



Grower Summary

BOF 077

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

Second annual report, December 2017

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

Project number: BOF 077

Project leader: Rob Lillywhite, University of Warwick

Report: Second annual report, December 2017

Previous report: First annual report, December 2016

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

Date project commenced: 1st January 2016

Date project completed (or expected completion date): 31st December 2019

GROWER SUMMARY

Headline

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

- Under laboratory conditions, thermal, ultra-violet radiation and chlorine dioxide treatments provided effective control of Fusarium spores
- Filtration and UV radiation equipment has been successfully retrofitted to a commercial HWT system
- Filtration proved difficult to implement under commercial conditions because most bioload was composed of very fine soil particles
- Field trials were established in Cornwall but as yet any benefit of treatments has yet to be determined

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added to HWT tanks as a general biocide i.e. to reduce inoculum in the tank water, however approval for formalin was withdrawn in 2008.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

This report covers the period January 2017 to December 2017 which is the second year of the four year project to investigate new or improved biocidal approaches in the hot water treatment of daffodil bulbs. This period saw the cessation of laboratory testing and the start of commercial on-farm testing. A literature review, details of the laboratory testing, including a feasibility study on microwave sterilization, and a feasibility and cost/benefit analysis of HWT modifications can be found in the 1st annual report.

Results from year 1 had shown that chloride dioxide was an effective biocide but also that tank bioload had a negative effect of its efficacy. Work in 2017 therefore concentrated on reducing tank bioload and examining if biocides were more effective under cleaner water conditions. The use of UV radiation was also investigated.

Filtration

The earlier laboratory work carried out during this project had established that for all chemical based fungicide or biocide treatments there was a negative effect of dirty water on the efficacy of the treatment. It is also well established that for UV sterilization to be effective the water needs to be of good clarity. This data, along with some of the feasibility work carried out, was presented to the growers at the AHDB growers meetings of spring 2017. There was a general agreement from the growers that cleaner tank water would be beneficial and a good level of interest in trialling some sort of filtration. Therefore work continued to establish what technology would be best suited to the continual clean-up of tank water.

This investigation into filtration had two aims: firstly a general clean-up of tank water to allow chemical treatments to work more effectively. It was assumed that filtration to a level of approximately 50-100µm would be effective to achieve this aim. Secondly to clean tank water to allow UV sterilization to be effective, which would require filtration to approximately the 5-10µm level. Given the high flow rate of some HWT systems, these parameters caused some problems in identifying suitable filtration equipment that the research team considered would be acceptable to growers and cost-effective. A full description is provided in the Science Section but in summary, it was decided to examine a set-up that comprised two different sized filters in series (150 and 25 micron) and to run two of these in parallel to cope with the flow rate. This system was installed at Carwin Farm in July 2017 in preparation for bulb dipping (Figure 1).



Figure 1. Retro-fitted filtration and UV system at Carwin Farm, Cornwall

The cost of the purchase and installation of the filtration system was £3,057 + VAT which was made up of four filters plus replacement screens (£1505), plumbing and fittings (£1072) and labour (£480).

The system was initially tested using fresh water without bulbs to ensure that it functioned properly. This initiated a considerable release of rust, which quickly clogged the filter screens and necessitated their removal and cleaning a number of times. It was noted that this rust deposit is normally removed by the first batch of bulbs.

The introduction of bulbs introduced organic and mineral contaminants into the system and the filters required cleaning multiple times in order to keep the water flowing through the system. The conclusion, at this early stage, was that unless there was a very clear benefit to filtration, a system that required stopping and the manual cleaning of filters during every run was not going to be commercially viable. In order to explore the effect of filter size on water flow, all the filters were changed to 150 µm which resulted in a marked improvement in water flow.

Whilst this filtration setup did remove some of the contaminants from the tank it was clearly not an appropriate set up to recommend without modification and further testing. With filter screens of pore size smaller than 100 μm (50, 25 and 10 μm) the flow of water was slowed to the point of not moving through the bulb tanks effectively and this problem became worse as the filters became clogged. Observations both on farm and in the lab suggest that filtering to 100 μm will only have a limited positive effect and will not be sufficient to clean the water adequately (to allow the use of UV sterilization although it may improve the efficacy of chlorine dioxide). To filter the water to a fine enough level it is likely that a different approach is required. One consideration was to install more powerful pumps, however, it is unclear at this stage whether greater pressure would improve the flow of water through the tanks and filters or stress the whole system somewhere else. Alternatively, a more sophisticated and expensive filtration system would be required that include automated removal of the collected contaminants and debris. This would be the preferred approach since it could be retrofitted without major modifications to the existing HWT system.

Further investigation on the suspended particle sizes present in tank water, revealed that a majority of them measured between 0.4 and 20 μm which is why the 150 and 25 μm filters had proved ineffective. At this size range it is likely that the particles are fine silt and/or clay particles released from any soil adhering to the bulbs rather than fragments of bulb scale; this was contrary to our assumption that bulb scale would make up a significant proportion of the suspended solids. While it is possible to obtain filtration systems to remove these particles, it would be a considerable investment and preventing more of the soil and scale from entering the tanks is likely be a more promising option.

At this stage of the testing, it is hard to draw any conclusions from the work on which recommendations to change of practice could be made. While it demonstrated that it is possible to retrofit filters (and a UV source) to an existing HWT system, the results of filtration were disappointing. It is planned to repeat these tests in Lincolnshire in 2018 to investigate if these results are particular to Cornwall or shared across different production areas. If similar results are observed, it is likely that a simple and inexpensive filtration system is unlikely to be the solution to dirty water in HWT systems and that if filtration is considered necessary, then more expensive and sophisticated systems will be required.

Ultraviolet radiation

Ultraviolet (UV) radiation is a proven sterilization technology that is often used to control pathogens in irrigation water. It is a non-chemical approach that is basically 'fit and forget' although its efficacy is known to be much severely reduced in dirty water conditions. A two-

fold approach was followed which used laboratory testing to assess its efficacy in clean water conditions, and this was followed by on-farm testing to judge whether it was viable under commercial conditions.

In laboratory tests, UV reduced the amount of *Fusarium* chlamydospores in water samples by 99% after two hours and to no detectable amounts after five hours. However, because UV operates on a continuous basis, we assume that there were still some viable spores circulating in the water early in the test before complete control was exerted. Following treatment, bulbs were air dried overnight before being incubated for 28 days at 25°C. After incubation the bulbs were dissected and scored from 0-10 for basal rot.

Although there was a significantly lower level of infection in the bulbs from the UV treated tank than in the non-UV treated tank the reduction was nowhere near as big as that seen in the water samples. This would suggest that even the very low level of *Fusarium* that was still present was sufficient to cause a substantial level of infection. Even though the level at which the tanks were inoculated was massively higher than would be expected in a commercial tank, the results were encouraging and show that the concept of using UV to control *Fusarium* was proven.

A commercial scale UV unit was trialled at Carwin Farm in August 2017. The cost of the purchase and installation was £2,450 + VAT. The combination of the filters and UV source was tested with one batch of bulbs and while it had some positive effects it was clearly not an appropriate set up to recommend without modification. With filter membranes of pore size smaller than 100 µm (50, 25 and 10 µm) the flow of water was slowed to the point of not moving through the bulb tanks effectively, this problem became worse as the filters became clogged. Because the UV technology relies on water flowing through the UV treatment tube, any reduction in water flow will reduce the control of water-borne pathogens. This is a double burden because clear water is required for effective UV treatment. Observations both on farm and in the lab suggest that filtering to 100 µm will only have a limited effect and will not be sufficient to clean the water adequately to allow UV treatment to be effective.

The key to improving water clarity and treatment efficacy is to reduce the amount of bioload entering HWT tanks. Even though bulbs are cleaned and graded prior to dipping, enough soil still adheres to them to produce sufficient suspended particles to dirty tank water. During 2018, it is hoped to examine if additional cleaning of bulbs prior to HWT can reduce tank bioload and improve the efficiency of filtration and therefore the efficacy of UV treatment.

Chlorine dioxide

Commercial testing of chlorine dioxide was undertaken at Carwin Farm in August 2018. Chlorine dioxide (in the form of Activ-ox, supplied by Feedwater) was added to the tank with in-tank levels quantified using a Palintest meter. Despite increasing levels of chemicals added (until the supply was exhausted) the in-tank level of chlorine dioxide did not at any point reach a detectable level on the measuring equipment. Consequently, it was decided not to run a load of bulbs as it was unlikely to give a meaningful result and the bulbs were unlikely to be protected from Fusarium. This result confirms the difficulties experienced by other researchers and has triggered a rethink of the approach. It is planned to test chloride dioxide again in 2018 but to use an automated dosing system supplied by the Scotmas Group to try and overcome some of the operational difficulties. However, despite these problems, chloride dioxide remains the most promising of the chemical biocides as it is widely used in other industries and its use is unlikely to be regulated in the foreseeable future.

Thermal treatment

Work in year one of the project demonstrated that thermal treatment was a very effective biocidal approach with complete control of Fusarium spores being achieved at temperatures above 60°C. The concept that supports this approach was that tank water could be either continuously or batch treated to provide another form of control. In practice, this would mean tank water being sterilised overnight, when empty of bulbs, by increasing the temperature to 60°C thereby ensuring that the water was sterile at the start of every day. This was tested at both Carwin and Bosahan Farms in Cornwall and was successfully achieved without any issues. The effectiveness of this approach is currently unknown although the treated bulbs will be monitored over the next two years to see if they show any advantage.

During the Narcissus Growers Workshops in May 2017 there was also interest in using thermal treatments on bulbs themselves. Although bulbs are normally dipped at 44°C to avoid tissue damage, while at the same time controlling stem nematodes and bulb scale mites, the effect of short-term dipping at 60°C or more was unknown.

Trials were undertaken at Carwin Farm and Warwick Crop Centre to investigate the effect of water temperature and dipping time on daffodil bulbs. Bulbs were dipped at either 60°C, 65°C or 70°C for either 3, 5 or 10 minutes. Bulb core temperature increased in line with water temperature and dipping time. However, it was noted that the data obtained at Carwin Farm shows a much higher core temperature than the equivalent data obtained at Warwick Crop Centre. This effect was more noticeable at the shorter dipping times. It may be that this is due

to the addition of bulbs to the small tanks at Warwick Crop Centre causing a drop in water temperature, which is likely to be less of an issue in commercial tanks.

Post-dipping, half of the bulbs were incubated at 25°C for 30 days while the other half were planted into individual pots and will be assessed in spring 2018.

Financial Benefits

At this stage of the project, it is not possible to make any assessment of the financial benefits arising from the results of the research. Although the costs of the different approaches are known, we cannot yet quantify any financial savings that might arise from the biocidal approaches.

Action Points

Growers should ensure that bulbs destined for hot water treatment are as clean as possible as that will reduce the build-up of tank bioload. Reduced bioload will increase the efficacy of both fungicides and chemical biocides.

Growers should be cautious of using manual chlorine dioxide treatments (premixed liquids) as repeated trials have shown that target concentrations cannot be achieved by single dosing.