Grower Summary

BOF 077

Narcissus: Investigation into the effects of a range of potential biocides in hot water treatment

Final Report 2020

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Project leader:	Rob Lillywhite, University of Warwick
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Key staff:	Mr Rob Lillywhite Dr John Clarkson Dr Adam Baker
Location of project:	Warwick Crop Centre, University of Warwick
Industry Representative:	Mr Julian Perowne, Jack Buck (Farms) Ltd, Green Lane, Moulton Seas End, Spalding, PE12 6LT Mr Andrew Richards, Carwin Farm, 16 Carwin Rise, Loggans, Hayle, TR27 5DG
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The results and conclusions in this report are based on an investigation conducted over a four-year period. The conditions under which the experiments were carried out and the results have been reported in detail and with accuracy. However, because of the biological nature of the work it must be borne in mind that different circumstances and conditions could produce different results. Therefore, care must be taken with interpretation of the results, especially if they are used as the basis for commercial product recommendations.

AUTHENTICATION

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

Rob Lillywhite

Assistant Professor

University of Warwick

Signature • Date 20/11/2020

[Name]

[Position]

[Organisation]

Signature .... Date ....

Report authorised by:

[Name]

[Position]

[Organisation]

Signature .... Date ....

[Name]

[Position]

[Organisation]

Signature .... Date ....

GROWER SUMMARY

Headline

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

Background

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

Other biocides have been considered, notably chlorine dioxide which was demonstrated to be effective against spread of Fusarium (Chastagner and Riley, 2002). Chlorine dioxide was assessed under UK conditions alongside a number of alternative biocides, but was not considered further as FAM 30 was found to be more effective (Lole, 2010). The use of chlorine dioxide was further reviewed in BOF 070 (Hanks, 2010) which suggested that additional investigation was required before it could be recommended to growers.

Other biocides previously examined include peroxyacetic acid (Hanks and Linfield, 1999), hydrogen peroxide and UV (Stewart-Wade, 2011) but tank bioload was again found to reduce their efficacy. Non-chemical biocidal approaches, e.g. UV and thermal treatment, have been used in other water-based treatment systems and appear to offer a viable alternative to chemical approaches but their efficacy of UV is known to very dependent of water clarity, which is a problem with high bioload HWT (Petit, 2016). The issue of high HWT tank bioload was reported in BOF 070 (Hanks, 2010) and identifying a solution to this issue is probably key in improving the efficacy of all biocides and biocidal approaches (and probably fungicides as well).

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

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

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

Summary

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

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

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

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

Laboratory tests

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

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

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

Small-scale tanks tests

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

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

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

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

Water turbidity (bioload), UV and filtration

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

Filtration and UV sterilisation were trialled on-farm to assess if retro-fitting the equipment was possible, and if so, if filtration could reduce tank bioload. Retrofitting proved possible and a centrifugal system was tested. Unfortunately, the very fine screens required to filter out the soil particles very quickly blocked the filters and the test had to be abandoned as water flow was restricted. UV sterilisation was tested at the same time.

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

Chlorine dioxide commercial trials

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

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

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

Thermal treatment

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

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

Fungicide concentrations in HWT tanks

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

The main result was that none of the growers managed to achieve their target concentration of either chemical at the start of the season (before bulbs were introduced into the system). Chlorothalonil concentrations were on average just 28% of the target value suggesting that either the wrong volume of fungicide was used, or more likely, that heat and other chemical reactions had already degraded the majority of the fungicide before bulbs had been added to the system. While it has long been suspected that active ingredients are lost from the circulating dip during HWT, this is possibly the first evidence of the magnitude of that loss at the start. Topping-up fungicide amounts did increase their concentration but none of the systems reached their target value during the testing period.

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

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

Financial Benefits

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

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

Running costs for chlorine dioxide depend on the dose rate required, which in the case of Jack Buck Farms was two litres per hour; this equates to a cost of £16 per hour of operation or approximately £7 per tonne. In comparison, we estimate the cost of FAM 30 is £4.45 per tonne.

Action Points

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

