Project title:	Daffodils: Developing alternatives to formalin. The concentration of chlorothalonil fungicide and iodophore biocide in HWT and cold dips
HDC project number:	BOF 61c
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Report:	Final Report (November 2010)
Previous reports:	Preliminary Report (June 2010)
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Location:	Commercial daffodil farms in Lincolnshire and Cornwall
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Date commenced:	1 May 2010
Date completion due:	30 September 2010
Keywords:	Basal rot, base rot, bulb dip, biocide, chlorothalonil, cold dip, daffodil, disinfectant, *Ditylenchus dipsaci*, fungicide, *Fusarium oxysporum f.sp. narcissi*, hot-water treatment, iodine, iodophore, *Narcissus*, stem nematode

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GROWER SUMMARY

Headline

As a follow up to recent HDC projects investigating Bravo 500 and FAM 30 as additives to bulb dips to minimise the spread of pests and diseases (including *Fusarium* basal rot and nematodes) within the treatment tank, detailed tests in commercial treatment tanks demonstrated a marked drop in concentration of both products soon after adding them to the tank. Improved tank recirculation and the use of suitable tank coatings are suggested as methods to minimise these issues.

Background and expected deliverables

Biocides

Until 2008 formalin (formaldehyde) was used routinely as a biocide (disinfectant) in the cold-dipping and hot-water treatment (HWT) of daffodil bulbs. It was an economical way to control fungal diseases, particularly *Fusarium* base or neck rot, and in HWT it augmented the control of stem nematodes. Although other biocides, like Jet 5 or glutaraldehyde, were tested at various times, there was little incentive to switch to using them.

A search for alternatives to formalin was necessitated in 2008, with the hurried withdrawal of its use in horticulture on grounds of risks to human health.

In HDC Projects BOF 61 and 61a, candidate biocides were tested in the laboratory for their efficacy against stem nematodes and base rot fungus in HWT. It was shown that heat alone was effective in killing stem nematodes, but an iodophore (iodine-based) biocide, FAM 30, was found to be:

- Effective against stem nematodes at cold dip temperatures (using a 3h treatment)
- Effective against the base rot fungus under HWT conditions
- Apparently free of adverse effects on the crop.

In follow-up work (BOF 71), FAM 30 was shown to be generally effective against base rot spores when used as a 3-hour cold dip.

Since small-scale experiments do not always translate into commercial-scale operations, field trials of FAM 30-treated bulbs were set up in 2008 and 2009 following treatment in commercial HWT tanks (BOF 61a and 61b). FAM 30 appears, so far, to be largely free of

adverse effects on the crop (though the full results will not be available until 2011). In the meantime, several questions were posed by the industry, and in 2010 this project was set up to address them:

- Is FAM 30/iodine stable under prolonged use in commercial HWT?
- Is it stable in commercial cold-dipping?
- What top-up regimes are required?
- Could its concentration be monitored easily on-farm?
- Does FAM 30 have a beneficial effect on hygiene, controlling 'bio-load'?

Fungicides

When growing daffodil cultivars susceptible to fungal diseases such as base rot, it is usual to add a fungicide to the HWT and cold dip tanks, in addition to a biocide. Following extensive trialling, a thiabendazole-based fungicide (Storite Clear Liquid) was mostly used (another product Tezate 220 SL is now also available). Since 2008 however the use of these fungicides on bulbs has been restricted to once per year and to using no more than a quarter-rate of the former rate used in bulb dipping, and they are not permitted on the Isles of Scilly.

As a result of these factors, some alternative fungicides were included in the HDC-funded projects mentioned above. Of the fungicides tested, chlorothalonil (as Bravo 500) was found to be:

- Effective against base rot spores in HWT and at cold-dip temperatures
- Apparently free of adverse effects on the crop.

Bravo 500 therefore joined FAM 30 in the field trials (Projects BOF 61a and 61b).

Thiabendazole is known to be rapidly 'lost' in HWT dips (BOF 64), and some HDC-funded analyses in 2009 suggested that losses of chlorothalonil were also occurring in HWT.

For the current project the following questions were addressed:

- Is chlorothalonil stable under prolonged commercial HWT?
- Is it stable in commercial cold-dipping?
- What top-up regimes are required?
- Are there ways of preventing the loss of fungicides in bulb dipping?

Summary of the project and main conclusions

Scope

To generate information quickly and realistically, the project was based on using commercial bulb dipping operations for testing and sampling. The operations chosen included:

- HWT of reclaimed (ex-forced) bulbs (often carried out in May or June, so providing results more promptly than waiting for regular HWT)
- Regular HWT
- Regular cold-dipping
- The use of FAM 30 and / or Bravo 500 where available.

Over the course of the commercial dipping operations, the concentrations of active ingredients were followed and the effects of treatments on bio-load were measured.

The dynamics of FAM 30 in dip tanks

Iodine concentrations in bulb dips with iodophore biocides were followed in six cases (three in HWT and three in cold dips). The initial iodine concentrations were 100 or 200ppm, depending on whether half- or full-rate dips were used. The findings showed a common pattern: high iodine concentrations (>80ppm) were found only within a short period of the biocide being added, up to a maximum of 1½ hours afterwards. Sometimes low levels of iodine were detectable up to 48 hours after the biocide had been added. Although topping-up took place as appropriate, this did not restore iodine concentrations to their target levels.

This pattern of response occurred in both HWT and in cold-dipping, in the HWT of reclaimed bulbs and of regular bulb stocks, when the biocide was being used alone or incombination with a fungicide (Bravo 500 or acidified Storite Clear Liquid), and in the one case where another iodophore biocide, Virudine, was used. As a result of these findings, HWT or dip tanks should not be set up with FAM 30 much in advance of actual use, and tanks should not be topped up at the end of the working day so the next load can be treated early the next morning using a timer switch.

Before an effective top-up procedure can be formulated the loss of iodine needs to be accounted for. The properties of iodophore biocides makes it unlikely to be the result of thermal inactivation or adsorption to organic matter, but inactivation through reaction with the metal of the tanks and components could be a possibility, though this needs to be confirmed.

Bulb dipping tanks are usually constructed of mild steel, but this would not necessarily exclude the use of such tanks or of these highly effective biocides, since it may be possible to apply an inert paint or coating to the metal of the tank to prevent these chemical reactions.

The pH value of dips

The pH of bulb dips is important because it may affect the stability and solubility of pesticides and biocides added to the tank; also, extreme pH values (below 2.0) may reduce subsequent daffodil growth, though to a minor extent (BOF 64). FAM 30 and Virudine are formulated in 20 to 30% acid, so a dip in pH will follow during operations. The pH fell rapidly to as low as 1.8 soon after the biocides were added, a borderline level for possible crop damage. However, this was followed by a loss of acidity (rising pH) over the next six hours, with the pH finally stabilising around 3 to 4.

When dipping bulbs in Storite Clear Liquid, acidification of the dip to a pH of 2.5 to 3.0 (by adding sodium bisulphate) is recommended (BOF 64); it is not clear at present whether using an iodophore and sodium bisulphate together would have an adverse effect on the bulbs, or whether using an iodophore could substitute for adding a straight acidifier. However, adding sodium bisulphate in subsequent top-ups did not greatly lower the pH of the dip compared to using FAM 30 or Virudine. Evans Vanodine (the product manufacturers) staff have suggested it may be possible to formulate a product more suitable (less acidic) for use in bulb-dipping operations.

Testing for iodine concentration

Iodine testing in this project was based on using ‘dip-sticks’ or colourimetric chemical tests, which were simple to conduct and robust enough for on-farm use. However, when using dip-sticks of various types the colour matches could be difficult to judge, and different dip-sticks often indicated different concentrations from each other or from other tests. One colourimetric test kit used was simple and effective, but it was found difficult to source supplies of reagent. An iodine-specific meter was also tested, but the disadvantage was that the dip samples had to be diluted 100-fold before it would give a reading, at which level the test responded to trace levels of chlorine in the water used to dilute the samples.

Effects of FAM 30 on bio-load

Microbiological growth (bio-load) in HWT and bulb dip tanks may, in the absence of formalin, result in the 'pollution' of the dips. This bio-load was measured in six HWT or cold-dipping operations, and it was found that the effects of adding FAM 30 had a common pattern. When measured as the number of bacterial colonies using a simple culturing method ('Petrifilm'), it was found that the 'plain' water in the tanks was highly polluted - which is understandable given the difficulties of thorough cleaning and decontamination of tanks and fittings. Very soon after the addition of FAM 30 (or Virudine) bacterial counts were reduced to zero. Thereafter, bacterial counts rose at a variable rate, beginning at various times after the start of testing. Treatment of the water with FAM 30 (or Virudine) was highly effective in controlling the initial pollution, but thereafter the extent of pollution varied, not surprisingly given the great variation in the state of bulb stocks, degree of cleaning and other factors at the various sites.

In one case virtually no bacterial pollution was found in HWT dips, and it is suggested that this was a consequence of the HWT system having been effectively disinfected by the use of chlorine dioxide (see Project BOF 70) in the days immediately before these tests.

Fungicide concentrations in bulb dips

In contrast to the findings for iodine, although chlorothalonil concentrations did fall rapidly soon after the start of HWT or cold-dipping, they did not 'disappear'. In the three cases where Bravo 500 was added, at the full- or half-rate (1.0 or 0.5kg/1000L), the concentration of chlorothalonil fell quickly over an initial period of about 12 hours, before levelling out and remaining more or less stable at about 100ppm thereafter. Expressed as the percentage of the initial (target) concentration of chlorothalonil remaining, the stable concentration was 20 to 30% of the starting concentration. This is similar to the findings for thiabendazole fungicide levels in HWT reported here and in BOF 64.

Possibly the initial loss of chlorothalonil, and the eventual lower but stable concentration, is a consequence of the sedimentation process and related to the sedimentation coefficient of the substance in water. (A suspension will in time attain an equilibrium, at which the rate of sedimentation of a substance is balanced by the rate of 're-uptake' from the settled material back into the circulating suspension). In the case of thiabendazole (as Storite Clear Liquid) in HWT, it was found that a reduced rate (equivalent to 25% of the original recommendation) was in fact adequate for base rot control BOF 64), and data from the current project showed

that similar considerations apply to cold-dipping. It is not yet known whether reduced-rate dipping is also applicable for chlorothalonil as Bravo 500.

For topping-up with Bravo 500 in HWT or cold-dipping, the method used here, i.e. topping-up at the same rate as used at the start whenever water levels are replenished, appeared adequate. The same would seem to apply to using acidified Storite Clear Liquid in cold-dipping.

Mitigation of the loss of fungicides such as Bravo 500

As a possible means of mitigation of the loss of active ingredients by settling-out on the floor of bulb-dipping tanks, additional agitation of the dip was created by using a powerful submersible pump working close to floor level in the middle of the tank, circulating the dip solution to the top ends of the tank. This failed to increase the concentration of chlorothalonil circulating in the tank. However it was observed that the area of the tank floor immediately in front of the the inlets where the heated dip was returned to the tank in the normal circulation, was relatively clear of sediment, so the case for increased agitation of the tank floor is not entirely ruled out. As chlorothalonil is regarded as a relatively stable compound, and if a reduced-rate application of chlorothalonil is found to be effective in controlling base rot, then the settling-out of active ingredient on the floor of the tank may not be too disadvantageous, since the sedimentation process should provide for it to be continually taken up into the circulating dip.

There are some rather obvious routes whereby fungicide sediments are lost to the dip. The movement of fork-lift trucks in and out of front-loading tanks when loading and unloading bins, removes a significant amount of the sediment that has settled on the tank floor. Potentially this could contaminate the environment, and it would be advisable (if sedimentation cannot be avoided) to contain this material by having the floor or roadway immediately in front of the tanks constructed to allow easy wash-down and containment.. Further removal of fungicide sediments will occur on the fabric of the bulb bins or crates (and, of course, on the bulbs themselves, a possible hazard to workers if working closely with them during handling and replanting). It may be possible to construct a balance sheet showing the amount of active ingredient that needs to be replaced after each dip.

Strategy for using pesticides in bulb dips

The results obtained in this project indicate that Bravo 500 appears suitable for use in bulb HWT and, by implication, in cold-dipping, despite the considerable loss of active ingredient that occurs. It would be useful, however, to determine the optimum and minimum chlorothalonil concentrations needed to control spores and other propagules of the base rot fungus (and a confirmation of the freedom from phytotoxic effects of chlorothalonil on daffodil crops is still awaited from field trials). Despite the high costs of thiabendazole fungicides, the alternating use of Bravo 500 and thiabendazole-based products is advised, in order to reduce the incidence of the base rot fungus developing resistance to specific fungicides.

The identification of fungicides from other mode-of-action groups that are effective against base rot would also provide further choice and better alternation of fungicide types, as well as removing the industry's dependence on one or two products. With the current high incidence of base rot in a wide range of daffodil cultivars, carrying out cold-dipping or HWT without adding a suitable fungicide can hardly be advised.

Strategy for using biocides in bulb dips

The need to use a biocide in bulb dipping should be re-evaluated, as it is clear from the tests with iodophore biocides reported here (and with chlorine dioxide in project BOF 70) that bulb dipping tanks regularly harbour a potentially huge bio-load or inoculum of microorganisms. Given the need for having clean bulb stocks and bulb handling that is hygienic for both bulbs and workers, there is justification for the inclusion of a biocide in dipping. Both iodophore biocides and chlorine dioxide (BOF 70) have the potential for use in bulb dipping. With iodophore biocides, the loss of active ingredient, probably by reaction with the metal of the tank, needs to be mitigated, while the use of chlorine dioxide awaits further testing and observations (in spring 2011) of stocks treated with the material.

Footnote

The use of biocides is governed by the EU Biocidal Products Directive (BPD). Before this project was approved, the HDC had received a written opinion from CRD that the use of biocides (such as iodophores) to achieve hygiene in crop production – as opposed to controlling a specific pathogen - fell outside the regulations. Further investigation of the BPD has indicated that this type of use fell within 'Product Type 2' (PT2), and that iodophore biocides were not being supported by the industry under PT2 in the EU's current product review. Discussions with the staff of Evans Vanodine, which is

supporting iodophores in the EU review process under other PTs, have produced divergent views on this issue. Clarification is being sought via the HDC; it will become urgent in the months preceding the next bulb harvest season.

Financial benefits

Bravo 500 has been confirmed as an effective fungicide for use in bulb HWT and cold-dipping; this material is relatively cheap, though it is suggested that its use should be alternated with that of a thiabendazole-based fungicide to avoid the likelihood of pathogens developing fungicide resistance. Yield losses due to base rot of 10% are not unusual, and some of the newer daffodil cultivars are now also showing base rot, so appropriate fungicide use (especially where there are doubts over the use of a biocide) should reduce these losses. Iodophore biocides, such as 'FAM 30', appear to have a role in general tank and bulb handling hygiene, and this should further reduce bulb losses.

Action points for growers

1. FAM 30, and probably some similar products, have a place in cold-dipping and HWT for the control of bio-load and achieving a good level of hygiene. However, due to its rapid loss from the dip, further work is needed before firm recommendations may be given. If used, it should be added to tanks immediately before use.

2. Half-rate 'Bravo 500' (0.5kg/1000L) can be used in daffodil HWT. Where there is a specific base rot problem, its use should be alternated with that of a thiabendazole-based product (either in alternate years or by using thiabendazole as a post-lifting bulb spray). These products should be topped-up regularly. In HWT, the recommended temperature, duration and other conditions should be adhered to. Steps should be taken to limit the spread of fungicide from the floor of tanks to the area surrounding the tanks.

3. A fungicide cold-dip should not be used without a biocide capable of controlling other pathogens and pests.

SCIENCE SECTION

Introduction

Biocides (disinfectants)

Until 2008 formalin (a.i., formaldehyde) had been used routinely for many years as a biocide in cold-dipping and hot-water treatment (HWT) of daffodil bulbs. It provided an economical means of controlling the spread of fungal diseases, particularly of *Fusarium* base and neck rots, and, in HWT, appeared to have augmented the management of stem nematodes, reputedly by killing those nematodes that escaped from the bulbs and which appear more difficult to control by heat alone than those remaining in the bulbs.

During the years that formalin was the preferred additive to bulb dips, relatively few problems were connected to it, provided precautions were taken to protect workers. Using too high a formalin concentration for bulb treatment could lead to damage to the base plate, and ADAS advisory information warned that suitable anti-corrosion paint should be used on the inside surfaces of HWT tanks to reduce the likelihood of loss of formalin through interactions with the mild steel of which the tanks are usually constructed. Long practice established formalin as working very effectively in this role, requiring only the regular topping-up of tanks with the usual strength formalin solution. In investigations where the formalin concentration in HWT tanks was followed over periods of several days, analyses confirmed that formalin was lost only slowly, and that the target concentration could be maintained through routine topping-up at the standard rate. Although some other biocides were tested at various times as alternatives to formalin, there was little or no incentive to switch to using them. Some interest was shown in peroxyacetic acid-hydrogen peroxide biocides (such as 'Jet 5') for use in HWT, but this material was never used on a large scale, and anecdotal information circulated in the industry that the active substance was 'lost' during the course of HWT.

A search for an alternative to formalin was forced on UK bulb growers in 2008, with the hurried withdrawal of its permitted use in agriculture and horticulture on grounds of possible risks to human health. In HDC-funded Projects BOF 61 and 61a, several candidate biocides were tested for their efficacy against stem nematodes and the base rot fungus under HWT conditions. In this case it was found that the heat of HWT alone was effective in killing stem nematodes, though an iodophore biocide, 'FAM 30', was found to be:

- ► Effective against stem nematodes at cold dip temperatures (using a 3h treatment)
- ► Effective against the base rot fungus at HWT temperatures
- ► Apparently free of adverse effects on the crop.

In follow-up work in HDC Project BOF 71, 'FAM 30' was also shown to be generally effective against base rot when used as a 3-hour cold dip treatment.

Since small-scale experiments do not always translate to commercial-scale operations, field trials of 'FAM 30'-treated bulbs were set up following treatment in commercial HWT facilities (Projects BOF 61a and 61b). 'FAM 30' has appeared, so far, to be free of any adverse effects on the crop, but the full results, due in 2011, are awaited for confirmation of these preliminary conclusions.

In the meantime, several questions were posed by the industry, and in 2010 Project BOF 61c was set up to address them.

- *Is 'FAM 30' – that is, its active substance, iodine – stable under prolonged (several days or weeks) use in HWT under commercial-scale conditions?*
- *Similarly, is it stable in commercial-scale cold-dipping?*
- *What top-up regimes are required for 'FAM 30' in HWT and cold dips?*
- *Can iodine concentrations be monitored easily on-farm using suitably robust methods?*
- *Does using 'FAM 30' in HWT and cold-dipping have a beneficial effect on hygiene, controlling bioload (microbial growth) in dip facilities and water?*

Fungicides

When growing daffodil cultivars susceptible to fungal diseases such as base rot, it is usual for growers to add a suitable fungicide to HWT and cold-dip tanks in addition to a biocide. Following extensive trialling, a thiabendazole-based fungicide ('Storite Clear Liquid'; 'Tezate 220 SL' is now also available) has been most used for this purpose, although it is relatively expensive. In addition, since 2008, the use of thiabendazole fungicides on flower bulbs has been restricted to once per year and to using, for bulb-dipping, no more than a quarter-rate of that formerly used, and their use is not permitted on the Isles of Scilly. For these reasons, alternative fungicides, as well as alternative biocides, were included in the HDC-funded projects referred to above. Of the candidate fungicides tested, chlorothalonil (as 'Bravo 500') was found to be:

- Effective against base rot spores in HWT and cold-dip temperatures.
- Apparently without adverse effects on the crop.

'Bravo 500' therefore joined 'FAM 30' in the field-scale trials of Projects BOF 61a and 61b; as is the case with 'FAM 30', full results will not be available until 2011.

Under some circumstances thiabendazole is known to be rapidly 'lost' in HWT dips, and some HDC-funded analyses in 2009 suggested that losses of chlorothalonil also occurred in HWT. Therefore, in Project BOF 61c the following questions were asked.

- *Is chlorothalonil stable under prolonged (several days/weeks) commercial use in HWT?*
- *Similarly, is it stable in commercial-scale cold-dipping?*
- *What top-up regimes are required for chlorothalonil in HWT and cold dips?*
- *Are there ways of preventing the loss of such fungicides in bulb dipping?*

In the case of chlorothalonil, on-farm measurement kits are unlikely to become available, so the project relied on standard laboratory analyses.

Project BOF 61c

In order to generate information as quickly and as realistically as possible, the project was based on using commercial bulb dipping operations as the means of testing and sampling. The operations chosen included the HWT of reclaimed (ex-forced) bulbs, as this is often carried out in May or June, in advance of the main HWT season (July and August), so would provide some results more promptly than waiting for regular HWT. In the main bulb lifting and HWT season, regular HWT and two cold-dip operations were used. Where practical, sites using 'FAM 30' and/or 'Bravo 500' were selected.

It is commonly observed that off-white sediments, presumably consisting largely of fungicide particles, accumulate on the floor and other, particularly horizontal, surfaces of HWT tanks. Presumably the deposits result from the normal process of sedimentation exhibited by solids suspended in water, possibly constituting to a significant loss of active ingredient. It may be possible to disperse sediments settling on the floor of the tank by introducing additional mixing and circulation across the tank floor, thereby increasing the concentration of fungicide 'available' and circulating around the bulbs. To test this, stand-alone supplementary pumping was introduced into one standard HWT facility and its effects on the concentration of circulating chlorothalonil measured.

Materials and methods

Hot-water treatment

HWT of daffodil bulbs refers to immersion of the bulbs in hot water - at 44.4°C for 3h is usual in the UK - to control stem nematode, base rot and other pests and pathogens. In summer 2010, regular HWT operations on commercial bulb farms in Lincolnshire were utilised to obtain samples of dip for the determination of active substance concentrations over time.

These included operations for both (1) the HWT of reclaimed (ex-forced) bulbs (often carried out in May or June), and (2) the regular HWT of bulb stocks in July or August.

Mutually suitable HWT facilities and programmes were discussed with growers, and arrangements agreed for access to obtain dip samples and other information. Key factors in deciding the suitability of a site included the use of either the candidate biocide ('FAM 30') or fungicide ('Bravo 500') in a regular HWT programme over a long-enough period, though in practice this was not always possible. It was agreed that each participating grower would receive a copy of the results of work on their farm, and that, when the overall findings were reported to the HDC, the identities of farms would be anonymised.

HWT facilities in the UK operate to a roughly uniform standard, but may vary considerably within that pattern, which could affect the results obtained. Details of the facilities, durations, temperatures, dip additives, concentrations, etc., are given in Tables 1 (reclaimed bulbs) and 2 (regular HWT). Testing started at the beginning of the growers' HWT programmes, using freshly made up dips (see dates and timings under 'Results'), and was continued at appropriate intervals without replacing the dips. Following the addition of chemicals initially or at topping-up, they were dispersed by pumping (e.g. from the holding tank to a treatment tank and back).

Table 1. Details of HWT facilities and conditions used in the project with reclaimed bulbs

	Case 1	***Case 2***
Tank type	Front-loading, Secker Welding	Front-loading, Secker Welding
Tanks used	1 treatment tank + holding tank	1 treatment tank + holding tank
System capacity (as used)	16,200L	36,000L
Load per tank	6 wooden bins holding *ca* 600kg each (3.6t)	12 wooden bins holding *ca* 750kg each (9.0t)
Water supply	Mains, direct	Mains, direct, fed to HWT tanks via a top-up tank
Test chemical and rate	'FAM 30' (8.0L product per 1000L water)	'Bravo 500' (0.5L product per 1000L water)
Wetter and rate	'Activator 90' (1.0L product per 1000L water)	'Activator 90' (0.3L product per 1000L water)
Other chemicals	'Fighter F' (anti-foam) (*ca* 0.025L product per 1000L water)	'Fighter F' (anti-foam) (0.03L product per 1000L water)
Regime	HWT 44.4°C for 3h	HWT 44.4°C for 3h
Top-up procedure	Before days dipping if required, with water and 'FAM 30' and 'Activator 90' at same rates as before	Before days dipping if required, with water and 'Bravo 500' and 'Activator 90' at same rates as before
Deviations and down-time	None	None

Table 2. Details of HWT facilities and conditions used in the project with regular bulb stocks

	Case 3	***Case 4***	***Case 5***
Tank type	Front-loading, Secker Welding	Front-loading, Secker Welding	Front-loading, van Rooyen
Tanks used	2 treatment tanks + holding tank	Treatment tank 1 + holding tank	2 treatment tanks + holding tank
System capacity (as used)	ca 18,000L (10,000L when using tank 1 only, otherwise 18,000L)	36,000L	10,000L
Load per tank	6 wooden bins holding *ca* 600kg each (3.6t)	12 wooden bins holding *ca* 750kg each (9.0t)	6 wooden bins holding *ca* 500kg each (3.0t)
Water supply	Mains, direct	Mains, direct, fed to HWT tanks via a top-up tank	Mains, direct
Test chemical and rate	'FAM 30' (7.5L product per 1000L water)	'Bravo 500' (1.0L product per 1000L water)	'FAM 30' + 'Bravo 500' (4.0L + 0.5L product per 1000L water)
Wetter and rate	'Activator 90' (1.0L product per 1000L water)	'Activator 90' (0.3L product per 1000L water)	'Activator 90' (0.3L product per 1000L water)
Other chemicals	None	'Fighter F' (anti-foam) (0.03L product per 1000L water)	None
Regime	HWT 44.4°C for 3h	HWT 44.4°C for 3h	HWT 44.4°C for 3h
Top-up procedure	When tank 2 was brought into use, water and 'FAM 30' (6.25L product per 1000L water) and 'Activator 90' (2.0L product per 1000L water) were added	Topping-up carried out at about 16:00h on the days before further HWT, with 'Bravo 500', 'Activator 90' and 'Fighter F' at the same rates as before	On the previous evening before dipping if required, with water and 'FAM 30' and 'Activator 90' at same rates as before
Deviations and down-time	For the first load only, used only treatment tank 1 and a half-load	During the HWT programme there were two intermissions (of 5 days and 7 days) during which the dip was held, uncirculated, in the holding tank, before the HWT programme was resumed	None

Cold-dipping

As a daffodil bulb treatment cold-dipping generally refers to a short (often 15min) soak treatment carried out at ambient temperatures shortly after the bulbs have been lifted from the field. With the addition of a suitable biocide and/or fungicide, cold-dipping particularly helps to control base rot. Similar arrangements to those described for HWT applied to cold-dipping, which is usually carried out in the same tanks as used for HWT. Details of the facilities, durations, dip additives, concentrations, etc., are given in Table 3. Testing started at the beginning of the growers' cold-dipping programmes, using freshly made up dips (see dates and timings under 'Results') and was repeated at appropriate intervals while the programme was continued, without replacement of the dip.

Table 3. Details of cold-dipping facilities and conditions

	Case 6	***Case 7***
Tank type	Front-loading, van Rooyen	Front-loading, Secker Welding
Tanks used	2 treatment tanks + holding tank	2 treatment tanks + holding tank
System capacity (as used)	10,000L	11,645L
Load per tank	6 wooden bins holding *ca* 500kg each (3.0t)	8 wooden bins holding *ca* 600kg each (4.8t)
Water supply	Mains, direct	Mains, direct
Test chemical and rate	'FAM 30' (4.0L product per 1000L water)	'Virudine' + 'Storite Clear Liquid' (5L + 1.25L product per 1000L water)
Wetter and rate	'Activator 90' (0.3L product per 1000L water)	A: None B: 'Activator 90' (0.65L per 1000L water)
Other chemicals	None	Anti-foam preparation used as required Sodium bisulphate not added at start-up but used at top-up
Regime	Cold-dip, ambient, 15min	Cold-dip, ambient, 30min
Top-up procedure	Before days dipping if required, with water and 'FAM 30' and 'Activator 90' at same rates as before	A: 'Virudine' at original rate and sodium bisulphate as required (see Table 11 for details) B: 'FAM 30' and 'Activator 90' at original rate and sodium bisulphate as required (see Table 12 for details)
Deviations and down-time	Not run over weekend	Dipping delayed by breakdown on 10 July

Dip additives used

The chemicals used in HWT and cold-dipping tanks were:

- ‘FAM 30’ (Evans Vanodine International PLC), an iodophore disinfectant containing 9.3% w/w sulphuric acid, 9.5% w/w phosphoric acid, 1 to 5% w/w iodine (mimimum available iodine at time of manufacture 2.75% w/w) and unspecified non-ionic surfactant
- ‘Virudine’ (Antec International – a DuPont Company), an iodophore disinfectant containing minimum 27% w/w available acids (as phosphoric acid) and minimum 2.7% w/w available iodine
- ‘Bravo 500’ (Syngenta Crop Protection UK Ltd) containing 500g chlorothalonil as a suspension concentrate (‘flowable’)
- ‘Storite Clear Liquid’ (Frontier Agriculture Ltd) containing 220g/L thiabendazole as a soluble concentrate
- ‘Activator 90’ (De Sangosse Ltd), a non-ionic surfactant/wetter containing 750g/L alcohol ethoxylates and 150g/L natural fatty acids
- ‘Fighter-F’ (De Sangosse Ltd), an anti-foam/defoamer containing 10% dimethylpolysiloxane
- ‘Dow Corning Antifoam RD Emulsion’ (Dow Corning S.A.), an anti-foam preparation containing 10% active silicone emulsion.

Dip sampling

Dip samples (minimum of 200ml each) were routinely taken from HWT or cold-dip tanks from near the top of the tank, excluding any foam or floating debris. Typically, samples were taken at the following stages of HWT or cold-dipping:

1. Tank filled with plain water shortly prior to the addition of any chemicals
2. After the addition of ‘FAM 30’, ‘Bravo 500’, etc. and allowing time for dispersal by pumping and circulating the dip
3. Shortly after the first load of bulbs has been covered with dip
4. For HWT, after 1 and 2h of treatment and at the end of treatment of the first load (usually 3h); for cold-dipping, at the end of treatment of the first load (15 or 30min)
5. For HWT, at the end of each further treatment made on the first day of HWT (often three per day); for cold-dipping, at the end of dipping for the middle and last loads of the first day of dipping
6. At the start and end of the following day’s HWT or cold-dipping and at suitable intervals; where the tanks were topped-up this was usually carried out before the start of the day’s HWT or cold-dipping

The exact details and sampling times for the different operations are given under ‘Results’. Samples were initially put in a cool place out of the sun and, where they were not to be analysed immediately, were refrigerated at *ca* 4°C. With the exception of fungicides, the test methods used were evolved as the project progressed.

Testing for iodine

The active constituent of iodophore biocides (such as ‘FAM 30’ or ‘Virudine’) is iodine. In previous projects ‘FAM 30’ has been used in bulb-dipping at ‘full’ and ‘half’ rates of 8.0 and 4.0L product per 1000L water (dilutions of 1:125 and 1:250, respectively). The ‘FAM 30’ label states that its iodine content is 1 to 5% w/w, with a minimum concentration of iodine at the time of manufacture of 2.75% w/w; for convenience, in this report dilutions of ‘FAM 30’ have been converted to parts per million of iodine (ppm, the same as mg/L), assuming an iodine content of 2.5% w/w, thus:

1:2500 dilution	≈	10ppm iodine
1:1500 dilution	≈	17ppm iodine
1:1000 dilution	≈	25ppm iodine
1:600 dilution	≈	42ppm iodine
1:400 dilution	≈	63ppm iodine
1:300 dilution	≈	83ppm iodine
1:250 dilution	≈	100ppm iodine
1:125 dilution	≈	200ppm iodine

Iodine biocides such as ‘FAM 30’ have a characteristic golden colouration, referred to under ‘Results’ as the ‘indicative colour’.

Iodine concentrations in HWT and cold-dip tanks were estimated using tests considered robust enough for on-farm use. Since tests for iodine may also respond to the presence of chlorine or bromine, including trace chlorine in tap and other water, it was necessary to carry out ‘control’ tests using plain water and deionised or distilled water. Where necessary samples were diluted to bring them within the range of tests (see below).

The test methods used are described overleaf.

Hanna Instruments (HI) Test Kit and Iodine Meter

The 'Iodine Test high-range Kit' (HI 3879; Hanna Instruments www.hannainst.com) consists of a pack with reagent sachets, a 5ml-capacity 'comparator cube' (for holding samples) and a plastic pipette. After shaking the sample, 5ml of it is pipetted into the comparator cube. The contents of one reagent sachet is tipped into the sample and the cube is capped and briefy shaken, then allowed to stand for 2min (this development time is critical). The liquid remains colourless if iodine is absent, turns a very faint pink if iodine is present at a concentration <1ppm, and if iodine is present the liquid develops a pink-red colouration that is proportional (over the range 1 to 5ppm) to the concentration of iodine. The colour becomes progressively deeper – darkening to a deep cerise - if the iodine concentration exceeds 5ppm.The colour of the test liquid is compared with the five colour 'swatches' (corresponding to iodine concentrations of 1 to 5ppm) printed on the side of the comparator cube; this is best done by holding the comparator cube about 10cm in front of a white surface.

The test was easy to use, but required samples to be diluted until they are within the rather narrow range of 1 to 5ppm. Under the conditions used, fresh mains water elicited a faint pink tinge, presumably due to chlorine, and this was largely eliminated in tap water allowed to stand overnight. Fresh supplies of both de-ionised and distilled water (The Water Company http://www.the-water-company.com) was used for diluting samples, but likewise elicited a weak pink colouration due to trace chlorine, but there was no way to avoid its use. Using a plain water 'control' enabled any background reading to be deducted from the observed reading.

Although the above test was simple and reliable, ongoing difficulties in sourcing supplies of reagent necessitated an alternative. Based on the same chemical reaction[1] as the Iodine Test Kit, an Iodine Meter (HI 93718; Hanna Instruments) was used; this is a hand-held colorimeter with an LED emitting at 555nm and reading iodine concentrations in the 0 to 12.5ppm range. A glass cuvette with a 10ml dip sample is placed in the meter and the instrument is 'zeroed'. The contents of one reagent sachet is then tipped into the sample and the cuvette is capped, briefy shaken and placed in the meter which then takes a reading after (at room temperature) 2.5min. From a practical viewpoint, the disadvantage of this method is that it will not accept even faintly coloured samples, so bulb dip samples typically needed to be diluted 100-fold with water before being readable, at which point many dip samples contained iodine at a concentration similar to that of trace chlorine in the de-ionised

[1] Adapted from *Standard Methods for the Examination of Water and Wastewater*, 18th edition, DPD method. Readings from the meter may be affected by the presence of ozone, oxidized chromium or manganese, or high alkalinity, acidity and hardness of water, as well as chlorine and bromine.

or distilled water used for sample dilution, so it is still necessary to deduct the 'background' reading from the reading obtained with samples. In discussions with Hanna Instruments technical staff it was claimed that any trace chlorine present in de-ionised or distilled water would give only a very low reading (<0.1ppm) on the meter, which would be within the accuracy of the device (±0.1ppm) and therefore irrelevant. However, further testing with a replacement meter using various sources of de-ionised/distilled water continued to result in readings of 0.3 to 0.6ppm for plain water.

Evans Vanodine (EV) Dip-sticks

'FAM 30 Check Strips' (Evans Vanodine International www.evansvanodine.co.uk) are 'dip-sticks' with a colour scale based on dilutions of 'FAM 30' rather than ppm of iodine. The swatches vary from a pale blue-green (representing a dilution of 1:2500) to black or very dark green (1:125, the usual maximum strength solution) in six steps. In the absence of iodine (or when iodine is below the limit of detection) the test pads remain yellow. After shaking the sample, a dip-stick is dipped into the sample for 2sec, removed, held horizontally, and the colour compared with the swatches after 2min; dipping and waiting times are critical.

It was found that, while the darkest colour was easy to see in fresh, strong solutions of 'FAM 30', colour-matching at higher dilutions was difficult and it was also difficult to determine the limit of detection. Serial dilutions of 'FAM 30' (from 1:125 to 1:4000) were made and tested, resulting in - at least under the conditions being used - what was considered a somewhat more distinct colour range. Under 'Results', this is referred to as the 'revised' EV method.

Industrial Test Systems (ITS) Dip-sticks

The above 'FAM 30 Check Strips' are produced by Industrial Test Systems (Rock Hill, SC 29730, USA) and repackaged by Evans Vanodine. On the packs used, the original label ('Iodine Test Strips') was present under the Evan Vanodine label; it showed two scales and two sets of colour swatches for low-concentration applications:

A. For 0 to 5ppm iodine from yellow to dark sage-green in seven stages, requiring a 5sec dip and a 30sec wait until colours are compared
B. For 0 to 0.4ppm iodine from yellow to dark sage-green in seven stages, requiring a 30sec dip and a 30sec wait until colours are compared.

Also available from Industrial Test Systems are the 'SenSafe Iodine Check' and 'Iodine Check High' dip-sticks. The former have the same two sets of swatches as described above, but slightly different instructions:

A. For 0 to 5ppm iodine use a 10sec dip, wait for 30sec, and match the colours within 15sec
B. For 0 to 0.4ppm iodine use a 30sec dip, wait for 30sec, and match the colours within 15sec.

The 'Iodine Check High' dip-sticks have a swatch with colours similar to those of the EV dip-sticks, with 14 stages from 0 to 300ppm. They are dipped for 2sec and then matched within 30sec.

Testing for chlorothalonil

Chlorothalonil is the active substance of 'Bravo 500' fungicide, and in bulb dipping it has been used at 'full' and 'half' rates of 1.0 and 0.5L product per 1000L water, equivalent to 500 or 250ppm (mg/L) of active ingredient, respectively. Samples were analysed by Eclipse Scientific Group www.eclipsescientific.co.uk using reverse-phase high-performance liquid chromatography with UV detection. Non-filtered samples were analysed so that the concentrations given are the total of dissolved and suspended chlorothalonil. As checks, 'blanks' and chlorothalonil solutions of known concentration were included amongst the samples analysed.

Testing pH

The pH of bulb dips is important, as pesticides may be inactivated or enhanced at different values of pH, while dips of very low (acidic) pH may be harmful to plants. For example, 'FAM 30' is formulated with sulphuric and phosphoric acids. The pH of dips was determined using a suite of narrow- and wide-range indicator papers ('Pehanon', Macherey-Nagel, Düren, Germany, and BDH, Poole, UK). This approach was chosen over using a pH meter because of the robustness of using indicator strips in an on-farm situation.

Testing bioload

Bacterial pollution in dip tanks was evaluated using 'Petrifilm' (3M Microbiology Products, St Paul, MN, USA). The 'film' consists of a paper containing a nutrient medium specific to aerobic bacteria and a transparent plastic cover. Having peeled back the cover sheet, a pipette is used to place a 1ml-aliquot of the well-mixed dip sample as a single drop in the centre of the 'Petrifilm'. The cover sheet is then gently replaced and the sample evenly spread across the film by pressure from a supplied plastic 'spreader'. The unit is left at room temperature for three days, after which bacterial growth on the film is assessed according to a visual scale provided by the manufacturer, ranging from 0 (not polluted) to 5 (very strongly

polluted). No bacterial growth is scored as 0, increasing numbers of distinct bacterial colonies from 1 (at which it is relatively easy to count the total number of bacterial colonies) to 4, with total coverage of the film by colonies scored 5. A score of 0.5 was used for very sparse colony numbers, and 0.1 where the number was less than ten.

General microbial pollution was determined in dip samples using the 'Clean-Trace NG Luminometer' system (Biotrace International PLC, Bridgend, UK). This is based on a luminescent reaction between a reagent on a 'measuring pen' and adenosine triphosphate (ATP, a compound produced by all living cells) in the sample. Essentially, the dip is sampled by dipping in the 'pen' (resembling that used forensically in swabs), replacing the pen in its holder (which mixes sample and reagents) and inserting it into a luminometer which gives a reading in arbitary 'relative light units' (RLU) within 30sec. The scale suggested by the manufacturer runs from 0 to 500RLU, meaning not or only slightly polluted, to >3,000RLU, meaning strongly polluted. Tests of fresh tap water, included as checks at the start and end of testing, typically gave values <20RLU. Because of the very high readings in highly polluted samples, ATP levels are shown in graphs on a logrithmic scale.

Both systems were relatively easy to use, though 'Petrifilm' might be difficult to use 'in the field' under adverse weather conditions. The 'Clean-Trace' system requires the use of a very controlled procedure if consistent results are to be obtained: the physical parameters need to be standardised, e.g. even to the size of the sample containers and the depth and position of dipping the 'pen' into the container. In the present study the technique included mixing the sample well, taking a sub-sample of *ca* 300ml and dispensing it equally to each of three *ca* 300ml beakers, usually sampling the three sub-samples individually and averaging the results. Any sub-sample can be tested only once, as reagent from the 'pens' may leach into the sample during dipping and affect subsequent results.

HWT with 'Bravo 500' and supplementary agitation of the dip

A commercial HWT facility treating regular bulb stocks with full-rate 'Bravo 500' was utilised for this test. Following previous HWT runs with 'Bravo 500' fungicide, a conspicuous white deposit was routinely evident on the tank floor once the treated bins had been removed. The grower's HWT programme had been started on 26 July 2010 and the test was carried out using the extant dip on 26 August 2010, following the usual top-up procedures (see Table 4 for details). This tank arrangement and the arrangement of bulb crates in the tank proved to be convenient for fitting a stand-alone pump and pipe-work temporarily to provide supplementary water circulation with minimal disruption to working practices. The

supplementary circulation was provided by a bore-hole pump delivering 800L/sec, mounted at the base of a vertical pipe, and held a few cm above the floor of the tank mid-way along one side wall. Dip was drawn in from floor of the tank and up the vertical pipe which branched through a T-junction to two horizontal pipes expelling the just dip below the water surface at either end of the tank. All pipework was made of glued 5cm-diameter PVC.

For the test, tank 1 was operated normally (with no additional circulation) until it had been at the target temperature for 1h (*ca* 1.5h after covering the bulbs with dip), when a dip sample was taken as described previously. The supplementary pump was then operated for 1h, following which a second dip sample was taken. A third sample was taken 1h later, at the end of the 3h-treatment. Testing and sampling was repeated over the next two HWT runs, in tank 2 and tank 1, respectively (following the usual procedure for this facility, the dip was pumped to the alternate tank for successive runs). On the third run the supplementary pump was operated for the last 2h of treatment. Chlorothalonil concentrations in the nine dip samples were analysed as described above.

Table 4. Details of HWT facilities and conditions used in the test of supplementary dip agitation

	Case 8
Tank type	Front-loading, Secker Welding
Tanks used	2 treatment tanks used alternately (no separate holding tank)
System capacity (as used)	Tank volume 11000L
Load per tank	10 metal crates holding *ca* 500kg each (5t)
Water supply	Mains, direct
Test chemical and rate	'Bravo 500' (1.0L product per 1000L water)
Wetter and rate	'Activator 90' (1.0L product per 1000L water)
Other chemicals	'Dow Corning Antifoam RD Emulsion' – only added as required (once during this test) (*ca* 0.025L product per 1000L water)
Regime	HWT 44.4°C for 3h
Top-up procedure	Before each day's dipping as required, with water and 'Bravo 500' (2.0L product per 1000L water)
Deviations and down-time	None

Results

Case 1 – Early HWT of reclaimed bulbs using 'FAM 30' biocide

In this operation reclaimed bulbs were hot-water treated with full-rate 'FAM 30' ('FAM 30' diluted 1:125 with water), dipping seven loads of bulbs over a 3-day period. A summary of the operations, times of taking dip samples, dip pH and iodine concentrations is given in Table 5.

Table 5. Case 1: Iodine concentrations and dip pH during HWT with reclaimed bulbs

Date	*Time (hh:mm)*	*Time since 'FAM 30' added (h)*	*Operations carried out*	*pH value*	*Iodine concentration (ppm)*		
					HI test	*EV sticks*	*Indicative colour*
19 May	12:30	-0.50	Treatment tank had been filled with mains water and brought to temperature	7.5	**<**	**<**	X
	13:00	0	'FAM 30' and 'Activator 90' had been added and dispersed by pumping to holding tank	1.8	**160**	**140**	✓
	13:15	0.25	Bulbs had been loaded; 'Fighter F' added and dip pumped back to treatment tank	2.1	**160**	**140**	✓
	14:30	1.50	Bulbs had been treated for 1h (after target temperature regained)	2.3	**<**	**<**	X
	15:30	2.50	Bulbs had been treated for 2h	2.4	**<**	**<**	X
	16:30	3.50	Bulbs had been treated for 3h, dip pumped to holding tank	2.4	**<**	**<**	X
	21:30	8.50	2nd load of bulbs had been treated	3.5	**<**	**<**	X
20 May	06:00	17.00	Tank had been topped-up ready for use	3.5	**<**	**<**	X
	21:30	32.50	3rd, 4th and 5th loads of bulbs had been treated	4.7	**<**	**<**	X
21 May	06:00	41.50	Tank ready for use (no further top-up)	4.7	**<**	**<**	X
	13:30	49.00	6th and 7th loads of bulbs had been treated	4.7	**<**	**<**	X

The symbol < means that iodine concentrations were below the limit of detection for a particular test, e.g. <1ppm for the HI test and <10ppm for the EV sticks. Where test results fell between gradations on a colour scale, the mid-point is quoted.

Iodine was measurable in the dip only in the first two samples following addition of 'FAM 30' to the HWT system, i.e. immediately after the addition and dispersal of the biocide in the holding tank and 25 minutes later when the first batch of bulbs had been loaded and the dip pumped to the treatment tank. Using both the colourimetric test and dip-sticks, the concentration of iodine at both sampling times was *ca* 150ppm, and it was only in these two samples that the indicative colour of 'FAM 30' could be observed.

In all subsequent samples, taken between 1.5 and 49.0h after the addition of the biocide, the iodine concentration was below the limit of detection (1 or 10ppm, depending on the test), notwithstanding that the system was topped-up at the start of the second day's work (17h after the original addition of biocide to the system). The rapid loss of the active substance, iodine, indicated that only the first load of bulbs will have been treated as expected. The top-up on day 2 had no observable effect on the iodine concentration of later samples. Whether any low, residual concentration of iodine would have had any meaningful effect on subsequent loads of bulbs is not known, though, from the bioload results of Case 3 (see below) there may well have been a general benefit of disinfection of the HWT system and water at the start of the operation. The reasons for the loss of iodine are being investigated (see 'Discussion').

The pH of the mains water supply was 7.5, and immediately following the addition of 'FAM 30' the pH of the dip was 1.8, not unexpected as 'FAM 30' contains sulphuric and phosphoric acids. Thereafter, dip pH increased slowly to 4.7 by the end of testing, presumably as a result of accumulating contamination of the dip with bulbs and soil and other debris.

Case 2 – Early HWT of ex-forced bulbs using 'Bravo 500' fungicide

In Case 2 reclaimed bulbs were HWT with half-rate 'Bravo 500', treating seven loads of bulbs over a 3-day period. A summary of the operations, times of taking dip samples and chlorothalonil concentrations is given in Table 6.

Table 6. Case 2: Chlorothalonil concentrations and dip pH during HWT with reclaimed bulbs

Date	*Time (hh:mm)*	*Time since 'Bravo 500' added (h)*	*Sample no.*	*Operations carried out*	*Dip pH value*	*Chlorothalonil concentration) (ppm)*
3 June	07:30	-	1	Holding tank had been filled with mains water	7.2	**<**
	07:45	0	-	'Bravo 500', 'Activator 90' and 'Fighter F' had been added and dispersed within the holding tank	-	**-**
	07:50	0.08	2	Sample 2 had been taken	6.7	**286**
	09:55	2.16	3	First load had been at target temperature in tank for 1h	6.7	**237**
	10:55	3.16	4	First load had been at target temperature in tank for 2h	6.7	**211**
	11:55	4.16	5	First load had finished treatment	6.7	**194**
	19:00	11.24	6	Second load (last of the day) had finished treatment	6.5	**102**
4 June	08:00	24.24	7	Tank had been topped-up and first load of day started	5.8	**82**
	17:00	33.24	8	Third load (last of the day) had finished treatment	5.1	**69**
5 June	12:00	52.24	9	Second load (last of the day) had finished treatment	4.9	**56**

The symbol < means that chlorothalonil concentration was below the limit of detection (<0.1ppm). Where test results fell between gradations on a colour scale, the mid-point is quoted.

The addition of 0.5L 'Bravo 500' (containing 50% w/w chlorothalonil) per 1000L dip should produce a concentration of the active substance of 250ppm (250mg/L). While the concentration determined for the first sample following adding the fungicide was 286ppm, this figure is probably acceptible, given the scope for variations in farm procedures, sampling and analysis. Thereafter chlorothalonil concentrations fell sharply over the first 12h before levelling out at around 60ppm (Figure 1). Fitting the data to a logrithmic trend-line provided a good fit, as indicated by the R^2 value (0.88) approaching 1.0.

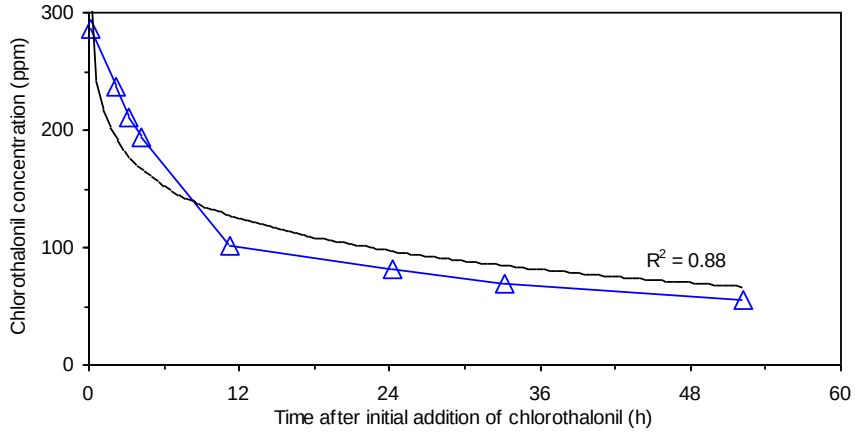


Figure 1. Chlorothalonil concentrations in HWT dip over a 3-day period (Case 2). Trend-line and R^2 value shown in black

The recorded drop in chlorothalonil concentration was in agreement with those of samples obtained by Dick Evenden for the HDC in 2009, and they are similar to the declines in the concentration of thiabendazole and benomyl previously recorded at Kirton. This may be connected with the settling of particles and the establishment of a sedimentation equilibrium, giving, within the body of the dip, around 20 to 25% of the original concentration. A means of preventing this loss is needed.

The pH value of the dip fell from 7.2 to about 6.7 after the chemical additions, then remained stable for the next 12h. Subsequently the pH fell to 4.9 to 5.1 by the end of the test period. The reason for this fall is not known, though it may be connected with the accumulation of soil and bulb debris.

Case 3 – Regular HWT using 'FAM 30' biocide

In this operation bulbs were hot-water treated with slightly less than full-rate 'FAM 30', treating five loads of bulbs over a 2-day period. A summary of the operations, times of taking dip samples, dip pH and iodine concentrations is given in Table 7.

The initial target concentration of iodine in this case was about 190ppm. Using the HI Meter, iodine concentrations of 80 to 90ppm were indicated during the first hour following the addition of 'FAM 30'. Thereafter, and despite the later addition of further 'FAM 30', the concentration fell quickly, though it remained detectable (at above 10ppm) until about 24h from initial addition. By the end of the run, about 30h after the start, the iodine concentration was below the limit of detection (<1ppm in this system). Using the EV dip-sticks, a concentration of about 140ppm was indicated in samples taken within the first 2h of adding the biocide, and thereafter iodine was undetectable by this method. Using two types of ITS dip-sticks, similar trends were indicated, with only very low concentrations detectable after the first 2h. Although dip-stick methods cannot be relied upon to give anything more than an indication that significant amounts of iodine are present, as presence-or-absence indicators or for the confirmation of results obtained by other means, they were useful.

Dip pH fell from an initial value of 6.0 – rather lower than expected, but perhaps related to the effects of residues remaining in the tanks from previous work – to 2.3 after 'FAM 30' had been added, drifting upwards only slowly to 3.7 by the end of treatment.

Bioload in the system was low, compared with some other cases. Using the 'Petrifilm' technique, only low scores for bacterial colonies were found, and then only in the samples

taken before ‘FAM 30’ was added and at the end of the run (Figure 2). Measured as ATP activity, the pre-‘FAM 30’ level was only 225RLU, and throughout this run activity was low, with values between about 1,500 and 3,000RLU (Figure 2). Although circumstantial, it is interesting to note that this HWT run was carried out immediately after using the facility to test another biocide, chlorine dioxide (see report on HDC project BOF 70). This would also explain the low initial pH of the water and the low levels of bioload detected.

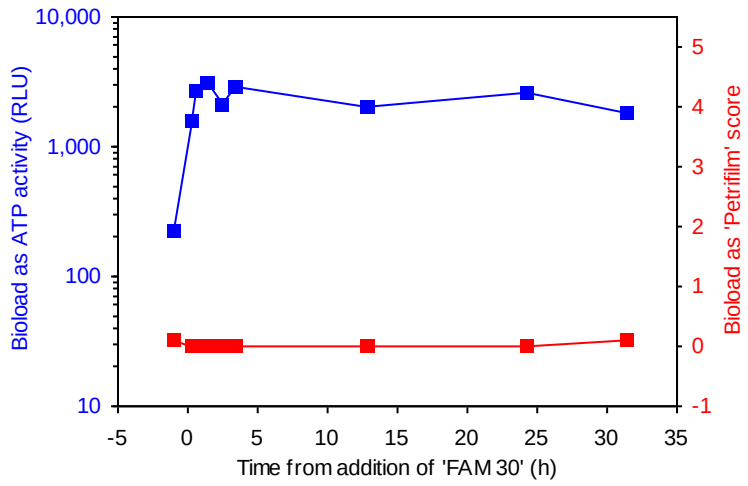


Figure 2. Bioload in HWT dip using ‘FAM 30’ over a 2-day period (Case 3)

Table 7. Case 3: Iodine concentration, dip pH and bioload during regular HWT

Date	Time (hh.mm)	Time since 'FAM 30' added (h)	Sample no.	Operations carried out	pH value	Iodine concentration (ppm)				In-dicative colour seen	Pollution	
						HI meter	EV sticks	ITS 'SenSafe' sticks	ITS high range sticks		ATP activity (RLU)	Bacterial activity (score)
7 Aug.	07:00	-0.08	1	Holding tank had been filled with mains water (10,000L)	6.0	**<**	**<**	**0.02 (B)**	**0**	X	225	0.1
	07:05	0	-	'FAM 30' and 'Activator 90' added	-	**-**	**-**	**-**	**-**	-	-	-
	07:20	0.25	2	Bulbs had been loaded, dip pumped from holding tank to treatment tank 1 (only tank 1 this load, thereafter both tanks)	2.3	**90**	**140**	**>5 (A)**	**20**	✓	1,564	0
	07:30	0.42	-	Dip at target temperature, 3h period started	-	**-**	**-**	**-**	**-**	-	-	-
	07:35	0.50	3	5min sample taken	2.3	**80**	**140**	**>5 (A)**	**15**	✓	2,693	0
	08:30	1.42	4	1h sample taken	2.3	**30**	**140**	**4 (A)**	**5**	✓	3,069	0
	09:30	2.42	5	2h sample taken	2.3	**20**	**<**	**2 (A)**	**0**	X	2,126	0
	10:00	2.92	-	Additional water (8,000L), 'FAM 30' and 'Activator 90' had been added to holding tank	-	**-**	**-**	**-**	**-**	-	-	-
	10:30	3.42	6	3h sample taken, treatment of first load completed (further loads used both tanks)	2.3	**10**	**<**	**0.2 (B)**	**0**	X	2,877	0
	20:00	12.92	7	Treatment of third load completed (last load of day)	3.0	**20**	**<**	**0.2 (B)**	**0**	X	2,024	0
8 Aug.	07:20	24.25	8	Sample taken before start of dipping (no further top-up)	3.0	**10**	**<**	**0.2 (B)**	**0**	X	2,597	0
	14:30	31.42	9	Sample taken at the end of dipping (after two loads)	3.7	**<**	**<**	**0.2 (B)**	**0**	X	1,821	0.1

The symbol < means that iodine concentrations were below the limit of detection for a particular test, e.g. <1ppm for the HI meter and <10ppm for the EV sticks. Where test results fell between gradations on a colour scale, the mid-point is quoted. (A) and (B) refer to the high- and low-range, respectively, of dual-range sticks.

Case 4 – Regular HWT using 'Bravo 500' fungicide

In this case bulbs were hot-water treated with full-rate 'Bravo 500'. The programme was as follows:

- ► Samples were taken over the first 5 days of HWT
- ► The programme was then held up for 5 days for more bulbs to be processed, during which time the dip remained uncirculated in the header tank
- ► Dip samples were taken before and after circulating the dip ready for a further run lasting 6 days
- ► The programme was held up for 7 days, with the dip uncirculated in the header tank
- ► Dip samples were taken before and after circulating the dip ready for a further run.

A summary of the operation, times of taking dip samples, chlorothalonil concentrations, dip pH and bioload is given in Table 8.

Chlorothalonil concentrations are shown in Figure 3. Full-rate 'Bravo 500' should produce a chlorothalonil concentration of 500ppm, though the first sample, taken 5min after the addition of chemicals, showed a concentration of 371ppm. Thereafter, despite regular topping-up, concentrations fell to 152, 128, 116, 68 and 110ppm by the end of work on days 1 to 5, respectively. This loss of active ingredient was similar to that seen in Case 2. After a 4-day pause in carrying out HWT, the chlorothalonil concentration was 105ppm; after a further 13 days, concentrations of 72 and 81ppm were recorded. As in Case 2, fitting the data to a logrithmic trend-line provided a good fit, indicated by the R^2 values of about 0.9.

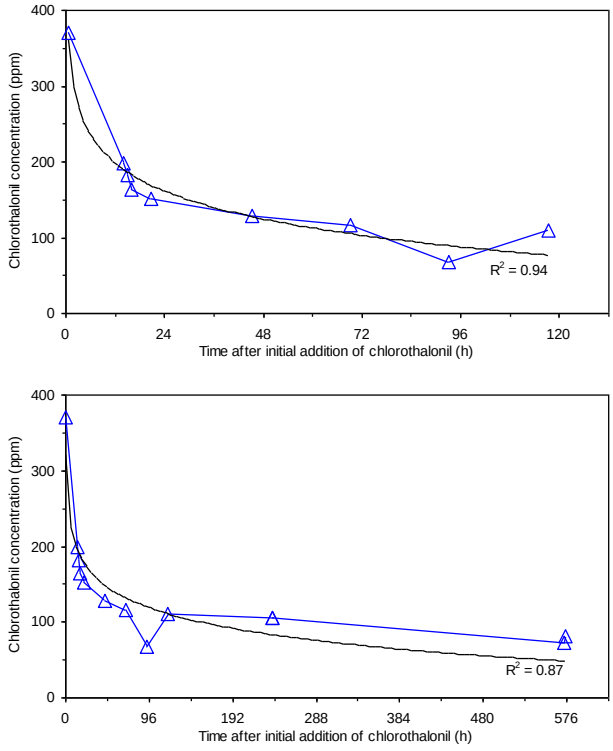


Figure 3. Chlorothalonil concentrations in HWT dip over a 24-day period (Case 4)

The upper figure shows the first five days, the lower figure the whole period

The initial pH of the plain water was 7.3, which fell slowly over the first day's treatments to end the day only slightly more acid at pH 6.5. The increase in acidification then continued slowly, reaching 4.0 (and then remaining stable) on day 4 of treatment.

Bioload, measured as bacterial activity by the 'Petrifilm' method, was very high in the plain water at the start of the run, and remained so (with some fluctuations) thereafter (Figure 4). In plain water at the start, ATP activity was low at only 278RLU, increasing to a very high value within 5min of pumping the dip round the system; thereafter, bioload activity remained high for the first 4 days, after which it appeared largely to have been controlled (Figure 4). The generally high levels of bioload were not unexpected as no biocide was used in this programme, only fungicide.

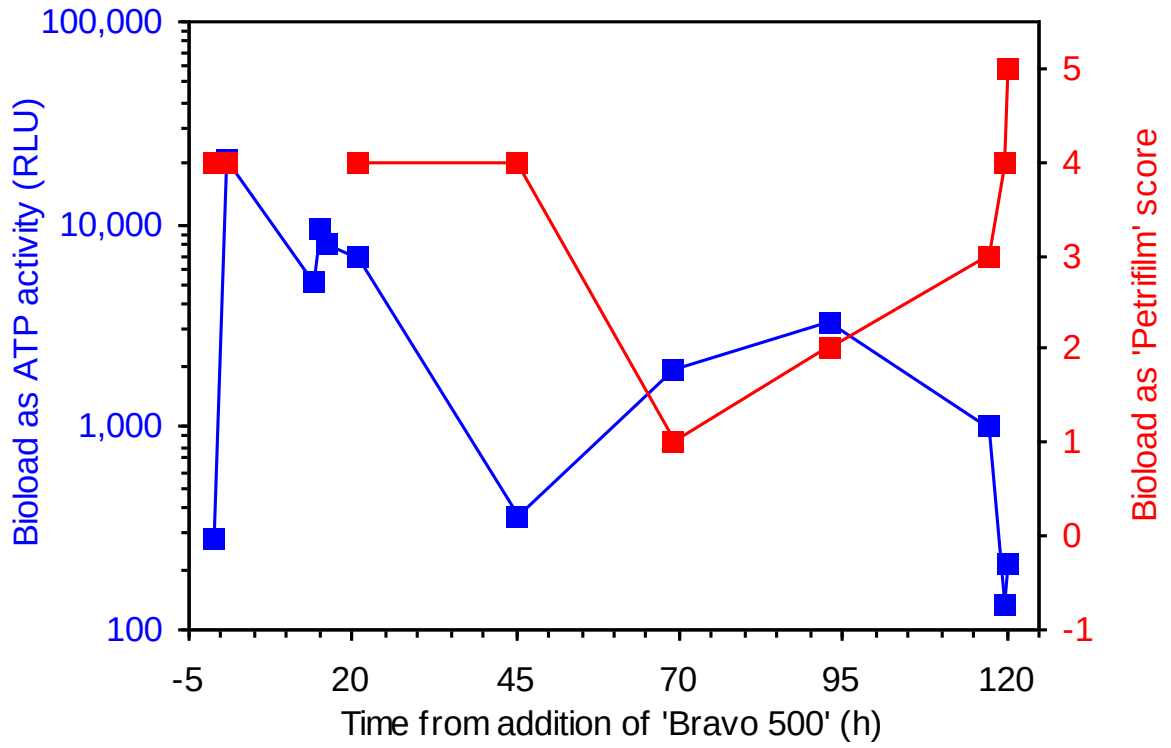


Figure 4. Bioload in HWT dip using 'Bravo 500' over a 5-day period (Case 4)

Table 8. Case 4: Chlorothalonil concentration, dip pH and bioload during regular HWT

Date	*Time (hh:mm)*	*Time since first 'Bravo 500' added (h)*	*Sample no.*	*Operations carried out*	*Dip pH value*	*Chlorothalonil concentration) (ppm)*	*Pollution*	
							ATP activity (RLU)	*Bacterial activity (score)*
1 Aug.	19:00	-0.08	1	Holding tank had been filled with water	7.3	**<**	278	4
	19:05	0	-	'Bravo 500', 'Activator 90' and 'Fighter F' had been added and dispersed within the holding tank	-	**-**	-	-
	19:40	0.58	2	Sample 2 taken	6.8	**371**	20,701	4
2 Aug.	09:06	14.01	3	Dip had been pumped to treatment tank, first load at target temperature for 1h	6.8	**199**	5,183	ND
	10:06	15.01	4	First load had been at target temperature in tank for 2h	6.5	**182**	9,591	ND
	11:06	16.01	5	First load had finished treatment	6.0	**164**	7,859	ND
	15:40	20.57	6	Last (third) load of day 1 had finished	6.5	**152**	6,813	4
3 Aug.	16:20	45.24	7	Last (third) load of day 2 had finished	5.2	**128**	359	4
4 Aug.	16:20	69.24	8	Last (third) load of day 3 had finished	4.5	**116**	1,947	1
5 Aug.	16:15	93.16	9	Last (third) load of day 4 had finished	4.0	**68**	3,271	2
6 Aug.	16:20	117.24	10	Last (third) load of day 5 had finished	4.0	**110**	998	3
7 Aug.	-	-	-	HWT programme held-up; dip held in holding tank, not circulated	-	**-**	-	-
11 Aug.	16:00	236.91	11	Sample taken from holding tank	4.0	**105**	131	4
	16:30	237.41	12	Tank had been topped-up and chemicals dispersed within the tank	4.0	**105**	215	5
12-17 Aug.	-	-	-	HWT continued at 3 loads per day (only 2 loads on 14 Aug.)	-	**-**	-	-
18 Aug.	-	-	-	HWT programme held-up as above	-	**-**	-	-
25 Aug.	16:00	573.41	13	Sample taken from holding tank	ND	**72**	-	-
	16:30	573.91	14	Tank had been topped-up and chemicals dispersed within the holding tank; HWT continued	ND	**81**	-	-

The symbol < means that chlorothalonil concentration was below the limit of detection (<0.1ppm). Where test results fell between gradations on a colour scale, the mid-point is quoted. ND means not determined

In this case bulbs were hot-water treated with a half-rate 'FAM 30' ***and*** 'Bravo 500'. Samples were taken over the first 8 days of HWT. A summary of the operation, times of taking dip samples, iodine and chlorothalonil concentrations, dip pH and bioload is given in Table 9.

High levels of iodine (30 to 50ppm) were found using the different tests (including the HI test, not shown in Table 9) only in the dip sample taken immediately after chemicals had been added to the tank. Subsequently, dip-stick tests failed to indicate positive levels of iodine, though the iodine meter sporadically showed iodine levels of 10 or 20ppm; however, in the strongly coloured solutions that developed later in the operation, meter readings may be unreliable. However, at this farm the addition of chemicals was always done the previous afternoon, in readiness for an early start. This meant that the dip solution was held for over 10h before use.

Chlorothalonil concentrations of 227ppm, close to the target concentration of 250ppm, were found in the sample taken soon after the chemicals had been added to the tank (Figure 5). By the next morning when HWT was about to begin, this had fallen to 147ppm. Concentrations at the end of the first, second and third days and at the end of the first week of dipping, were 106, 66, 60 and 95, respectively, again indicating a rapid loss followed by a much slower loss, as in previous examples (Cases 2 and 4), though in this instance with an enhanced concentration after a further period, perhaps indicating a more effective topping-up regime. Fitting the data to a logrithmic trend-line provided a reasonably good fit, with an R^2 value of 0.74, lower than in the previous cases.

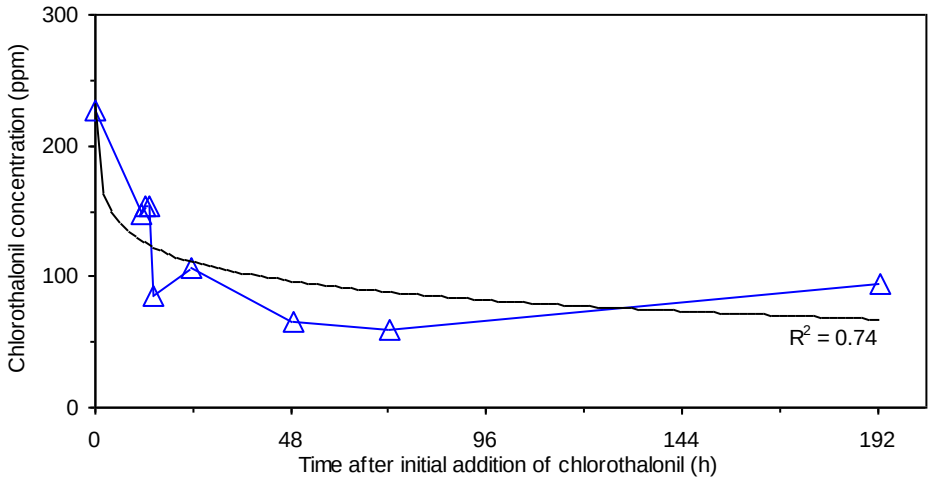


Figure 5. Chlorothalonil concentrations in HWT dip over a 8-day period (Case 5)
Trend-line and R^2 value shown in black

The initial pH of plain water in the tank was 5.5, perhaps indicating the effect of residues left in the tank from previous treatments with ‘FAM 30’. This fell to 2.4 after the addition of chemicals and was 3.0 by the start of HWT the next day. During the first day of dipping dip pH rose to 3.9 and was then stable.

Bioload, assessed as bacterial growth using the ‘Petrifilm’ method, was very high (score of 5.0) in the plain water at the start, but fell to zero once the chemicals had been added and remained at that level overnight. During the first week’s HWT, the score increased slowly, reaching only 1.5 by the end of the first week. Assessed as ATP activity, bioload was extremely high in the plain water, but fell greatly by the following morning after the chemicals had been added. Bioload then increased to a very high level by day 3 of the programme, subsequently falling (Figure 6).

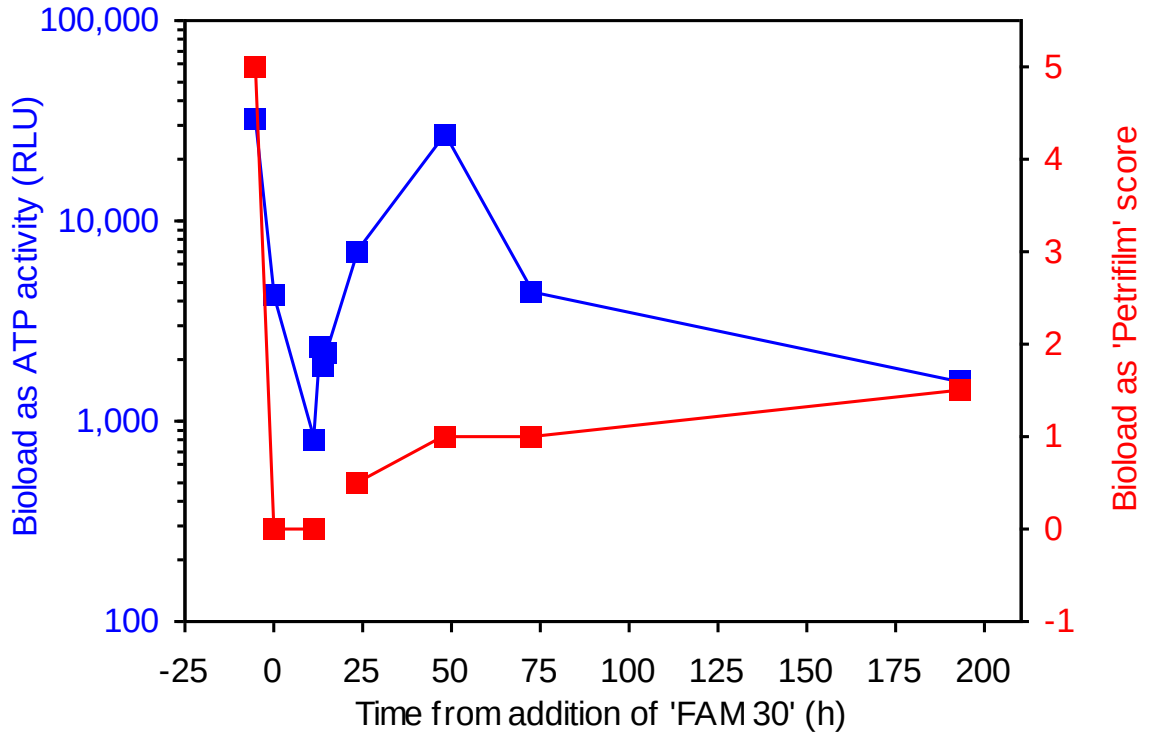


Figure 6. Bioload in HWT dip using ‘FAM 30’ and ‘Bravo 500’ over an 8-day period (Case 5)

Table 9. Case 5: Iodine and chlorothalonil concentrations, dip pH and bioload during regular HWT

Date	Time (hh:mm)	Time since 'FAM 30' added (h)	Sample no.	Operations carried out	pH value	Chlorothalonil concentration (ppm)	Iodine concentration (ppm)				Pollution	
							HI meter	ITS 'SenSafe' sticks	ITS high range sticks	In-dicative colour	ATP activity (RLU)	Bacterial activity (score)
28 June	17:00	-0.42	1	Holding tank had been filled with water	5.5	**<**	**<**	**< (B)**	**<**	X	32,293	5.0
	17:25	0	-	'FAM 30', 'Bravo 500' and 'Activator 90' had been added and allowed to disperse	-	**-**	**-**	**-**	**-**	-	-	-
	17:30	0.08	2	Sample taken	2.4	**227**	**30**	**50 (A)**	**50**	✓	4,247	0
29 July	04:50	11.33	3	Chemicals had been dispersed, still in holding tank	3.0	**147**	**<**	**0.01 (B)**	**<**	X	809	0
	05:00	11.50	-	Bulbs had been loaded and dip pumped from holding tank to treatment tank	-	**-**	**-**	**-**	**-**	-	-	-
	06:00	12.50	4	1h into treatment of first load	3.9	**154**	**20**	**0.01 (B)**	**<**	X	2,305	ND
	07:00	13.50	5	2h into treatment of first load	3.9	**154**	**<**	**0.01 (B)**	**<**	X	1,863	ND
	08:00	14.50	6	End of treatment of first load	3.9	**85**	**10**	**0.01 (B)**	**<**	X	2,163	ND
	17:00	23.50	7	End of third treatment of day (last load of day)	4.0	**106**	**20**	**0.01 (B)**	**ND**	X	7,088	0.5
30 July	05:00	35.50	8	Tank had been topped-up the previous evening, first treatment of day started	3.9	**ND**	**10**	**< (B)**	**<**	X	ND	ND
	18:00	48.50	9	End of third treatment of day	4.0	**66**	**20**	**0.01 (B)**	**ND**	X	26,738	1.0
31 July	05:00	59.50	10	Tank had been topped-up the previous evening, first treatment of day started	4.0	**ND**	**<**	**0.01 (B)**	**ND**	X	ND	ND
	18:00	72.50	11	End of third treatment of day	4.0	**60**	**<**	**0.01 (B)**	**ND**	X	4,362	1.0
1 – 4 Aug.	-	-	-	HWT continued as above (3 loads per day)	-	**-**	**-**	**-**	**-**	-	-	-
5 Aug.	18:00	192.50	12	End of first week of HWT (3 loads per day)	3.9	**95**	**ND***	**0.01 (B)**	**<**	X	1,576	1.5

The symbol < means that concentrations were below the limit of detection for a particular test, e.g. <1ppm for the HI meter and <0.1ppm for chlorothalonil analysis. Where test results fell between gradations on a colour scale, the mid-point is quoted. (A) and (B) refer to the high- and low-range, respectively, of dual-range sticks. ND means not determined, ND* means meter could not read sample.

Case 6 – Cold-dipping using 'FAM 30' biocide

In this operation freshly lifted bulbs were cold-dipped with half-rate 'FAM 30' ('FAM 30' diluted 1:250 with water), treating 12 loads of bulbs over a 4-day period (interrupted by the weekend). A summary of the operations, times of taking dip samples, iodine concentrations, dip pH values and bioload is given in Table 10.

Iodine was measurable by the three methods used only in the three samples following its addition:

- 50 to 200ppm (depending on test method) by the time the solution had been held in the holding tank for about 45min
- 25 to 100ppm about 5min after the solution had been pumped to the first load of bulbs
- 10 to 50ppm about 15min later (at the completion of dipping the first load of the day)

These three samples also showed clearly the indicative colour of 'FAM 30'. Low concentrations of iodine were detectable by at least one of the test methods in the next two samples, extending to the completion of the second load, but iodine was below the limit of detection in later samples. The top-up on day 4 did not show up in the subsequent iodine results.

The pH of the mains water supply was 7.0; the pH of the dip was 2.4 shortly after the addition of 'FAM 30', thereafter increasing slowly to 5.0 by the end of the operation.

Despite the loss of iodine over the course of a few hours, the addition of 'FAM 30' to the system had a marked effect on bioload generally (Figure 7):

- Using the 'Petrifilm' method, the bacterial content of the dip was scored as 'polluted' in the initial sample, i.e. of plain water being held in the holding tank in readiness for dipping, prior to 'FAM 30' being added. After the biocide had been added, there was little or no evidence of bacterial pollution until half-way through day 4; from that point, pollution levels increased markedly.
- Using the ATP method gave a low value of only 65RLU from the plain water prior to the start of dipping. After adding 'FAM 30' and wetter, the reading increased to 795RLU, perhaps as a consequence of disturbing any sediments when pumping the tank to disperse the chemicals. Thereafter, pollution levels exceeded 6,000RLU, except following the top-up on day 4 which appeared to cause a temporary fall in the pollution reading.

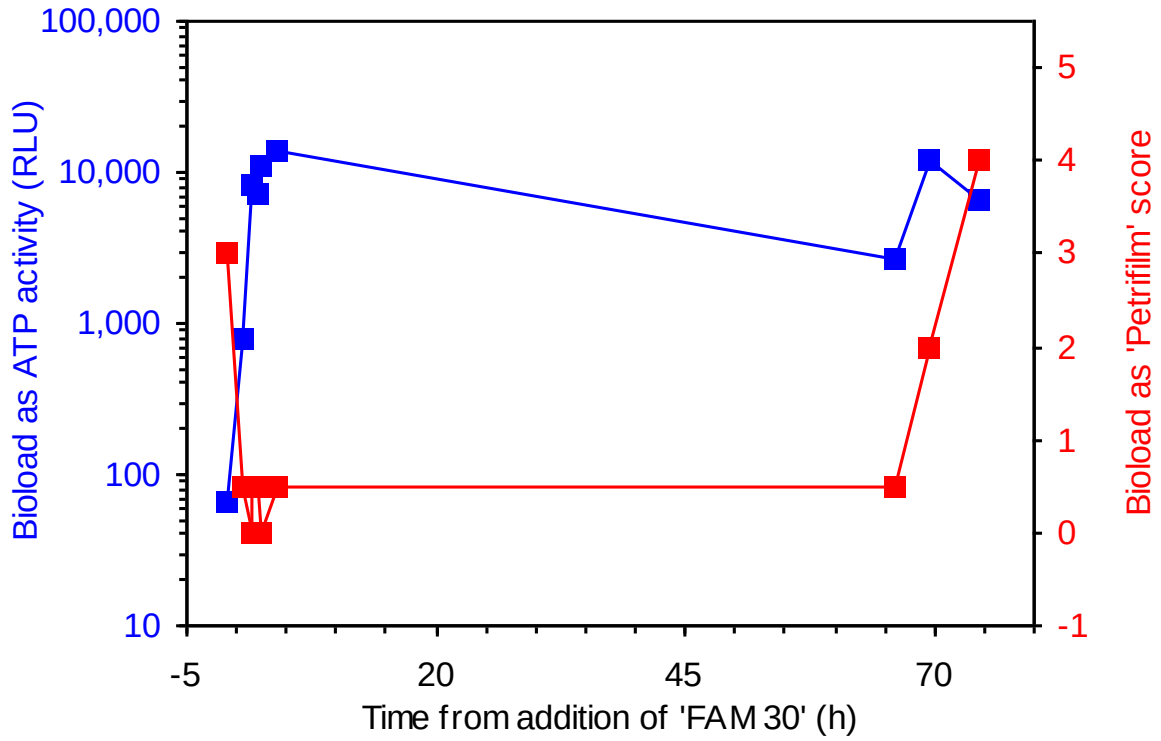


Figure 7. Bioload in cold dip using ‘FAM 30’ over an 4-day period (Case 6)

The loss of iodine in this case was substantial from the start, but detectable levels remained for longer than in Case 1 where no iodine was detectable 25min after it had been added to the tank. This may simply be a result of a faster loss at the higher dipping temperature. As in Case 1, the top-up had no observable effect on iodine concentration in subsequent samples, confirming the need to discover the reason for the apparent loss. However, it was clear in Case 3 that there was a general benefit of disinfection of the HWT system (of the equipment and/or water) by adding ‘FAM 30’.

Table 10. Case 6: Iodine concentration, dip pH and bioload during cold-dipping

Date	*Time (hh:mm)*	*Time since 'FAM 30' added (h)*	*Sample no.*	*Operations carried out*	*pH value*	*Iodine concentration (ppm)*				*Pollution*	
						HI test	*EV stick*	*ITS stick*	*In-dicative colour*	*ATP activity (RLU)*	*Bacterial activity (score)*
25 June	13:55	-0.02	1	Holding tank had been filled with mains water	7.0	**<**	**<**	**< (B)**	X	65	3.0
	13:56	0	-	'FAM 30' and 'Activator 90' had been added and dispersed within the holding tank	-	**-**	**-**	**-**	-	-	-
	14:22	0.43	2	-	2.4	**200**	**52**	**50 (A)**	✓	795	0.5
	15:01	1.37	3	Bulbs had been loaded and dip pumped from holding tank to treatment tank	2.5	**100**	**31**	**25 (A)**	✓	8,305	0
	15:16	1.62	4	Treatment of first load completed	2.7	**50**	**13**	**25 (A)**	✓	8,325	0.5
	16:00	2.07	5	Bulbs had been loaded and dip pumped from holding tank to treatment tank	3.5	**<**	**<**	**25 (A)**	X	7,035	0.5
	16:15	2.32	6	Treatment of second load completed	3.1	**<**	**13**	**25 (A)**	X	10,906	0
	17:00	3.07	-	Bulbs had been loaded and dip pumped from holding tank to treatment tank	-	**-**	**-**	**-**	-	-	-
	17:50	3.90	7	Treatment of third load completed {last load of day)	3.2	**<**	**<**	**ND**	X	13,651	0.5
26 – 27 June	-	-	-	Not in use	-	**-**	**-**	**-**	-	-	-
28 June	08:00	66.07	8	Tank had been topped-up and first batch of day loaded	3.8	**<**	**<**	**ND**	X	2,633	0.5
	11:25	69.49	9	First three loads of day completed, awaiting loading of further batch	4.5	**<**	**<**	**ND**	X	11,922	2.0
	16:30	74.57	10	Six further loads completed	5.0	**<**	**<**	**ND**	X	6,687	4.0

The symbol < means that iodine concentrations were below the limit of detection for a particular test, e.g. <1ppm for the HI test and <10ppm for the EV sticks. Where test results fell between gradations on a colour scale, the mid-point is quoted. (A) and (B) refer to the high- and low-range, respectively, of dual-range sticks. ND means not determined.

Case 7 – Cold-dipping using 'Virudine'/'FAM 30' biocide and 'Storite' fungicide

Two cold-dipping programmes were studied in two consequetive weeks, the first (run A) using 'Virudine' biocide and the second (run B) using 'FAM 30', and both using acidified 'Storite Clear Liquid' as the fungicide.

Case 7A

In run A, dips were studied over a three-day period, and a summary of the operations, times of taking dip samples, iodine and thaibandazole concentrations, dip pH values and bioload is given in Table 11.

Appreciable concentrations of iodine, and the indicative colour of the iodine biocide, were found only in the sample taken within 15 minutes of the biocide being added to the tank and allowed to disperse. Depending on the method used, the estimated iodine concentration was between 84 and 200ppm. Lower concentrations of iodine (about 20ppm) were indicated in the next two samples, both taken within about 30 minutes of the original addition, but thereafter iodine was below the limit of detection.

In a dip sample taken about 15 minutes after the addition of 'Storite Clear Liquid', a thiabendazole concentration of 397ppm was found, falling to 328ppm some 30 minutes later. By the end of the first, second and third days' dipping, this level had fallen to 288, 238 and 175ppm, respectively (Figure 8). This loss of thiabendazole is of a similar order as previously observed in HWT (e.g. in HDC project BOF 64).

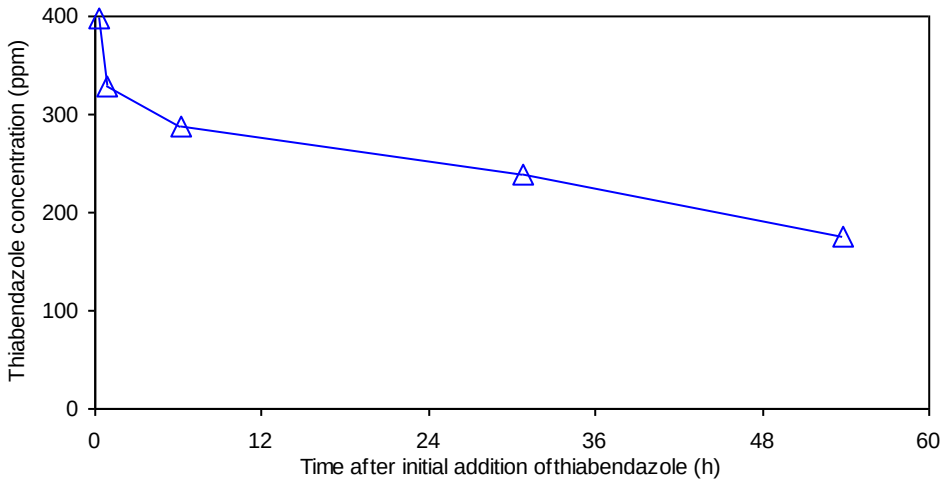


Figure 8. Thiabendazole concentrations in cold dip over a 3-day period (Case 7A)

In this programme, the initial pH value of the ‘clean’ water used to fill the tanks was unexpectedly low at 3.8; no definite explanation for this has been found, but it may have been due to acidic contaminants remaining in the HWT system from previous use of acidified dips. After ‘Virudine’ addition, dip pH fell to 1.8. Despite the regular addition of acidifier (sodium bisulphate) in this facility, the pH value subsequently drifted upwards to reach about pH 4.0 by the end of the 3-day run.

Bioload in the ‘plain’ water at the start was moderately high when measured as bacterial contamination using the ‘Petrifilm’ system, falling to zero thereafter and remaining so for a few hours at least (Figure 9). By the end of the first day’s dipping, samples were highly polluted. Measured as ATP concentrations, a very low concentration was recorded in the plain water, rising to a moderate level within about 15 minutes of ‘Virudine’ addition (and the start of circulation) but then falling back to a low level within a further 15 minutes or so (Figure 9).

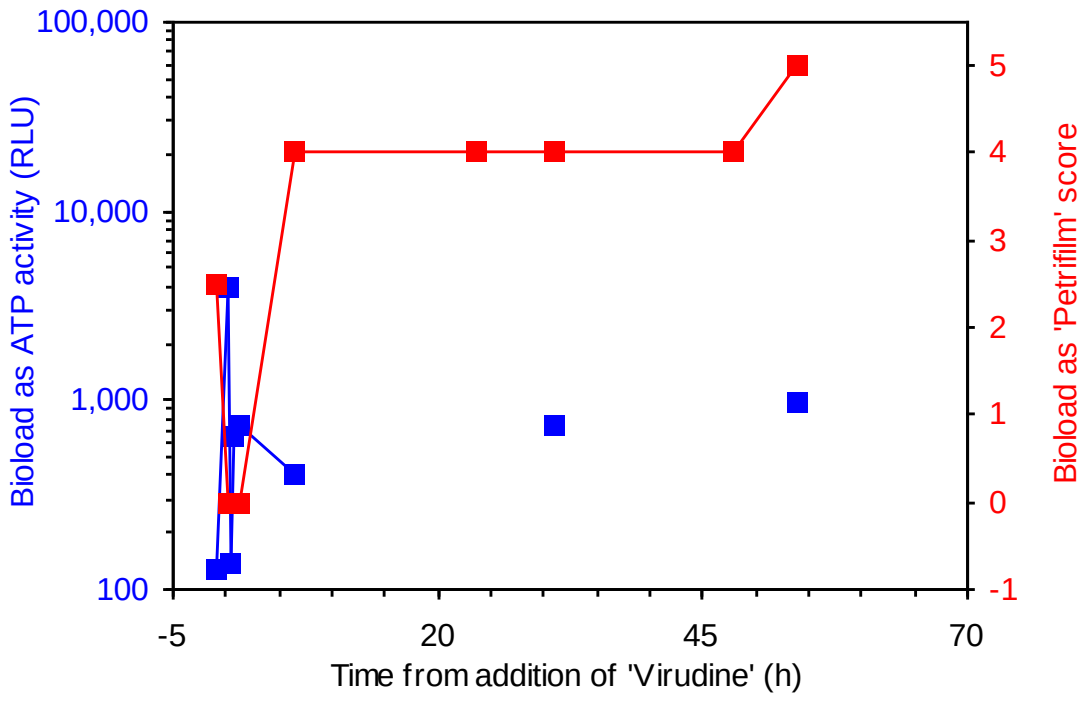


Figure 9. Bioload in cold dip using ‘Virudine’ and acidified ‘Storite Clear Liquid’ over a 3-day period (Case 7A)

Case 7B

The observations from this 8-day run are summarised in Table 12.

The iodine concentrations found in this case differred from all of those reported above, as iodine appeared undetectable using the HI Test, and indeed an iodine concentration was found only in the sample taken some 15 minutes after adding and dispersing the 'FAM 30', and then only when using 'dip sticks' and only at the low concentration of 25ppm. The characteristic 'FAM 30' colour was only weakly observed, and there were no other positive results. The failure to detect iodine using the HI Test suggests that some component in the dip was interfering with the normal chemical reaction, but this has not been confirmed. It did not appear related to pH levels, since, apart from a somewhat lower pH than in Run 7A (5.0 instead of 3.8), pH again rapidly fell to 1.8 and then drifted upwards to remain at around pH 3.0 to 4.0 for the remainder of the run.

As in run 7A, bioload in the 'plain' water at the start was moderately high when measured as bacterial contamination, falling to zero thereafter and remaining so for a few hours at least (Figure 10). By the end of the first day's dipping pollution levels were rising, though here they were lower than in the earlier case. Measured as ATP concentrations, levels were again generally low, with a small rise after dispersion (Figure 10).

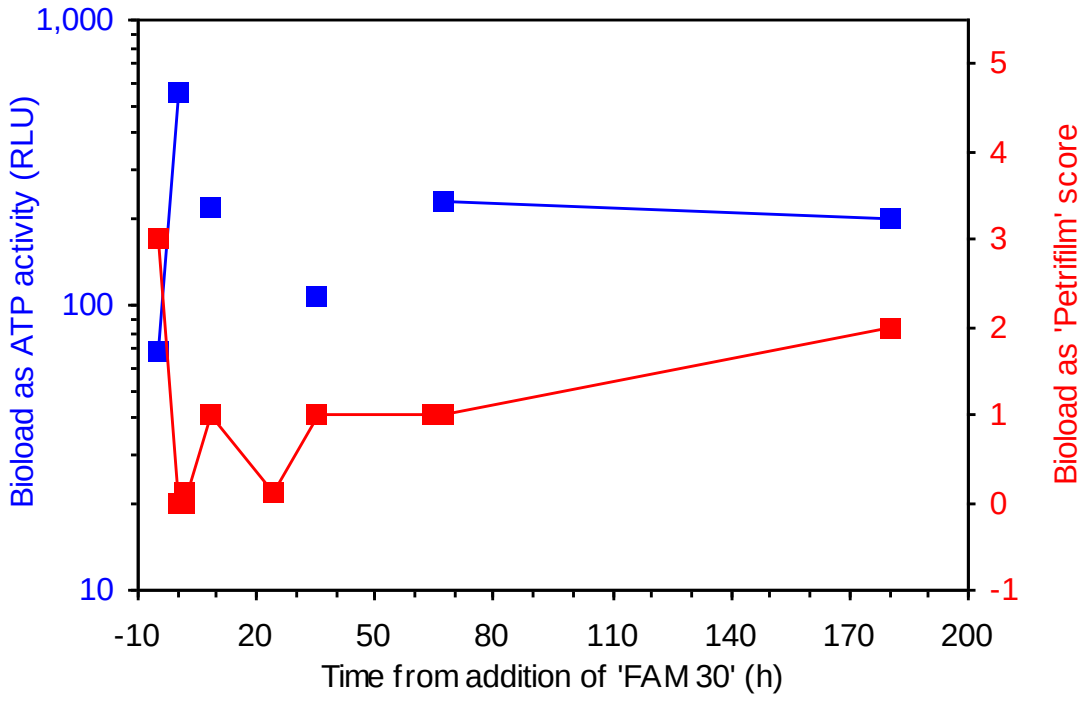


Figure 10. Bioload in cold dip using 'FAM 30' and acidified 'Storite Clear Liquid' over an 8-day period (Case 7B)

Table 11. Case 7A: Iodine and thiabendazole concentration, dip pH and bioload during cold-dipping

Date	*Time (hh:mm)*	*Time since 'Virudine' added (h)*	*Sample no.*	*Operations carried out*	*pH value*	*Thiabendazole concentration (ppm)*	*Iodine concentration (ppm)*				*Pollution*	
							HI test	*EV stick*	*EV stick (revised)*	*In-dicative colour*	*ATP activity (RLU)*	*Bacterial activity (score)*
2 July	08:30	0	1	Holding tank had been filled with mains water and sample taken before 'Virudine' added	3.8	**ND**	**<**	**<**	**<**	X	130	2.5
	08:45	0.25	2	'Virudine' had been dispersed within the holding tank and sample taken before 'Storite Clear Liquid' added	1.8	**ND**	**100**	**200**	**84**	✓	3,988	0
	09:00	0.5	3	'Storite' had been dispersed within the holding tank	2.1	**397**	**<**	**30**	**19**	X	137	0
	09:05	0.58	-	Bulbs had been loaded and dip pumped from holding tank to treatment tank	-	**-**	**-**	**-**	**-**	-	-	-
	09:10	0.66	4	First load 5min into cold-dip	2.2	**ND**	**<**	**17**	**<**	X	638	0
	09:40	1.17	5	End of first cold-dip	2.4	**328**	**<**	**<**	**<**	X	740	0
	10:30	2.0	-	System topped-up	-	**-**	**-**	**-**	**-**	-	-	-
	14:55	6.42	6	End of last (fourth) dip of day	3.8	**288**	**<**	**<**	**<**	X	413	4
3 July	08:15	23.75	7	Sample taken before topping-up	3.5	**ND**	**<**	**<**	**<**	X	ND	4
	13:10	28.67	-	System topped-up	-	**-**	**-**	**-**	**-**	-	-	-
	15:30	31.00	8	End of last (sixth) dip of day	4.0	**238**	**<**	**<**	**<**	X	738	4
4 July	08:20	47.83	9	Sample taken before topping-up	3.2	**ND**	**<**	**<**	**<**	X	ND	4
	14:30	54.00	10	End of last (sixth) dip of day (half-load for sixth dip)	3.7	**175**	**<**	**<**	**<**	X	985	5

The symbol < means that iodine concentrations were below the limit of detection for a particular test, e.g. <1ppm for the HI test and <10ppm for the EV sticks. Where test results fell between gradations on a colour scale, the mid-point is quoted. ND means not determined.

Table 12. Case 7B: Iodine concentration, dip pH and bioload during cold-dipping

Date	*Time (hh:mm)*	*Time since 'FAM 30' added (h)*	*Sample no.*	*Operations carried out*	*pH value*	*Iodine concentration (ppm)*				*Pollution*	
						HI test	*EV stick*	*EV stick (revised)*	*In-dicative colour*	*ATP activity (RLU)*	*Bacterial activity (score)*
7 July	07:00	0	1	Holding tank had been filled with mains water and sample taken before 'FAM 30' added	5.0	**<**	**<**	**<**	X	69	3.0
	07:05	0.08	2	'FAM 30' had been dispersed within holding tank and sample taken before 'Storite Clear Liquid' added	1.8	**<**	**<**	**25**	✓ (weak)	556	0
	07:20	0.33	3	'Storite' had been dispersed and sample taken before wetter added	2.2	**<**	**<**	**<**	X	ND	0
	07:30	0.50	4	Wetter had been dispersed and sample taken	2.2	**<**	**<**	**<**	X	ND	0
	08:30	1.50	-	Bulbs had been loaded and dip pumped from holding tank to treatment tank	-	**-**	**-**	**-**	-	-	-
	08:35	1.58	5	First load 5min into cold-dip	2.5	**<**	**<**	**<**	X	ND	0.5 (trace)
	09:00	2.00	6	End of first cold-dip	2.65	**<**	**<**	**<**	X	ND	0
	14:55	7.92	7	End of last (eighth) dip of day	3.5	**<**	**<**	**<**	X	223	1.0
8 July	07:00	24.00	8	Sample taken before top-up	3.1	**<**	**<**	**<**	X	ND	0.5 (trace)
	11:45	35.45	9	End of last (third) dip of day	3.35	**<**	**<**	**<**	X	108	1.0
9 July	13:00	64.20	10	Sample taken before top-up	3.5	**<**	**<**	**<**	X	ND	1.0
	15:45	66.95	11	End of last (second) dip of day	3.5	**<**	**<**	**<**	X	233	1.0
10-13 July	-	-	-	Further topping-up (5 times in all) and cold-dipping (11 loads in all)	-	**-**	-	**-**	-	-	-
14 July	07:00	178.20	-	Further topping-up	-	**-**	-	**-**	-	-	-
	09:00	180.20	12	End of last (second) dip of day (last load was a half load)	3.5	**<**	**<**	**<**	X	203	2.0

The symbol < means that iodine concentrations were below the limit of detection for a particular test, e.g. <1ppm for the HI test and <10ppm for the EV sticks. Where test results fell between gradations on a colour scale, the mid-point is quoted. ND means not determined.

Case 8: HWT with 'Bravo 500' and supplementary agitation of the dip

It is common to find there is a conspicuous white deposit on the floor of HWT tanks following treatment with fungicide. The aim of this exercise was to provide additional agitation of this sediment to return any settled fungicide to circulation within the tank. A successful treatment would be expected to result in increased concentrations of circulating fungicide and less sediment on the tank floor.

The results of sampling and analysing dip before and after supplementary water circulation are shown in Figure 11. Disproving expectations, all nine samples had very similar concentrations of chlorothalonil, allowing for the gradual fall in concentration over the day, consistent with other findings. The mean concentration was 179ppm, with a standard deviation of 20.0 and a range of 154 to 205ppm. Although this was an inadequate number of samples for statistical analysis, and the exercise was conducted only once, this conclusion was convincing.

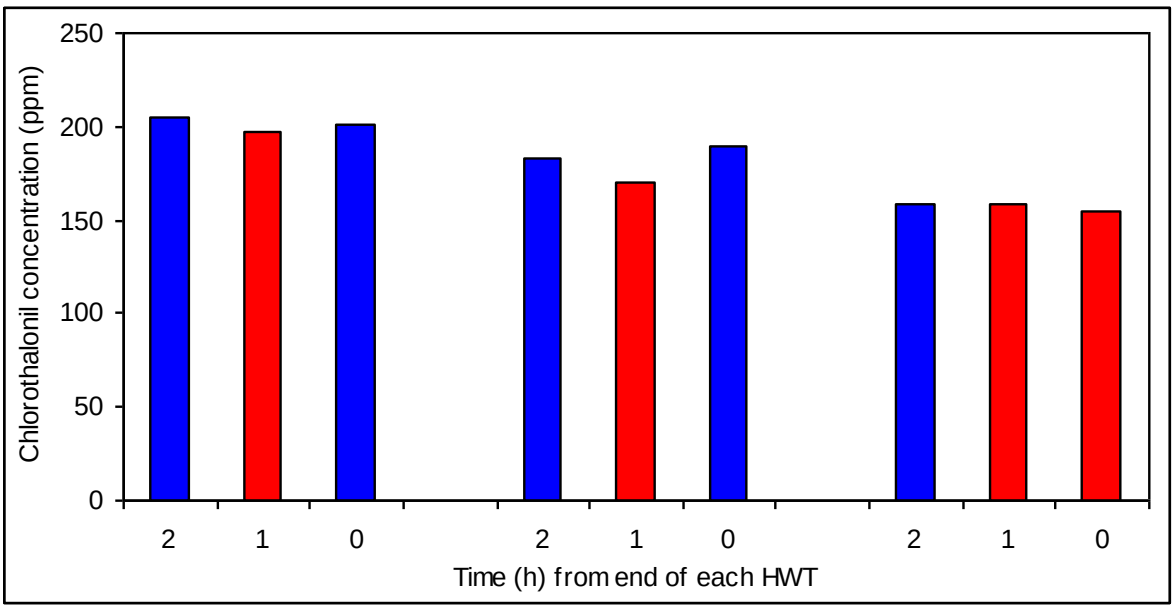


Figure 11. Chlorothalonil concentrations in three consequetive HWT dips; at sampling, supplementary circulation had been either off (blue bars) or on (red bars) during the previous 1h period

This conclusion was backed up by observations of the amount and pattern of tank floor sediments following the three HWT runs. The appearance of this sediment after HWT was inconsistent. After run 1 there were areas of the floor relatively clear of sediment, though this

was not so after run 2 and after run 3 there were some clear areas of the floor. At the end of testing there was a series of small (2 to 3cm-diameter) clear patches on some parts of the floor, perhaps related to areas of differential corrosion, but the floor was clearest of sediment directly in front of the main water return (from along the back wall of the tank), indicating that the primary circulation of the tank was effective in scouring sediment from the tank floor. It was noted that the entrance and exit of the fork-lift truck (to remove or load bulb crates) removed much of the white deposit under the path of the tyres, possibly a significant source of loss. In all cases a white deposit could be wiped from the tank floor and from horizontal surfaces of the metal bulb crates, there were obvious white deposits on horizontal ledges of the tank and on the upper surfaces of treated bulbs, and thinner deposits were evident on vertical surfaces.

Discussion

The loss of iodine using iodophore biocides in HWT and cold dips

Work in Projects BOF 61a and 71 showed that the iodophore biocide 'FAM 30' was effective against both stem nematode and the *Fusarium* rot pathogen in bulb dipping. Following concerns that some other chemical additives to bulb dips apparently exhibit a loss in concentration with ongoing dipping – relatively slowly in the case of formaldehyde, but relatively rapidly in the case of the tested benomyl, thiabendazole and chlorothalonil fungicides – the in-dip concentration of iodine from iodophore biocides was monitored during typical, commercial bulb dipping operations. Iodine concentrations were followed in six cases, three in HWT and three in cold-dipping. The initial and target iodine concentrations were 100 or 200ppm, depending whether half- or full-rate dips were being used (the full-rate being taken as 8L 'FAM 30' per 1000L water, the usual highest concentration recommended by the manufacturer).

The findings showed a common pattern: high iodine concentrations (say, 80ppm or more) were recorded only within a relatively short period of the biocide being added to the system at start-up – up to ¼ to 1½ hours later in the various cases. In some instances lower levels of iodine were still detectable up to 48h after the biocide had been first added. In all cases the biocide was topped-up regularly, but this did not apparently restore iodine concentrations to the target level in any noticeable degree. The obvious response is to question the usefulness of adding the biocide. However, at this point it is unknown (a) whether any low or undetectable level of iodine remaining might still provide some useful biocidal effect to the treated bulbs, or (b) whether there are practical benefits to bulb health of the initial

disinfecting of the tanks and dip water, even if effective concentrations of iodine do not persist long into the treatment period.

This pattern of iodine loss occurred both in HWT and cold-dipping and in the HWT of both reclaimed bulbs and regular bulb stocks; it occurred when the biocide was being used alone or incombination with a fungicide ('Bravo 500' or acidified 'Storite Clear Liquid'), and in the one instance where an alternative product, 'Virudine', had been used instead of 'FAM 30'. Exceptionally, there was just one example - Case 7B - where virtually no iodine was detected in ***any*** of the samples taken, even in the earliest samples following the initial addition of the biocide; despite discussions with the staff involved on the exact protocols used, no convincing explanation for the complete absence of positive tests for iodine in this case can be suggested. The failure of the test method, perhaps through chemical interference, needs to be considered, as well as possible failings on-farm. In no case did measurable iodine concentrations persist for a substantial time into the HWT or cold-dip operation.

In use, biocides can become inactivated for several reasons:

- *Thermal inactivation* Iodophore biocides are considered thermally stable at the temperatures being used, and the same loss was found in both HWT and cold-dipping
- *Adsorption to organic matter* Iodophore biocides are claimed to be less affected by the presence of organic matter than some other biocides
- *Chemical inactivation* Iodophore biocides are regarded as relatively stable.

Although thermal inactivation is unlikely, adsorption to organic matter and chemical inactivation should be considered further.

Large amounts of organic matter will be present in bulb dip tanks – not only the bulbs themselves, but also soil (or growing media in the case of reclaimed bulbs – the bulbs in Case 1 contained significant amounts of peat), other debris, and wooden bulk bins. It would be useful to measure the amount of iodine in these components after dipping. However, a substantial loss of iodine in dips occurred even before the dip was pumped to the tank containing bulbs, so absorption on organic matter cannot be the whole explanation.

Inactivation of the biocide through chemical reaction with the metal of the tanks (and metallic components in the associated pipe-work, valves, heat-exchanger, etc.) is considered the most likely reason for the loss of iodine. This explanation needs to be confirmed experimentally. Although bulb dipping tanks are usually constructed of mild steel, this would not necessarily exclude the use of such tanks with the highly effective and (to humans)

relatively benign iodophore biocides, since it may be possible to apply an inert paint or coating to the metal of the tank and largely prevent the expected chemical reactions.

On a practical note, it is common for bulb growers to top-up HWT tanks at the end of the working day, so that the first load of the next day can be treated early next morning using a timer switch. Given the rapid loss of iodine in tanks, this is .

Before recommendations can be made on initial rate of use of iodophore biocide, and of appropriate topping-up procedures, it is necessary to understand ***how*** the loss is occurring and how it might be mitigated.

The measurement of iodine concentrations in HWT and cold dips

The iodine tests used in this project were simple or relatively simple to conduct and would be generally robust enough for on-farm use, provided there was a convenient supply of clean water for rinsing glassware between measurements, and some minimal flat surface on which to work. However, when testing covers the lower range of iodine concentrations, samples need to be diluted until they are in range, which is not a convenient operation in a farm situation.

Despite this, a number of problems occurred.

- ► Using dip-sticks of various types, colour matches were often difficult to judge. The different types of dip-sticks sometimes indicated concentrations different from each other, or from other test results. Some problems may hinge on ***exactly*** how the dip tests are conducted – dipping time, waiting time before the stick is read, how the stick is held in the test solution and after dipping - but they could be used as simple 'presence or absence' tests on undiluted samples.
- ► The HI Test Kit proved simple and effective, though with a restricted range of operation sometimes requiring dilution of samples before testing. However, it was found that supplies of reagent could be difficult to source, and for ongoing commercial use, other tests or suppliers need to be investigated.
- ► The HI Iodine Meter was only slightly less simple to use, and removed some of the subjectivity of the test kit. Unfortunately, the meters used would not read even slightly coloured samples, so samples from bulb dips needed usually to be diluted 100-fold to be on-scale. And, by diluting dip samples 100-fold, low concentrations of iodine may produce similar results to that of trace chlorine present in the distilled or deionised water used to make the dilutions. The suppliers of the meter state that any response to trace

chlorine should be within the variability of the meter, ±0.1ppm, and therefore undetectable, but tests in this project produced a much higher reading for trace chlorine, and the discussion is ongoing.

Should the use of iodophore biocides in bulb dipping prove feasible, it would be necessary to resolve the issues of these false readings and of the supply of reagent, or find an alternative test kit. 'Dilution testing kits' (dip-sticks) are now available for other iodophore biocides, and could also be tested.

Effects of iodophore biocides on bioload

Bioload was measured in six of the eight HWT or cold-dipping operations with iodophores, and, although a first-look at the results might suggest a great variety of responses, the underlying trends showed a common pattern.

When measured as the number of bacterial colonies using the 'Petrifilm' method, the general result (in Cases 5, 6, 7A and 7B) was that the plain water after filling was highly polluted – not unexpectedly, since thorough cleaning and decontamination of the tanks and associated fittings is difficult. Very soon following the addition of 'FAM 30' (or 'Virudine'), bacterial counts were reduced to zero, and thereafter bacterial counts rose at a variable rate beginning at various times after the start of testing. Treatment of the water with an iodophore biocide was evidently highly effective in controlling this initial pollution; thereafter, the time and rate of increasing bioload varied between cases, again not surprisingly, given the great variation in the state of bulb stocks, degree of bulb cleaning, etc.

One case was an exception to this pattern: in Case 3 virtually no bacterial pollution was indicated throughout, and it was suggested that this was a consequence of the HWT system having been effectively disinfected by the just prior use of chlorine dioxide (see report on HDC Project BOF 70). In contrast to these results, in Case 4 no biocide was added to the system and pollution levels remained high throughout testing.

Measured more generally as ATP levels, bioload was typically low in the plain water filling the tanks, but increased greatly once the dip chemicals had been added and dispersed through the system by circulation, presumably as residues in the system were disturbed. Thereafter, ATP levels varied, often increasing and then sometimes falling, again, probably depending on factors such as the state of the bulb stocks, biocide concentrations, and top-up practices.

Effects of iodophore biocides on dip pH

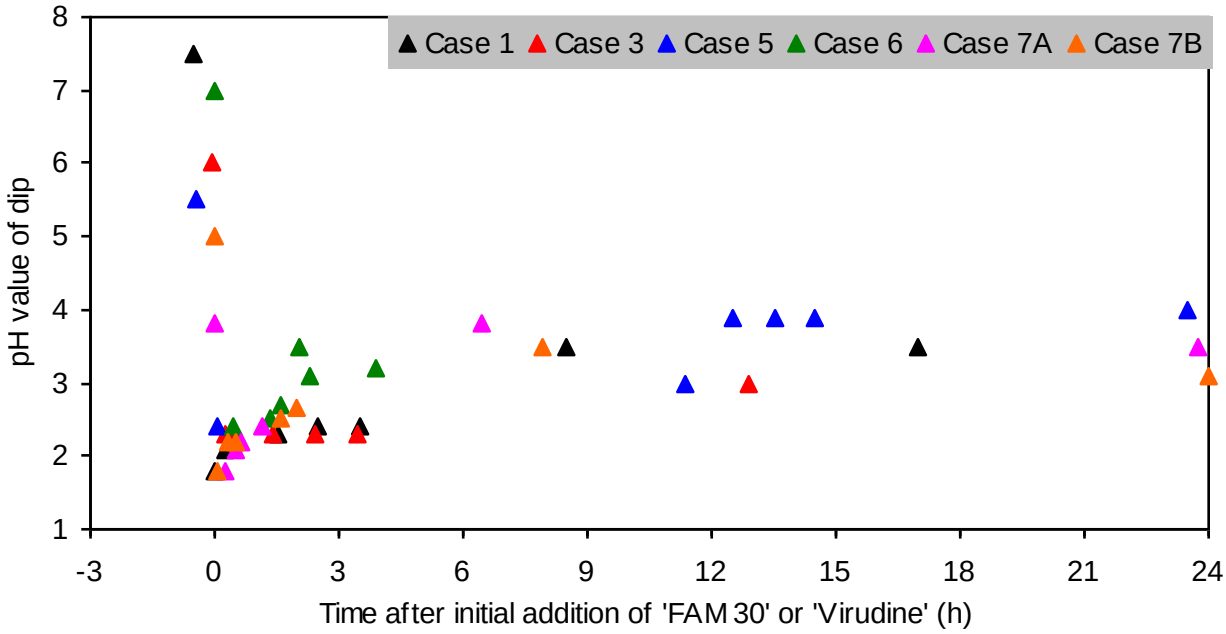
The time-course of dip pH values for all six cases where 'FAM 30' or 'Virudine' were used are shown in Figure 12. This shows the rapid drop in pH following addition of the biocide, to as low as 1.8, a borderline damaging level (see Projects BOF 43 and 43a), followed by a relatively slow loss of acidity (rising pH) over the next six hours, and finally remaining stable at a pH of 3 or 4 (despite regular topping-up).

The pH of bulb dips is important because:

- ► pH may affect, adversely or beneficially, the stability and solubility of pesticides added to the tank (e.g. thiabendazole needs an acidic environment to maintain high solubility, while many pesticides are hydrolysed under alkaline conditions)
- ► Extreme pH values (below about pH 2.0) may restrict subsequent daffodil growth.

Since 'FAM 30' and 'Virudine' are formulated in 20 to 30% w/w sulphuric/phosphoric acids, it is not surprising that the pH of dips fell rapidly to as low as 1.8 soon after the biocides were added. Discussions with Evans Vanodine (ongoing at the time of writing) have indicated that it may be possible to formulate a more suitable (less acidic) product for use in bulb-dipping operations.

When dipping bulbs in 'Storite Clear Liquid', acidification of the dip to a pH of 2.5 to 3.0 is recommended, usually by the addition of sodium bisulphate (BOF 43, 43a and 64). It is not clear whether using an iodophore and sodium bisulphate together would have an adverse effect on the bulbs or the process, or whether using an iodophore biocide could substitute for adding a straight acidifier. However, in Cases 7A and 7B, adding sodium bisulphate in subsequent top-ups did not greatly lower the pH of the dip compared to using 'FAM 30' or 'Virudine'. In these cases 'Storite Clear Liquid' had been routinely used at the site with an acidifier, and residues may have been responsible for the lower-than-expected pH values of the 'plain' water when the tanks were filled.



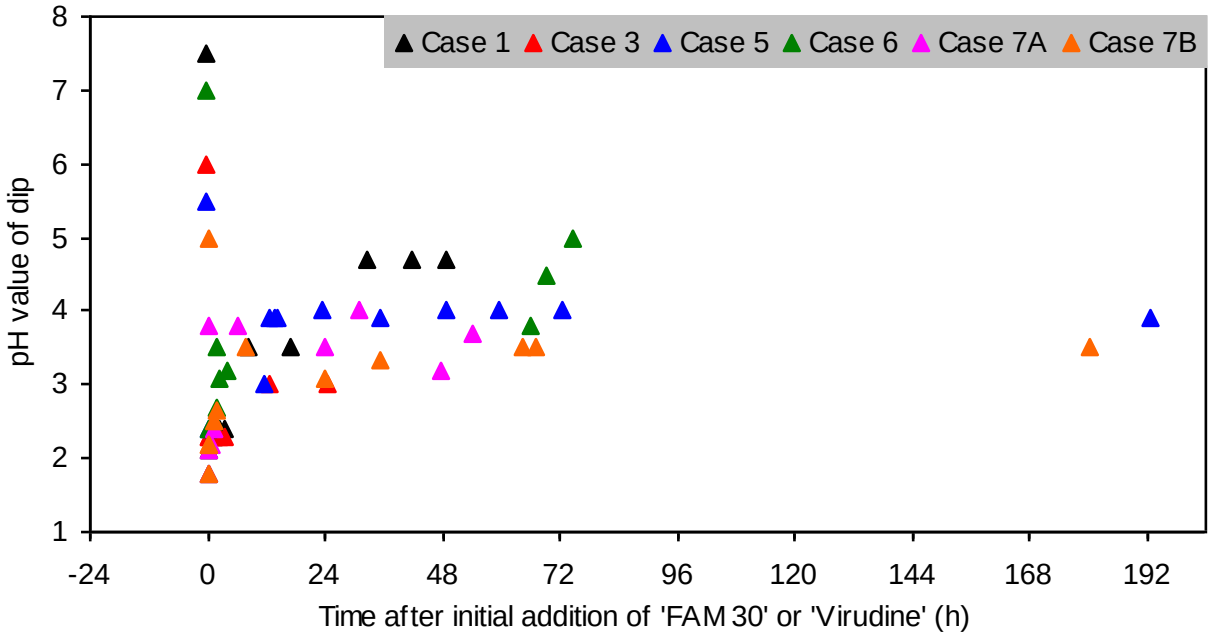


Figure 12. Scatter plots showing changes in dip pH during HWT or cold-dipping with 'FAM 30' or 'Virudine'. The upper figure shows the changes over the first 24h; the lower figure shows the longer term changes

Chlorothalonil and thiabendazole concentrations and topping-up

In contrast to the findings for iodine, although chlorothalonil concentrations did fall rapidly soon after the start of HWT or cold-dipping, they did not 'disappear'. Figure 13 shows the combined results from the three cases where 'Bravo 500' was added, in one case at the full-rate (1.0kg product per 1000L dip) and in the other cases at the half-rate (0.5kg/1000L). In all three cases the concentration of chlorothalonil fell quickly over a period of about 12h, before levelling out - and remaining more or less stable - at about 100ppm thereafter. Plotted as the percentage of the initial (target) concentration (see lower part of Figure 13) it shows that the stable concentration is 20 to 30% of the starting concentration. This is similar to the findings for thiabendazole fungicide levels in HWT reported elsewhere (BOF 64) and for cold-dipping in Case 7A (Figure 8).

Possibly the initial loss of chlorothalonil, and the eventual lower but stable concentration, is a consequence of a settling process governed by the sedimentation coefficient of the substance in water. At equilibrium, the rate of sedimentation will be balanced by the rate of de-sedimentation back into the bulk of the suspension. In the case of thiabendazole (as 'Storite Clear Liquid') in HWT it was found that a reduced rate (equivalent to 25% of the original recommendation) was in fact adequate for base rot control (BOF 64), and the data from Case 7B indicate that similar considerations apply to cold-dipping. It is not yet known whether reduced-rate dipping may also be applicable for chlorothalonil products.

Regarding the procedures for topping-up with 'Bravo 500' for HWT and cold-dipping, the method used here, i.e. topping-up at the same rate as used at the start whenever water levels are replenished, appear adequate. The same would seem to apply to using acidified 'Storite Clear Liquid' in cold-dipping.

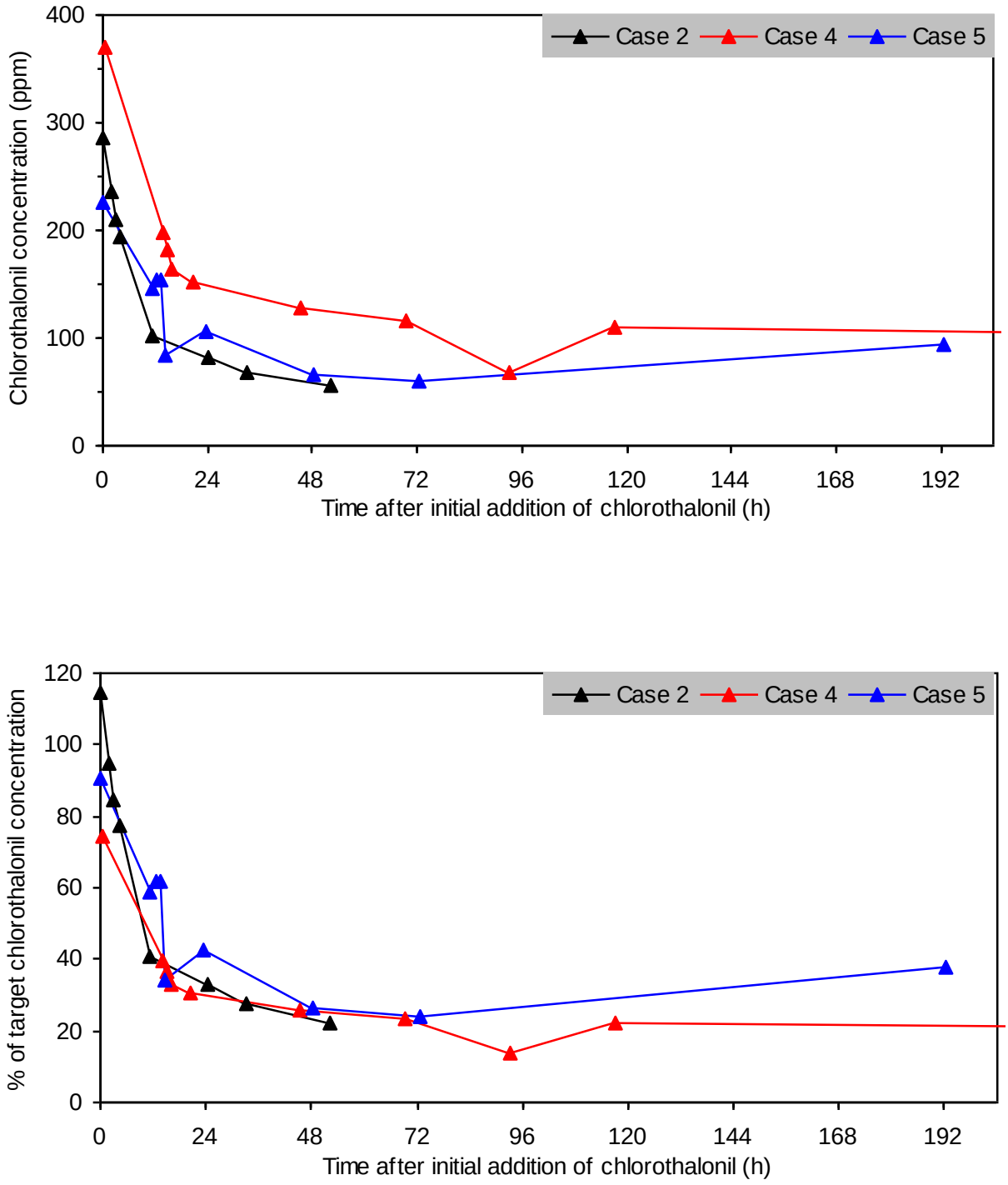


Figure 13. Changes in dip chlorothalonil concentration over time during three cases of bulb dipping. Upper figure: actual concentrations; lower figure: concentrations as percentage of target

Mitigation of the loss of chlorothalonil in bulb dipping

As a possible means of mitigation of the loss of active ingredients by settling-out on the floor of bulb-dipping tanks, in Case 8 additional agitation was introduced via a powerful submersible pump working close to floor level in the middle of the tank. However, this failed to increase the concentration of chlorothalonil circulating in the tank and therefore regarded as 'available' to the bulbs. At the same time, it was observed that the area of the tank floor immediately in front of the the water inlets where the heated dip was returned to the tank was relatively clear of sediment, so the case for increased agitation of the tank floor is not entirely ruled out. As chlorothalonil is regarded as a relatively stable compound, and ***if*** a reduced-rate application of chlorothalonil is effective in controlling base rot, then the settling-out of the material on the floor of the tank may not be too disadvantageous - sedimentation physics should allow it to be continually re-incorporated into the circulating dip, maintaining a stable concentration.

There are some rather obvious routes whereby fungicide sediments are lost to the dip. For example, observations showed that the movement of fork-lift trucks in and out of front-loading tanks when loading and unloading bins, removes a significant amount of the sediment from the tank floor. Potentially, this will could contaminate the environment, and it would be preferable (if sedimentation cannot be avoided) either (a) to contain this material by having the floor or roadway immediately in front of the tanks constructed to allow easy wash-down, or (b) assess the possibility of hosing these sediments into the outlet of the tanks in order to prevent loss.

Further removal of fungicide sediments will occur on the fabric of the bulb bins or crates (and, of course, on the bulbs themselves). It may be possible to construct a balance sheet showing the amount of active ingredient that needs to be replaced, so improving top-up procedures.

Biocide and pesticide legislation

Before this project was approved, the HDC had received a written opinion from the Chemical Regulation Directorate (CRD) that the use of biocides to achieve hygiene in crop production – as opposed to controlling a specific pathogen – "fell outside the [pesticide] regulations". Further investigation of the review of the EU's Biocidal Products Directive (BPD) indicated that this type of biocide use fell within 'Product Type 2', and that iodophore biocides were not

being supported under PT2. Discussions with the staff of Evans Vanodine, who are supporting iodophores in the EU review process under other PTs, have produced divergent views on this issue. At the time of writing clarification of this issue is being sought via the HDC; it will become urgent in the months before the 2011 bulb harvest season.

The legislation regarding pesticides is better understood by the horticultural industry, and does not at the present time place any difficulties in the way of using 'Bravo 500' in bulb dipping, either in HWT or cold-dipping. The same applies to some other pesticides used in bulb dips – 'Storite Clear Liquid' and 'Tezate 220 SL' for base rot control, and 'Pyrinex 48EC', 'Cyren' and 'Alpha Chlorpyrifos 48EC' for large narcissus fly control. These materials are all currently usable via SOLAs. (The fungicide 'Cercobin WG' also has a SOLA for bulb dipping, but its use is limited to a 30-minute dip.)

Strategy for using pesticides and biocides in bulb dips

The results obtained in this project indicate that 'Bravo 500' appears suitable for use in bulb HWT and, by implication, in cold-dipping, despite the considerable loss of active ingredient that occurs, though even the fungicide that has settled out on the dip tank floor (and other surfaces) may remain 'available' through the normal physical processes of sedimentation. It would be useful, however, to determine the optimum and minimum chlorothalonil concentrations needed to control spores and other propagules of the base rot fungus, as a reduced-rate usage might be possible. Additionally, a confirmation of the freedom from adverse effects of chlorothalonil on the crop is still awaited from field trials, though crop growth in the first year of field trials was encouraging (HDC Project BOF 61b). Despite the high costs of the other fungicides, the alternating use of 'Bravo 500' and of thiabendazole-based products is still advised, to reduce the incidence of the base rot fungus developing resistance to specific fungicide groups. The identification of fungicides from other mode-of-action groups effective against base rot would also provide further choice and better alternation of fungicide types, as well as removing the industry's dependence on one or two products. With the current high incidence of base rot in a range of daffodil cultivars, carrying out cold-dipping or HWT without an added fungicide cannot be advised.

A biocide may be used to control a crop pest or disease only if it is approved as a pesticide; otherwise it may be used only as an agent for general hygiene or disinfection. Before the current regulatory framework came about, formalin was used in bulb dipping to control base rot and other fungi and to augment the kill of stem nematodes.

Recent work indicates that it is feasible to use fungicides to control base rot, and that in HWT (in BOF 61a), the heat alone may be sufficient for killing all stages of the stem nematode. The need to use a biocide in bulb dipping should therefore be re-evaluated, and it is clear from the tests with iodophores reported here (and with chlorine dioxide in project BOF 70) that bulb dipping tanks regularly harbour a potentially huge bioload or inoculum of microorganisms. Given the need for having clean bulb stocks and bulb handling that is hygienic for both bulbs and workers, there is justification for the inclusion of a biocide in bulb dipping. Leaving aside regulatory issues, both iodophores (this project) and chlorine dioxide (BOF 70) have the potential for use in bulb dipping. With iodophore biocides, the loss of active ingredient (iodine), probably by reaction with the metal of the tank, needs to be mitigated, while the use of chlorine dioxide awaits further testing and observations (in spring 2011) of stocks treated with the material.

Any take-up of these strategies by bulb growers will need to comply with the regulatory position at the time.

Technology transfer and R&D needs

The following information should be promoted to growers, dependent on a successful outcome from Project BOF 61c in 2011.

1. Half-rate ‘Bravo 500’ (0.5kg/1000L) can be used in daffodil HWT. Where there is a specific base rot problem, its use should be alternated with that of a thiabendazole-based product (either in alternate years or by using thiabendazole as a post-lifting bulb spray). These products should be topped-up regularly. In HWT, the recommended temperature, duration and other conditions should be adhered to. Steps should be taken to limit the spread of fungicide from the floor of tanks to the surrounding area.
2. A fungicide cold-dip should not be used without a biocide capable of controlling other pathogens and pests.
3. ‘FAM 30’, and probably some similar products, have a place in cold-dipping and HWT for the control of bioload and achieving a good level of hygiene. However, due to its rapid loss from the dip, further work is needed before firm recommendations may be given. If used, it should be added to tanks immediately before use.
4. ‘FAM 30’ could form part of the clean-up of bulb dipping facilities before the season.

The following questions suggest R&D needs that would allow full exploitation of these results:

1. Is chlorothalonil (as 'Bravo 500') effective against *Fusarium* rots in bulb dipping at reduced rates?
2. Are there other fungicides that could be used as alternatives to chlorothalonil and thiabendazole in bulb dipping, to reduce reliance on just two active substances?
3. Are there ways in which fungicide settled on the floor and other surfaces of dip tanks can be returned to circulation?
4. Can the loss of iodine from iodophore biocides in dip tanks be mitigated, e.g. by coating the tank walls with an inert paint?
5. Is iodine adsorbed on bulbs and other debris to a measurable or useful extent during dipping?
6. What is the concentration/time effect and minimum effective dose of 'FAM 30' on stem nematode (active and wool stage) and *Fusarium* (conidia, chlamydospores and mycelium) survival?
7. Discussions with biocide manufacturers and with regulators (CRD) should be continued to clarify the legal position of iodophore biocides in bulb dipping.

Acknowledgements

The author thanks Dick Evenden and the HDC for sharing data collected from bulb dips in 2009, Mr Lin Secker (Secker Welding Ltd) for helpful discussions of HWT and for his work on Case 8, Evans Vanodine International PLC for helpful discussions and for the supply of 'FAM 30 Check Strips', and Mr Mark Clark (Grampian Growers Ltd) for reviewing the text.

The HDC and the author are grateful to the growers who provided access to their HWT and cold-dipping operations.

