bespoke and will require consultation to prescribe the optimal design.

Small scale tank tests

Production of Fusarium chlamydospores.

Chlamydospores of *Fusarium oxysporum f.sp. narcissus* isolate 139 were prepared by using the method described in the 2016 report for this project. All samples were pooled and the concentration of spores was measured using an improved Neubauer haemocytomer. The samples averaged 87 x 10^4 spores per ml of suspension giving final number of 1131 x 10^4 spores used per treatment.

Chlorine dioxide reaction kinetics

Chlorine dioxide was produced for these small tanks tests using Cidox 300 tablets (Scotmas). Cidox 300 is a trade name of a dissolvable tablets that deliver small scale amounts of ClO_2 in a safe and fast manner. Trials were run to establish the concentration and dissociation time of the tablets.

A water bath was set up at 44.4°C and a series of tests were run to establish the number of tablets and timing of addition in order to achieve 5ppm and 10ppm concentration in a single and continuous delivery. Measurements of the chlorine dioxide levels were performed using ChlordioXense CS300 device from Palintest Water Analysis Technologies (Palintest, 2018). The water baths are made by Clifton Range and hold 35L of water (Nickel, 2018). They provide a precise temperature regulation and water circulation.

Bulb dipping

Daffodil bulbs, cultivar Actaea, were excavated from experimental fields at Warwick Crop Centre on 12-06-2018 and set to air dry in single layer (Figure 8). Prior to dipping, the bulbs were gently brushed by hand to remove the dried soil and loose dried scales. Twenty bulbs were used per each bath.

Figure 8 Daffodil bulbs set to dry.

The following treatments were set up, each containing twenty bulbs with addition of:

T1 - Clean water

T2 – Clean water and FON spores

T3 – Clean water, FON spores and single application of 5ppm ClO_2

T4 – Clean water, FON spores and maintained level of 5ppm ClO_2

T5 – Dirty water, FON spores and single application of 5ppm ClO_2

T6 – Dirty water, FON spores and maintained level of 5ppm ClO_2

T7 – Clean water, FON spores and single application of 10ppm ClO_2

T8 – Clean water, FON spores and maintained level of 10ppm ClO_2

T9 – Dirty water, FON spores and single application of 10ppm ClO_2

T10 – Dirty water, FON spores and maintained level of 10ppm ClO_2

Experiments were run over two days. Clean water treatments (T1, T3, T4, T7 and T8) were run on 04-07-2018 while the dirty water treatments (T2, T5, T6, T9 and T10) were run the next day (Figure 9).

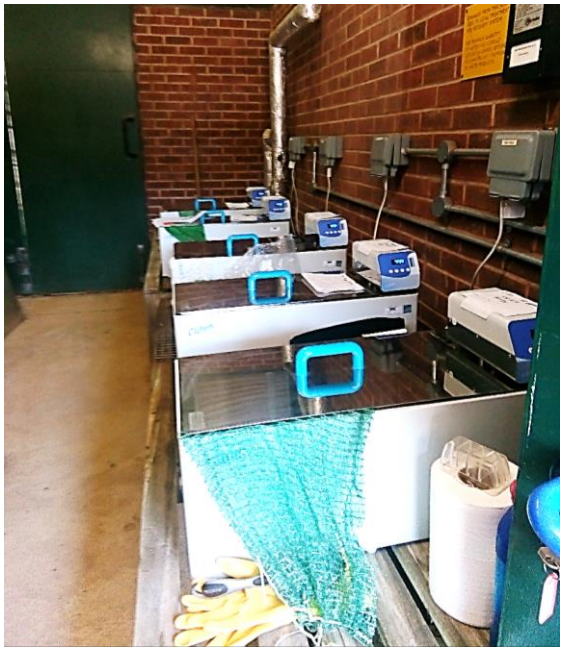

Figure 9 Baths during the experiment on day one. The green net visible at the front holds the daffodil bulbs.

At the start of each day, all baths were cleaned with bleach, filled with 35 litres of fresh tap water and set to warm up to 44.4°C. Treatment bulbs were immersed into each bath and time was noted. There was no drop in temperature of the bath after adding the bulbs. The spore suspensions were poured into treatments where appropriate immediately after adding the bulbs. After five minutes of mixing a sample of around 30ml of water was taken from each bath for serial dilutions. The appropriate number of Cidox 300 tablets were then added in single burst or maintained as per treatment requirement. After 180 minutes from immersion of bulbs, another water sample was taken for serial dilution, bulbs were packed into individual clean boxes and moved to dry overnight.

Figure 10 Inside the water bath on day two – 'dirty' water treatment. The green net holds the daffodil bulbs. The white foam residue is from dissolving of Cidox 300 tablets.

For dirty water trials, soil was added to the tanks (Figure 10). In previous project work ground-up daffodil bulb scales were used to create the dirty water. However, after investigating samples from a commercial farm it was

noted that the composition of the particles suspended in water consisted of soil rather than plant material. Consequently, 1.6g/L was used to produce 'dirty' water for this experiment. 56g of autoclaved M2 Levington compost was suspended in water for each of the treatments T5, T6, T9 and T10. The soil was added after the first water sample was taken and before the ClO_2 was applied.

The bulbs were then air dried overnight before being incubated for 28 days at 25°C. After incubation the bulbs were dissected and scored from 0-10 for basal rot.

Serial dilutions of the water samples were then performed and each dilution (100 µl) plated in duplicate. Plates were incubated at 18°C (+/- 2) for five days, the number of colony forming units (cfu) recorded and the concentration of viable spores/ml calculated.

Results

Culture from water samples

Treatment T1 (no spores added) did not show any CFU at the beginning or the end of the HWT. That indicated that there were no fungal pathogens present in the tap water and if any amount of pathogen was rinsed out from the bulbs it was controlled by the temperature alone. The bulb infection results confirm this as innate infection level was low in all the bulbs.

Total reduction of FON spores during the hot water treatment with addition of ClO_2 was observed for treatments T4, T5, T6, T9 and T10. Treatment T2 had no ClO_2 added and the reduction was partial from 1087.5 to 127.5 average CFU/ml (Figure 11).

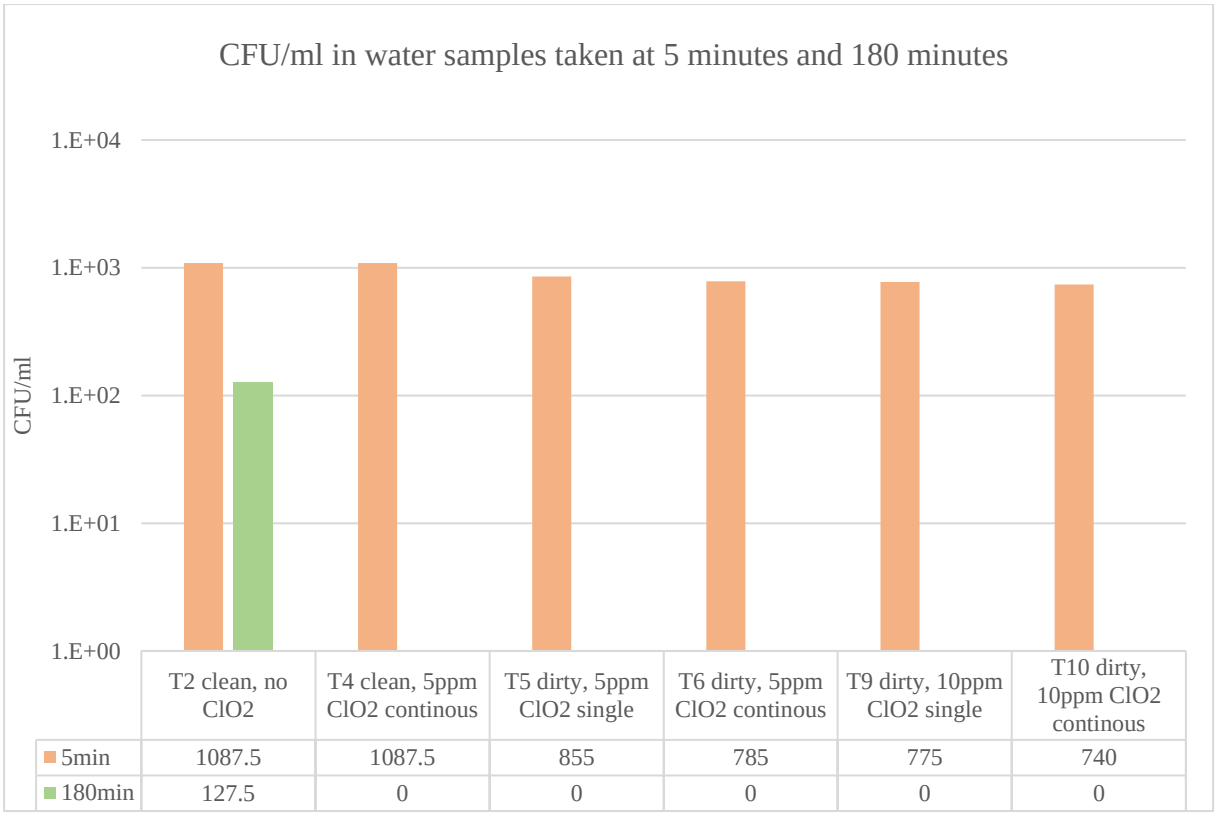

Figure 11 Average number of CFU/ml of water before and after treatment.

Bars present average numbers of CFU/ml in water taken after 5 minutes of mixing and at the end of 180 minutes of each treatment. The conducted experiment confirmed that the viability of FON spores was reduced to 0 with addition of ClO_2 both in clean and dirty water but the thermal treatment (44.4°C) alone was not sufficient to control the FON spores in water.

Bulb infection results

After four weeks of incubation all bulbs were dissected and scored according to ten point scale. Bulbs that scored 0-2 were categorised as low severity infection, bulbs that scored 3-5 were categorised as medium severity infection and those that scored 6-10 were categorised as high severity infection (Figure 12).

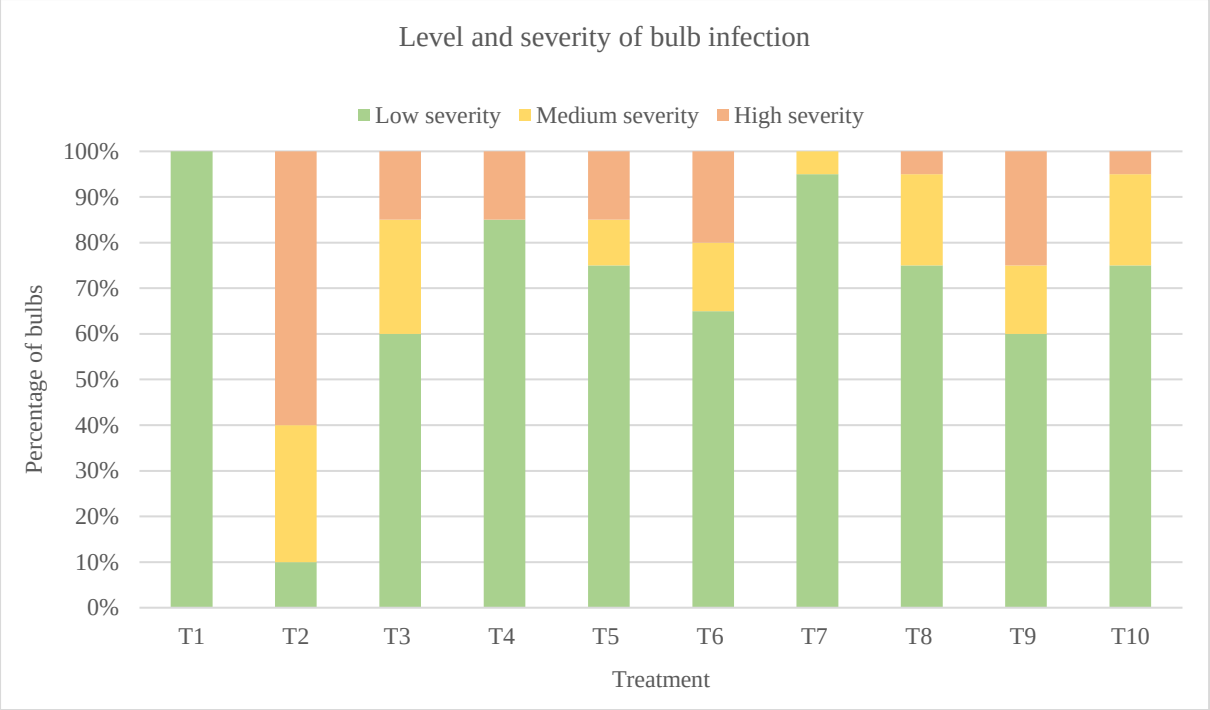

Figure 12 Percentage of bulbs with low, medium and high severity symptoms of Fusarium basal rot after 28 days of incubation.

In addition to scoring the bulbs as low, medium or high severity, bulbs were also assessed for any sign of infection (Table 5). This allows for some evaluation of the effectiveness of either single or maintained dosing of ClO_2 and also differences that might arise from the use of clean and dirty water. T1, the control, and T2, no biocide, were as expected, at different ends of the infection spectrum. Two treatments types (T3 v T4 and T9 v T10) suggest that maintained dosing is better than a single dose, while one (T7 v T8) was neutral and one (T5 v T6) that single dosing was better than maintained. Dosing in clean water was better than dirty water in all four treatment types.

Table 5 Percentage of bulbs with zero infection.

	T1	T2	T3	T4	T5	T6	T7	T8	T9	T10
Percentage of bulbs with a score of 0	65	5	50	70	40	35	65	65	50	55

Discussion

The results from serial dilutions show that HWT alone lowers the amount of viable spores of FON. While HWT is known to be effective in controlling the stem and bulb nematode and incidental control of other pathogens (Hanks, 2013) it is not enough to control FON. This result confirms the need for additional treatment to eradicate FON from the bulbs.

The innate level of bulb infection was very low and the highest infection levels were detected in the treatment without chlorine dioxide, providing suitable background for the comparison. The bulb infection

results from all ClO_2 treatments showed reduced level of bulb infection compared to the control treatment.
Both the serial dilution and bulb infection results confirm the ability of ClO_2 to control FON at both concentrations 5ppm and 10ppm. This supports results from the *in vitro* studies reported previously. Additionally chlorine dioxide proved effective in dirty water conditions which increases its usefulness for commercial situations.

The results contradict the dismissal of ClO_2 as an effective biocide (Lole, 2010). In his investigation stabilised ClO_2 product Harvest Wash was used that is different to the Cidox 300 tablets. The tablets release highly oxidising ClO_2 when dissolved in water during treatment providing high level of control. This difference in outcome suggests that the chlorine dioxide delivery system is a significant factor in its efficacy as a biocide.

Commercial trials of Chlorine Dioxide

Following the successful outcome of the laboratory and small tank tests, it was decided to initiate on-farm commercial trials using chlorine dioxide as a HWT biocide. An agreement was reached with Scotmas Ltd (Ewan Cameron, Agriculture Director) to carry out trials during the 2018 dipping season. Julian Perowne of Jack Buck Farms agreed to host the trials and the work was carried out over a number of visits during July and August 2018.

The delivery of ClO_2 was based on a precursor – sodium chlorite and acid activator. The precursor and activator are mixed in a reaction chamber to produce chlorine dioxide gas that is then dissolved directly in the water of the HWT vessel. Due to the highly oxidising nature of ClO_2 a level of dosing where ClO_2 can still be detected after reaction indicates that the maximum biocidal effect has been achieved. This allows the detection of a residual level of ClO_2 to act as a real time proxy measure for sterilisation, water samples were also taken and processed for microbiological analysis (cfu measurements).

Residual ClO_2 levels were monitored throughout the testing using a Palintest ChlordioX Plus ClO_2 meter and adjustments were made to the flow rate to achieve a stable residual value. The size of the reaction chamber was found to play an important role in allowing the full activation of the sodium chlorite at differing rates of flow and chambers of different sizes were trialled on different test days to allow full activation at the different flow rates. The automated ClO_2 system is illustrated in Figure 13.

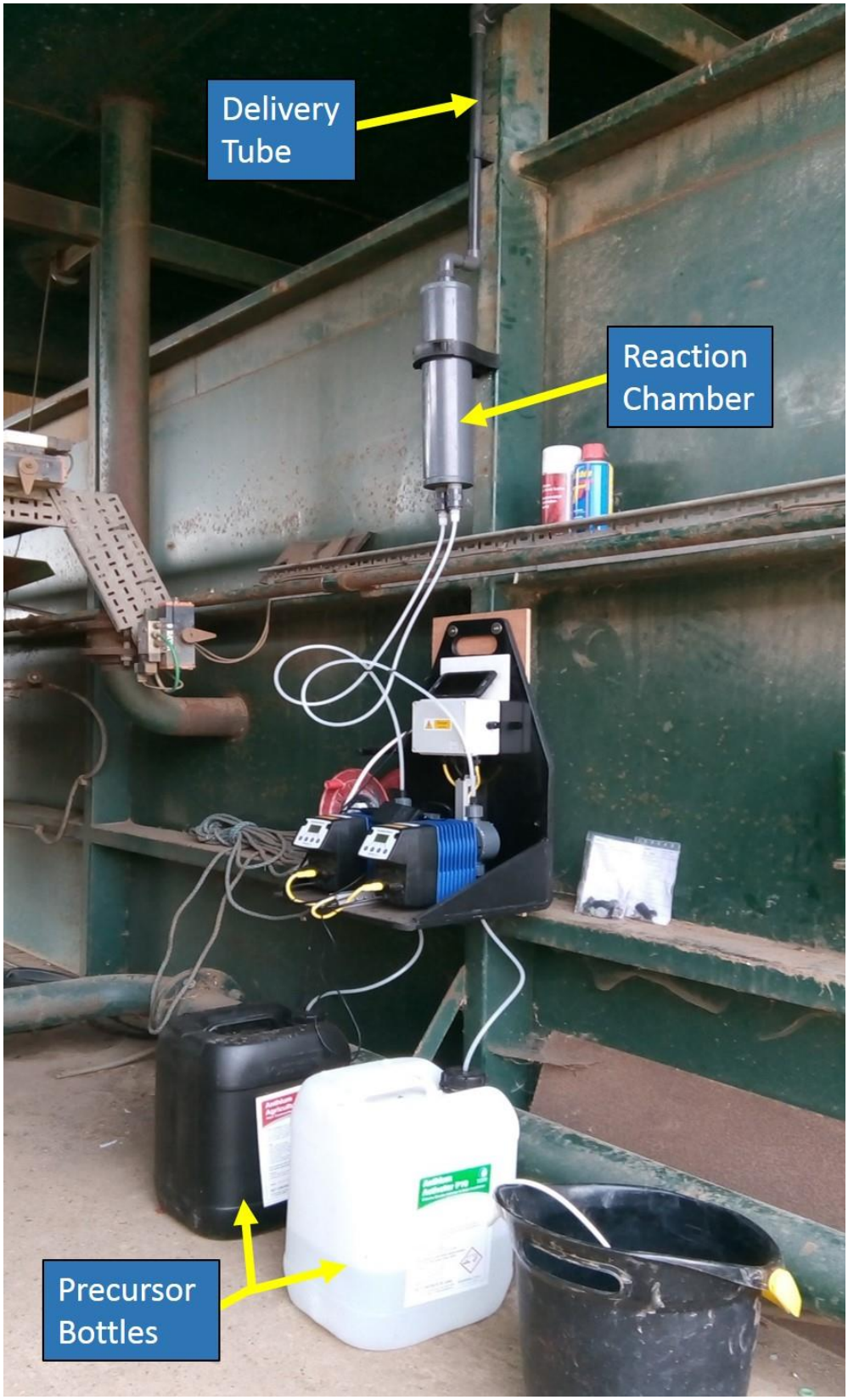

Figure 13 Automated chlorine dioxide delivery system installed on farm. Visible are two precursor ingredient bottles pumped into a grey reaction chamber from where the chlorine dioxide gas flows to the hot water tank.

Trials were continued until a steady residual level of ClO_2 could be detected (in this case 1.6ppm). It was noted that during the trials where fungicide levels were topped up as per standard farm practice a residual level of ClO_2 was not achieved even at high dose rates. When these trials were repeated without the fungicide top ups a residual could be detected at the same dose rates, this would suggest some interaction between the ClO_2 and the added fungicide. The relationship between the ClO_2 flow rate, residual ClO_2 ppm and cfu is shown in Table 6 and Figure 14.

Table 6 Residual ClO_2 levels and measured cfu at various precursor dosing rates

Dosed Chlorine Dioxide Precursors (Litres/hr)	**Residual Chlorine Dioxide (ppm)**	**Cfu/ml**	**Comments**
0.5	0.02	25300	manual dosing no fungicide
0.75	0.04	6200	
0.86	0.03	4800	
1	0.04	12000	Automated dosing with fungicide present
1.5	0.06	5100	
2	0.08	2400	
1	0.1		Automated dosing no fungicide
1.5	0.3	300	
2	1.1	186	
2.2	1.6	36	

The residual value of 1.6ppm that was achieved in the final iteration of the test returned a value of 36 cfu/ml (reduced from the initial values of over 25,000 cfu/ml). Within the complex and fluid environment of a dipping tank, this is likely to be as good as it gets and represents a greater than 99% reduction of viable bacterial and fungal cells. This is considered to be a very good result and vindication of the approach.

The treated bulbs were replanted in September 2018 and will be assessed in the spring 2019 and again in 2020. This will allow assessment of effects of ClO_2 on bulb vigour and subsequent flowering.

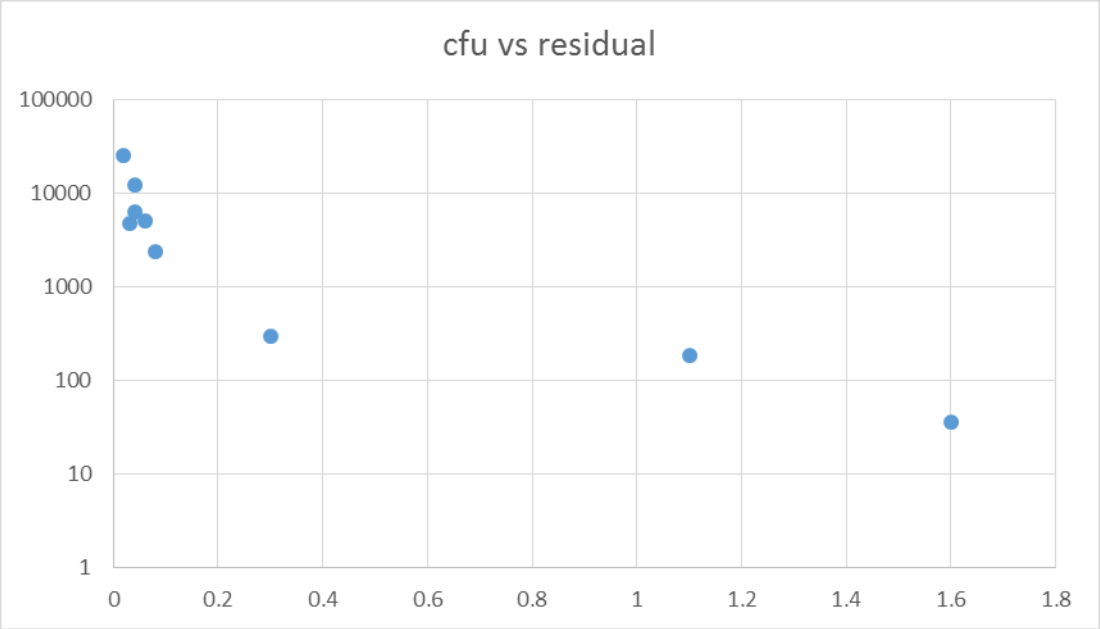

Figure 14 The relationship between residual ClO_2 level and measured cfu/ml in water samples

Financial considerations

The cost of the chloride dioxide system will vary depending on the setup at each site and is ultimately a commercial decision; however, following testing and optimisation at Jack Buck Farms, an estimation of the cost of using chlorine dioxide as a tank biocide is possible. This section provides an estimation for running a single 15,000l tank. The costs at other locations would vary depending on the optimized delivery rate although the assumption is that as a rule of thumb, the cost would scale in a linear fashion depending on the tank size.

The likely up-front cost of the chlorine dioxide delivery system, including pump, analytic unit and installation, is £5-£10k. This figure is Scotmas' own and takes into account the fact that there are likely to be different equipment requirements for different sites. Individual quotes would be required for each site but this figure suggests that the set up costs are unlikely to be prohibitive for most operators.

Running costs for chlorine dioxide depend on the dose rate required, which in the case of Jack Buck Farms was two litres per hour; this equates to a cost of £16 per hour of operation. At Jack Buck Farms the tanks are normally utilised for four dips per day with a treatment time of three-and-half hours per dip. This equates to a treatment time of fourteen hours per day that translates into a running cost of £224 per day. At eight tonnes per tank this gives a cost for chlorine dioxide of £7 per tonne (plus initial equipment and installation cost).

In order to offer some context, the running costs of the biocide FAM 30 for the same 15,000l tank can be calculated based on a dose rate of 6l FAM 30 per 1000l tank water and a cost of £95 per 25l FAM 30. The tanks are filled at the start of the season at a cost of £342 (90l FAM 30 in 15,000l). In-season top ups of 10,000l are also made, each at a cost of £228, it is assumed that these are made every five

dipping days (approximately weekly). Each individual batch also receives a top up of 1000l costing £22.80 per batch for FAM 30. With the initial cost of dosing plus in-season top-ups averaged over the batches and added to the every batch top-up this gives a per tonne cost for FAM 30 of £4.45 (Table 7).

Table 7. A comparison of running costs for chloride dioxide and FAM 30.

	Chlorine Dioxide	FAM 30
Start of season fill up	N.A.	£342
In-season top-up	N.A.	£684
Every batch top-up	N.A.	£22.80
Daily cost (4 dips/day)	£224	£91
Cost per season (650 tonnes)	**£4,550**	**£2,895**
Cost per tonne (32 tonnes/day)	**£7**	**£4.45**

Further testing in 2019

Work is continuing at Scotmas to investigate interactions between common fungicides and both ClO_2 and the Palintest meter. Also variations in the dispersal method within the tank are being looked at. Further on farm testing is planned for the 2019 season with a number of sites having put themselves forward to host trials.

HWT chemical analysis

In 2018, AHDB commissioned some additional work to examine the degradation of fungicides within HWT of bulbs. That work is reported her for convenience.

Analysis of HWT fungicide concentration

During spring of 2018 a number of growers from different regions were asked to take part in a monitoring trial to examine the concentration of fungicide in their bulb dipping tanks. Gowers were sent pre-numbered collection tubes and a protocol for sampling. Sampling began on the first day of dipping and continued at regular intervals for the next week. The plan was to track how fungicides concentrations varied over time using the grower's normal starting and refill quantities of fungicides. The samples were then returned to Warwick Crop Centre and levels of chlorothalonil (Bravo 500) and thiabendazole (Storite or Tezate) were analyzed, using HPLC with UV detection. Standards of known concentration were run alongside the samples to allow standardization of the results.

The recommended rate for chlorothalonil is 500ppm (equivalent to 1l Bravo 500 per 1000l water) although half-rate is sometimes used. Thiabendazole is normally used (in the presence of an acidifier) at 275ppm (1.25l Storite per 1000l water).

The results from each participating grower are presented below as case studies. The sampling protocol and schedule is included as an appendix at the end of the report.

Grower 1

The HWT set up includes 4 tanks (2 pairs each with a slave tank) and chlorothalonil is used as the tank fungicide. Bravo 500 was added at a rate of 0.5 litres per 1000 litres (half rate) giving a target rate of 250ppm of chlorothalonil. The intention was to maintain this target concentration throughout the dipping season by topping up at the same rate daily. The results are presented in Table 8 and Figure 15.

Table 8 Chlorothalonil levels measured in HWT samples from Grower 1

Tube Number	Dipping Date	Number of runs	Comments	Chlorothalonil mg/l (ppm)
1, 2	31/07/2018	0	After charging with chemicals but before addition of bulbs	98.9
3, 4	01/08/2018	1	End of first run (before any top up)	13.3
5, 6	01/08/2018	1	Start of second run (after top up)	67.5
7, 8	02/08/2018	2	End of second run (before any top up)	68.1
9, 10	02/08/2018	2	Start of third run	74.5
11, 12	03/08/2018	3	End of third run	69.4
13, 14	03/08/2018	3	Start of fourth run	75.6
15, 16	06/08/2018	4	End of fourth run	89.2
17, 18	13/08/2018	9	End of second week	41.9

The first observation is that tank concentration never matched the target concentration; in fact, the highest concentration achieved was at the start of the process before bulbs were dipped and even then the concentration was only about 99ppm or 40% of the target. This suggests that either the wrong volume of fungicide was used, or more likely, that heat and other chemical reactions had already degraded three-fifths of the fungicide that had been added. While it has long been suspected that active ingredients are lost from the circulating dip during HWT, this is possibly the first evidence of the magnitude of that loss (HDC Factsheet 10/13).

At the end of the first run, the concentration of chlorothalonil had fallen to just 13 ppm, or just 5% of the target value. The action of heat and chemical reactions, and bulb adsorption had removed 95% of the chlorothalonil from the system.

Following this large initial loss, regular topping-up maintained the chlorothalonil concentration mostly between 65ppm and 90ppm (26-36% of target rate) although one sample did return a particularly low value of 42ppm. These results are broadly in agreement HDC Factsheet 10/13 which states that about 25% of the active ingredient will remain in the tank. This shows that after the initial losses, the topping up programme was capable of maintaining a fairly consistent, if low, concentration. The efficacy of chlorothalonil at under 100 ppm to control plant pathogens is unknown.

These levels are similar to those measured for grower 3 but lower than those of grower 2. Grower 1 is the only grower to using chlorothalonil at half rate.

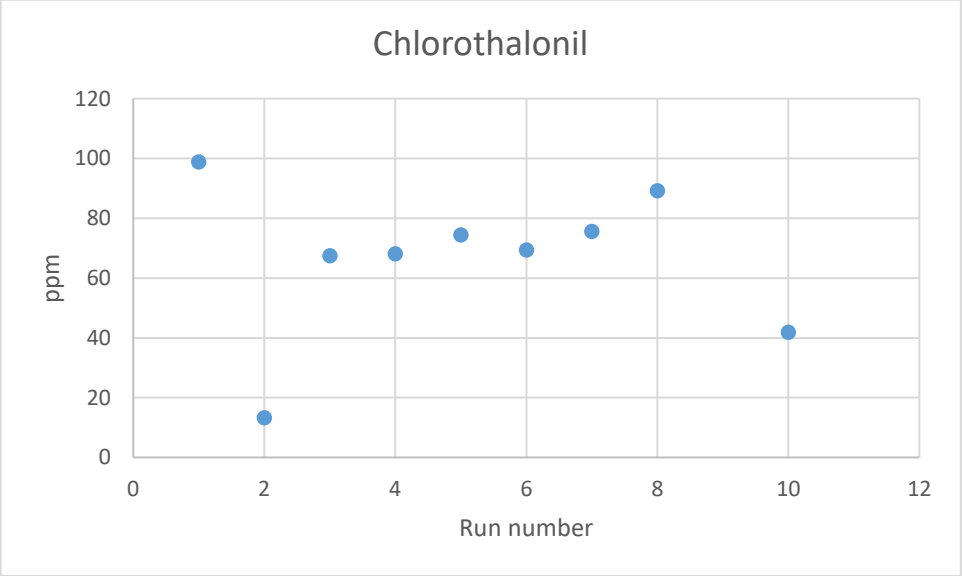

Figure 15 Chlorothalonil levels measured in HWT samples from Grower 1

Grower 2

The HWT set up includes two front loading tanks and chlorothalonil is used as the tank fungicide. Bravo 500 was added at a rate of 1 litre per 1000 litres of water giving a target rate of 500ppm of chlorothalonil. The main tanks are topped-up as required from a slave tank which contains 3000l of water with 3l Bravo 500. The results are presented in Table 9 and Figure 16.

The same big initial drop in concentration that occurred with grower 1 also occurred with grower 2. In fact it was worse in that the first sample only found 32% of the added chlorothalonil and that value dropped to 11% at the end of the first day in which three batches of bulbs were treated. Subsequent topping-up restored, and even increased, the concentration of chlorothalonil and for most of the process (tube numbers 3 through 19) levels were between 115 and 210ppm (23-42% of target rate). In general terms, this pattern of results is similar to grower 1 it is just that a larger amount of chlorothalonil was used.

Table 9 Chlorothalonil levels measured in HWT samples from Grower 2

Tube Number	Dipping Date	Number of runs	Chlorothalonil mg/l (ppm)
1	31/07/2018	0	159.9
2	01/08/2018	3	53.9
3	02/08/2018	6	176.4
4	03/08/2018	9	154.7
5	06/08/2018	12	210.1
6	07/08/2018	15	162.8
7	08/08/2018	18	173.0
8	09/08/2018	21	163.0
9	10/08/2018	24	154.9
10	13/08/2018	27	154.1
11	14/08/2018	30	161.9
12	15/08/2018	33	134.2
13	16/08/2018	36	197.6
14	17/08/2018	39	139.7
15	20/08/2018	42	122.1
16	21/08/2018	45	129.5
17	22/08/2018	48	115.4
18	23/08/2018	51	129.7
19	24/08/2018	54	129.7
20	27/08/2018	57	65.2
21	28/08/2018	60	84.3
22	29/08/2018	63	100.8
23	30/08/2018	66	92.1
24	31/08/2018	69	93.2

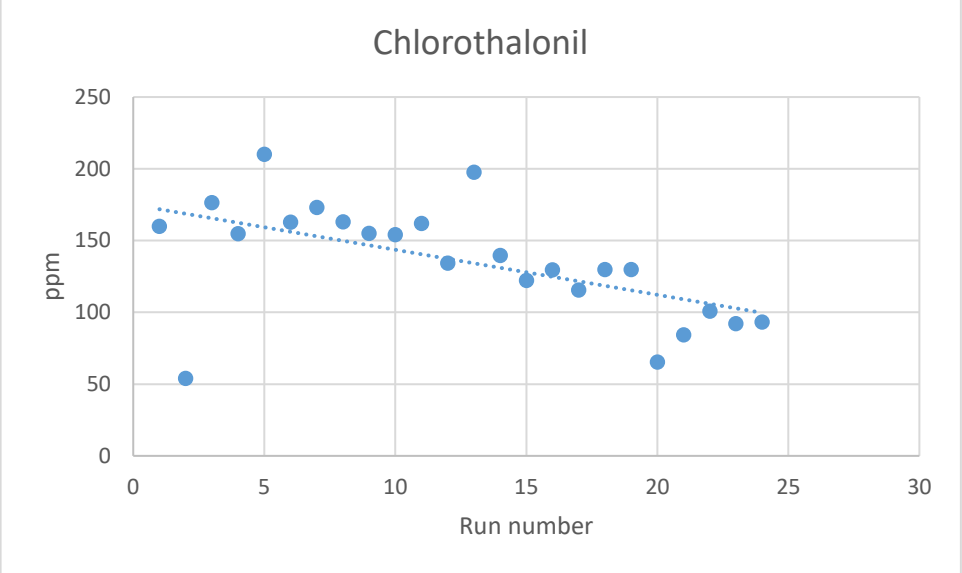

Figure 16 Chlorothalonil levels measured in HWT samples from Grower 2

Grower 3

The HWT set up includes four top loading tanks and both thiabendazole and chlorothalonil were used as tank fungicides. Bravo 500 was added at a rate of 1 litre per 1000 litres of water giving a target rate of 500ppm of chlorothalonil. Storite Clear Liquid was added at 1.25 litres per 1000 litres of water giving a target rate of 275ppm of thiabendazole. Each tank is topped up to maintain a 6000l volume using both fungicides at the same rates. The results are presented in Table 10 and Figure 17.

Table 10. Fungicide levels measured in HWT samples from Grower 3

Tube Number	Dipping Date	Number of runs	Thiabendazole mg/l (ppm)	Chlorothalonil mg/l (ppm)
1	17/07/2018	1	136.6	60.7
2	18/07/2018	4	68.9	97.2
3	23/07/2018	7	39.6	54.6
4	30/07/2018	10	31.8	72.2
5	31/07/2018	13	27.7	36.0
6	02/08/2018	16	25.7	42.2
7	06/08/2018	19	35.2	27.6

Thiabendazole

In the first sample (taken after one dip) the level of thiabendazole was approximately half of the target rate. This level is then reduced again by about 50% by the second sample (day 2, after 4 dips). Although there is some variability in the samples taken over the following 5 days of dipping (these days

are spread over the following two weeks) the concentration remained fairly constant at approximately 30ppm.

The main result is undoubtedly the initial fall in concentration over the first two observations. While some loss of the active ingredient is expected, and assuming that it is bulb adsorption rather than tank degradation, the results suggest that the first one or two batches of bulbs are being treated with luxury levels of the active. Although the concentration does stabilise at about 30ppm this only represents 11% of the target value. While it suggests that thiabendazole loss through bulb adsorption and tank degradation are matched by the top-up regime, there is no way of knowing if 30ppm is a sufficiently high residue to provide control of plant pathogens.

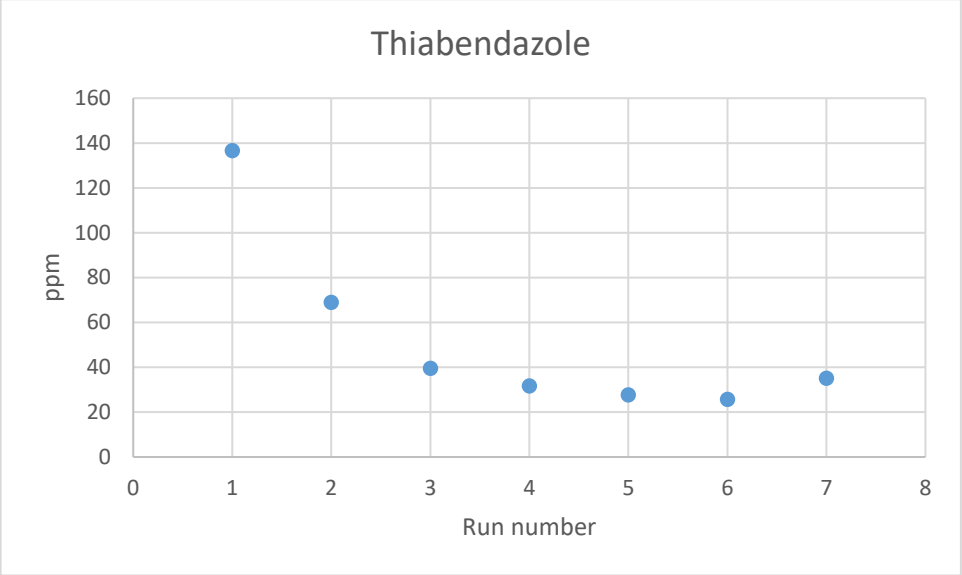

Figure 17 Thiabendazole levels measured in HWT samples from Grower 3

Chlorothalonil

Chlorothalonil levels detected in the samples are consistently low compared to the target rate. A large loss occurred after the first dip when the concentration had dropped to 61pm which is just 12% of the target rate. Like thiabendazole, some loss is expected but this does seem very high. Unlike the other systems examined in this study, the concentration of chlorothalonil mostly continued to fall over the time period of the observations. This suggests that more chlorothalonil is being broken down or removed from the tank than is being replaced by the top-up regime; this is in contrast to growers 1 and 2, where the concentration stabilised. There is no obvious cause to explain the large initial drop in concentration or continued decline. It may be that using both chlorothalonil and thiabendazole resulted in an antagonistic reaction or an acidifier was not being used which changed the pH of the dip.

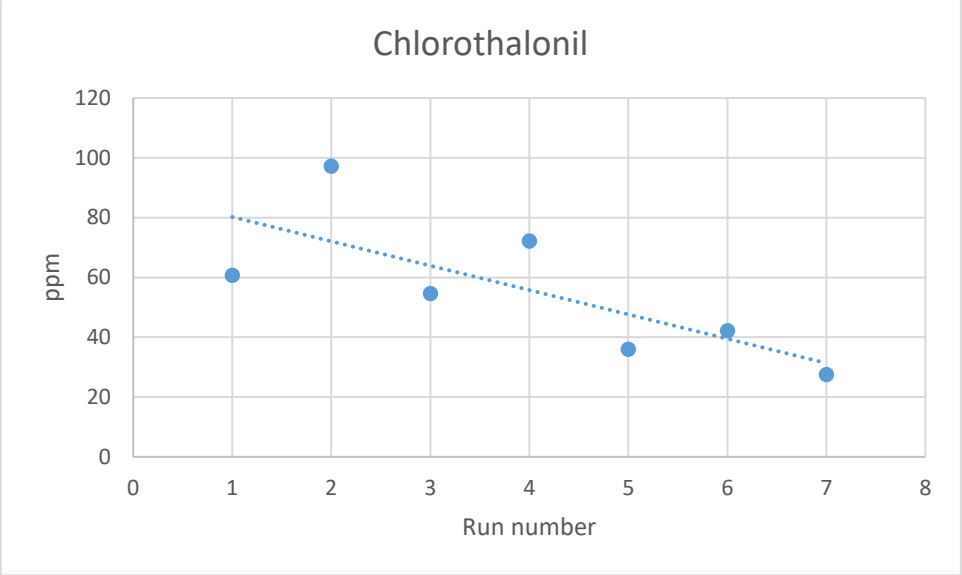

Figure 18 Chlorothalonil levels measured in HWT samples from Grower 3

Analysis of tank sediment

In addition to water samples, sediment samples were taken from each of four treatment tanks at the end of the dipping season. The tanks were drained and then samples were taken of the remaining sediment. These samples were then analysed for the presence of both thiabendazole and chlorothalonil.

The samples were left to settle in a cold room for several days after which any liquid was drained off. A sub-sample (10g) was then made up to a volume of 50 ml with methanol and the samples were agitated for 30 minutes on a rotary shaker. The samples were then centrifuged for 10 minutes and the supernatant was analysed, using HPLC, for both thiabendazole and chlorothalonil. Due to the high levels of fungicide detected in these first samples this extraction was repeated twice more i.e. the supernatant was removed and the sediment was resuspended to a volume of 50ml methanol, the samples were agitated and centrifuged again before being analysed. These extractions represent dilutions of 1:5, 1:25 and 1:125. A separate extraction using 0.5g sediment again made up to 50ml methanol (1:100 dilution) was also analysed for each sample.

The initial analysis of these samples gave mean values of 7100ppm for chlorothalonil and 1100ppm for thiabendazole. In both cases these values are considerably higher than the dosed rates suggesting that the sediment acts as a significant sink for both active ingredients. Extracting using methanol as a solvent is expected to be significantly more effective at removing the fungicides from the sediment than water would be and therefore the likelihood is that little of the fungicide sequestered by the sediment would be available to act in the normal running of the HWTs.

Results

All three surveyed growers used chlorothalonil as their main fungicide and it was interesting to note that no grower, after two days, had a measurable level that was even 30% of the dosed rate. After two days, growers 1, 2 and 3 had 69ppm (28%), 138ppm (27%) and 47ppm (9%) of their target values, respectively. The findings from Growers 1 and 2 supports the findings of the HDC Factsheet 10/13 which states that a stable concentration of about 25% of the target concentration will be achieved after two days. However, the performance of Grower 3 was poor in comparison and their loss of chlorothalonil was relatively much greater than Growers 1 and 2. One possible cause might be the use of thiabendazole as well or another the use of top-loading tanks which might result in accumulation of more sediment than front loading tanks.

Grower 2 had the highest levels measured and these were maintained well into the season, it may be relevant that the samples from Grower 2 were also consistently the clearest in terms of visual appearance/sediment level. Earlier work in BOF 61c also showed relatively low levels of chlorothalonil compared to dosed rate and it was suggested that this was in part due to the sedimentation of the chemical, this is certainly backed up by the very high levels of chlorothalonil detected in the end of year tank sediment.

Thiabendazole (used only by Grower 3) showed a similar pattern to chlorothalonil with an initial value of approximately 50% of dosed rate which then stabilized at a lower level after the first two days of dipping.

Overall, the results support the findings published in the HDC Factsheet 10/13 that active ingredients are ‘lost’ from the circulating dip during HWT. To some extent this is as expected, as fungicides will only provide protective control of pathogens if they are adsorbed by the bulb or adhere to the bulb surface. However, loss of active ingredients also occurs through heat and chemical degradation and through sequestration into tank sediments. The ratio across these different losses is unknown although it may be possible to reduce any negative impact through improved understanding of the chemical interactions between different fungicides, biocides and acidifiers. However, this is difficult as the continuing loss of active ingredients, and the different rates used, make this a never-ending task. Minimising tank sediments and bioload through improved bulb cleaning is easier to achieve.

Conclusions

The third year of the project saw the completion of the experimental work and continuation of the monitoring and evaluation programme. A number of themes have run through much of this research and this section will try and bring them together.

The first is cleanliness of the HWT system and bulb stocks. It has long been known that bioload, predominantly soil and bulb fragments, reduces the efficacy of chemical treatments. This was examined during the first year of the project when filtration, along with UV sterilisation, was tested commercially. Unfortunately, the filtration system used was unable to satisfactory remove the fine soil

particles that make up most of tank bioload. Despite these issues, some testing was completed and the first year assessment of the replanted bulbs showed that neither filtration nor UV sterilisation had any negative effects on flower production. Interestingly but unsurprisingly, dipping in clean water did give better results. The second year flowers will be assessed in spring 2019.

A parallel study into the loss of active ingredients in HWT showed some differences in the clarity of water within HWT tanks suggesting that management, predominantly cleaning, of bulbs prior to dipping could reduce tank bioload. In top loading tanks where bioload is allowed to settle into sludge, our results showed very high levels of active ingredients within the sludge suggesting that it removes the actives from circulation. As in previous years, we recommend that growers should ensure that bulbs are as clean as possible before dipping.

The second theme is thermal treatment, of both tank water and bulbs. Previous work has shown that temperatures of 60°C will control Fusarium with HWT tanks. This suggests that thermal treatment might be an effective control method. For sterilising tank water, commercial trials have shown this to be easily achievable but it is very difficult to assess how effective a strategy it is as has to be undertaken at night which subsequently allows batch-to-batch transfer of pathogens and infection during the day.

Thermal treatment of bulbs was also trialled and showed that short duration (under five minutes) at temperatures of 60 to 65°C did not induce physiological damage. While this is conceptually interesting, the logistical difficulties of short duration dipping are considerable which may rule it out commercially. Additionally, thermal treatments are unlikely to provide the curative or protective benefits that come from using biocides and fungicides.

The third theme was the use of chlorine dioxide as a chemical biocide. Laboratory testing has confirmed its effectiveness against Fusarium but its efficacy within HWT was mostly unknown. Previous work had demonstrated that it was fickle to work with under commercial conditions which is why it had not been recommended before.

Commercial testing by Scotmas Ltd during summer 2018 examined an automated dosing system using concentration monitoring which delivered variable amounts of chloride dioxide to achieve a set residual value within the tank. The rationale is that if this level of residue is present then all pathogens have been destroyed. The tests were successful and the treated bulbs was replanted in September 2018. Monitoring will take place in spring 2019 and 2020. At this stage, the approach is consider a success.

Appendix 1. Instructions for taking tank water samples.

Hot Water Tank Sampling

Thank you for agreeing to be involved in this survey. We hope that you will find the sampling straight forward. Your results will be returned to you at the end of the season, we hope you find them useful.

Enclosed are 24 sampling tubes (20 for scheduled sampling and 4 spare), please fill two tubes at each sampling point.

Please take all samples from the same dipping tank.

When sampling please submerge the tube avoiding any surface scum, which may not be representative of the bulk of the tank.

Enclosed is a sampling schedule, the bulk of the sampling is carried out in the first two days of dipping. Please fill in the dipping date and if a sample is missed then please manually correct the information on the schedule. If you feel that additional samples would be useful then please use the spare tubes and fill in the details at the bottom of the schedule.

After sampling please store samples in a cool dark location, freezing is preferable but away from direct sunlight is the most important factor.

Once the first 16 tubes have been filled please contact me and I will arrange for pick-up of the samples. Please include the schedule to that point with your samples.

Knowledge and Technology Transfer

Grower events. Details of the project and its objectives were presented at two grower meetings organised by AHBD:

- Spalding, Lincolnshire, 6th September 2018
- Redruth, Cornwall, 18 September 2018
- AHDB promotional video

Articles:

Lillywhite RD. (2018). Current issues on hot-water treatment in daffodil. RHS Daffodil, Snowdrop and Tulip Yearbook 2018

Scibisz N. (2018). Investigation of the efficacy of chlorine dioxide to control *Fusarium oxysporum f.sp. narcissi* in the hot water treatment of daffodils. Unpublished MSc dissertation. University of Warwick.

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Hanks GR. (2010). Daffodils: Chlorine dioxide – a potential biocide for use in hot-water treatment and cold dips. AHDB BOF 70 final report.

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Hanks GR & Linfield CA. (1999). Evaluation of a peroxyacetic acid disinfectant in hot-water treatments for the control of basal rot and stem nematode in Narcissus. Journal of Phytopathology 147: 271-279.

Lole M (2010). Narcissus: Alternatives to the use of formaldehyde in HWT tanks for the control of stem nematode and *Fusarium* basal rot. AHDB BOF 61a Final Report.

Pettitt T. (2016). Methods of water treatment for the elimination of plant pathogens. Factsheet 22/15, AHDB Horticulture.