Project title:	Narcissus: Investigation into the effects of a range of potential biocides in hot water treatment
Project number:	BOF 077
Project leader:	Rob Lillywhite, University of Warwick
Report:	Final report, April 2020
Previous report:	First annual report, January 2017 Second annual report. January 2018 Third annual report, January 2019
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Date project duration:	January 2016 to March 2020

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The results and conclusions in this report are based on an investigation conducted over a four-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.

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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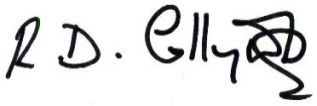
Signature  Date 20/11/2020

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CONTENTS

GROWER SUMMARY

Headline

- Laboratory and on-farm testing of chlorine dioxide has shown it to be an effective and safe biocide for use in hot-water treatment of daffodils
- Field trials have shown chlorine dioxide to have no adverse effects on flower production
- Growers should minimise the amount of soil entering HWT tanks to improve efficacy of biocides and other chemical treatments

Background

Hot water treatment (HWT) of narcissus bulbs is used to control pests and diseases, notably stem nematodes, bulb scale mites and basal rot caused by *Fusarium oxysporum f.sp. narcissi*. This has been the standard approach for at least 70 years. For most of that time, formaldehyde was added to HWT tanks as a general biocide i.e. to reduce the inoculum load in the tank water, however approval for formaldehyde was withdrawn in 2008. Work in 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 formaldehyde so as a consequence, growers do not always use it at the required rate. This issue is exacerbated since FAM 30 rapidly depletes in tanks under a high bioload (soil and bulb scale) which describes most HWT tanks during bulb dipping.

Other biocides have been considered, notably chlorine dioxide which was demonstrated to be effective against spread of Fusarium (Chastagner and Riley, 2002). Chlorine dioxide was assessed under UK conditions alongside a number of alternative biocides, but was not considered further as FAM 30 was found to be more effective (Lole, 2010). The use of chlorine dioxide was further reviewed in BOF 070 (Hanks, 2010) which suggested that additional investigation was 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. 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 of UV is known to very dependent of water clarity, which is a problem with high bioload HWT (Petit, 2016). The issue of high HWT tank bioload was reported in BOF 070 (Hanks, 2010) and identifying a solution to this issue is probably key in improving the efficacy of all biocides and biocidal approaches (and probably fungicides as well).

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.

This was a wide-ranging project that evolved through its duration. The results are reported under the following headings:

1. Review of existing practice, possible solutions and feasibility of retrofitting biocide delivery systems to existing HWT tanks
2. *In vitro* laboratory tests
3. Small-scale tank tests
4. Water turbidity (bioload), UV and filtration
5. Chlorine dioxide commercial trials
6. Thermal treatments
7. Fungicide concentration in HWT tanks

Summary

Review of existing practice, possible solutions and feasibility of retrofitting biocide delivery systems to existing HWT tanks

This study examined the feasibility of employing ultra-violet (UV) disinfection, chlorine dioxide or hydrogen peroxide dosing, pre-filtration and discussed the implications of retro-fitting to existing HWT systems. The feasibility of making physical changes to the HWT tank (e.g. insulation) were investigated to assess to impact on cost and operation using simple alterations.

Selected growers were interviewed and their existing HWT systems were inspected to understand how different HWT systems are constructed and used. The results showed that tanks were supplied by two main manufacturers but that modifications and self-build were also present. The retrofitting of biocidal delivery systems was considered to be feasible in most cases. Growers considered that a combination of both pre-filtering and non-chemical disinfection would be most suitable for improving operational efficacy and changing chemical regulations.

Of the growers that used biocides, and not all did, there was a general consensus that an alternative to FAM 30 would be welcome as the availability of chemical treatments was reducing and grower's feared that regulation and/or the cost of treatments might make bulb dipping unviable in the future. Likewise, there was interest in non-chemical treatments including UV and thermal.

Laboratory tests

Laboratory tests were used to examine the efficacy of different chemical candidate biocides to control *Fusarium oxysporum f.sp narcissi* (FON) causing basal rot and *Ditylenchus dipsac*i (stem nematode). The chemical biocides were chlorine dioxide, hydrogen peroxide and didecyl dimethyl ammonium chloride (DDAC); thermal treatment was also examined.

In clean water and under laboratory conditions, all the biocides and biocidal approaches provided greater control of Fusarium in comparison to a clean water control. Of the chemical treatments,

almost complete control was provided by chlorine dioxide at 5ppm or greater, by hydrogen peroxide at 1.5% or greater and by DDAC/Boot at 0.5% or greater. However, in dirty water (bioload) the efficacy of all these biocides was reduced dramatically. These results suggested that some form of filtration or separation should be examined alongside the other treatments.

Thermal treatment was a very effective approach with complete control achieved at temperatures above 60°C, however, there are two main practical difficulties involved in its use on-farm. Firstly, many HWT systems have neither the heating nor storage capacity to allow it to work and secondly, since the water temperature cannot be raised with bulbs in-situ, control can only be exercised between batches of bulbs, rather than within batch meaning that Fusarium spores can freely circulate within one batch.

Small-scale tanks tests

Small-scale tank tests were used to scale up the testing to imitate commercial tanks and to allow bulbs to be introduced into the testing regime (the laboratory tests only examined the use of biocides on Fusarium spores).

Testing involved dipping 20 bulbs at a time into tanks maintained at 44.4°C for 180 minutes. Each test was replicated three times. Tanks contained FON spores (apart from the control, T1), two concentrations of chlorine dioxide (5 and 10 ppm), and tests were run using both clean or dirty (bioload) water. Water samples were taken after 5 and 180 minutes and tested for the presence of viable Fusarium spores.

The results show that the viability of FON spores was mostly unaffected after 5 minutes but that control with chloride dioxide was total after 180 minutes. This was the case in both clean and dirty water and did not change with either single (at the start) or continuous dosing. Hot water, in the absence of chloride dioxide, did reduce pathogen numbers but on its own was not sufficient to control all the FON spores in water.

After dipping, bulbs were incubated at 25°C for 28 days to accelerate the development of any diseases. The results show that infection results from all ClO_2 treatments were less than the control. Overall these tests showed that chlorine dioxide was an effective biocide in water and could reduce subsequent infection in bulbs.

Water turbidity (bioload), UV and filtration

The treatment of bulbs in a HWT tank introduces bioload (soil and bulb scale particles) into the water system and its presence is known to reduce the efficacy of some treatments, particularly biocides and UV, so preventing or overcoming bioload was of great interest in this study.

Filtration and UV sterilisation were trialled on-farm to assess if retro-fitting the equipment was possible, and if so, if filtration could reduce tank bioload. Retrofitting proved possible and a centrifugal system was tested. Unfortunately, the very fine screens required to filter out the soil

particles very quickly blocked the filters and the test had to be abandoned as water flow was restricted. UV sterilisation was tested at the same time.

Laboratory testing of tank water revealed that the suspended solids were between 0.4 and 20µm in size, and therefore very small. It is likely that at this size range these particles are mainly soil based fine silt or clay particles rather than fragments of bulb scale. Any filter system designed to remove these particles will need a pore size of less than 20µm and preferably 10µm or lower. This would likely require a larger, more sophisticated and expensive system and wasn't investigated any further.

Chlorine dioxide commercial trials

In 2017, initial single dosing of farm tanks had shown that chloride dioxide could be an effective biocide so further trials were undertaken using a Scotmas automated dosing system.

In 2018, testing took place over a three week period where different delivery systems were tested to assess the flow rate required to reach an acceptable residual level. Early testing resulted in residual levels of approximately 1 ppm which was considered too low. A change to a larger reaction chamber allowed the residual level to reach an acceptable 4 ppm chlorine dioxide. Three full batches of bulbs (six boxes containing approximately three tonnes of bulbs) were treated with chlorine dioxide and replanted as per commercial practice.

Flower and stem assessments took place in March 2019 and March 2020 and the results showed there were no significant differences in stem weight, stem length and trumpet depth between the treatments. All flowers appeared healthy and the grower confirmed that they could not distinguish between the treated and untreated plants. In summary, treatment with chloride dioxide had no negative effect on any of the measured parameters in comparison to farms normal biocide treatments; if anything, there was a slight positive effect.

Thermal treatment

Laboratory testing had shown that thermal treatment, raising the water temperature to 60°C, was a very effective way to control Fusarium spores. On-farm trials showed that existing equipment was capable of raising the temperature of tank water to 60°C (and reducing it back to 44° by the following morning) but this approach only provided a one-time sterilisation and still allowed reinfection between batches of bulbs during the following day.

Growers also expressed interest in short dips in very hot water (>60°C). Small-scale and on-farm tank tests were used to determine a dose rate response prior to field testing. Bulbs were placed in a water bath at temperatures from 60°C to 70°for durations between 3 and 10 minutes. The interior temperature of the bulbs was measured using a thermal probe at 10mm depth, 20mm depth and bulb centre. The results suggested that short dips at 60°C did not cause physiological damage but that longer and hotter dips did. Ten minutes at 70° was enough to raise core bulb temperature above 50°C with damaging effects (rots after incubation and blind stems).

Fungicide concentrations in HWT tanks

During 2018 a number of growers from different regions were asked to take part in a monitoring trial to examine how the concentration of chlorothalonil (Bravo 500) and thiabendazole (Storite or Tezate) varied over time, using the grower's normal starting and refill quantities of fungicides. Although the relevance of this work is a little diminished by the subsequent withdrawal of the active ingredients, the principles of the work remain sound.

The main result was that none of the growers managed to achieve their target concentration of either chemical at the start of the season (before bulbs were introduced into the system). Chlorothalonil concentrations were on average just 28% of the target value suggesting that either the wrong volume of fungicide was used, or more likely, that heat and other chemical reactions had already degraded the majority of the fungicide before bulbs had been added to the system. 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 at the start. Topping-up fungicide amounts did increase their concentration but none of the systems reached their target value during the testing period.

Analysis of tank sludge at the end of the dipping season revealed mean values of 7100ppm for chlorothalonil and 1100ppm for thiabendazole. These very high values suggest that the sludge acts as a significant sink for both active ingredients.

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

The cost of purchasing, installing and running an automated chloride dioxide delivery system will vary depending on the sophistication of the system chosen and is therefore a commercial decision. An example is provided here for a single 15,000L tank.

The purchase cost to include pump, analytic unit and installation will be between five and ten thousand pounds.

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 or approximately £7 per tonne. In comparison, we estimate the cost of FAM 30 is £4.45 per tonne.

Action Points

- Growers should ensure that bulb stocks are as clean as possible prior to dipping to reduce tank bioload and formation of sludge
- Growers can consider the use of an automated chlorine dioxide dosing system as a viable alternative to other chemical biocides
- Growers should be aware that target concentrations of fungicides in HWT tanks are rarely met or maintained

SCIENCE SECTION

Introduction

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, however approval was withdrawn in 2008. The study of biocides in HWT is a very specialised area of research and the majority of evidence generated since then is to be found in AHDB commissioned research, namely BOF 61a (Lole, 2010) and BOF 70 (Hanks, 2010) and BOF 70a (Hanks, 2012) and the HDC Narcissus Manual (Hanks, 2013).

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 chemical biocide alternatives have been considered, notably chlorine dioxide which was demonstrated to be effective against spread of *Fusarium* (Chastagner and Riley, 2002). However, in AHDB Horticulture projects BOF 61 and 61a, chlorine dioxide was assessed alongside a number of alternative biocides, but was not considered further as FAM 30 was more effective. The use of chlorine dioxide was further reviewed in BOF 70 which recommended that further investigations were required before it could be recommended to growers.

Other biocides examined previously include peroxyacetic acid (Hanks and Linfield, 1999), hydrogen peroxide and UV (Stewart-Wade, 2011) but require further commercial-scale testing before they can be recommended. Stewart-Wade (2011) reviewed a number of biocides and technological innovations and discussed the advantages and disadvantages of a number of approaches and her work is used to inform this project. 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 very dependent on water clarity, which is a problem with high bioload HWT. The issue of high HWT tank bioload was reported in BOF 70 and finding a solution to this issue is probably one of the key objectives in improving the efficacy of all biocides and biocidal approaches (and probably fungicides as well).

The aim of the project was to examine alternative chemical and non-chemical biocidal approaches and to investigate the most promising in commercial-scale trials. The candidate biocides were divided into chemical and cultural approaches:

- chlorine dioxide, hydrogen peroxide and didecyl dimethyl ammonium chloride (DDAC)
- thermal and ultra-violet (UV)

Review

A review was undertaken in the first year of the project to examine the alternatives to, and feasibility of, using alternative chemical and cultural biocides, and cost of retrofitting biocide delivery systems to existing HWT tank systems. A number of growers were contacted and interviewed and their views form part of the conclusions of this review. The full review was reported in the annual report of 2016 with a summary here.

In general, HWT employs the submersion, heating and chemical dosing of a quantity of bulbs, or batch, in solution. A typical HWT system is characterised by a large steel tank with access via the top or side of the system (Figure 1). The system is heated using light oil burners and is pumped with water using externally connected pumping systems. The tanks are often chemically dosed by hand and contain temperature and process monitoring devices that vary in their degree of complexity between operators. Bulb batches are placed into the container in boxes or cages that permit a degree of water flow and can be easily removed and transported.

Figure 1. Typical tank size and location.

Current operational guidelines recommend a system renewal of approximately 8-10 volumes per hour, thus requiring high flow rates and relatively large pumping systems. Further, the relatively large operating volumes demand large quantities of fuel to heat the water to the required temperature. As such the installations are typically large (over 2 m high) and have numerous connecting pipes and equipment. The different demands and scale of commercial growers has resulted in a number of bespoke tank designs and treatment systems.

The control of stem nematodes requires that each batch of bulbs should be heated to 44.4°C for three hours total to ensure that the centre of every bulb has reached the prescribed temperature. In practice, the batch process can take up to four hours as extra time is required to bring the bulbs up to temperature, and to allow for loading and uploading. This energy intensive process demonstrates numerous opportunities for improving both the treatment efficacy and efficiency.

In contrast to the stem nematode, fungal base rot can be treated using surface-acting fungicides and disinfectant chemicals. These chemicals need to be circulated around the bulbs to ensure effective surface contact with the entire batch. This process relies more heavily on the flow of water and on the efficacy of tank recirculation. Note that, in some cases where stem nematodes do not present a problem, fungicidal treatment can be applied more quickly in cold-water treatment; eliminating heating requirement and lengthy treatment times.

Ongoing changes to chemical regulations and the increasing cost of chemicals means that the environment for growers is continually changing and that alternative and more efficient treatment processes need be investigated. There is also motivation to improve the treatment of stem nematodes given the lengthy and expensive process of HWT. Pertinent to this is the need to determine and explore the current sources of ineffective and inefficient treatment.

There are two potentially significant sources of inefficiency associated with HWT. Firstly, the process requires large quantities of heat employed in an outdoor environment, subject to ambient conditions. Many HWT systems are thin, mild-steel and frequently lack sufficient insulation or container lids to minimise ambient heat losses. Moreover, the bulbs are stowed in large bins that may inhibit the flow of heat and water through the container reducing efficiency. Therefore, heat loss, inefficient heat exchange and heat flow through the bulbs all contribute to energy waste. Secondly, high water turbidity (bioload) as a result of soil sediment and loose bulb scale increases chemical demand through adsorption and bio-loading.

There is therefore scope for HWT process adaptation and alterations to ensure that profitability is maintained whilst adhering to chemical regulations; keeping-pace of technological changes and adapting to variations in pest species.

This review described the main alternatives and assessed the feasibility for retrofitting proposed alternative technologies and novel HWT operations. In doing so, an analysis of each technology was conducted in terms of applicability and cost-benefit. The results confirm that there is growing demand for an alternative treatment technology or processes that overcomes the changes, and potential changes, to chemical regulations and the relatively high cost of FAM 30, and a range of alternative chemical and technological biocidal fixes have been examined.

This study examined the feasibility of employing ultra-violet (UV) disinfection, chlorine dioxide or hydrogen peroxide dosing, pre-filtration and discusses the implications of retro-fitting to existing HWT systems. An idealised conceptual model of the HWT tank system was proposed and three case studies examined. In addition, physical changes to the HWT tank (e.g. insulation) were investigated to assess the impact on cost and operation using simple alterations.

While the HWT system for each case study was regarded as fuel inefficient, given the limited degree of insulation and location in ambient conditions, the idealised conceptual HWT model showed that improved thermal insulation provides only minimal savings, in terms of costs, relative to the cost of

the current and proposed chemicals. Apparent in all case studies was the need for a holistic approach for retro-fitting and adapting current operations to the novel techniques. Each operator has a separate HWT design and thus a different set of requirements and applicable technologies. An identified limitation, apparent in the current operations with FAM 30 and expected using the proposed techniques, is the potential bioload of the recirculating water, as a result of the continued addition of bulb-based sediment to the HWT system. Filtration or separation within the HWT system was therefore regarded as a potential method for reducing both chemical costs and improving treatment efficiency by reducing biological demand. Co-treatment is also considered to be an appropriate method for reducing cross-contamination between batches and is potentially a key requirement of using chlorine dioxide and UV systems. Moreover, the relatively low capital costs for installing a sediment separation or filtration system are favourable given the potential improvements to the treatment process.

The conceptual model showed that high temperature HWT (greater than 44.4°C) would result in a small saving in fuel given the significant reduction in treatment time. However, the impact on the durability and quality of the bulb is unknown. The general consensus from the growers contacted was that a combination of both pre-filtering and non-chemical disinfection is most suitable for improving operational efficacy and changing chemical regulations. The recommendation following from this, is that HWT systems incorporate some form of easy-to-fit, vortex separation and utilize relatively cheap UV disinfection devices; thus minimising the available bio-load and potential for cross-contamination from the water column.

In vitro laboratory testing

In vitro laboratory tests were used to examine the efficacy of the different chemical candidate biocides to control *Fusarium oxysporum f.sp narcissi* (FON) causing basal rot and *Ditylenchus dipsaci* (stem nematode). The chemical biocides were chlorine dioxide, hydrogen peroxide and didecyl dimethyl ammonium chloride (DDAC); thermal treatment was also examined. The purpose of this screening exercise was three-fold: to provide a baseline for future investigations; to confirm current knowledge; and to examine new biocides and physical approaches. All tests were replicated to provide evidence on natural variation. This work was undertaken in the first year of the project and full details of the approach and methodologies can be found in the 2016 Annual Report. A summary is provided here.

In clean water and under laboratory conditions, all the biocides and biocidal approaches provided greater control of Fusarium in comparison to a clean water control. Of the chemical treatments, almost complete control was provided by chlorine dioxide at 5ppm or greater, by hydrogen peroxide at 1.5% or greater and by DDAC/Boot at 0.5% or greater. However, in dirty water the efficacy of these biocides was reduced dramatically which illustrates the detrimental impact of tank bioload. These results might go some way in explaining why chlorine dioxide, in particular, has not been adopted more widely despite promising results in the past. Its efficacy under laboratory conditions is not in doubt but its efficacy on-farm has been found to be variable especially under high bioload conditions.

These results suggest that some form of filtration or separation should be examined alongside the chemical biocides (and UV and thermal treatments) as evaluation of any biocide (or indeed fungicide) under on-farm commercial conditions cannot be consistent when the amount and influence of bioload cannot be assessed. Since the efficacy of all these biocides (and fungicides) is likely to be improved by reduced bioload/higher clarity water, it was an obvious starting point.

Thermal treatment was a very effective biocidal approach with complete control achieved at temperatures above 60°C, however, there are two main practical difficulties involved in its use on-farm. Firstly, many HWT systems have neither the heating nor storage capacity to allow it to work and secondly, since the water temperature cannot be raised with bulbs in-situ, control can only be exercised between batches of bulbs, rather than within batch meaning that Fusarium spores can freely circulate within one batch.

Continuous thermal treatment is conceptually possible using similar technology to a flash pasteuriser/separate water heater but this, and UV, cannot guarantee to treat all of the water, unlike a chemical approach, and therefore some spores may remain viable. Continuous thermal treatment may also make maintaining a constant water temperature in the treatment tank more difficult since relatively warm water might be re-entering the tank.

UV treatment was not assessed under *in vitro* conditions because controlling the intensity and duration of UV exposure was felt to be inconsistent, however, it was included during the small-scale tank testing phase. However, as noted earlier, both thermal and UV approaches suffer from only treating partial volumes of tank water at any one time, unlike chemical approaches which treat the whole volume.

In terms of identifying the next generation biocide, the results were mixed. All the biocides and biocidal approaches show promise but no individual one was outstanding. Filtration or separation to reduce the bioload and improve water clarity appears to be the obvious first step that will benefit the chemical biocides and UV treatment while the practicality and effectiveness of thermal treatments requires further investigation.

Small-scale tank testing of chlorine dioxide

In vitro studies demonstrated that the three chemical biocides all offered control of Fusarium spores. After review, the next stage was to scale up the investigation and move to small-scale tank tests. These used water baths to imitate commercial tanks and allowed bulbs to be introduced into the testing regime. This was also an opportunity to test UV sterilisation and to undertake further the work on high bioload waters. After a review of the candidate biocides, this stage focused on just chloride dioxide.

Testing involved dipping 20 bulbs at a time into tanks maintained at 44.4°C for 180 minutes. Each test was replicated three times. Tanks contained FON spores (apart from the control, T1), two concentrations of chlorine dioxide (5 and 10 ppm), and tests were run using both clean or dirty (bioload) water (Table 1). The full approaches and methodologies were reported in the 2017 annual report. Post dipping, water samples were taken for analysis of pathogens and bulbs were incubated to accelerate development of disease.

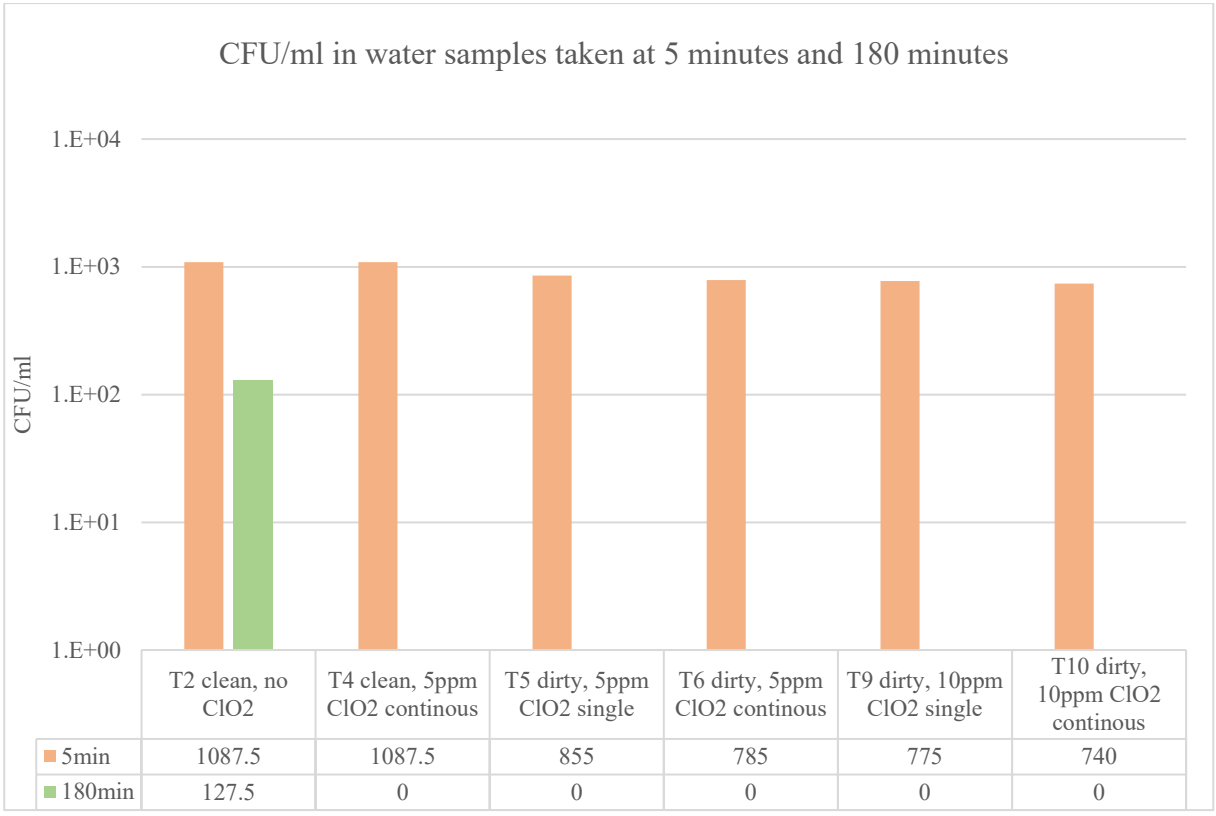
Table 1. Treatments for small-scale tank testing

Treatment number	Treatment type
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

Analysis of the water samples post-testing showed that the control treatment (T1, no spores added) revealed no CFUs of Fusarium at either the beginning or the end of the HWT. This 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.

Figure 2 shows the mean numbers of CFU/ml in water taken after 5 minutes (orange) of mixing and at the end of 180 minutes (green). This shows that the viability of FON spores was mostly unaffected after 5 minutes but that control with chloride dioxide was total after 180 minutes. This was the case in both clean and dirty water and did not change with either single (at the start) or continuous dosing.

The result for T2 shows that hot water, in the absence of chloride dioxide, did reduce pathogen numbers but on its own was not sufficient to control all the FON spores in water.



	T2 clean, no ClO2	T4 clean, 5ppm ClO2 continous	T5 dirty, 5ppm ClO2 single	T6 dirty, 5ppm ClO2 continous	T9 dirty, 10ppm ClO2 single	T10 dirty, 10ppm ClO2 continous
5min	1087.5	1087.5	855	785	775	740
180min	127.5	0	0	0	0	0

Figure 2. Average number of CFU/ml of water before and after treatment.

After four weeks of incubation all bulbs were dissected and scored according to ten point scale (Figure 3). 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.

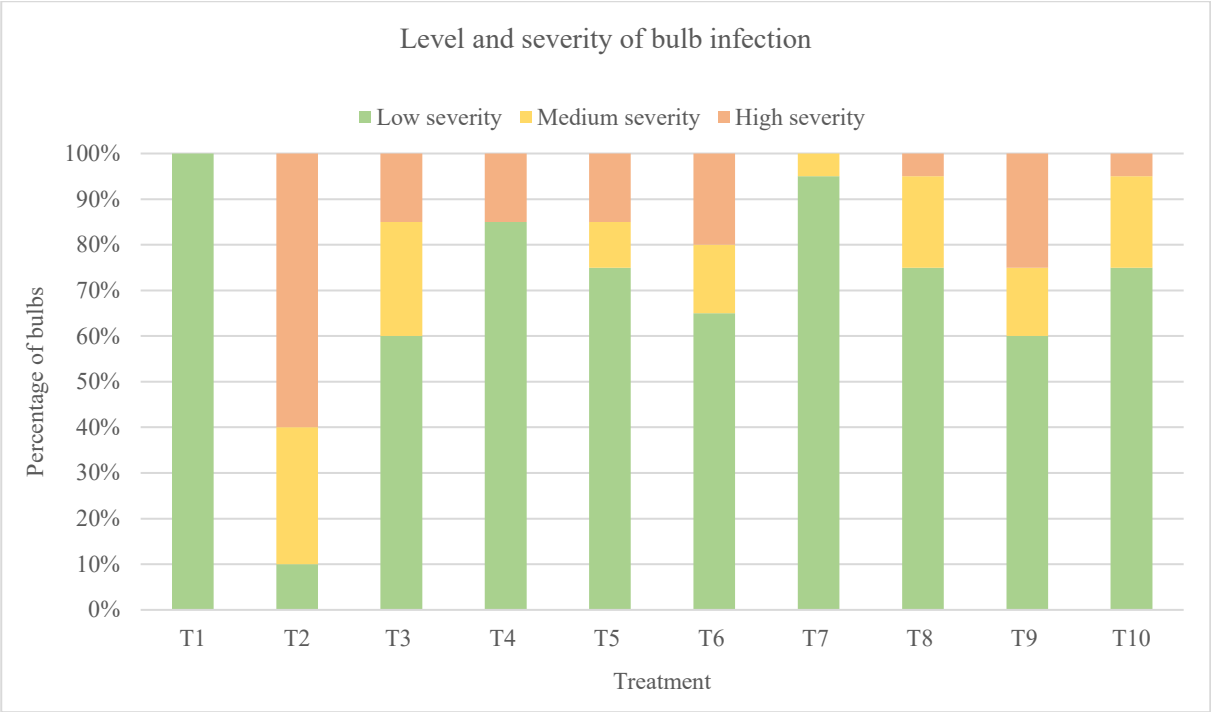


Figure 3. Percentage of bulbs with low, medium and high severity symptoms of Fusarium basal rot after 28 days of incubation.

Hot water alone is known to control stem and bulb nematodes; however, it will only reduce but not control water-borne pathogens. These results confirm the need for additional treatment to eradicate FON and demonstrate that chloride dioxide is an effective biocide.

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 that investigation, the stabilised chlorine dioxide product 'Harvest Wash' was used which is different to the Cidox 300 tablets used in this trial. Cidox 300 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.

Water turbidity (bioload), UV and filtration

The treatment of bulbs in a HWT tank introduces bioload (soil and bulb scale particles) into the water system and its presence is known to reduce the efficacy of some treatments, particularly biocides and UV, so preventing or overcoming bioload is a key objective in this study. Unfortunately, preventing bioload, principally soil, from entering the tanks is difficult. 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.

During the course of this study, 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 and progression in appearance depending on the site of collection and stage of dipping (Figures 4 and 5).

Figure 4. Examples of tank water: fresh, after 1 dip, after 2 dips and end of season (left to right)

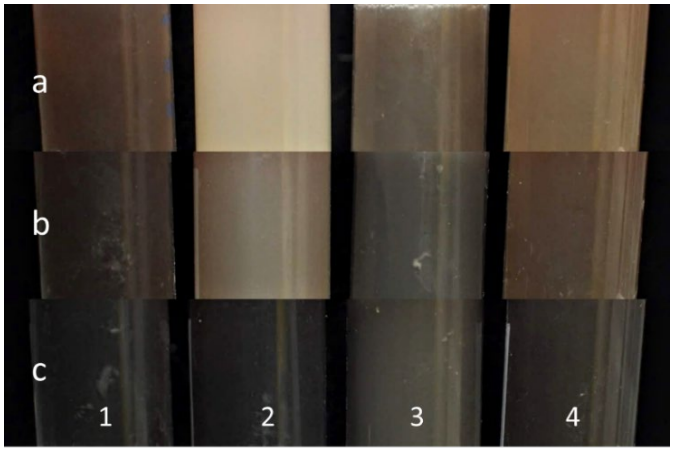


Figure 5. Water samples collected from the dipping tanks of four different growers (1-4). Samples 1a-4a were taken after the first run of the season, samples 1b-4b were taken on the second day of dipping and samples 1c-4c were taken late in the dipping season.

Filtration

Early laboratory work had established that for all chemical based biocide treatments there was a negative effect of dirty water on the efficacy of the treatment. It is also well established that for UV sterilization to be effective, the water needs to be of good clarity. This data, along with some of the feasibility work carried out, was presented at the AHDB Narcissus Growers meetings in spring 2017. There was a general agreement from growers that cleaner tank water would be beneficial and there was a good level of interest in trialling some sort of filtration.

This investigation into filtration had two aims: firstly, general clean-up of tank water allowing chemical treatments to work more effectively. It was assumed that filtration to a level of approximately 50-100µm would be effective to achieve this aim. Secondly in order to clean up the tank water to allow UV sterilization to be effective, which would require filtration to approximately the 5-10µm level.

Small-scale tank testing of UV.

The testing of UV was undertaken in parallel to the filtration studies since they shared many of the same requirements. Small-scale tank testing of an UV source had showed promise since it achieved a 99.9% control of FON spores in a tank before the addition of the bulbs, and zero Fusarium spores being detectable by the end of an experiment.

Results showed that there was a significantly lower level of infection in the bulbs from the UV treated tank than in the non-UV treated tank although the reduction was nowhere near as dramatic as that seen in the water samples. This suggested that even the very low level of Fusarium that was still present when the bulbs were added the test tank was sufficient to cause a substantial level of infection. The possibility of latent infection was considered by the use of a control treatment. The level at which the tanks were inoculated was far higher than would be expected in a commercial tank and so the level of control achievable may be more impressive than shown in these tests.

Despite these setbacks, the test demonstrated that UV is an effective biocidal approach but that there is a delay between the bulbs entering the water and full control being implemented. As a proof of concept, the results are compelling but further testing at spore levels closer to those found commercially was required before any further conclusions can be drawn.

Commercial scale testing of filtration and UV

Commercial scale testing of filtration and UV sterilisation were carried at Carwin farm in Cornwall during 2017. The test setup included centrifugal filtration, UV sterilization (and chlorine dioxide). The equipment was retro-fitted to one of the tanks (Figures 6 and 7).

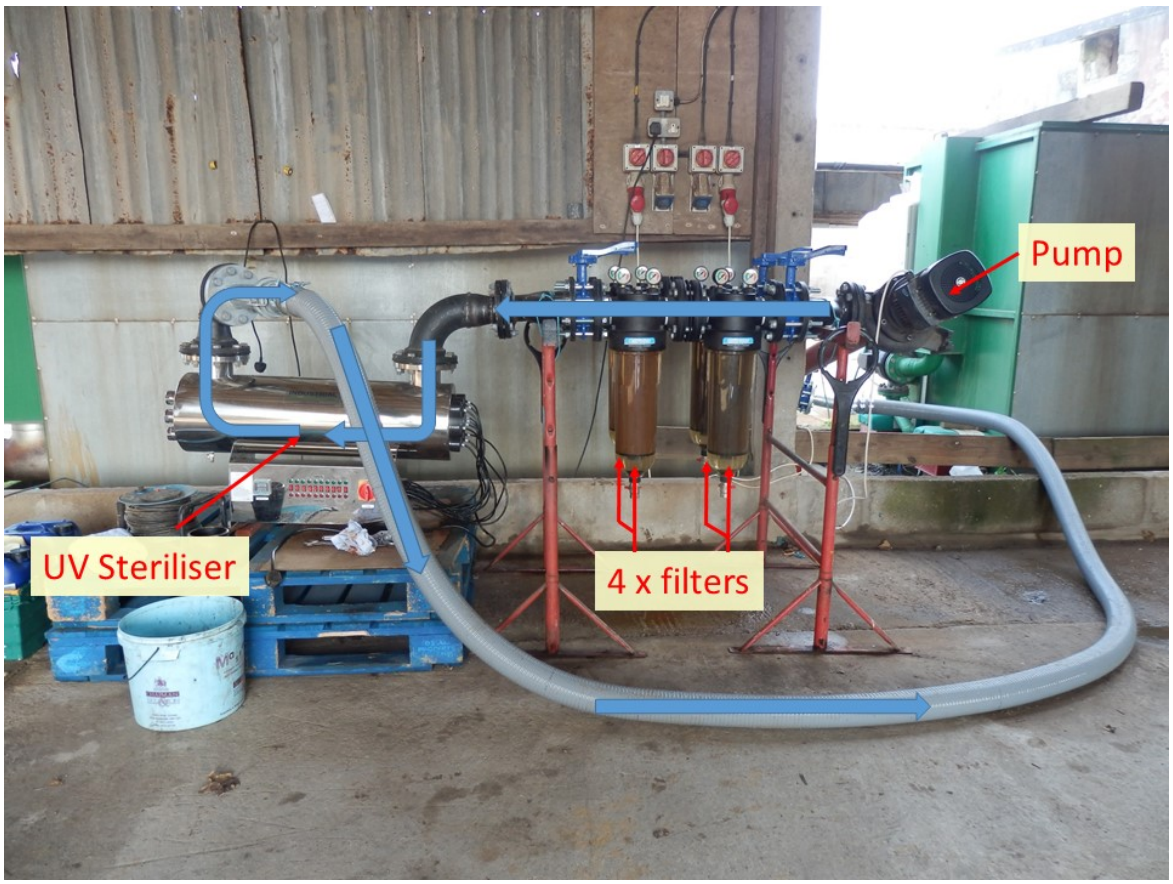


Figure 6. Retro-fitted filtration and UV system at Carwin Farm, Cornwall

Figure 7. Retro-fitted filtration system at Carwin Farm, Cornwall

The use of filter screens with pore sizes smaller than 100 µm (50, 25 and 10 µm) reduced the flow of water to the point where we judged that it was not moving through the bulb tanks effectively, and this problem became worse as the filters became progressively more blocked. On-farm and laboratory observations lead us to believe that filtering to 100 µm will only have a small positive effect and will not be sufficient to clean the water adequately to allow UV treatment to be effective.

One solution to obtain cleaner water would be to specify higher pressure pumps, however, it is not clear at this stage whether the additional pressure would result in improved flow through the HWT system or simply reduce the amount of time to block the filters. Our trial required filters to be manually cleaned so higher pressure pumps would also require auto cleaning filters to avoid imposing too great a workload on the operator.

The nature of UV sterilisation, like that of chemical biocides, means that there is no observable effect of the treatment. However, bearing in mind the fact that filtration appeared to provide only a minor improvement in water clarity, we assume that dirty tank water would severely restrict the ability of the UV light to penetrate through the sterilization chamber and as such no broad conclusions can be drawn from these results. The tests revealed a number of limitations so further work would be required on screen sizes and flow rates before any recommendations can be made. The conclusion was that filtration may be possible at farm scale but that it might be easier to further clean bulb stocks prior to dipping as that would improve the efficacy of the whole system.

Estimation of suspended solids

The assumption at the beginning of the test was that the majority of the particulates in tank water would be derived from bulb scale and therefore be relatively large. However as testing progressed, it became apparent that this was not the case and that a greater understanding of particle size and origin was required.

A water sample was taken four weeks after the start of dipping season and analysed by passing it through a series of filters starting with a milk filter (pore size ~250 µm, followed by 125, 80, 20 and finally 0.4 µm filters). The sample passed through each of the filters from 250-20 µm without the removal of any solids. On passing through the 0.4 µm filter a layer of silt was deposited on the filter and the liquid appeared to be clear of any further suspended material.

All of the suspended solids came out at between 0.4 and 20µm and are therefore very small particles. It is likely that at this size range these particles are mainly soil based fine silt or clay particles rather than fragments of bulb scale, whereas the expectation was that bulb scale would make up a significant proportion of the suspended solids. It is therefore important to find that in fact the size of suspended particles are very much concentrated in the very fine range. Any filter system designed to remove these particles will need a pore size of less than 20µm and preferably 10µm or lower.

Field trials

On farm trials to examine both filtration and UV sterilization were carried out in September 2017 at Carwin Farm in Cornwall. The 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. These trials were described in detail in the 2017 Annual report.

Summary

Filtration of tank water is conceptually sound but proved to be difficult to implement. The very small particles of mainly soil that are present in HWT tanks proved difficult to remove with typical water industry filtration. This suggests that if clean water is a pre-requisite for future treatment, then more sophisticated filtration systems will be required to achieve it. To use 5-10 µm screens for successful UV operation, would likely require (new) higher pressure pumps to maintain an adequate flow rate through the tanks, and auto-cleaning filters. These systems would be more expensive than the test system and more difficult to retrofit to an existing HWT system making them unattractive to growers unless they are installing a new system. Further details can be found in the annual report for 2017 and 2018.

Chlorine Dioxide commercial trials

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.

The first preliminary work was undertaken alongside the filtration and UV testing at Carwin Farm in Hayle in 2017. The advice from Feedwater Ltd, who supplied the chlorine dioxide, was that to work effectively an automated dosing system was required to provide a steady supply of low levels of chlorine dioxide and that if the water was dirty the efficacy would be lessened. At this proof-of-concept stage, we chose to manually dose the HWT tanks, rather than install an automated dosing system, but the results from pathogen testing the tank water was still very promising, showing a greater than 99% reduction in Fusarium CFUs (pathogen counts using Colony Forming Units). It is not clear however whether this was the effect of a one-off sterilization of the tank water when the chemicals were added or whether there would be any lasting effect over the course of a three hour run. The decision was taken to repeat the trials in 2018 with an automated system.

Agreement was reached with a commercial firm, Scotmas Ltd, to carry out trials during the 2018 dipping season at Jack Buck's Farms in Spalding. The delivery of chlorine dioxide to the HWT tanks was based on two precursors, sodium chlorite and an acid activator, which are mixed in a reaction chamber to produce chlorine dioxide gas that is then dissolved directly in the water. Because chlorine dioxide oxidises fairly easily, dosing quantity is judged by the level of chlorine dioxide residual that remains in the tank; a residual indicates that the maximum biocidal effect has been achieved, with higher residuals providing more confidence in the result. This allows the detection of a residual level of chlorine dioxide to act as a real time proxy measure for sterilisation. Water samples were also taken and processed for microbiological analysis (cfu measurements). The automated chloride dioxide system is illustrated in Figure 8. Full details can be found in the annual report for 2018.

Testing took place over a three week period where different delivery systems were tested to assess the flow rate required to reach an acceptable residual level. Early testing resulted in residual levels of approximately 1 ppm which was considered too low. A change to a larger reaction chamber allowed the residual level to reach an acceptable 4 ppm chlorine dioxide. Three batches of bulbs were dosed with batch 3 being considered the best with a residual level of 4 ppm.

The treated bulbs were replanted in September 2018 and where assessed in April 2019 and March 2020 to provide an estimation of stem and flower performance.

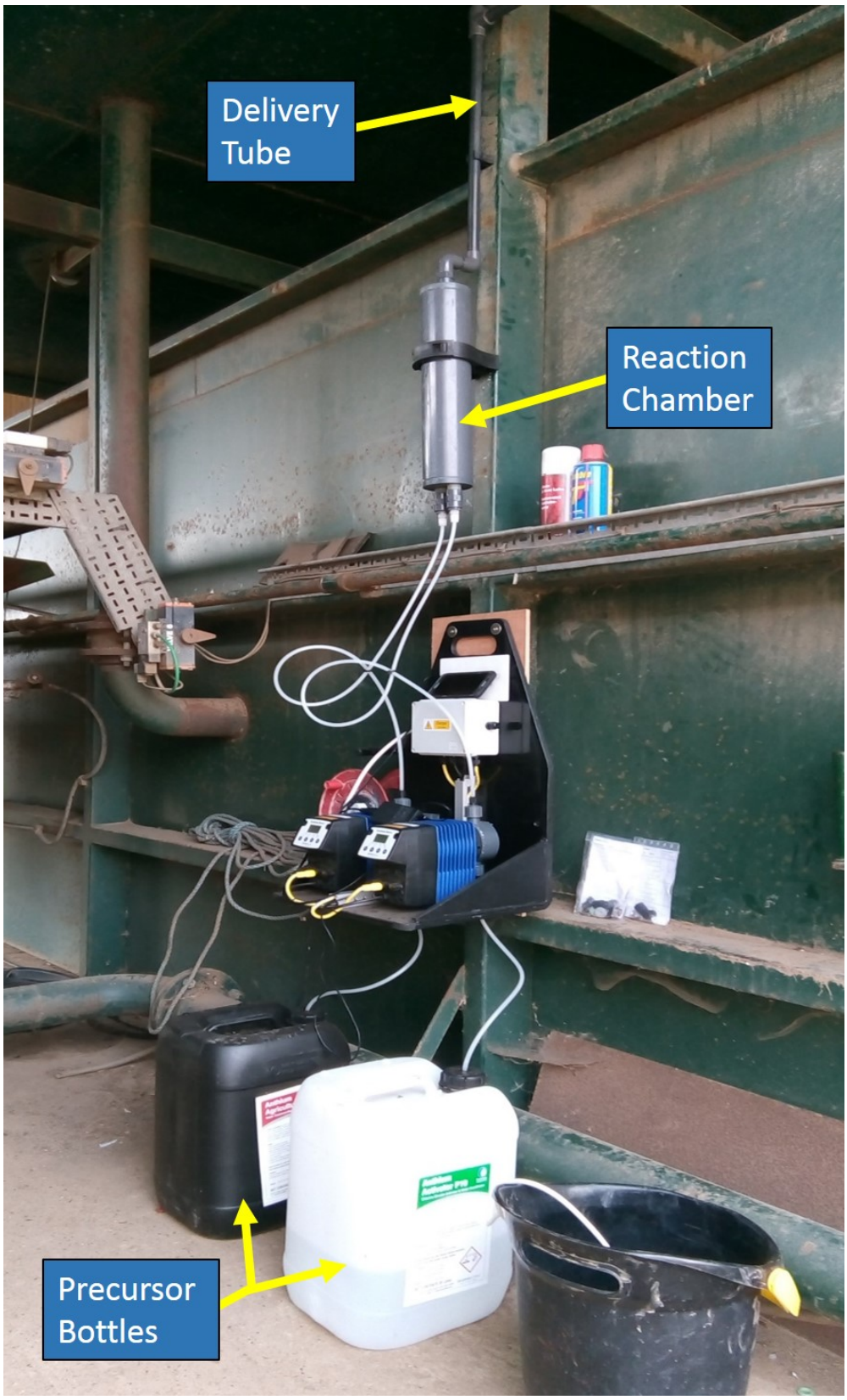


Figure 8. Automated chlorine dioxide delivery system installed on farm. Visible are two precursor ingredient bottles pumped into a grey reaction chamber from where the chlorine dioxide gas flows to the hot water tank.

Flower assessment

The initial assessment took place on the 20th March 2019 (Table 2). All four batches (three treated with chlorine dioxide and one control) were assessed even though only Batch 3 had been considered to be a success; 20 stems were harvested from each batch and stem length measured. The results are presented in Table 18 and show that there was no significant difference between the batches (the p-value is greater than 0.05). Although this was only a snapshot of first season flowers it suggested that there was no detrimental (or indeed, positive) effects of chlorine dioxide treatment.

Table 2. Jack Bucks flower assessment March 2019 (first year down)

Treatment	Stem length (cm)
Batch 1	40.75
Batch 2	41.55
Batch 3	40.90
Control	41.00
p-value	0.617
standard error of difference	0.637

The second assessment took place on the 23th March 2020. Approximately 300 stems were harvested from an area of treated (Batch 3) and control bulbs (three replicates (rows) of 50 stems per treatment). The results are presented in Table 3 and show that there were no significant differences in stem weight, stem length and trumpet depth between the treatments. All flowers appeared healthy and the grower confirmed that they could not distinguish between the treated and untreated plants.

Table 3. Jack Bucks flower assessment March 2020 (second year down)

Treatment	Stem weight (g)	Stem length (cm)	Trumpet depth (mm)
Batch 3	18.67	41.72	46.90
Untreated	17.23	40.01	46.50
p-value	0.018	0.004	0.156
standard error of difference	0.606	0.598	0.287

The results suggest that treatment with chloride dioxide had no negative effect on any of the measured parameters in comparison to the farms normal biocide treatments; if anything, there was a slight positive effect.

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 15,000 L 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 15,000 L tank can be calculated based on a dose rate of 6 L FAM 30 per 1000 L tank water and a cost of £95 per 25 L FAM 30. The tanks are filled at the start of the season at a cost of £342 (90 L 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 4).

Table 4. 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**

Thermal treatment

In vitro testing had shown that thermal treatment, raising the water temperature to 60°C, was a very effective way to control Fusarium spores. Although thermal treatment of bulbs was not part of the project remit, there was some interest from growers as to whether it would be a viable approach, so further testing was undertaken to provide more evidence

Small-scale tank tests were used to determine a dose rate response prior to field testing. Bulbs were placed in a water bath at temperatures from 60°C to 70°for durations between 3 and 10 minutes. The interior temperature of the bulbs was measured using a thermal probe at 10mm depth, 20mm depth and bulb centre.

The results show, as expected, that with an increased water temperature or with longer dipping times the core temperature of the bulbs increases (Figure 9). It is interesting to note that the data obtained on farm showed a much higher core temperature than the equivalent data obtained at Warwick. This effect was more noticeable at the shorter dipping times. The difference may be due to the addition of bulbs to the small tanks at Warwick causing a drop in water temperature, which is likely to be less of an issue in commercial 6000L tanks.

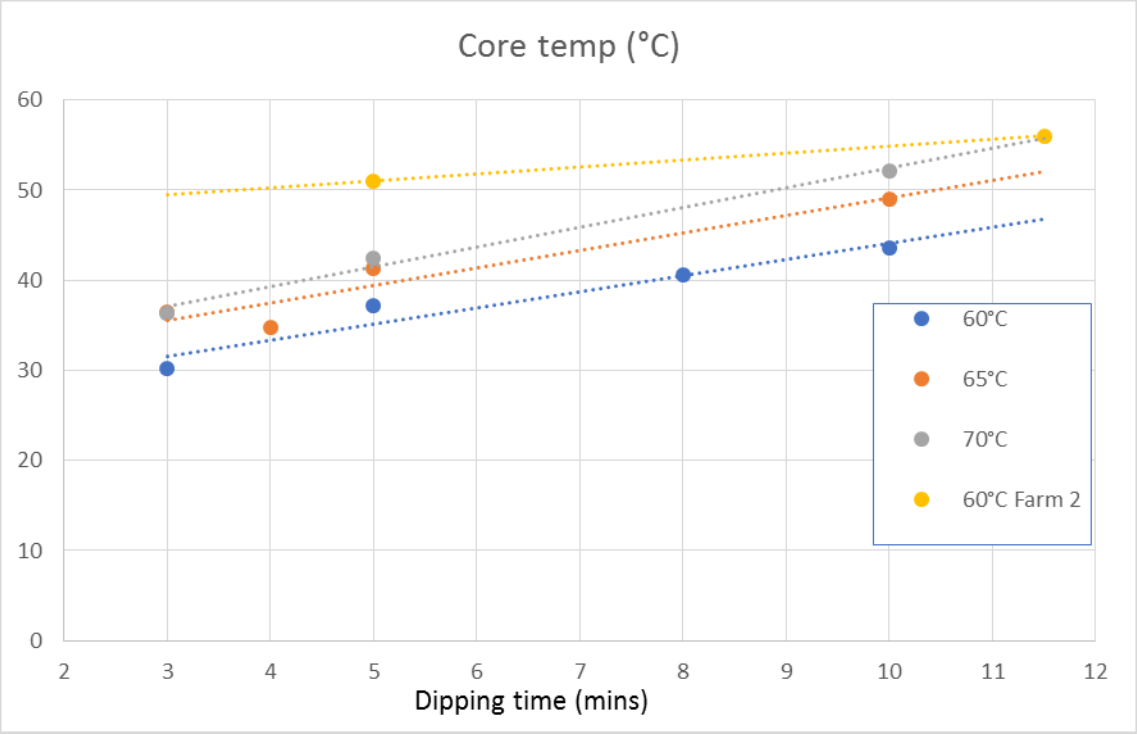


Figure 9. Bulb core temperature at different dipping temperatures and durations

Once treated these bulbs were placed in an incubator at 25°C. After a month the bulbs were dissected and basal rot was scored from 0 to 10. Scores were then grouped to give low, medium and high basal rot categories as indicated in the scoring method (Figure 10).

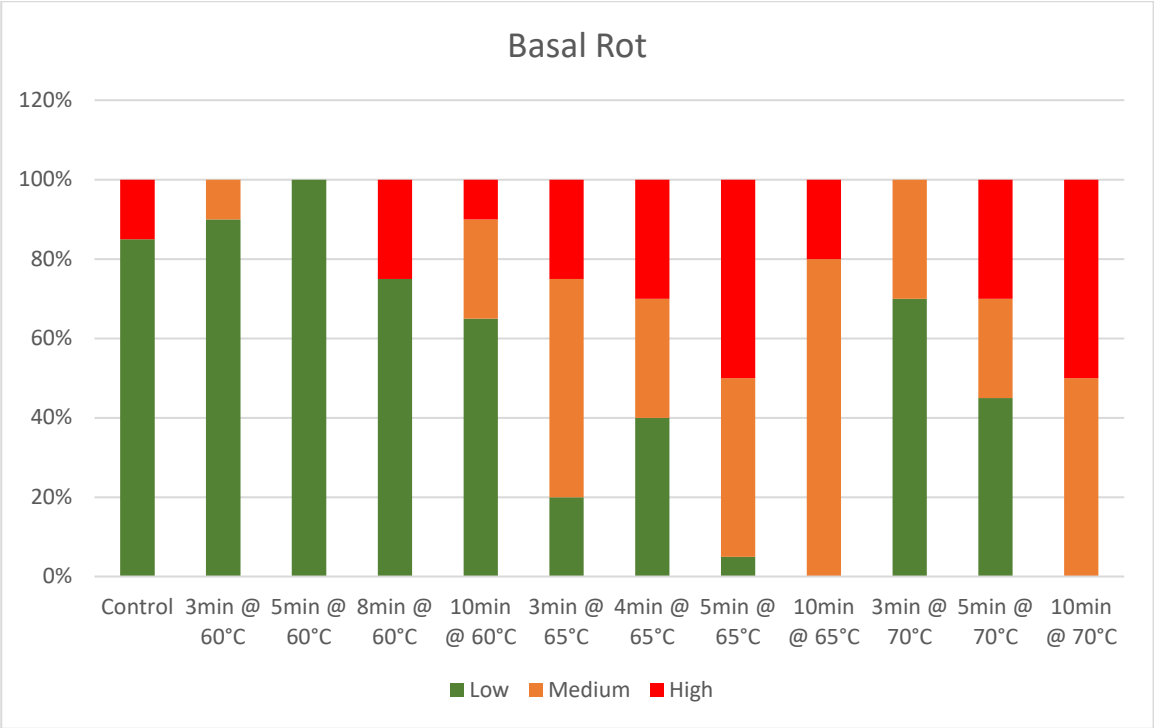


Figure 10. Incidence of basal rot following incubation (Warwick thermal trial)

Where bulbs were dipped at 60°C, the core temperature never exceeded the industry standard dipping temperature of 44.4°C and this is reflected in the incubation results that showed the incidence of rots were in line with the control. However, at higher temperatures and longer durations, the incidence of rots was considerably higher and the assumption is that at these temperatures, the bulbs are sustaining physiological damage leading to bulb decay.

On-farm feasibility

Feasibility studies were carried out at two farms in Cornwall. At Farm 1, raising the water temperature to 60°C overnight was easily achieved using their standard equipment. Water heating was turned off once 60°C was reached and allowed to cool. The temperature was found to have dropped to 50°C by morning but the addition of top up water (4000L/day) and bulbs brought the water temperature back down to 44°C at the start of treatment. This suggests that batch sterilisation of water is possible without the addition of bulbs.

At Farm 2, raising the tank temperature to 60°C was also achieved without problem and the opportunity was taken to dip some bulbs at this raised temperature. Two varieties (Finland and St Patrick's Day) were used as they were found to have significant base rot and so of little commercial value. The bulbs were either control (no dip), dipped for 5 minutes at 60 °C or for 11.5 minutes at 60°C. During dipping the core temperature of a sample of the bulbs was measured. Dipping for 5 and 11.5 minutes raised the core temperature to 51 and 56°C respectively (Figure 9). Post-dipping, the bulbs were planted as normal.

Two sub-samples of bulbs were dissected to assess the level 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 rot (Figure 37). Following incubation the level of rot had increased in all test groups. For Finland the increases in rots for the control and 5 minute dip groups were modest (25% to 30% and 20% to 27.5% respectively) however the increase in the bulbs dipped for 11.5 minutes was more significant (25% to 67.5%). This suggests that for the Finland bulbs a 5 minute dip at 60°C was either neutral or slightly beneficial but the longer 11.5 minute dip actually increased the level of rot in the bulbs.

For the St Patrick's Day bulbs, which had shown slightly higher levels of basal rot in the initial observations, the levels observed after incubation were consistently high. Control bulbs showed 65% damaged up from 30% initially, although most of these showed moderate rather than the most severe damage. The 5 minute and 11.5 minute dipped bulbs both showed 92.5% damage (up from 25% and 35% respectively).

The dipping of bulbs for short periods at 60°C, whilst successful from a technical point of view, gave mixed results. In the case of the Finland bulbs those dipped for 5 minutes at 60°C showed the lowest levels of rot both initially and after incubation. However these results were only slightly lower than for the control bulbs. The 11.5 minute dip actually increased the levels of rot damage after incubation. The longer dip at high temperature would be expected to reduce the level of viable Fusarium, however it would seem that any such effect was more than counteracted by some secondary effect, most likely a softening or damage to the bulb tissue allowing a more rapid spread of surviving spores.

In the case of the St Patrick's Day bulbs the levels of rot present, particularly after incubation, were so high that it is hard to draw any conclusions beyond the fact that neither of the dips appeared to have any beneficial effect.

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 (Figure 11). 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 negative effect, suggesting that a dip of this length is damaging to the bulbs.

Work in year 1 showed 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.

Figure 11. Assessment of year 1 flowers for heat treated bulbs. a-c Finland, a – control, b – 5 minute dip, c - 11 minute dip. d-f Variety St Patrick's Day d – control, e – 5 minute dip, f – 11 minute dip.

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.

Microwave technologies

The use of microwaves to heat bulbs has been proposed as an alternative method to HWT. The theoretical advantage to this approach is that immersion in hot water for 180 minutes would be avoided. This would increase the speed and reduce the cost of treatments to control stem nematodes.

In theory, bulbs, once cleaned and graded could be continuously treated on a conveyor belt system where they enter a sealed unit and are irradiated using microwaves until their core temperature reaches 44.4ºC. The major advancement in employing such a process is to reduce the treatment time per bulb from 180 plus minutes to, potentially, a number of minutes (depending on the bulb size). However, bulbs would still require a fungicidal treatments to control diseases but this could be performed using cold spraying or dipping which is far less time consuming in comparison to HWT.

Microwave disinfection is employed in the medical industry as a method for sterilising surgical instruments and equipment. In this environment, the material is placed within a sealed unit to ensure high safety for the operators. Sanitec Industries manufacture microwave disinfection systems for medical waste. The waste is loaded into the machine and ground into a manageable size before entering a sealed screw conveyor system where the waste is irradiated with microwaves. Other manufacturers of microwave sterilisation include Sterilwave by Bertin Technologies and OptiMaser by SS Medical Systems; although both systems are a batch process design.

The concept of using microwaves to control stem nematodes *in-situ* is based on the fact that targeted microwaves may be able to increase the temperature of the pest to a lethal level, but do so without damaging the plant tissues. Microwave technology is energy and cost-effective. It is highly reliable and has been successfully applied in the food industry. However, to date, there exists no theoretical concept or experimental evidence to suggest that microwave technology can be used for pest control in bulbs. Therefore, the objective of this study was to establish a theoretical basis and generate robust experimental evidence to prove or disprove the hypothesis that microwave can be used to control stem nematodes.

The use of microwave technology to control stem nematodes was the subject of a masters research project at the University of Warwick. Experimental work was undertaken by the Microwave Process Engineering Research Group at the University of Nottingham and focused on two frequencies: 910 and 2470 MHz as representative the industrial and domestic used microwave frequency. The specific objectives of this study were to (1) determine dielectric properties of *Narcissus* bulbs as a function of temperature and frequency, (2) set up treatment trials to demonstrate the effects of microwave on bulbs, (3) set up treatment trials to demonstrate the effects of microwave on nematodes, (4) compare the effects of HWT and microwave heating treatment.

Results

The efficiency of heating using microwaves is determined by the physical and thermal properties of the material being heated. Higher wattage microwaves can heat more quickly and penetrate more deeply but the risk of tissue damage is also greater. Different combinations of power (10, 20, 30 and 40W) and frequency were examined to establish bulb temperature, penetration depth and tissue damage. Power and bulb size were found to be the main influence in determining bulb temperature. Penetration depth was greater at 910 MHz.

The threat of tissue damage was a major concern and considerable time was spent examining the effect of different wattage microwaves on bulb tissue. Treatments focused on achieving different temperatures using different powers and a damage scale was developed to aid assessment (Figure 24). The results show that a temperature of 44°C could be achieved using any one of four wattages (10, 20, 30 & 40 W) but that tissue damage increased with wattage. Tissue damage was rated at 8/9 at 40W but only 1 at 10W (Figure 10). A second series of tests revealed that temperatures of between 35 and 50°C could be reached using 10 or 20W but that damage at 20W was rated 4/5/6 compared to 1 at 10W. A final assessment treated 49 bulbs at 10W to reach 44°C and resulted in 46 bulbs being rated at 1 and only 3 suffering greater damage. Based on this evidence, it was concluded that treatment at 10W is unlikely to cause tissue damage.

A simple pot experiment was established to examine the effect of microwave treatment on subsequent plant and flower performance. Two treatments were imposed on 20 bulbs: microwaved and non-microwaved. Post treatment the bulbs were individually grown on in pots outdoors and assessed for stem length and overall quality in spring 2017. The results show that microwaving at 10W had no detrimental effect on plant growth or quality, and perversely may have improved performance. Visual assessment confirmed that microwaving bulbs at low power (10w) does not reduce flower performance).

Despite the positive results, caution should be used in interpreting these results as this was only a small-scale trial. Whether microwaves are a useful long-term solution will depend on greater understanding the issues and implications and any physiological damage that occurs. Despite this, the concept of microwave heating to control stem nematodes was proven in this study although further work is required to understand the subtleties of its use. Dry microwave treatment is undoubtedly easier to manage than wet HWT and likely to be more energy and cost-effective in the long-term. Further details can be found in the annual report for 2016.

Fungicide concentration in HWT tanks

Over the course of the project, there was considerable interest from growers in the dosing and stability of fungicides. Although it was not within the project remit, the opportunity was take to collect water samples from two commercial growers to quantify fungicide levels under different circumstances. Samples were tested for levels of thiabendazole and chlorothalonil by high performance liquid chromatography (HPLC). The results are presented in Table 5.

Table 5. In-tank fungicide levels at different water status (Farm 1)

Sample number	Water status	Thiabendazole (ppm)	Chlorothalonil (ppm)
1	Full strength chemicals at ambient temperature	533	351
2	After heating water to 60°C	363	55
3	After one load	413	187
4	After 9 loads	510	144

Treatment at 60°C lowered the levels of detectable thiabendazole by approximately 30% and chlorothalonil by approximately 85%. Topping up the tanks after each load recovers some of this activity particularly in the case of thiabendazole. For thiabendazole we can accurately predict the level after nine loads if we assume that each top up increases the tank concentration by the same amount as the first one (~50ppm) and that heating to 60°C after every three loads (nightly) reduces the level by approximately 30% (Figure 12). Perhaps due to the very high level of degradation of chlorothalonil it is not possible to produce a similar model that fits the data for this chemical.

At Farm 2, water samples were collected at different times and levels of thiabendazole (Storite) and chlorothalonil (Bravo) quantified by HPLC. Levels of thiabendazole decreased fairly uniformly with each run which suggests that it is lost, either through adherence to the bulbs, degraded through heat action or adsorbed to soil particles, at a greater rate than it was topped up. Levels of chlorothalonil decreased by 14ppm and then 15 ppm for each of two runs. Overall levels were well below the recommended rates (thiabendazole 275ppm and chlorothalonil 500ppm).

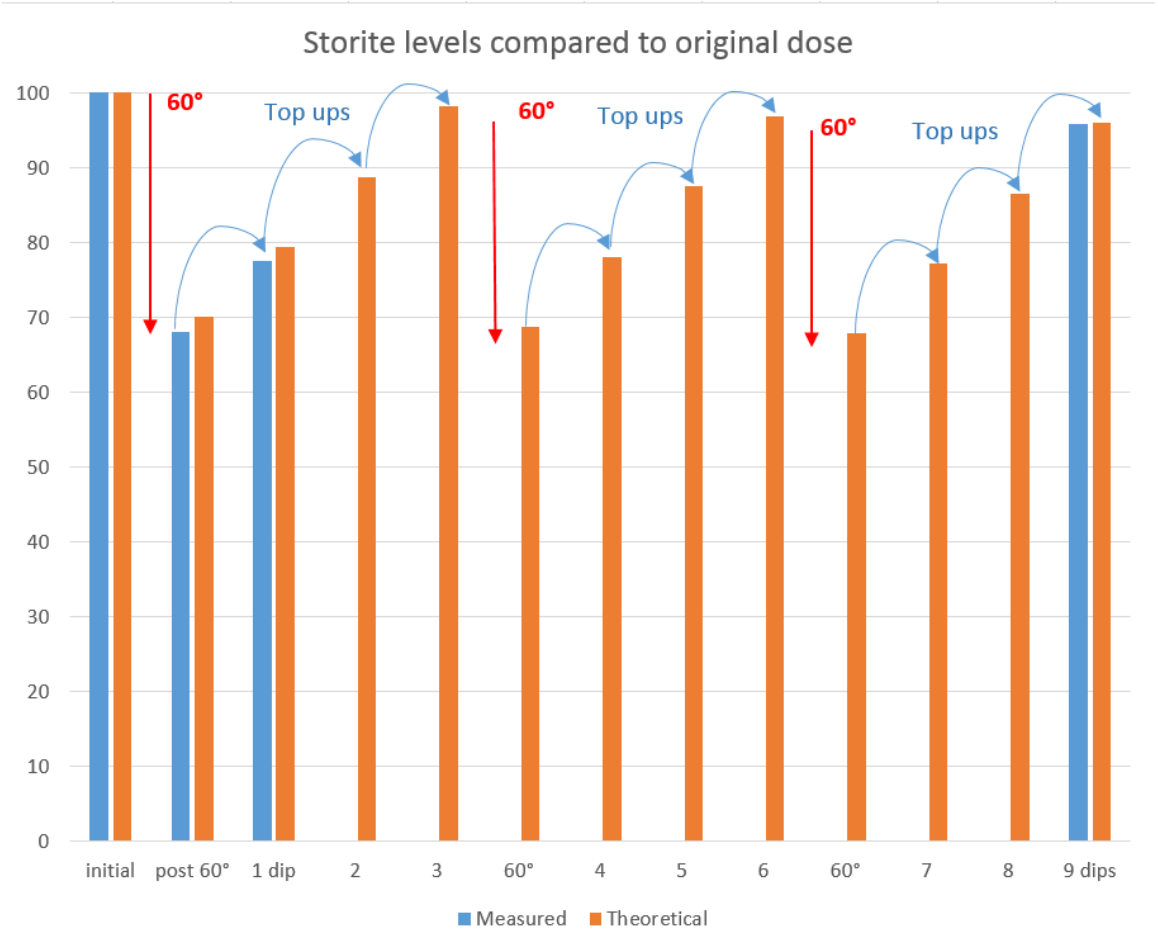


Figure 12. An illustration of how levels of thiabendazole (Storite) vary under temperature and top-up regimes. These theoretical values are based on a 30% loss on heating to 60° and 50ppm increase of concentration for each top up.

This ad-hoc testing of tank fungicide levels revealed some unsettling but perhaps not unsurprising results. Although all growers strive to ensure that levels of fungicides are at the recommended rates at the start of the dipping season, and will estimate how much topping up is required during the season, they have no way of monitoring levels and therefore no way of knowing the amount of active ingredients that are lost through adherence to bulbs or degraded through thermal action. The results of this work suggested that active ingredients are typically below recommended rates but the effects of this are mostly unknown.

Interest in this work from growers prompted AHDB to commission a follow-up study.

Analysis of HWT fungicide concentration

During 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 record 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 analysed, using HPLC with UV detection. Standards of known concentration were run alongside the samples to allow standardization of the results. 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.

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).

Grower 1

The HWT set up includes four 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 6 and Figure 47.

Table 6. Chlorothalonil levels measured in HWT samples from Grower 1

Tube Number	Dipping Date	Number of runs	Comments	Chlorothalonil mg/ (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 (Figure 13). 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.

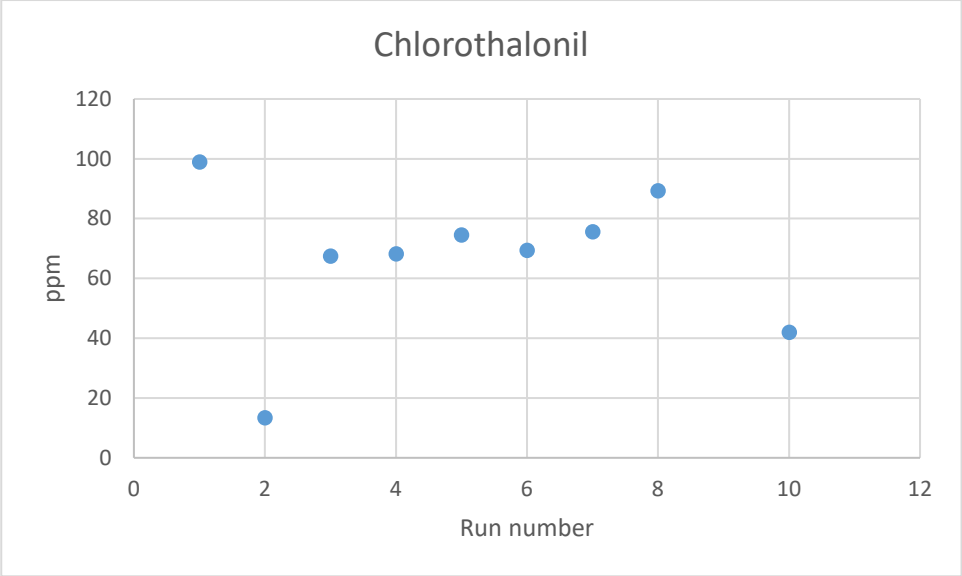


Figure 13. Chlorothalonil levels measured in HWT samples from Grower 1

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 7 and Figure 14.

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.

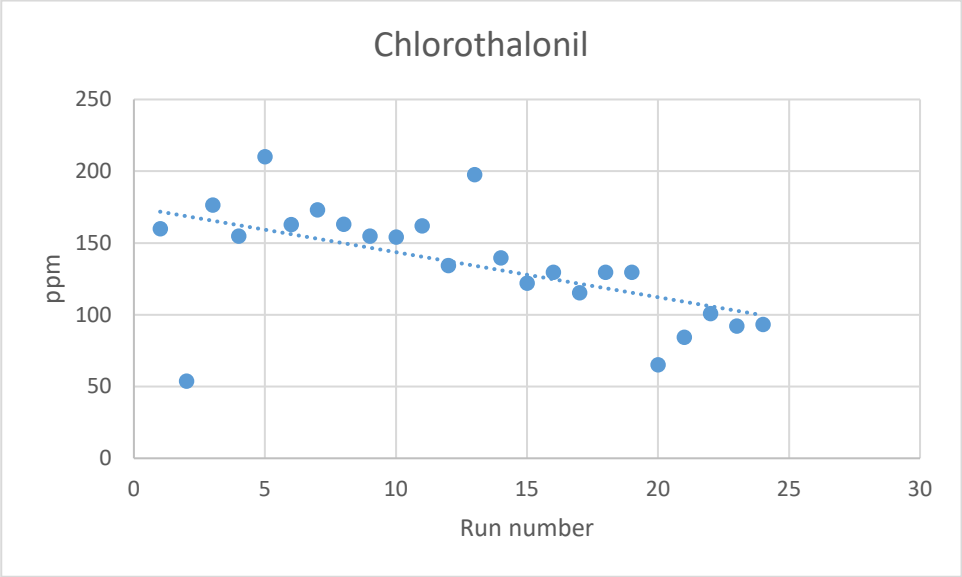


Figure 14. Chlorothalonil levels measured in HWT samples from Grower 2

Table 7. 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

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 8 and Figure 15.

Table 8. 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

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.

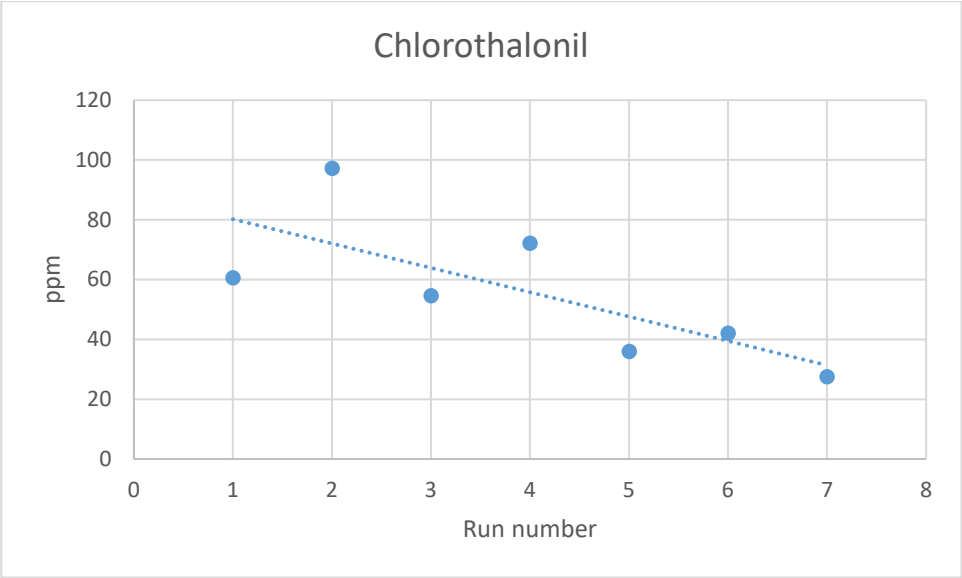


Figure 15. Chlorothalonil levels measured in HWT samples from Grower 3

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 results are presented in Table 10 and Figure 16.

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.

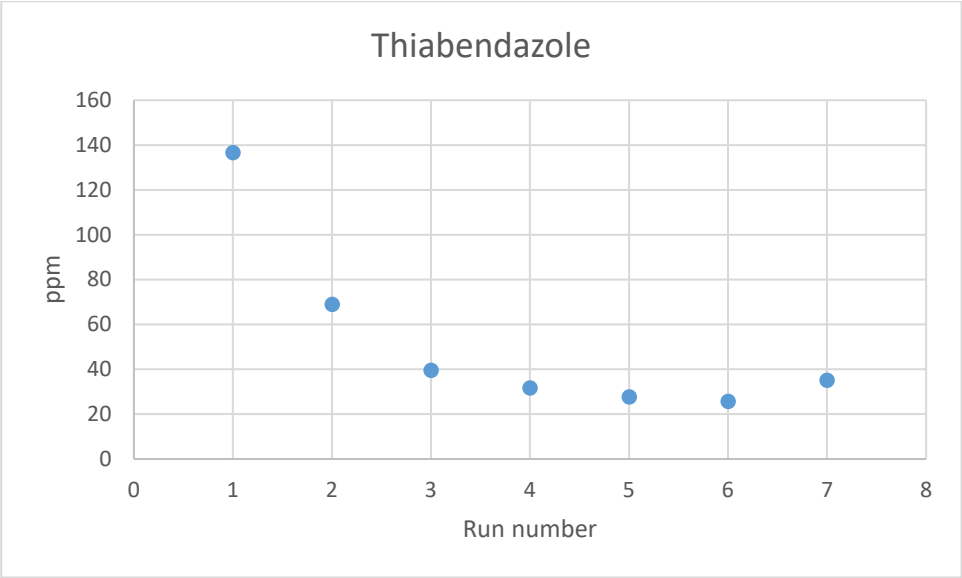


Figure 16. Thiabendazole levels measured in HWT samples from Grower 3

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 was 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

This was a wide ranging project that over four years examined a number of different biocidal or pathogen control methods. Initial laboratory work confirmed the efficacy of a number of different chemical biocides, including: chlorine dioxide, hydrogen peroxide and didecyl dimethyl ammonium chloride (DDAC). *In vitro* tests also confirmed that thermal treatment (heating to 60°C) was capable of full control of Fusarium spores and that bioload (or dirty water) reduced the efficacy of the chemical controls but not thermal control.

Small-scale tank were used to examine the efficacy of chlorine dioxide at different concentrations, for different time durations, and in both clean and dirty water conditions. Results showed good control at both 5 and 10 ppm and that single batch or continuous dosing did not seem to make a big difference. Ultra-violet (UV) light was tested and found to be an effective control measure of Fusarium spores. However, the efficacy of chlorine dioxide and UV was reduced in dirty water conditions

Dirty tank water reduces the efficacy of chemical biocides but is the inevitable consequence of dipping field condition bulbs. Filtration was tested but the very small size of soil particles meant that it was not a success. Unless more sophisticated, and expensive, alternatives are used, filtration, and as consequence, UV is unlikely to be a viable option.

On-farm testing of chlorine dioxide showed that an automated dosing system could be integrated into a typical HWT tank system and that consistent residual levels could be obtained that confirmed the efficacy of the treatment. Two years of subsequent flower production showed that chlorine dioxide had no negative effect on flower performance. The research results, and subsequent testing and implementation by Scotmas, provide confidence that growers can use chlorine dioxide as an alternative to FAM30.

On-farm testing of thermal treatment showed that existing HWT systems were capable of raising the water temperature to 60°C. Short duration testing showed that up to a 5 minute dip did not cause physiological issues in bulbs or flowers but that longer, or hotter, dips would cause serious damage. For surface sterilisation, this could be effective but handling difficulties mean that it is probably only viable in top-loading tanks.

Thermal treatment using microwaves was evaluated and while it showed promise, like wet thermal treatment, the margin between success and disaster was too narrow for serious consideration at this time. However, dry and quick thermal treatment would offer an advantage over the existing slow and wet thermal treatment in the future.

Fungicide concentrations in HWT tanks over the dipping period were measured and show large variation. In general, concentrations were below recommended levels and topping up procedures could be improved.

Knowledge and Technology Transfer

- AHDB Narcissus Growers Workshops, Spalding and Cornwall, 12 and 18 May 2016
- AHDB Narcissus Growers Workshops, Cornwall and Spalding, 17 and 25 May 2017
- AHDB The Grower Magazine, July/August 2017. Clearer future for bulb treatment
- AHDB Narcissus Growers Workshops, Lincolnshire and Cornwall, 6 and 18 September 2018
- AHDB The Grower Magazine, Feb/March 2019. In hot water.
- RHS Yearbook 2019. Current issues on hot-water treatment in daffodil
- AHDB Narcissus webinar: Use of chlorine dioxide in bulb dipping, 12 November 2020

Two masters students contributed to the project:

- Chow E. (2016). Alternative pest control in Narcissus – microwave technologies. University of Warwick unpublished master's dissertation.
- Scibisz N. (2018). Investigation of the efficacy of chlorine dioxide to control Fusarium oxysporum f.sp. narcissi in hot water treatment of daffodils. University of Warwick unpublished master's dissertation.

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

Stewart-Wade SM. (2011). Plant pathogens in recycled irrigation water in commercial plant nurseries and greenhouses: their detection and management. Irrigation Science 29: 267-297.

Acknowledgements

The author would like to thank all of those individuals who contributed to this project, including:

Mr Andrew Richards and Mr Julian Perowne who acted as industry representatives for Cornwall and Lincolnshire, respectively. They allowed their land, bulb stocks, equipment and time to be used in this project and it would not have been possible without them.

Mr Mark Eves, Mr Chris May, Ms Sylvie Pearson, Mr Allen Scrimshaw, Mr Wolfe Scrimshaw and Mr Mark Vandervleit for providing valuable industry knowledge and for allowing access to their farms, equipment and knowledge.

Mr Ewan Cameron of Scotmas Ltd who brought valuable commercial knowledge of chlorine dioxide to the project.

