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Project number:	BOF 077
Project leader:	Rob Lillywhite, University of Warwick
Report:	1st annual. December 2016
Previous report:	N/A
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Location of project:	Warwick Crop Centre
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Date project commenced:	1st January 2016
Date project completed (or expected completion date):	31st December 2019

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

Rob Lillywhite

Assistant professor

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Signature . Date. 8th February 2017

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GROWER SUMMARY

Headline

Both chlorine dioxide and hydrogen peroxide can be used to control the spread of Fusarium in hot water treatment of Narcissus bulbs, however, their efficacy is severely compromised by tank bioload and neither can be recommended at this time. Therefore, it is recommended to reduce tank bioload to improve the efficacy of chemical biocides (and fungicides). The installation of separation and/or /filtration systems is likely to be straight forward.

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added to HWT tanks as a general biocide i.e. to reduce inoculum in the tank water, however approval for formalin was withdrawn in 2008.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 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 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 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

The study of biocides in HWT is a very specialised area of research and the majority of evidence is to be found in AHDB commissioned research, namely BOF 61a (Hole, 2010) and BOF 70 (Hanks, 2010) and BOF 70a (Hanks, 2012) and the HDC Narcissus Manual (Hanks, 2013). No new scientific articles on this topic have been published since Chastagner and Riley in 2002. Therefore there is no new direct evidence which this project can build on. However, the literature on biocides is slightly more forthcoming as they are used as disinfectants in a number of industries. Perhaps the most useful of these is the treatment of irrigation water, particularly in recirculating systems, where the control of pathogens is critical. 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. At this early stage in this project, given that all the chemical biocides under investigation have some issues relating to either their use or efficacy, the focus will be to consider

combinations of treatments, e.g. filtration with UV and/or a chemical biocide, rather than repeating earlier work.

In vitro laboratory testing of candidate biocides

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 must 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 is the obvious starting point. Filtration and/or separation will be examined during the second year of the project, under both laboratory and commercial conditions.

Thermal treatment is 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 exercised between batches of bulbs, rather than within batch meaning that Fusarium spores can freely circulate within one batch.

Continuous thermal treatment is feasible 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 impractical, however, this will be undertaken during tank scale testing in early January 2017 to provide a comparison. 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. So, even if UV is shown to be an effective treatment, some doubts must remain whether it could be successfully used at a commercial scale.

In terms of identifying next generation biocides, this work provides mixed results. 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.

Assessment of the feasibility and cost of retrofitting biocide delivery systems

The assessment provides a number of clear outcomes. Firstly, while tank insulation is cheap and feasible for all systems, the potential savings are minor when compared to the overall operational costs. This said, tank insulation can reduce heat-up times and has the potential to reduce the total daily operation time.

Secondly, alternative biocides and biocidal approaches, namely the chemical biocides and UV radiation, demand good and very good, respectively, water clarity to be effective and reducing the tank biological load (bioload) is, we believe, key to the effective use of these approaches. Reducing tank bioload to improve water clarity is likely to be a key step in the use of biocides and probably fungicides. Since it is also a one off cost, it is also likely to be value-for-money. It is therefore recommended that a method of turbidity reduction be employed, through filtration or, preferable, vortex separation. Vortex separation is suitable given the low cost and accessibility of maintenance, although additional pumping may be required to maintain hydraulic pressure throughout the system.

Three case studies were undertaken to assess the viability of retro-fitting dosing and filtration equipment to existing tank systems. All three systems were different and employed different levels of sophistication, but in all three cases, retro-fitting of new equipment was assessed to be straightforward with little, or no, loss in system performance. The only instance where performance might be reduced was using a vortex separator in HWT systems where pump performance /flow rate was only just adequate in the absence of the separator. The capital costs for retro-fitting alternative disinfection methods and improvements to the operational efficiency (e.g. insulation or separation) are relatively small, one-off costs in the order of £10,000 to £20,000 per technology (inclusive of installation) depending on the size and complexity of the chosen devices.

Overall conclusion

The results from the biocide investigation confirm that all the approaches have value to commercial growers in terms of control of Fusarium and stem nematodes in tank water, and ease of operation.

In terms of chemical control, chlorine dioxide, hydrogen peroxide and Boot are all effective biocides in clean water, however, all three have issues as their efficacy is greatly reduced in dirty water. Although Boot is an effective biocide, concerns have been raised in the past about its phytotoxicity effect and it will not be examined any further. Of the remaining two, chlorine dioxide is possibly the better option in the long term since more is known about its use and efficacy in HWT systems, however, both it and hydrogen peroxide will undergo further testing before a final evaluation is undertaken. The key result from this stage of the project is the negative influence of tank bioload on the efficacy of all three chemical biocides. This is undoubtedly a major obstacle in the use of chemical biocides (and probably other biocidal and fungicide approaches) so we suggest that examining the introduction of some form of filtration/separation will be required as a starting position for the next stage of investigations.

Thermal treatment is undeniably effective and its efficacy probably unaffected by tank bioload. The difficulty arises in its implementation since increasing the temperature of tank water either continuously or in batches introduces new problems. Continuous thermal treatment may only provide selective treatment allowing some Fusarium spores to remain untreated while batch treatment will only prevent batch to batch infection and not reinfection within an existing batch.

Both UV and microwave treatment have promise and both will be examined in more detail next year. The efficacy of UV treatment is known to be affected by water clarity which adds weight to filtration/separation being the starting position for many, if not all, of these treatments.

The practicality of retro fitting biocidal technology was also examined. Most of the approaches do not create any special difficulties as automated dosing systems are available for chlorine dioxide and hydrogen peroxide, and the installation of filtration and UV lamps requires only minor pipework modifications.

Financial Benefits

At this stage, it is not possible to make any assessment of the financial benefits arising from this project.

Action Points

No actions should be taken at this time beyond exploring the possibility of fitting filtration or separation units to hot water tank systems.

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 for formalin was withdrawn in 2008. Work in BOF 61 (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 currently used by American narcissus growers. 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. 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 of water clarity, which is a problem with high bioload HWT. The issue of high HWT tank bioload was reported in BOF 70 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).

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.

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Objective 1. Review of the literature

The study of biocides in HWT is a very specialised area of research and the majority of evidence 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). No new scientific articles on this topic have been published since Chastagner and Riley in 20002. Therefore there is no new direct evidence which this project can build on. However, the literature on biocides is slightly more forthcoming as they are used as disinfectants in a number of industries. Perhaps the most useful of these is the treatment of irrigation water, particularly in recirculating systems, where the control of pathogens is critical. 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. At this early stage in this project, given that all the chemical biocides under investigation have some issues relating to either their use or efficacy, the focus will be to consider combinations of treatments, e.g. filtration with UV and/or a chemical biocide, rather than repeating earlier work.

The literature that informed the different aspects of the work is detailed within the relevant sections.

Objective 2. In vitro laboratory tests

Materials and methods

In vitro laboratory tests were used to examine the efficacy of the different biocides to control *Fusarium oxysporum f.sp narcissi* (FON) causing basal rot and *Ditylenchus dipsac*i (stem nematode). 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.

Production of Fusarium chlamydospores

Chlamydospores of *Fusarium oxysporum f.sp. narcissus* isolate 139 were prepared by using a method based on that of Bennett and Davis (2013) and Dhingra and Sinclair (1985). Clean water produced through the process of reverse osmosis (RO) was added to Levington M2 compost 4:1 (v/w) and left to stand for 36 hours. Large particles were filtered using a 2mm sieve and then the remaining liquid filtered through 10 layers of cheesecloth. The resulting soil broth was then autoclaved twice (121 °C, 15 mins) and the sediment remaining in the broth allowed to settle before decanting off the clear fraction for use. FON isolate 139 was grown at 22°C on Potato Dextrose Agar (PDA) plates, for two weeks, after which 2ml sterile distilled water was added to each plate which were then scraped to yield a conidial suspension; this was then filtered to remove mycelium fragments, and 2ml of spore suspension added to petri dishes containing 20ml sterile soil both. Plates were incubated for seven days at room temperature and prior to use in experiments the chlamydospore cultures were macerated in a lab blender for 10 minutes, and then filtered to remove mycelium.

Maintenance of stem nematodes

Narcissus bulb nematodes (*Ditylenchus dipsaci*) were maintained using a standard carrot disc culture method. Whole carrots were soaked in a 10% sodium hypochlorite solution for approximately one hour and removed using sterile forceps. The carrots were then placed into sterile distilled water and soaked for a further hour. The water was then drained off and in a laminar flow cabinet using sterile scalpel and forceps the carrots were peeled (in a clean petri dish) and cut into approximately 1cm discs.

Approximately 20 carrot discs (or to fill) were placed in a clean petri dish and 100µl of nematode solution pipetted onto each disc (approximately 100 nematodes per disc), the dishes wrapped in parafilm and incubated at 25°C in the dark. When carrot discs started to turn brown in colour as nutrients were used up, and nematodes started to emerge (6-8 weeks), they were sub-cultured onto fresh carrots. To harvest the nematodes for use in experiments the carrot discs are placed into a sieve lined with milk filters inside a plastic box, the plastic box filled with water and left for approx. 1h, or overnight to allow the nematodes to swim out of the discs into the plastic box. The water was then poured from the plastic box into a beaker nematodes allowed to settle for approx. 1h before water was decanted off to leave approximately 100ml. This was then transferred into 50ml falcon tubes (x2) and the nematodes allowed to settle again, after which they were visible as a cream coloured layer at the bottom of each tube.

Testing of thermal treatments (Fusarium and nematodes)

<u>Fusarium:</u> chlamydospores of FON 139 (500µl) in 1ml eppendorfs suspended in polystyrene floats were placed in water baths at 44.4°C, 50°C, 60°C, 70°C and 80 °C for 2, 15, 30, 60, 120 and 180 minutes. At the end of each time duration, spores were removed and placed immediately on ice. A serial dilution of the spore suspension was then performed and each dilution (100 µl) plated onto three replicate plates. 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.

Stem nematode: 50 nematodes were counted out into 1ml eppendorfs, re-suspended in 1ml of sterile distilled water and placed into polystyrene floats in water baths at 44.4°C, 50°C, 60°C, 70°C and 80 °C for 5, 15, 30, 60 and 180 minutes. At the end of each time duration, tubes were removed and placed into a water bath at 18°C, to allow the nematodes to recover. Assessments of nematode mortality were then carried out one and three days after treatment, by observation under a dissection microscope; nematodes were considered to be alive if they responded to a light touch with a dissection needle.

Testing of didecyl dimethyl ammonium chloride (Fusarium)

Chlamydospore suspensions (1 ml) of FON isolate 139 were placed in 2ml tubes and the required amount of didecyl dimethyl ammonium chloride (BOOT, 120 g/L a.i.) added to achieve 0.1%, 0.2%, 0.5%, 1% and 2% final concentration product. Tubes were then floated in a water bath at 44.4 °C for 5, 15, 30, 60 and 180 minutes. At the end of each time duration, spores were removed and an equal volume of sterile distilled water added, followed by vortexing and centrifugation at 13,000 rpm for one minute. To wash the spores free of product, the supernatant was then removed and the pellet re-suspended in 1ml of SDW, vortexed and centrifuged for 1 min at 13,000 rpm. This process was then repeated twice after which the spore suspension was used to perform a serial dilution, onto three replicate plates (100 µl per plate). Plates were incubated at 18°C for five days after which the number of colony forming units (cfu) recorded and the concentration of viable spores/ml calculated.

Testing of Chlorine Dioxide (Fusarium)

A stock solution of 1000ppm of ClO_2 was created from 10ml Activ-Ox10 and 10ml Activ-8 in 80ml of water, and then diluted to achieve 1.0, 2.5, 5.0, 7.5 and 10.0 ppm ClO_2. Chlamydospore suspensions of FON isolate 139 were placed in 2 ml tubes and then centrifuged to a pellet. The spores were then re-suspended in 1ml of ClO_2 at each of the five concentrations and tubes floated at 44.4 °C for 5, 15, 30, 60 and 180 min. At the end of each time duration, the spores were removed and an equal volume of sterile distilled water was added, vortexed and centrifuged at 13,000 rpm for one minute. Spores were then washed three times as described above by centrifugation. As before, spore suspensions were used to perform a serial dilution, onto three replicate plates (100 µl per plate). Plates were incubated at 18°C for five days after which the number of colony forming units (cfu) recorded and the concentration of viable spores/ml calculated.

Testing of Hydrogen Peroxide (Fusarium and nematodes)

<u>Fusarium</u>: Chlamydospore suspensions of FON isolate 139 (1 ml) were placed in 2ml tubes and the required amount of H_2O_2 added to achieve 0.1%, 0.5%, 1%, 1.5% and 2% final concentration after which tubes were floated at 44.4 °C for 5, 15, 30, 60 and 180 minutes. At the end of each time duration, the spores were removed and an equal volume of sterile distilled water was added, vortexed and centrifuged at 13,000 rpm for 1 minute. Spores were then washed three times as described above by centrifugation. As before, spore suspensions were used to perform a serial dilution, onto three replicate plates (100 µl per plate). Plates were incubated at 18°C for five days after which the number of colony forming units (cfu) recorded and the concentration of viable spores/ml calculated.

Stem nematode: Fifty nematodes were counted out into 1ml eppendorfs and re-suspended in 1ml of sterile distilled water. The required amount of H_2O_2 was then added to the tubes to achieve 0.1%, 0.5%, 1%, 1.5% and 2% final concentration and tubes floated in a water bath at 18°C for 5, 15, 30, 60 and 180 minutes. At the end of each time duration, nematodes were pipetted into 50 ml of sterile distilled water and allowed to settle for 1 hour, after which 40 ml of the water was removed and replaced with 40 ml of fresh sterile distilled water and left overnight. The following morning 40 ml of water was again removed and replaced with another 40ml of fresh sterile distilled water and allowed to settle for one hour. After this water was carefully removed to leave 5ml which was then transferred to a 4cm petri dish for examination under the microscope. Assessments on nematode mortality were carried out one and three days after treatment (DAT), by observation under a dissection microscope; nematodes were considered to be alive if they responded to a light touch with a dissection needle.

Testing of chlorine dioxide and hydrogen peroxide in bioload 'dirty' water

The efficacy of selected chlorine dioxide and hydrogen peroxide treatments were tested in 'dirty' water with a bioload to compare with the results using clean laboratory water described above.

To produce water with a bioload which could be replicated experimentally, loose dried narcissus bulb scales were removed from bulbs, ground in a coffee grinder to a fine powder, passed through a 1mm sieve and autoclaved to prevent the introduction of any diseases. 1ml of chlamydospores of FON isolate 139 were placed in 2ml tubes and centrifuged at 13,000 rpm to pellet the spores and the supernatant removed. Ground narcissus scale (40 mg) was then added to the tubes and the spores and scale mix re-suspended in 1 ml of sterile distilled water and vortexed vigorously. The required amount of chlorine dioxide or H_2O_2 was then added to achieve 7.5ppm, 10ppm and 1.5%, 2.0% final concentration respectively to tubes containing the spores and scale mix and the tubes were floated in a water bath at 44.4°C for 60 and 180 minutes. At the end of each time duration, the spores were removed and an equal volume of sterile distilled water was added, vortexed and centrifuged at 13,000 rpm for one minute. Spores were then washed three times as described above by centrifugation. As before, spore suspensions were used to perform a serial dilution, onto three replicate plates (100 µl per plate). Plates were incubated at 18°C for five days after which the number of colony forming units (cfu) recorded and the concentration of viable spores/ml calculated.

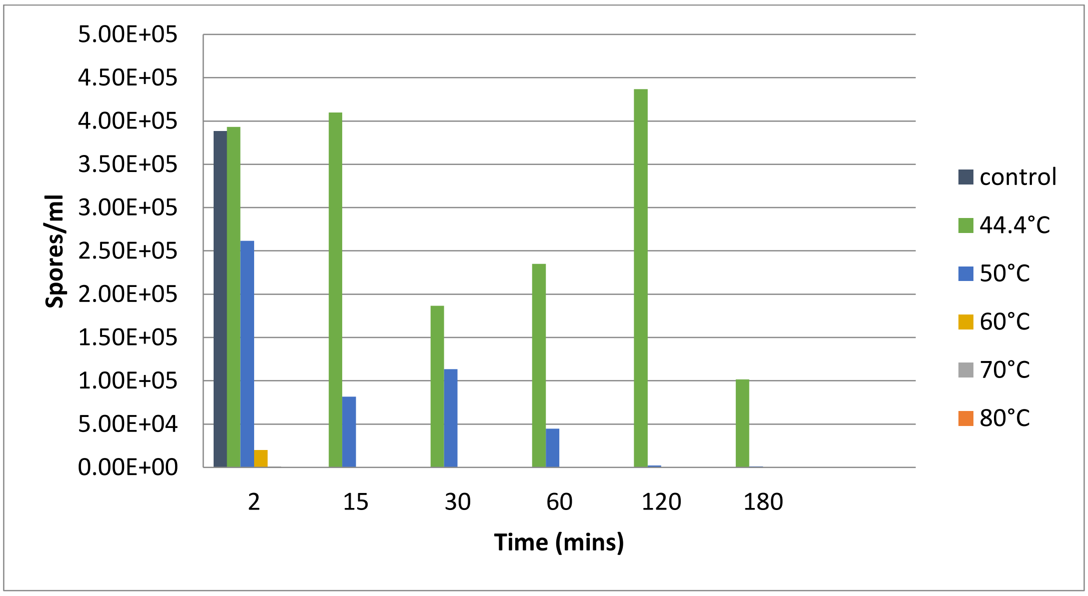
The production of a bioload to replicate the dirty water found in commercial HWT tanks is far from an exact process. In addition to macerated bulb scales, the bioload from farm tanks contains some soil and other biological materials, and possibly pesticide residues. In order to provide consistency across treatments, the decision was taken not to replicate this exactly, so the manufactured bioload used in the project contains only macerated bulb scales.

Results

Thermal treatments (Fusarium and nematodes)

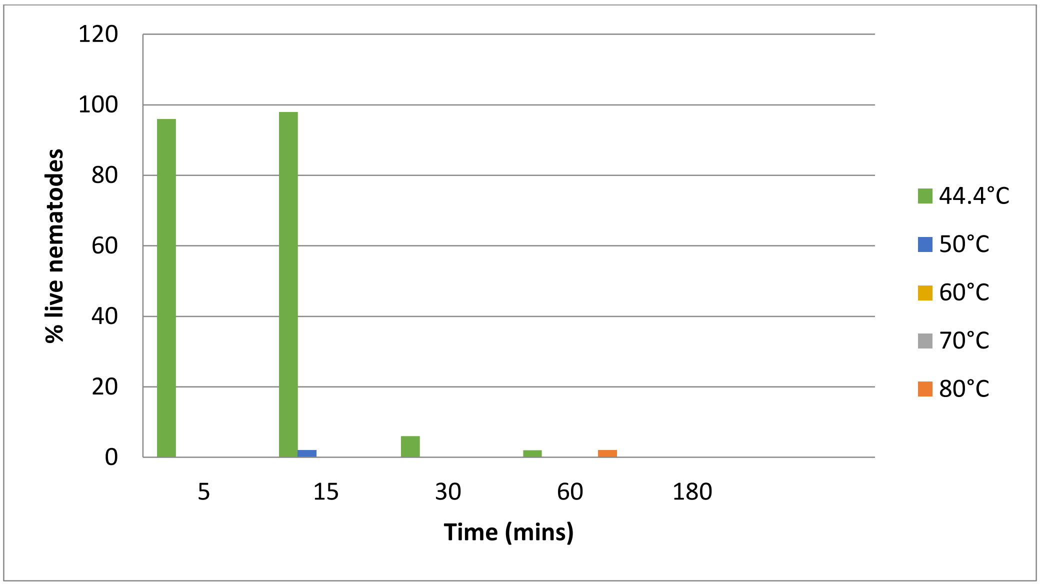
The results show that raising the temperature of water above the 44.4°C routinely used for narcissus bulb dipping reduces the viability of FON chlamydospores. At 44.4 °C, the number of viable spores was only slightly reduced from 4 x 10^5 spores / ml to 1 x 10^5 spores / ml confirming that some form of biocide is required at the recommended dipping temperature (Figure 1). Increasing the water temperature to 50°C resulted in total loss of spore viability after 180 minutes while at 60°C most spores were killed within 2 and all within 15 minutes. Temperatures of 70 and 80°C were lethal to FON spores within 2 min exposure (Figure 1).

Figure 1. Effect of temperature treatment and duration on viability of FON spores



Increasing the temperature of the water was also effective in controlling nematodes (Figure 2). The current recommendation is 44.4°C for 180 minutes and this combination provided complete control, however, there is a definite tipping point at which nematodes succumb to increasing temperature, which appears to be between 15 and 30 minutes. Although it should be remembered that these results were generated in vitro and that nematodes in bulbs will behave differently. Increasing the water temperature to 50°C and above shortened the time taken to achieve complete kill although occasionally a single nematode survived at higher temperatures. At 60°C complete control was achieved at all time durations.

Figure 2. Effect of temperature treatment and duration on survival of nematodes



Didecyl dimethyl ammonium chloride (DDAC) (Boot)

In clean water, DDAC reduced the viability of FON spores at all the concentrations tested with 0.5%, 1% and 2% levels resulting in complete mortality at all the durations tested (Figure 3). Concentrations of 0.1% and 0.2% were also completely effective for treatment durations over 30 minutes. However, the efficacy of DDAC was severely reduced by the presence of a bioload with efficacy dropping from complete mortality at the highest dose / longest duration (2% for 180 minutes) combination in clean water to around 60% reduction in viability in bioload water. (Figure 4). Bioload water also introduced some difference in control at the 2% concentration which is possibly explained by the soil and organic matter in the bioload water.

Figure 3. Effect of DDAC concentration and duration on viability of FON spores in clean water

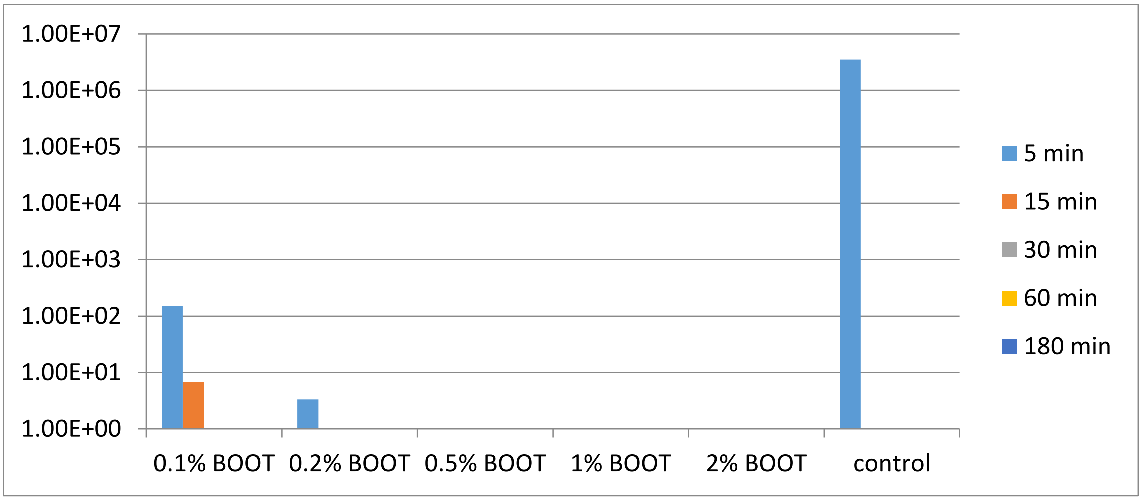
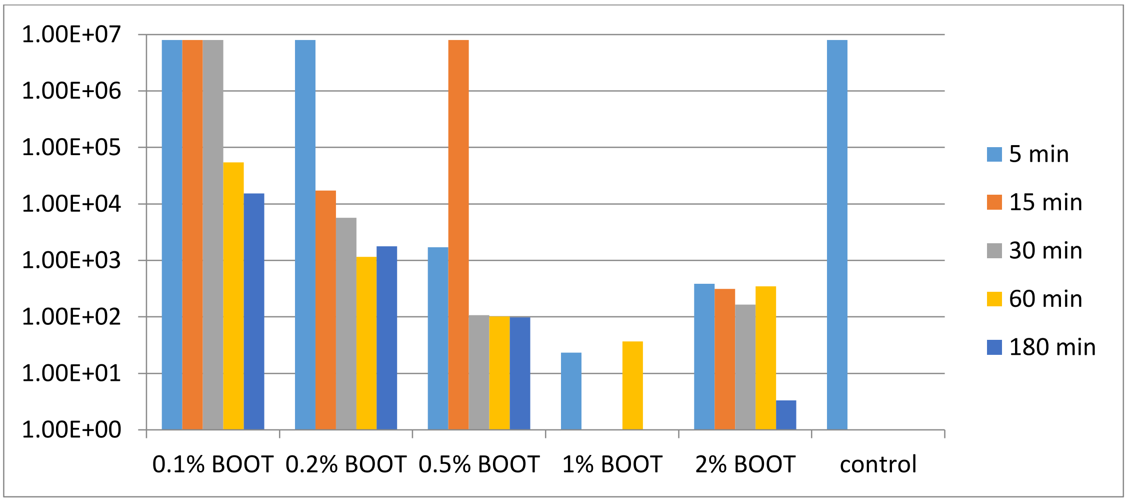


Figure 4. Effect of DDAC concentration and duration on viability of FON spores in dirty water



Chlorine Dioxide (Fusarium)

In clean water, the efficacy of chlorine dioxide in reducing FON spore viability increased with concentration and duration. Concentrations of 1 and 2.5 ppm were ineffective but at 5ppm and above, spore viability was reduced substantially. Complete mortality was obtained for a concentration of 10ppm for a duration of 180 minutes (Figure. 5). However, efficacy of chlorine dioxide was severely reduced when the tests were repeated using dirty water; for the 10ppm treatment, spore viability was reduced from 1 x 10^7 cfu / ml (control) to 2 x 10^5 cfu / ml after 180 minutes duration (Figure 6).

Figure 5. Effect of chlorine dioxide concentration and duration on viability of FON spores in clean water (spores/ml)

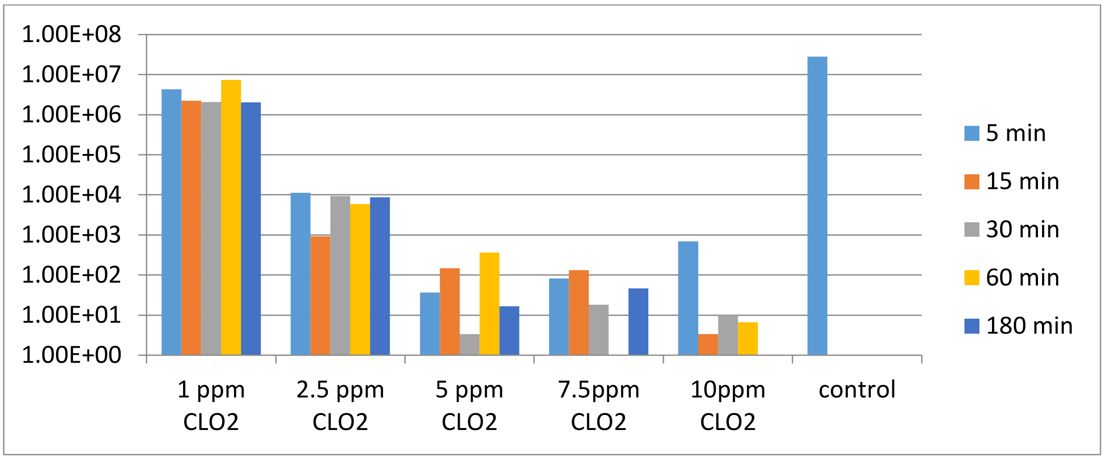
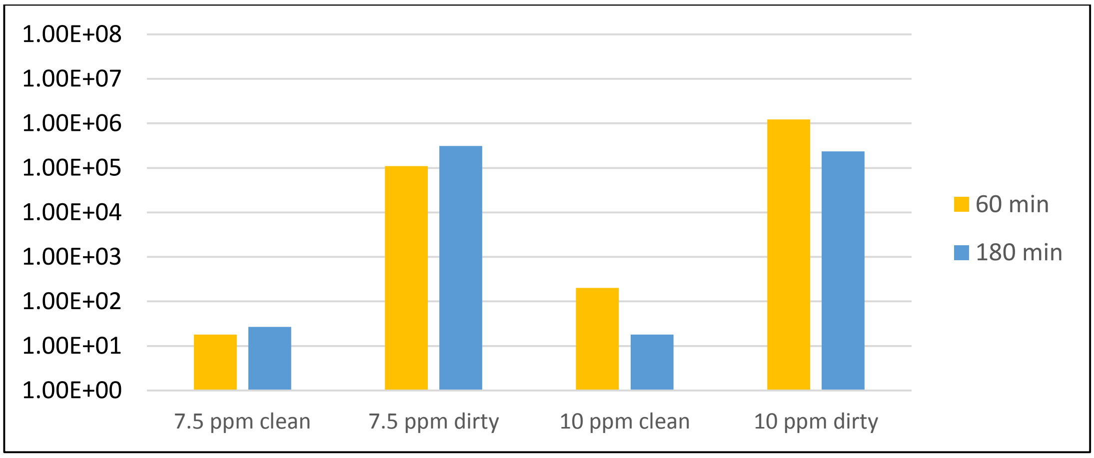


Figure 6. A comprison on the effect of chlorine dioxide concentration and duration on viability of FON spores in clean and dirty water (spores/ml)

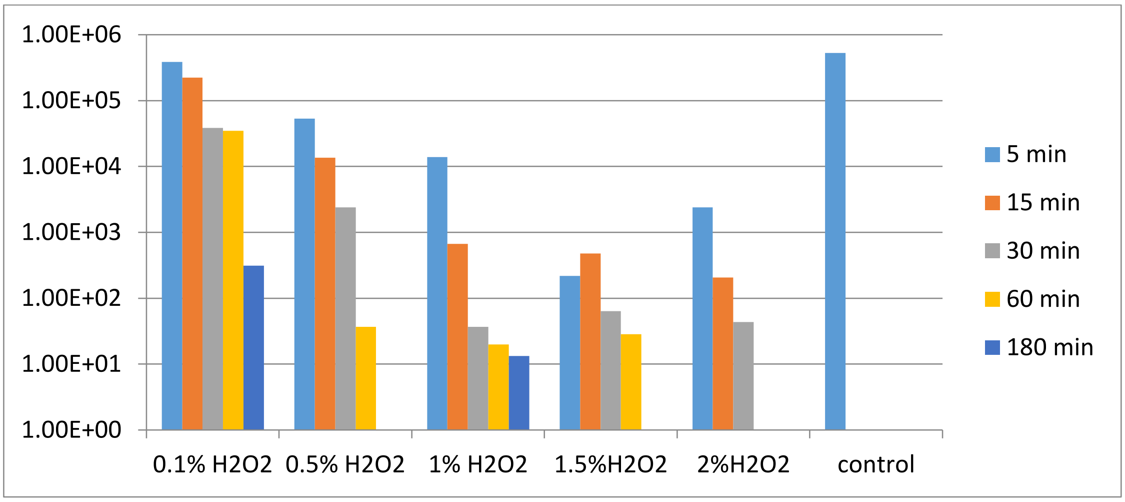


There are some differences between the clean water values at 7.5ppm and 10ppm between Figures 5 and 6. These were separate experiments and the differences are likely to be natural variation.

Hydrogen Peroxide (Fusarium and nematodes)

In clean water, the efficacy of hydrogen peroxide increased with concentration and duration. At 1.5% concentration, FON spore mortality was 99.9% after 60 minutes with complete mortality after 180 minutes and for 2.0%, 99.9% mortality was achieved after 30 minutes with complete mortality after 60 minutes (Figure 6). The efficacy of hydrogen peroxide was reduced when the tests were repeated using dirty water. At 1.5%, efficacy was reduced four-fold at 60 minutes at 44.4°C and by 5.8-fold for 2.0% after 60 minutes. However the performance of 1.5% and 2.0% H_2O_2 after 180 minutes remained unaffected (data not shown).

Figure 6. Effect of *hydrogen peroxide* concentration and duration on viability of FON spores in clean water



A proportion of nematodes survived all hydrogen peroxide treatments at all durations although there was a reduction in the number of live nematodes at the highest concentration/longest duration combination. Nematodes which did survive were more sluggish to respond to touch at the higher concentrations. These experiments were carried out at 18°C, which is well below the recommended temperature to control stem nematodes, therefore it is very likely that all nematodes would have been controlled at the recommended temperature of 44.4°C for 180 minutes.

Discussion

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 must 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 is the obvious starting point.

Thermal treatment is 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 exercised between batches of bulbs, rather than within batch meaning that Fusarium spores can freely circulate within one batch.

Continuous thermal treatment is feasible 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, this will be undertaken during tank scale testing in early January 2017 to provide a comparison. 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, this work provides mixed results. 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.

Objective 3 - Assess the feasibility and cost of retrofitting biocide delivery systems to existing HWT tanks

The Hot Water Treatment (HWT) process

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 container (approximately 10 m^3 in volume) with access via the top or side of the system (e.g. **Figure** 7). 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 7. Common 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.

Control of stem nematodes requires that each batch of bulbs should be heated to 44.4ºC for three hours total treatment to ensure that the centre of every bulb has reached the prescribed temperature. In practice the batch process can take approximately four hours when considering the temperature drop caused by the subsequent batch and turnover times. 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; namely prochloraz, thiabendazole or FAM30. Similarly to HWT, fungicidal 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 is conducted using cold-water treatment; eliminating heating requirement and lengthy treatment times.

The need for alternative technologies

Changes to chemical regulations and the increasing costs for permitted chemicals demand that alternative and more efficient treatment processes 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 as a result of soil sediment and loose bulb peel increases chemical demand through adsorption and bio-loading.

There is therefore large 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.

Aim

This objective seeks to assess 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. In addition, the impact on HWT infrastructure was chemically assessed using a controlled laboratory study to provide advice on alternative fungicidal treatments.

Alternative Technologies

The proposed alternative technologies were selected to provide viable alternatives that can be retrofitted onto existing HWT systems; or can be included in system operations whilst demanding only minor alteration to the HWT process. In every case below, the technology is described in terms of application within the HWT, infrastructural requirements, and the specific target pathogen(s). It should be noted that all of the following proposed alternative treatment methods and improvements have been tested, individually, in terms of efficacy under controlled laboratory conditions.

I. Chlorine Dioxide disinfection

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 application of dissolved ClO_2 for wider disinfection has not been extensively used in more industrial applications (e.g. where the required concentrations are significantly higher); however, its use in sewage treatment is increasing, giving encouragement for its application to systems demanding high concentrations – such as agricultural disinfection and sterilisation.

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. 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. It should be noted that one current drawback of on-site preparation is the current limitation when attempting to dose at higher concentrations using currently available injection techniques.

The suggested method for ClO_2 treatment is to dose the HWT water using an automated dosing system at the tank inlet. Concentration would be continuously recorded using chemical probes or real-time chemical analysis; stipulating that the concentration detector is located far from the dosing inlet.

A number of uncertainties currently limit the employment of ClO_2 into current HWT facilities. Firstly, the impact of oxidisation on HWT infrastructure needs to be investigated. Secondly, the efficacy of application in concurrence with hot water treatment has not been sufficiently investigated to assess potential gaseous losses due to high temperatures and open-air treatment. Additionally and most importantly, the required dosing rates need to be adjustable for varying biological demand; which subsequently varies between bulb batches and turbidity levels. It follows that achieving the desired concentration of ClO_2 using commercially available dosing systems may not be viable for agricultural applications.

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.

II. Hydrogen Peroxide disinfection

Hydrogen Peroxide, H_2O_2, is an oxidiser and chemically reacts in a similar manner to ClO_2 forming ions of oxygen and hydrogen when in aqueous solution. Hydrogen Peroxide's application is therefore viable for pathogenic treatment – indeed H_2O_2 has been used as a steriliser in high-grade bio-chemical and other scientific experimentation.

Hydrogen Peroxide can be dosed in the same manner as ClO_2 employing an automated dosing system and real-time concentration monitoring. It can also be commercially purchased as a synthesised solution – negating the need for an on-site mixing system. However, H_2O_2 is chemically highly reactive and entails safety considerations as well as regulation for industrial applications.

Hydrogen Peroxide presents a major benefit to the HWT process given the chemical by-product of water (H_2O). Similarly to ClO_2, the application of H_2O_2 is limited by the current knowledge of its efficacy in higher temperature conditions; by its impact on HWT steel infrastructure and by its efficiency in turbid conditions.

III. Ultra-Violet disinfection

Ultra-violet (UV) disinfection is currently employed by the potable and wastewater industry as a final treatment or "polishing" method. UV disinfection irradiates the water column using high power UV incandescent tubes submerged within the water.

The suggested implementation of UV is to install in-line UV chambers located within the water pumping system or within the HWT slave system (if applicable). The application of UV is suggested for a combinational use with alternative treatment methods or chemicals. For example, UV radiation may provide additional system sterilisation – reducing the potential for cross-contamination and the persistence of Fusarium within the recirculating water.

Pipe installation represents the most viable option as it increases the radiation dose per unit volume, increasing the treatment potential. Moreover, the lack of chemical requirements bypasses chemical regulation and by-product treatment. The potential application to agricultural disinfection is therefore promising given that HWT system are recirculating; thus increasing the radiation dose and potential for elimination of pathogens.

Ultraviolet disinfection may, however, be limited by the turbidity of the water – where the radiation dose is directly affected by the absorbance of the water column. As such, filtration or separation methods may be required to ensure that UV disinfection is effective (see below).

IV. Pre-treatment filtration and separation

Obviously chemical challenges are apparent in the HWT process associated with the biological load of sediment, waste material and other pathogens. Significant sediment levels accumulate in the treatment tanks during a season; absorbing and adsorbing increasing levels on anti-fungal chemicals. Screening, separation and filtration of the re-circulating water is a potential method for reducing the overall bio-load. Reducing water turbidity may also improve the efficacy of other proposed treatment methods such as UV, ClO_2 and H_2O_2 disinfection.

There is a range of proposed methods of reducing water turbidity depending on the required water clarity. Screening of the water is common to many HWT tanks – removing bulb husk and large vegetation matter. Effective management and removal of debris often limits the efficacy of screening allowing the hot water to breakdown material into smaller sediment. Mechanical filtration is a viable method for reducing turbidity. On-line or parallel filters can be easily installed onto existing HWT tanks. Although, given the high turbidity levels, mechanical filtration using filter media (e.g. porous material) may not be an effective method for two reasons. Firstly, the filter media quickly fills with sediment requiring regular maintenance and expenditure on parts. Secondly, water pressure can drop dramatically across the filter when blocked with sediment, thus reducing the flow of hot water through the batch. Filtration devices and media are, relatively cheap and accessible making it potentially more favourable to some growers.

HWT using vortex separation presents the most suitable approach to reducing turbidity. Vortex separation reduces turbidity by manipulating the flow of water within a device such that the particles are forced out of the water column. The efficacy of this process clearly depends on particle size and flow rate through the device but is an attractive option given the absence of filter media. The separated particles can be collected in a separate unit and removed quickly and easily to maintain efficacy.

V Thermal insulation and efficiency improvements

HWT systems are location in open-air environments and are therefore subject to ambient temperatures and fluctuations. Apparent in many designs is the lack of thermal insulation and potential operational inefficiencies. Investing in thermal insulation could increase thermal efficiency – therefore reducing costs. Such an approach is applicable given the range of bespoke HWT tanks and the relative costs of thermal insulation compared to other technologies. It’s uptake by growers is subject to the overall costs on fuel.

Conceptual Desk Study Analysis

An idealised HWT system was used to perform a conceptual desk study of the feasibility for the proposed alternative treatment technologies. In doing so it is acknowledged that each HWT system is specific to a particular grower and thus feasibility will vary in practice. The conceptual desk study does, however, enable the impact and efficacy of the proposed technologies to be investigated for a ubiquitous design and compared to a standard model without the need to provide a bespoke solution for each grower.

Where possible, each technology was modelled in terms of cost – capital and operational – to assess the net savings that result. Each technology is analysed with the inclusion of quotes and manufacturers.

Standard Model HWT

The idealised HWT system is a 10,000 l (10.0 m^3) with a height, width and depth of 2.0, 2.0 and 2.5 m, respectively. The tank is constructed from 4 mm mild steel, with steel bracing along the tank vertices. The tank is open-top with a free-water surface of 5 m^2, a steel surface of 23 m^2 with a total surface area of 28 m^2. The tank is filled with water at an assumed 20ºC and heated to 44.4ºC using a light oil gas burner – located directly beneath the system – capable of supplying 120 kW of heating. Ambient temperature is taken as 15ºC being the mean, outdoor temperature over June, July and August for Cornwall. The water within the tank is recirculated using a pump and the pump intake and outlet are located at the diagonal vertices of the tank.

Narcissus bulbs are inserted into the tank in half tonne, porous boxes (500 kg), two at a time, and are treated at 44.4ºC for 3 hours. Water is forced through the bulbs using the recirculating pumping system and the temperature is maintained at 44.4ºC using temperature controlled oil burning. The Narcissus bulbs cause a drop in the temperature of the system so an additional 0.5 hours is required to allow the system to reach 44.4ºC. The time for one full treatment is estimated as 4 hours and thus 3 treatments can be achieve in one day. The Conceptual Heat Model is provided in Appendix 1 and provides an estimated total heat input per day and approximate cost. The following assumptions about the system are made:

- The water heats proportionally to power input and disproportionately to the temperature difference between the system and ambient conditions;
- The water-steel and steel-air interfaces are in thermal equilibrium with each other when the tanks is kept at a steady 44.4ºC;
- The two major sources of heat loss are convection through the open surface area and black-body radiation through the steel area;
- The thermal conductivities of the material are not functions of temperature;
- A maximum power input of 120 kW is supplied from the light oil burners;
- The time taken to change Narcissus batches is 15 minutes.

The model outputs show that:

- The time taken to heat to 44.4ºC is 50 minutes (assuming minimal heat losses during heating);
- Assuming a fuel output efficiency of 95% (provided by the manufacturer), a fuel price of £0.265/litre, the initial heating cost is £2.26, the cost for one cycle is £2.76 and the daily costs are £3.84;
- The total daily fuel consumption is approximately 19.3 litres.

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 chlorine dioxide 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 chlorine dioxide 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 Chlorine Dioxide.

Using a 15 m^3 volume example, 37 ml and 74 ml of pure chlorine dioxide is required to yield 2.5 and 5.0 ppb concentration, respectively. However, BOF 61a (Lole, 2010) recommends a working, steady concentration of 50 ppm implying that an initial input of 740 ml of ClO_2 is required. Clearly the rate and dosage of ClO_2 is dependent on the biological load of the water, and also on temperature and pH, although a dosing system delivering 100 g/hr is deemed sufficient.

A number of considerations need to be taken into account when assessing the feasibility of chlorine dioxide 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.

The ChloriDos Lite is a chlorine dosing system manufactured by Water Hygiene Services Limited capable of delivering up to 100 m^3 of chlorine solution per hour. Rough estimates put the capital costs for such a design at approximate £5000 (note that this value does not include the cost for telemetry and monitoring).

Alternatively, the Feedwater Activ-Ox Chlorine Dosing system is capable of delivering chlorine dioxide between 10-100 g/hr. The Feedwater designs provides chlorine dioxide in solution without a gaseous stage by mixing two chemicals together, Activ Ox and Activ 8, which may be performed on-site. This method could be employed using the HWT tank as the chemical mixer minimising capital costs. Activ Ox and Activ 8 are priced at £7.24 and £2.60 per kilogram (2016 prices), respectively, and assuming a conversion rate per part per million concentration of 50 ml per cubic meter, the initial cost of dosing to reach 5 ppm is £36.90.

The rate of dosage during the treatment process cannot be estimated given the need for accurate quantification of the decay rate of chlorine; which is, in turn, dependent on the biological loading of the system and potentially sediment concentration. The decay rate may also be variable with operation time.

Consultation with Grundfos estimates the installation costs for a ClO_2 dosing system at between £12-15k with an additional £3-5k required for monitoring and analysis for the ChloriDos Lite design. The capital costs for the Feedwater ActivOx system are lower than the ChloriDos Lite system, costing £4k for the dosing infrastructure and £3.5k for the chemical detection and control system. However the ActivOx chemicals are more expensive than ChloriDos and so overall cost implications will need to be examined before any decisions can be made.

Hydrogen peroxide disinfection

Hydrogen peroxide is a strong oxidiser, bleaching agent and disinfectant. It also demonstrates broad-spectrum efficacy against viruses, bacteria, yeast and bacterial spores. It is regarded as a potentially environmentally friendly alternative to chlorine-based bleaches as it degrades into water and oxygen. It has been used in water treatment services, e.g. irrigation lines, at concentrations around 1,000 to 3,000 ppm (Stewart-Wade, 2011).

Linfield (1991) found that Fusarium spores were eradicated after 80 minutes using a 0.5% (5,000 ppm) solution of hydrogen peroxide and peracetic acid. Increasing the concentration reduced time to eradication. Based on a tank size of 15 m^3, 75 litres of hydrogen peroxide would be required to provide a concentration of 5,000 ppm. Hydrogen peroxide can be purchased commercially for approximately £0.5 – 1.00 per litre available in a range of pre-made aqueous solution concentrations. The decay rate of hydrogen oxide in HWT tanks is unknown so dosing strategies and costs need further work before any recommendation could be made.

Ultra-violet (UV) disinfection

Ultra-violet disinfection is employed as a “polishing” method in potable and waste-water treatment services. The radiation delivered by UV lamps destroys the cell wall of the bacterium or fungus and is therefore highly suitable for treating water-borne pathogens. Paramount to UV disinfection efficacy is the water turbidity. Higher turbidity will attenuate the radiation power and will reduce the overall dose that any one bacterium or fungus receives when passing the UV lamp.

The conceptual UV design is to install UV lamps within the recirculating pipe network such that each parcel of water is regularly irradiated during the treatment cycle. The recirculating nature of the HWT process lends itself to UV treatment and also allows the lamp power to be reduced given the total dose that a pathogen might receive. High radiation power is employed in the water treatment industry given the high flow rates and therefore the requirement to provide sufficient radiation as the water passes into the distribution network. In HWT, the treatment water needs to be recycled at up to 10 times per hour requiring, for the idealised system, a mean flow rate of approximately 27 l/s or 100 m^3/hr.

A number of UV in-pipe lamp systems are available depending on the overall power that is required. However, the S008-T5225 UV Water Filter from IWE is designed to treat up to 227 litres per minute (4 l/s) and costs £1392.60; while the larger model is suitable for flows of up to 9 l/s, costing £2552 (Note that costs are quoted from Industrial Water Treatment Ltd). There are a number of producers of enclosed UV disinfection for water treatment, including:

- Trojan UV – manufacture UV disinfection for a range of applications and flow rates delivering equipment with monitoring capabilities. The Trojan UV Max Pro is a suitable device that can be installed into the HWT system in-line or in-parallel with the treatment process.
- Sita DS range – manufacture for larger scale applications capable of operating under higher pressures (necessary to force water through the bulbs). The DS 400 can treat at flow rates of up to 100 m^3/hr and is equipment with 1, 400 W UV lamp with a 1400 hour lifetime costing approximately £8000 (and £11,298 for 2x400 W lamps). Note that the Sita AM24 can treat flow rates of up to 50 m^3/hr and costs £5326 for the model with 2x200 W lamps, also at up to 9 bar.
- S Line UV Water Filters – manufacture for medium sized operations of up to 21 m^3/hr. These enclosed units can provide 110 to 550 W of UV radiation, costing between £825 and £ 3958, respectively.

Given the required operational flow rates of up to 100 m^3/hr (27 l/s) a larger device is required with a minimum flow capacity of 50 m^3/hr. Such a model is estimated to cost at least £5000 based on initial research. A capital investment of up to £12,000 would allow for a large model to be installed capable of handling particularly high tank circulations rates.

Pre-treatment sediment filtration/separation

Sediment separation has been employed in high-end manufacturing processes as a method for separating material from lubricants. It is proposed that an in-line or parallel sediment filter or separator could be employed within the HWT system to provide continuous filtration during operations. In-line or parallel filtration/separation can be easily retro-fitted onto existing HWT systems given the relative size and manoeuvrability of the proposed units.

For the self-contained, idealised HWT system, a sediment separator can be installed in-line with the recirculating pumping system to ensure sufficient pressure through the device. Such an installation is relatively easy given the access to the piping network and cost of additional piping.

Given the high levels of turbidity and debris associated with HWT, filtration is not recommended for two reasons. Firstly, the filters will become readily clogged requirement frequent maintenance and replacement; and secondly, filter blocking causes a reduction in water pressure, reducing the flow rate through the bulbs.

Vortex separation may also be installed in parallel with a slave tank system. This approach would require a separate pumping system but would allow the water to be separated during batch change over.

Bell and Gossett manufacture Sediment Removal Separators up 1 m in height, requiring an operating pressure of 125 psi. Nikuni manufacture small scale dynamic vortex separators that may easily be installed onto existing systems using a piping re-arrangement. Such a system comes with a dumping value allowing the waste material to be easily removed from the separator making such a model suitable for environments where maintenance needs to be straight-forward.

The approximate cost for both of the above devices starts at £8000. Such a capital cost is regarded as a relatively small investment given the potential gains for removing sediment and the low maintenance when compared to conventional filtration. Note that installation of a sediment separation system is recommended in combination with a novel disinfection system to reduce the overall biological load within the water column, improving chemical disinfection such as chlorine dioxide, or turbidity, improving the efficacy of radiation treatments such as UV.

HWT thermal insulation and operational improvements

The idealised HWT system heating and costing model was re-evaluated for four modified scenarios; a closed lid system (with steel), open lid system polystyrene insulation, a closed-lid system with polystyrene wall insulation and a closed lid system with polystyrene insulation on all surfaces. Recall that the estimated first-cycle and daily costs for the open top steel system are £2.76 and £3.84, respectively (note that first-cycle includes the initial heating requirements from 20ºC to 44.4ºC). In every case, the polystyrene was chosen to be 25 mm based on commercially available products.

Note that such a model should be used for comparative means and should not be used as a method to precisely predict the daily fuel costs for specific site operators. However, the estimated heating time for the idealised system of 50 minutes agrees well with the most similar installation (Hayle, Cornwall) where a heating time of 45 minutes was verified.

Firstly, the model showed that the enclosure of the tank system using a steel lid made from the same steel and thickness as the tank walls reduced the first-cycle and daily costs, by a minimal amount, to £2.69 and £3.61, respectively; representing a total daily increase of £0.23. Note that this estimation assumes that the lid of the tank is in contact with the water surface and thus the rate of heat loss through the surface is over-estimated by neglecting the water-air-steel-air interfaces.

Secondly, the open-lid system with polystyrene insulation reduced the first-cycle and daily costs to £2.48 and £2.97, respectively; representing a total daily saving of £0.87. Thirdly, the closed steel-lid system with polystyrene insulation on the walls also reduced the first-cycle and daily costs to £2.41 and £2.73, respectively; providing a daily saving of £1.11. Finally, as expected, the completely insulated scenario with insulated lid reduced the first-cycle and daily costs to £2.35 and £2.54, respectively; representing a maximum saving of £1.30.

Table 1 compares the first-cycle, daily and seasonal fuel and volume cost savings compared to the base scenario for the four proposed insulation methods. Evident from the four scenarios is that, firstly, full polystyrene insulation both on the tank sides and lid reduces daily fuel costs by 33% when compared to the base scenario; and secondly, the absolute fuel costs of a 70 day season cycle is relatively small compared to the potential insulation costs at £91 for the most insulating scenario.

Table 1. Cycle, daily and seasonal fuel costs and volume differences for the four insulations scenarios compared to the open-lid, steel sided base case.

Fuel Scenario	First-Cycle cost (£)	Daily cost (£)	Daily fuel use (l)	70 day cost (£)	70 day Season Saving (£)
Base	2.76	3.84	14.5	269	-
1	2.69	3.61	13.6	252	17
2	2.48	2.97	11.2	208	61
3	2.41	2.73	10.3	192	78
4	2.35	2.54	9.6	178	91

The benefits of tank insulation are, however, that the costs are one-off, requiring minimal and accessible maintenance if needed to the insulation material used. Secondly, the thickness of the insulation will increase the costs saving. For example, a doubling of the proposed thickness to 50 mm could seasonally save £101, potentially paying back the investment within 2 seasons.

However, given the relative cost of fuel, insulation is regarded as relatively a minor option for improving the efficacy of HWT and should, therefore, be used in combination with other alternative technologies.

High temperature HWT

There is some interest in short-duration high-temperature HWT as it would allow a greater throughput of bulbs. Although the effect of HWT at 80°C on bulb physiology and nematode control is unknown (although probably detrimental), it was felt useful to include some discussion here.

Heating tank water to the proposed 80ºC operating temperature will clearly increase the initial heating costs, although the rapid treatment time of 1 minute may reduce the costs per cycle (e.g. bulb batch). The idealised heating model was run for an 80ºC tank temperature, at 1 minute dipping duration with the same ambient and initial tank temperatures. The inter-dipping time was maintained at 15 minutes given that the physical process of loading the bulbs would remain the same.

The 80ºC scenario drastically increased the initial heating cost from £2.26 to £5.56, giving a first cycle cost of 6.83 compared to £2.76, and increased the initial heating time from 50 minutes to 120. However, the high temperature method hugely increases the daily batch rate given the shorter dipping time. Assuming that 4 batches can be achieved in one hour, and that the system is operated for 12 hours in a day, the daily number of treatments under the higher temperature scenario could increase from 3 to a minimum of 40 treatments. Such a scenario I estimated to result in a daily cost of £9.33 and an average cost per treatment of £0.23. This is a significant saving on the base case scenario where the estimated average cost per treatment is £1.28, representing a seasonal saving of approximately £220. (Moreover, with total insulation on the tank walls and lid, the potential seasonal saving – 210 batches – could be £239).

Case Studies

The following case studies briefly describe the current operations used by three Narcissus growers; Richards and Sons (Cornwall), PS & JE Ward (Lincolnshire) and Jack Buck Farms (Lincolnshire). Recommendations for improving the efficacy and cost of operations are provided based on a feasibility assessment of the alternative technologies. The case studies should be used as a qualitative assessment for retro-fitting the proposed alternative treatment methods.

Richards and Sons, Hayle, Cornwall

This business employs bespoke, individually circulating, hot water tanks that are open-top, allowing for easy batch change over. The system consists of three 7 m^3 tanks which provides a total operating capacity of 21 m^3. Each tank is insulated on either side with polystyrene (25 mm) between sheets of mild steel (4 mm) (**Figure** 8a); although the tanks ends and base are not insulated. A slave water tank system is not employed and thus each tank operates in isolation throughout the dipping period.

Figure 8. side view of hot water tank system showing flume for heating exhaust and internal view of HWT with expanding pipe-work for heat transfer.

Water is heated using two light oil burners (model: Reillo 40 G5) located beneath the tanks in a sealed chamber of pipes designed to promote heat transfer (7b). The heating exhaust is ejected from the system through an insulated flume located at the opposite end from the burner input. The treatment water is recirculated around the tank and forced through the bulb bins using a Grundfos pump (9a) and the pump inlet and outlet are located at the same end of the tank, but separated by the bulb bins, in an effort to promote circulation through the bulbs (**Figure** 9b). Note that light oil (Kerosene) is relatively cheap and so has stopped the motivation for installing tank lids and further insulation (the cost of kerosene in early 2016 was at £26.5 per l)[1].

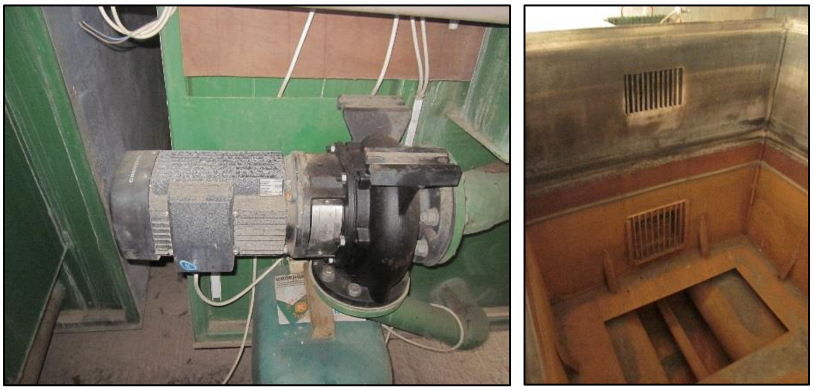


Figure 9. Channel inlet and outlet located at the surface and bottom of the tanks, respectively. Water is circulated using a Grundfos pump (model unknown).

The tanks are designed to house two bins at a time, equivalent to 2.5 tonnes of bulbs per treatment. The bulb bins and pumping system are designed to promote flow down through the bulbs before being re-circulated through a porous mesh located at the bottom of the bin. Metal screens are positioned at the pump inlet and outlet to prevent any large debris from entering the pump (**Figure** 9b).

The efficacy of water circulation through the bulbs using this design is unknown; however, the loose stacking of the bulbs within the bins may prevent effective circulation if the bulbs are not correctly graded or if the pump pressure is insufficient.

The system temperature of the treatment water is controlled automatically using a thermostat positioned at the pumping end at the water surface. The thermostat switches on the light oil burners when the temperature drops below 44.4ºC; although the thermostat's location at the surface may not provide a sufficient description of the variation in temperature within the tank. Note that the burners are also set to eject their maximum power throughout the operation. Under these conditions it takes approximately 45 minutes to heat the system from ambient to operating temperature. However, placement of the bulbs causes the temperature to drop requiring a total

[1]According to the Reillo data sheet, the model G5 produces a maximum heat output of 60 kW and, at such an output, consumes approximately 5.12 kg/h of kerosene.

treatment time of 3.5 hours per batch. Under these operations it is possible to treat three batches of bulbs per day; equivalent to 7.5 tonnes.

The system is dosed with one 250 l container of treatment chemical located above the tank and is injected manually. This chemical is dosed at the beginning of the treatment season and serves the entire season.

Heating analysis using the conceptual heating model developed in the previous section estimates approximately 38 minutes required to heat the system from ambient to operating temperatures, indicating that the modelling procedure operates to scale.

Operational Recommendations

The tanks are poorly insulated and the heat exchange process could be improved. However, the relatively cheap cost of fuel does not provide the operators with sufficient financial motivation to improve heating efficiency, and so any proposed recommendations need to improve the treatment efficacy of operations. That considered, the price of installing tank lids would reduce fuel costs and may increase the speed of the treatment process by minimising the drop in temperature between batches. This may also reduce chemical evaporation from potential volatile substances.

The potential for retro-fitting a filtration/separation system, considered as an integral component for reducing cost and improving efficacy, may require the tanks to be connected in series. The installation of a Nikuni Dynamic Vortex Filter is recommended and would require minimal alterations to the system given the device's small size and the relatively low cost for additional piping (as expressed by the operator of the site).

The site has sufficient space for alterations and installing additional equipment. Additionally, alternative disinfection processes (e.g. UV disinfection) may be easily installed in series with the recirculating tank system to provide further and enhanced treatment.

High temperature treatment requires an increase in the initial heat-up time to at least 1.5 hours and demands a constant boiler input of approximately 14 kW, although this does not take account of the exhaustive losses so a recommended input of 20 kW is necessary. The growers currently employ 2x Reillio 40 G5 burners; the higher temperature operations are still capable of achieving with the current heater system but will reduce daily operations due to longer heat up time. The estimated costs, in fuel, for one dipping cycle of 15 minutes, including initial heating, are £3.93 given a fuel economy of 26.5 p/l. This compares to an estimate cycle cost of £2.05 for the 3.5 hour treatment at 44.4C. However, considering the potential to increase the number of daily dips at the higher temperature, the daily estimate fuel costs are £3.07 and £5.31 for 3 and 10 dips respectively, for the low and high temperature treatment. This gives the daily fuel price per dip of £1.02 and £0.53, respectively, approximately half the price. A breakdown of the estimation process is given in the table below. These estimations assume an ambient temperature of 15C, typical of the mean temperature in Cornwall in the months of June to August.

Table 2. Comparison of costs between low and high temperature treatment (Richards)

Operation	**Low temperature**	**High temperature**
Temperature (deg. C)	44.4	80
Fuel costs (£/l)	26.5	26.5
Dipping time (hours)	3.5	0.25
Number of dips (dips/day)	3	10
Daily fuel cost (£)	3.07	5.31
Cost per dip (£/dip)	1.02	0.53
Capital requirements	None	Potential burner purchase/more rapid steel degradation

The higher operating temperature will increase the steel degradation rate given in terms of rust. This analysis does not take into account the efficacy of the higher temperature process. Although it has been shown that high temperature is more effective at killing the nematodes, the previous analysis does not consider the time taken for the hotter water to circulate around the bulbs. The bulbs are loaded in 1.25 tonnes bins and remain loose within them.

The team dose each batch of new bulbs with 1.5l of FAM30 with the aim to hold a concentration of 4.0l FAM30 for every 1000 litres water. There is potential for an increase in chemical evaporation given that the tanks are open top and that the chemical volatility will increase at higher temperatures. Further, the efficacy of the hydrogen peroxide or chlorine dioxide is not understood at the higher temperatures.

This analysis does not therefore recommend that the higher treatment temperatures be employed given the relatively low cost of fuel compared to the other operating costs. Over the whole operating season the total fuel costs are only estimated to be £215 and £140 for the low and high temperature treatments, respectively, indicating minimal savings compared to the risk to infrastructure and chemical volatility.

The site operator stressed the importance of having an effective fungal treatment system given that nematodes are not currently considered to be an issue. Chlorine dioxide has been more widely used in the water treatment industry and there is greater advice and manufactures of equipment. Chlorine dioxide is therefore an obvious choice when seeking an alternative dosing chemical. However, the volatile properties due to temperature and treatment efficacy at 44.4-80ºC are not well known. Additionally, the rate of dosing needs to be more thoroughly investigated as higher than normal dosing rates will be required if the bioload is significantly greater than potable water treatment.

Filtration pre-treatment is regarded as a viable option for improving the operational efficacy of the HWT and can easily be retro-fitted onto the current systems.

PS & JE Ward, King's Lynn

This dipping system at Wards is more complex compared to some others. Three sets of bulb boxes can be dipped at a time, into two HWT tanks, providing an operating capacity of almost 6 tonnes per treatment.

The total volume of each tank is 8000 litres and one of the tanks can be separated in half to provide a 2850 l or 5000 l volume if needed (e.g. 1 or 2 bins). The systems are fully automated and are dipped from the top on cages that are raised after the designated 3.5 hours dipping at 44.4°C. Water is recirculated via an inlet and outlet located at the top and bottom of the tanks and undergoes coarse screening (15 mm) in the pumping unit and within the tank itself. Water is heated via a controlled heat exchange system using piping that circulates hot air generated by a renewable biomass boiler (combusting wood-chip and waste husk) – controlled automatically to ensure efficient burning and temperature control.

The tank water reaches operating temperature in 20 minutes when the biomass boiler supplies heat at 1 MW (990 kW). The water is recirculated through a stirring system to pre-dose the tanks with JET5 disinfectant to treat Fusarium. Each treatment takes 4 hours; accounting for raising and draining of the bulb bins. The treatment water is left in the tanks for the length of the dipping season and has relatively low turbidity due to the high silt content of the local soils.

The system employed at Ward is relatively advanced and requires little improvements to increase operating efficiency, however, insulation of the steel tanks using polystyrene and installing tank lids would reduce heat loss and could reduce the overall time per treatment.

A concern highlighted by the operators is the need to develop alternative disinfection methods given the changes to chemical regulations. Pre-treatment separation and alternative disinfection is therefore highly viable at the site given these desires.

The pipes serving the HWT tanks are plastic and can be easily altered for retro-fitting. There is also sufficient space on site to house additional equipment and adjust the current operations depending on the requirements. The site has exceptionally clean bulbs and has little need for fungal treatment. The site also has sufficient space and machinery to create a conveyor belt system allowing for a microwave lines to be installed.

Operational Recommendations

Initial testing of an inline vortex separation device and subsequent UV filtration is highly feasible at Wards. The vortex separator could be rapidly installed to provide pre-treatment filtration; thus reducing the potential bioload associated with sediment. However, given the relatively clean tank water, a UV disinfection system may be sufficient. Screening of the recirculating water using UV radiation would help to prevent cross-contamination between batches and may reduce the overall impacts of the Fusarium.

The current design lends itself for retro-fitting and alterations given the plastic pipe-work and available space. Chlorine Dioxide could be also easily dosed into the system; accessed through the malleable pipe-work. This, in combination with UV disinfection would provide a viable and effective disinfection method that can eradicate Fusarium both on the bulbs and within the water column.

Jack Buck Farms, Spalding, Lincolnshire

Jack Buck Farms is a fairly advanced system although it employs a lower degree of automation than Wards. Jack Buck Farms utilise a drive in system using two complimentary, side-by-side HWT tanks, with one tank acting as the slave for the other. The two, 15,000 l tanks are heated using kerosene oil burners via an efficient heat exchanger. The water is stored in the slave tank while the other is unloaded and then re-loaded; allowing the operators to perform four treatments per day. The tanks are located outside, subject to ambient conditions, thus requiring up to 60 minutes of heating to achieve the desired operating temperature of 44.4°C. As such, each treatment batch can take up to 4.5 hours to complete; however, turnover between batches is rapid thanks to the side loading system. The water is also heated using an exchange system, running from an enclosed, kerosene light oil burner capable of consuming up to 1500 litres of kerosene a week (quoted at £0.30 per l). The system volume is recirculated at 3 volumes per hour. The operators do not employ FAM30 as a biocide given its rapid breakdown time and corrosive effect on infrastructure. Seasonal costs for fuel and chemical disinfectants are quoted as £3000 and £4000, respectively.

The side-loading tanks are also open-top and constructed from mild steel. Inspection of the system found that sides of the tanks were warm to the touch indicating low thermal insulation.

Operational Recommendations

The tanks are located outside and are subject to ambient conditions and convective cooling from wind. Thermal insulation using polystyrene and tank lids is regarded as a cheap and effective means for reducing both the fuel costs and the time taken to reach operating temperature. The conceptual model describe in the previous section showed that full insulation of the tank system has the potential to reduce fuel costs by up to 33%. As is the case for most operators, fuel represents a relatively minor contribution to net costs, however, the increase in heating time is regarded as an additional benefit. Complete system insulation and lid installation is highly recommended as a cost effect means for reducing costs and reducing heat-up time.

Chlorine dioxide dosing is recommended and should be considered ahead of UV disinfection given the potential for the turbid water to attenuate radiation. It was observed that the tank water was relatively turbid, giving greater need for pre-treatment separation/filtration to reduce biological loading within the recirculating system. Although, disinfection using UV radiation could be performed continuously given the use of the slave tank, helping to reduce cross-contamination between batches and provide maximal disinfection potential.

The service pipes are steel but are accessible and have sufficient space to be cut and retro-fitted with alterative treatment technologies. As such, a parallel vortex separation system is recommended, in-line with a UV disinfection unit.

Concluding remarks

The results from this study confirm that there is growing demand for an alternative treatment technology or process 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. However, the feasibility of uptake and installation of such proposals has not been sufficiently explored. Therefore, this study examined the feasibility of employing ultra-violet (UV) disinfection, chlorine dioxide or hydrogen peroxide dosing, pre-filtration using vortex separation, 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 to 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 sediment to the HWT system. Filtration or separation within the HWT system is therefore regarded as an effective 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 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 requires further investigation. The general consensus is that a combination of both pre-filtering and non-chemical disinfection is most suitable for improving operational efficacy and changing chemical regulations. It is recommended 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.

Finally, the capital costs for retro-fitting alternative disinfection methods and improvements to the operational efficiency (e.g. insulation or separation) are in the order of £10,000 to £20,000 per technology (inclusive of installation) depending on the size and complexity of the chosen devices.

Alternative pest control in Narcissus – 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.

It is not feasible to estimate the costing for microwave heating given the bespoke nature of the methodology and the relative age of this new technology in an agricultural application. The method is however considered highly feasible given the current operations and facilities at most HWT sites. There is sufficient space and equipment to operate a conveyor belt design and so the capital costs for changing methodology would be associated only with purchasing the microwave unit. The Sanitec screw sterilisation system is most suitable given its production line style design and may be modifiable for a more coarse agricultural application.

The use of microwave technology to control stem nematodes was the subject of a University of Warwick funded four-month investigation. This was undertaken as part of a MSc degree by Emily Chow at the universities of Warwick and Nottingham. The full dissertation is available by request. A short summary is presented here.

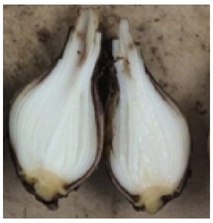
The concept of using microwaves to control stem nematodes in-situ is based on the fact that targeted microwaves may be able to increase the temperature of the pest to a lethal level, but do so without damaging the plant tissues. Microwave technology is energy and cost-effective. It is highly reliable 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.

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.

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 10). 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 with 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.

Figure 10. Damage scale for treated bulbs.


Untreated bulb

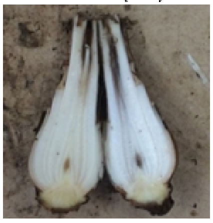
Damage Scale: 1

Sticky sap exuded when bulbs were cut into half. A slight browning was shown around area where FO temperature probe inserted into. Scales remained fresh and white in colour. The colour of the basal stem remained greenish tallow.


Treated bulb (2-3)

Damage Scale: 2-3

Sticky sap exuded when bulbs were cut into half. A slight browning was shown around area where FO temperature probe inserted into. Part of the scales (in the middle of the bulb) became slightly transparent the colour changed from white to light brown. The colour of the basal stem remained greenish tallow.


Treated bulb (4-5)

Damage Scale: 4-5

Sticky sap exuded when bulbs were cut into half. A slight browning was shown around area where FO temperature probe inserted into. All scales remained fresh. Small brown spot formed near the basal stem. The colour of the basal stem remained greenish yellow.


Treated bulb (6-8)

Damage Scale: 6-8

Sticky sap exuded when bulbs were cut into half. A slight browning was shown around area where FO temperature probe inserted into. All scales remained fresh. Big brown spot formed on the centre of the bulb. The colour of the basal stem remained greenish yellow.


Treated bulb (9-10)

Damage Scale: 9-10

No sticky sap exuded when bulbs were cut into half. A slight browning was shown around area where FO temperature probe inserted into. All scales became soft and transparent. The colour of the basal stem changed from greenish yellow to brown.

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 shown that microwaving at 10W had no detrimental effect on plant growth or quality, and perversely may have improved performance (Table 3).

Table 3. Bulb quality following microwave treatment

Treatment	**Stem height (mm)**	**Relative quality score**
Non-microwaved	125	5.8
Microwaved	147	6.7
Both treatments, p-	0.085	0.420

Visual assessment in March 2017 confirms that microwaving bulbs at low power does not reduce flower performance (Figures 11 and 12).

Figure 11. Untreated bulbs in microwave trial

Figure 12. Treated bulbs in microwave trial

However, caution should be used in interpreting these results as this was only a small-scale trial. But, the approach does show promise and further investigations will be undertaken if funding is available.

The ability of HWT and microwaves to control stem nematodes were studied in-vitro. Nematodes were placed in glass tubes and then heated or microwaved to reach different temperatures. Mortality counts were undertaken using a dissecting microscope. The results shown that HWT was more effective than microwaving. HWT resulted in 100% mortality at 44°C while it required 55°C to achieve the same using microwaves. Although this result is somewhat surprising, it was achieved under laboratory conditions using small sample sizes and may not be representative of a commercial setup.

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.

Conclusion

The results from the biocide work confirm that all the approaches have value to commercial growers in terms of control of Fusarium and stem nematodes, and ease of operation.

In terms of chemical control, chlorine dioxide, hydrogen peroxide and Boot are all effective biocides in clean water, however, all three have issues as their efficacy is greatly reduced in dirty water. Although Boot is an effective biocide, concerns have been raised in the past about its phytotoxicity effect (BOF 061a) and it will not be examined any further. Of the remaining two, chlorine dioxide is possibly the better option in the long term since more is known about its use and efficacy in HWT systems, however, both it and hydrogen peroxide will undergo further testing before a final evaluation is undertaken. The key result from this stage of the project is the negative influence of tank bioload on the efficacy of all three chemical biocides. This is undoubtedly a major obstacle in the use of chemical biocides (and probably other biocidal and fungicide approaches) so we suggest that examining the introduction of some form of filtration/separation will be required as a starting position for the next stage of investigations.

Thermal treatment is undeniably effective and its efficacy probably unaffected by tank bioload. The difficulty arises in its implementation since increasing the temperature of tank water either continuously or in batches introduces new problems. Continuous thermal treatment may only provide selective treatment allowing some Fusarium spores to remain untreated while batch treatment will only prevent batch to batch infection and not reinfection within an existing batch.

Both UV and microwave treatment have promise and both will be examined in more detail next year. The efficacy of UV treatment is known to be affected by water clarity which adds weight to filtration/separation being the starting position for many, if not all, of these treatments.

The practicality of retro fitting biocidal technology was also examined. Most of the approaches do not create any special difficulties as automated dosing systems are available for chlorine dioxide and hydrogen peroxide, and the installation of filtration and UV lamps requires only minor pipework modifications.

Future work

Objective 4, to assess the effect of the chemical treatments has been underway since June 2016 and will be assessed in Spring 2017. The original assessment date was September 2016 but this has been extended to allow the maximum possible time for any corrosive effects to become visible.

Objective 5, the tank scale tests, is currently underway and will hopefully be completed by January 2017. The delay was due to the difficulty is producing sufficiency large quantities of chlamydospores in time. We will also investigate a completely non-chemical approach using a combination of separation and/or heat and UV.

Objective 6, to carry out commercial scale testing. It is planned to start this in July 2017 following discussion at the AHDB 2017 Narcissus Growers Workshops.

Knowledge Transfer

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

- Spalding, Lincolnshire 12th May 2016)
- Redruth, Cornwall (18th May 2016)

Results from the project so far will be presented at AHDB grower meetings at the same locations during May 2017.

Visits were made to six growers during the year. The discussions generated the three case studies reported here.

An article is under preparation for the July 2017 issue of AHDB Horticultural News.

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