

<b>Project title:</b>	Narcissus: the use of FAM 30 disinfectant as a cold dip treatment for <i>Fusarium</i> basal rot
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## AUTHENTICATION

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

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# CONTENTS

<b>Grower Summary .....</b>	<b>1</b>
Headline.....	1
Background and expected deliverables .....	1
Summary of the project and main conclusions .....	1
Financial benefits .....	3
Action points for growers .....	4
 <b>Science Section .....</b>	 <b>5</b>
Introduction .....	5
Materials and methods .....	5
Results.....	11
Discussion .....	20
Conclusions .....	23
Glossary.....	23
References .....	24
Appendices .....	24

## **GROWER SUMMARY**

### **Headline**

FAM 30 (an iodophor disinfectant) diluted at 1:125 kills *Fusarium oxysporum* f.sp. *narcissi* and when recently-lifted narcissus bulbs are dipped in treated water at ambient temperature for 25 minutes the incidence and severity of Fusarium basal rot during subsequent storage can be significantly lower than in bulbs left undipped.

### **Background and expected deliverables**

Basal rot of narcissus remains a major disease problem; any infection within a bulb basal plate potentially leads to a rotten bulb. Retail stock with an incidence of 5% of bulbs affected is usually unsaleable, 2% is the accepted limit. For planting stock, anything over 10% leads to a potential problem at lifting after two years.

Previous work (BOF 61a) showed that the disinfectant FAM 30 (an iodophor disinfectant) was effective against *Fusarium oxysporum* f. sp. *narcissi* and can be used to replace formaldehyde in hot water treatment tanks. The emergence and flowering of bulbs was unaffected by treatment with FAM 30 in hot water.

However, the efficacy of FAM 30 at ambient temperature against *Fusarium* is unknown, there is therefore a need to determine whether or not a cold FAM 30 dip can control *F. oxysporum* f. sp. *narcissi* after 15-25 minutes immersion so that it could be used by growers immediately after the lifting of narcissus bulbs to prevent fungal invasion of wounds.

### **Summary of the project and main conclusions**

The initial stage of this project (BOF 71) showed that FAM 30, used at the label dilution rate of 1:125 *in vitro* at ambient temperature for 15-25 minutes was effective against resting spores of *Fusarium oxysporum* f. sp. *narcissi*. Half this rate of FAM 30 (1:250) was also effective in preventing the growth of *Fusarium*. However, no control was achieved when the disinfectant was unable to penetrate inert material to reach resting spores inside.

In the second part of the project (BOF 71a) a 25 minute ambient temperature dip of cv. Carlton narcissus bulbs in FAM 30 at the 1:125 dilution was carried out. To ensure disease presence the bulbs came from a field with a history of basal rot. Eight replications of 25 recently harvested bulbs without any external basal root rot symptoms were dipped in FAM

30 under laboratory conditions, or left undipped, and then stored for 9 weeks. Dipping in FAM 30 treated water significantly reduced the incidence and severity of *Fusarium* basal rot in the lifted naturally infected narcissus bulbs.

After 7 weeks storage, 19% of undipped bulbs felt soft, compared with 4.5% of bulbs in FAM 30 treated water. The dipping reduced the incidence of dark brown rotting, which covered over 15% of the internal surface, from 47% to 5.5% of the bulbs (see table on following page).

After a further 2 weeks, following storage in warm humid conditions to encourage the visible growth of *Fusarium oxysporum*, 84% of undipped bulbs were rotting, compared with 60% of bulbs from treated water. FAM 30 significantly reduced the incidence of *Fusarium* infection.

Much greater incidences of *Fusarium* browning than at the earlier assessment indicate that dipping in FAM 30 had only delayed symptom development and expression in many bulbs. Treatment success may have been through FAM 30 preventing the entry of infection to harvest wounds. It is likely that some of the apparently healthy bulbs selected for storage were already infected, but not visibly, and the FAM 30 was not be expected to kill *Fusarium* already in the tissue.

The majority of dipped bulbs affected by *Fusarium* had rotted after 9 weeks. A white *Fusarium* growth predominantly affected bulbs which had been dipped in FAM 30 treated water, whereas salmon pink *Fusarium* growth, which usually covered a more extensive area than the white, was more common in undipped bulbs. Commercially any rotting would cause rejection and it is likely that the level of internal rotting would have increased during further storage.

*Effect of FAM 30 at 1:125 dilution on growth of F. oxysporum f.sp. narcissi from resting spores, and development of Fusarium rot in narcissus bulbs*

Assessment	FAM 30 at 1:125	FAM 30 not used
Growth of Fusarium from resting spores (% agar plate cover)	0.0	100.0
Bulb rot after 7 weeks storage (% bulbs soft)	4.5	19.0
Internal browning by probable Fusarium after 7 weeks storage (% of bulbs affected)	5.5	47.0
Fusarium sporulation confirmed on cut bulbs, after 2 weeks warm, humid incubation post-storage (% of bulbs affected)	60.0	84.5
Extent of bulb scales and basal plate area affected by Fusarium after post-storage incubation (mean % internal surface area affected)	15.1	49.5

## Financial benefits

The principal benefit of using FAM 30 would be the acceptance of bulbs from a crop which without the dipping could otherwise be totally rejected for retail sale. Commercially, a 5% disease incidence would risk retail rejection, if bulbs with this potential level of disease incidence were treated with FAM 30 in the dip water then infection could be reduced, permitting retail industry and PHSI limits for export to third countries of 2% Fusarium to be attained. Similarly, bulbs with a potential incidence above the 10% limit advisable for bulbs to be planted for lifting could be saved from rejection.

In terms of the approximate treatment cost, FAM 30 costs around £43 per 1,000 litres of treated water when used at a dilution rate of 8 litres per 1000 litres of water. If it takes 1,900 litres of water to treat 1 tonne of bulbs then the chemical cost of treatment is £82 per tonne of bulbs (not allowing for re-use of the dip treatment).

### **Action points for growers**

- The incidence of narcissus bulb infection by *Fusarium* basal rot can be reduced when freshly lifted bulbs are placed in cold water with FAM 30 at a 1:125 dilution. This was seen after bulb immersion for 25 minutes.
- Bulbs should be thoroughly mechanically cleaned to remove spore-contaminated soil before dipping because FAM 30 can have difficulty disinfecting when chlamydospores are held within suspended inert material.



## SCIENCE SECTION

### Introduction

Formaldehyde can no longer be used in hot water tanks which may increase the risk of spread of stem nematode (*Ditylenchus dipsaci*) and basal rot (*Fusarium oxysporum* f. sp. *narcissi*) during bulb dipping. BOF 61a investigated alternatives to formaldehyde in hot water tanks within a standard 3 hour exposure period, and showed that FAM 30, an iodophor disinfectant, added to hot-water at 44.4°C killed *F. oxysporum* in its resistant chlamydospore stage. Emergence and flowering of bulbs was unaffected by this treatment.

Some growers treat bulbs immediately post-harvest to control basal rot. A cold water 15 minute formalin dip was also previously used immediately post-harvest. With the loss of formaldehyde, thiabendazole (TBZ) is currently the only fungicide approved for this use. However, TBZ can only be used once on any narcissus crop because of the resistance management strategy for MBC fungicides. If it is intended to use it as an additive during later hot-water treatment then an alternative is required to its use as a cold spray straight after harvest.

HNS 147 tested FAM 30 at ambient temperature for disinfection of used plant containers; *Pythium* with resting oospores was killed after 15 minutes immersion. *Fusarium* was not, however, tested. It is not known whether *F. oxysporum* chlamydospores would be killed at ambient water temperature following 15 minutes exposure to FAM 30. It is possible that heating the water in BOF 61a might have aided the activity of the disinfectant, such as by inducing germination of chlamydospores to expose the more susceptible fungal germ tube.

This project initially aimed to determine the efficacy of FAM 30 to treat water at the label rate *in vitro* at ambient temperature for 15 minutes against spores and mycelium of *F. oxysporum* f. sp. *narcissi*. Following confirmation of efficacy at the 1:125 dilution rate, this rate of FAM 30 was tested on uncleaned bulbs taken from a field with a history of *Fusarium* basal rot.

### Materials and methods

#### *In-vitro* testing

Chlamydospores of *F. oxysporum* f. sp. *narcissi* were prepared by the method described in Hanks & Linfield (1999). The fungus was isolated from several samples of infected narcissus bulbs and grown on modified Nash medium (Nash & Snyder, 1962). Colonies

produced were then transferred to Potato Dextrose Agar (PDA) and grown in the dark at 20°C for 10 days. Conidia and mycelium was then washed from the cultures into heat-sterilised fine talc (catalogue ref. 33122; Merck Ltd, Poole, UK). The infested talc paste was left to dry for 3 weeks, in which time chlamydospores (resting spores which can survive several years if kept dry) formed and were visible by microscope examination. The dried talc paste was pulverised and stored at ambient temperature in lidded universal tubes. Before use in experiments a sample of the infested powder was scattered onto PDA to confirm chlamydospores viability.

The concentrations of FAM 30 tested (Table 1) were 1:125 as in BOF 61a and half this rate (1:250) in order to match work being carried out under BOF 61c. In addition, a more concentrated rate of 1:50 was tested because tests in BOF 61a had allowed a 3 hour contact period for the *Fusarium* spores and it was possible that the 15 minutes to be used in the current project would be insufficient at the 1:125 dilution. High rates of 1:62.5 and 1:20 FAM 30 are used in the livestock industry for tuberculosis prevention.

**Table 1.** Dilution rates of FAM 30 tested as a disinfectant in cold water

Treat-ment	Active	Product	Dilution Rate	%	Final dilution of product
T1	—	Sterile Tap Water	—	—	—
T2	Iodophor disinfectant	FAM 30	1:125	0.8	0.8 ml / 100 ml water
T3	Iodophor disinfectant	FAM 30	1:250	0.4	0.4 ml / 100 ml water
T4	Iodophor disinfectant	FAM 30	1:50	2.0	2.0 ml / 100 ml water

The test procedure used was based on Hanks & Linfield (1999) and as carried out in BOF 61a. A stock suspension of *Fusarium oxysporum* chlamydospores was made by mixing 1 g of the infested talcum powder per 10 ml sterile distilled water (SDW) just before use. Microscope examination of a drop (about 20 µl) of stock suspension showed a count of about 80 chlamydospores.

All work with open tubes of spore suspension was carried out under sterile conditions. Solutions of FAM 30 (Batch 92357 expiry date 31 May 2012, as also used in BOF61c) were made up at double the concentrations required for the tests as they were to be diluted by half by the spore solution. Dilutions were made with sterile tap water (STW). The diluted FAM 30 solutions were kept for no more than two hours in sealed glass containers before

use. Evans Vandoline International FAM 30 check strips were dipped into the concentrated solutions for 2 seconds and read after 30 seconds to confirm that the solution was at least as concentrated as the highest (darkest) indicator shown (1:125) on the reference chart.

Records were taken of the pH of the water and FAM 30 stock solutions used and also of the solutions after addition of the spore stock solution (Table 4). Duplicates of the spore dilutions were made to provide sufficient volume required for the Hanna H198128 probe. To avoid contamination of the spore suspension for culturing, Evans Vandoline International FAM 30 check strips were also used in the duplicate spore / FAM 30 dilution series.

For each concentration of FAM 30 used (Table 1), 5 ml of the double-strength FAM 30 solution was put into each of 5 lidded sterile transparent polystyrene universal tubes of 30 ml capacity. Five ml of STW were also added to each of a further 5 tubes, to act as undipped controls. This gave a total of 20 tubes, although it was necessary to divide the replication over two days (with Replicate 1 only carried out on one day) because of the length of the whole procedure for each replicate.

The tubes were placed in racks in a water bath at 18°C (ambient temperature). A thermometer was placed in an extra tube of water alongside the other tubes and when the temperature within the tubes had equalised with that of the water bath, a 5 ml aliquot of the stock *F. oxysporum* spore suspension was added to each tube, diluting the chemical already in the tube to the required concentration. Spore suspension was also added to the control tubes containing only STW. 20 ml of spore suspension was made up for each replicate and withdrawn by syringe in close succession for each of the treatments in turn, replacing the tube lids immediately. All tubes were agitated well initially by hand and then again at intervals throughout the test in order to keep the talc and spores from settling into a layer which might reduce surface contact with the FAM 30.

After 15 minutes in the water bath, tubes from the same replicate were removed simultaneously, given a final agitation and then placed immediately into a centrifuge operating at 2,000 rpm. The tubes were centrifuged for 3 minutes to concentrate the spores in the bottom of the tubes. To remove the FAM 30 the supernatant was quickly removed using a fresh sterile pipette for each tube and the tubes refilled to 20 ml with STW. Tubes were shaken and then immediately re-spun in the centrifuge. The supernatant was again removed to leave about 3 ml of solution containing the rinsed 'plug' of spores and talc.

Two ml of 0.1% w/v cold tap water agar (TWA) was then added to each tube and agitated to re-suspend the talc and spores. One ml of this dilute agar suspension was then taken from each tube by pipette and spread out over the surface of Nash medium in a 9 cm round Petri dish. One dish was used per replicate of each treatment, giving a total of 20 dishes. These dishes were incubated at 20°C in 12 hr dark : 12 h light with assessments after 3, 7 and 10 days initially recording colony diameter and then, following the growth of colonies towards each other, the % coverage of the plate by *F. oxysporum* mycelium.

On some treated spore plates, where surviving spores started to produce colonies, sections of agar plate containing apparently dead spores were cut out and sub-cultured. This allowed records to be made of any delayed spore germination without the area becoming overgrown by the early produced colonies.

### *Bulb dipping*

Narcissus cv. Carlton were lifted commercially on 13 July 2010 in the Boston area from a field with a history of Fusarium basal rot. Four sacks were filled from the harvester in the early afternoon and stored sheeted overnight in a barn. Bulbs were selected the next day for treatment from each of the bags. The bulbs were selected to be visibly healthy i.e. to have no external fungal growth and to have a full ring of fleshy fresh white roots around the base plate. Bulbs with roots missing or hidden under mud were avoided. The bulbs were used without cleaning and dipped on 14 July starting at midday and finishing at 17:00 h, so completing all eight replicates within 28 hours of lifting.

400 visibly healthy bulbs were taken from the harvest bags just before use to prevent them drying out. The bulbs were divided into 16 batches of 25, with 200 bulbs allocated to T1 (control) and 200 to T2 (FAM 30 1:125). Bulbs for each replicate were taken from the same harvest bag (2 replicates from each of 4 bags with a record kept of their source).

Each batch contained bulbs representative of the size range in the bag including a representative proportion of double nosed bulbs. Each batch thus had 20 single nosed and 5 double nosed bulbs.

Each of the 8 replicate samples of 25 bulbs was immersed in FAM 30 for 25 minutes. A separate dip solution was used for each sample. Bulbs which remained undipped were placed directly from the harvest bag into a slatted sterilized produce tray at the same time as bulbs for the same replicate were taken for treatment. Following the advice of the HDC panel the control bulbs were not given a plain water dip.

**Table 2.** The two treatment options (undipped and dipped) given to the narcissus bulbs

Treatment	Active	Product	Rate of active	Rate of product
T1	–	–	–	No water dip
T2	Iodophor disinfectant	FAM 30	0.8% (0.8 ml in 100 ml) 1:125	24 ml / 3L tap water per replicate

T1: Trays 1, 3, 5, 7, 9, 11, 13 and 15      T2: Trays 2, 4, 6, 8, 10, 12, 14 and 16

The FAM 30 dip was carried out in new plastic 10 L buckets with the solution temperature recorded at intervals using a Hanna H198128 probe. The first batches were at 19.8°C and the solution temperature rose slightly during the afternoon to 21°C. The tap water was pH 7.12, the diluted 1:125 solution of FAM 30 was pH 3.08 and the solution post-dipping was pH 2.88. The solution was made up freshly for each bucket. 3 L of solution was used as this totally immersed the bulbs. The bulbs were placed into the bucket and the solution added.

The bulbs were supported on a net which was briefly lifted up and down three times at intervals to raise the bulbs and aid solution circulation around them to ensure full surface contact with the disinfectant.

After treatment, the bulbs were not rinsed but were spread out and left to drain in a clean plastic seed tray for about 30 minutes. Once the bulbs had drained they were placed in a single layer without touching in an unlined slatted sterilised produce tray. Two replicates were fitted in the same tray. The same treatments were placed in the same tray because of the risk of cross-contamination from the undipped or fumigant effect from the bulbs which had been in the FAM 30 disinfectant solution. A cardboard insert was placed between the two batches to divide them.

The trays were stored un-stacked on wooden racks in a darkened dry equipment storage barn at ambient temperature for 7 weeks. They were shielded from light with a paper cover. The control treatment undipped bulb trays were arranged in the same ambient temperature storage area in-between the trays containing bulbs which had been in the FAM 30 disinfected water. Temperature and humidity around the bulbs was recorded.

#### *Assessment of bulb rot after 7 weeks*

The bulbs were assessed after 7 weeks incubation on the 2<sup>nd</sup> and 3<sup>rd</sup> September 2010. Records were kept separate for each bulb and the bulbs replaced in order after assessment. Each batch was replaced in a separate sterilised tray.

Each bulb was cut in half from nose to base, sterilising the knife between bulbs with 75% Industrial Methylated Spirit (ethanol) to avoid cross-contamination. Any debris was wiped off the knife before dipping in the ethanol. The outermost skin of the bulbs was removed and discarded before cutting because it fell away from the bulb. In the undipped the outermost layer often had some mud on it and its disposal also stopped contamination of the incubation trays by saprophytic fungi

For double nosed bulbs comprising a mother with a nearly separate daughter, an overall assessment was made of the total internal area affected (which was analysed), and a separate assessment made for each.

Assessments were made of the following:

- 1) The presence of any fungal sporulation visible externally or internally (the brown skin hindered any recording of browning from rotting).
- 2) Any softness when held and pressed with the thumb
- 3) The total % surface area of browning of the internal faces
- 4) The position/s of rotting for each bulb i.e. nose, middle or base plate.

Bulb halves were kept together from each bulb and were placed in a sterilised tray with the cut bulb surface uppermost. They were laid out in the order of assessment, ensuring neighbouring bulbs did not touch. Each tray was lined with dampened paper towel.

Once filled, the trays were sealed in a transparent plastic bag to raise the humidity and encourage fungal mycelial growth. The trays were placed in a glasshouse under natural daylight conditions in order to aid *Fusarium* sporulation. The trays were placed in two randomized stacks with a blank top tray to protect the stack from direct overhead sunlight and left to incubate for 2 weeks. Temperature and humidity was recorded around the bulbs.

#### *Identification and assessment of fungal rot after 9 weeks*

After 9 weeks from dipping, the incubated bulbs were assessed and recorded on 17th September 2010 in the same order as the previous fortnight. Both pink and white *Fusarium* was recorded, with a separate score for each as these were never on the same bulb. Bulbs were not inspected on the undersides. Mother and daughter bulb records were made as two weeks earlier.

Assessments were made of the following

- 1) The presence of any white fungal sporulation internally
- 2) The presence of any salmon pink fungal sporulation internally

- 3) The % surface area of white fungal growth on the internal faces
- 4) The % surface area of salmon pink fungal growth on the internal faces
- 5) The position/s of each colour fungal growth across each bulb i.e. nose, middle or base plate.

Analysis of the difference between means was carried out by Analysis of Variance for records of the % of internal surface area with either brown rot or *Fusarium* growth. Non-parametric Regression Analysis was used where the % of bulbs with or without browning, *Fusarium*, or *Penicillium* was calculated from the number of bulbs showing symptoms.

## Results

The total immersion time of the *F. oxysporum* spores was 25 minutes from adding the spores to the first rinse after centrifuging. However the 15 minute standing time in the water bath was the period in which the spores were kept suspended in the FAM 30 and thus in full contact.

Plates with untreated chlamydospores were soon totally covered by mycelial growth of *F. oxysporum* as the chlamydospores germinated and colonies merged, with the plates developing a pale pink colouration.

FAM 30 at 1:125 gave complete control of *F. oxysporum* at 18°C, with no growth occurring during 10 days incubation. Zero growth occurred in four replicates of half-rate (1:250) FAM 30. However, in Replicate 1 at the 1:250 dilution after three days threads of mycelium were seen to originate from pin-head sized lumps of the infested talc. The *Fusarium* grew quite quickly and the first replicate plate was soon covered by pale pink or white mycelial growth from these lumps (Table 3). The lumps were within the pulverised dried *Fusarium* infested talc slurry used for the tests, the majority of which had been broken down to a powder. .

The most concentrated dilution of FAM 30 (1:50) allowed chlamydospore growth (Table 3). However, all colony growth clearly originated from talc lumps. This treatment received spore suspension from the bottom of 20 ml stock tubes (to which lumps had settled). , There were no lumps on the plates of the other treatments taken from higher up the tubes (except Replicate 1, T3). Plate cover was lower in 1:50 than in the untreated because growth from the untreated inoculum originated from spores in powder dispersed over the plate, not from lumps.

**Table 3.** *Fusarium oxysporum* f.sp. *narcissi* growth on 90 mm diameter agar plates after *in-vitro* spore treatments at 18°C for 15 minutes

Treatment	FAM 30 dilution	Replicate	Day 3 colony diameter (mm)			Day 3	Day 7	Day 10
						% cover		
T1	Water only	1	90			100	100	100
		2	90			100	100	100
		3	90			100	100	100
		4	90			100	100	100
		5	90			100	100	100
T2	1:125	1	0			0	0	0
		2	0			0	0	0
		3	0			0	0	0
		4	0			0	0	0
		5	0			0	0	0
T3	1:250	1	t			t	98	100
		2	0			0	0	0
		3	0			0	0	0
		4	0			0	0	0
		5	0			0	0	0
T4	1:50	1	0			0	75	100
		2	17	8	15	10	95	100
		3	13	16		10	70	95
		4	17	12		10	85	95
		5	9	10		5	50	75

t = threads of mycelium seen, but not forming a distinct colony

On Day 4, agar covered by spore solution but without *F. oxysporum* growth was excised from plates of T4 replicates 2 – 5 before it became overgrown from elsewhere on the plate. There was still no mycelial growth by Days 7 and 10 from these areas of 1:50 treated spore solution. Some small lumps were present on one excised piece and there was no growth from these.

**Table 4.** Records of the pH of the test solutions at 18°C used in *in vitro* testing

STW	SDW	Spore stock in SDW	Double concentration FAM 30 (pre-dilution to the ratios shown)			FAM 30 at the dilutions tested after spore stock solution addition		
			1:250	1:125	1:50	1:250	1:125	1:50
8.3	8.9	8.4	2.8	2.6	2.4	3.1	2.8	2.5

The FAM 30 was acidic and dilutions with spore stock solution for the *in vitro* work remained acidic (Table 4).



The Evans Vandoline International FAM 30 check strips used are sold to monitor the decline in activity of the solution over time during its re-use in dip tanks. All strips turned black when put into the double concentrated FAM 30 solutions, showing that the concentration of 1:125 was exceeded. There were difficulties in interpreting the indicator strips in FAM 30 dilutions with the infested talc solution. With 1:125 the centre of the indicator window was black and the inner paler, and for 1:250 the indicator was a yellower green than shown on the reference chart, but the strength of colour was equivalent to that shown. The 1:50 dilution was, as expected, off the reference scale of 1:125 to 1:2500. When the test strips were used in the bulb dipping experiment the indicator strips were dark blue green but not as dark as should have been indicated for 1:125 although the solution was fresh. These results indicate that colour indication by the strips was not consistent and could not be used in this work as a scientific tool to assess the active strength of the FAM 30. However, all solutions were freshly prepared for all experiments using precise dilutions to ensure target concentrations were achieved.

#### *Bulb dipping*

Infection by *Fusarium* was confirmed and the greater incidence was easily visible in the undipped bulbs after 7 weeks when first cut open to show browning (Fig. 1) and then again after 9 weeks when incubated to encourage the *Fusarium* to grow out and sporulate (Figs 2 & 3). The temperature range during unheated barn storage of the intact bulbs was 17°C to 21°C with relative humidity between 66 and 74. Cut bulbs in sealed trays in an unheated glasshouse were incubated at between 11°C to 30°C and relative humidity of 94 to 97.

#### **After 7 weeks storage**

After 7 weeks dry storage, a mean 19% of undipped bulbs felt soft, significantly more than the 4.5% following FAM 30 treatment (Table 5). It was not possible to detect all infected bulbs by their appearance. Few bulbs had external signs of brown rotting as discolouration was not recognizable on the tan brown dry outer scale.

**Table 5.** Effect after 7 weeks on bulbs of having been in FAM 30 treated water on the incidence of bulb soft rot (presumed *Fusarium* rot), compared with undipped bulbs

<b>Dip treatment</b>	<b>Mean % of bulbs soft externally</b>
Undipped	19.0
FAM 30	4.5
s.e.d.	3.69
P	0.001
d.f.	14

Internal discolouration was found when bulbs were cut open for inspection, with a significantly greater area affected in undipped bulbs (25%), than in the dipped (6%) (Table 6). Many bulbs had dark brown staining inside. This browning often occurred on the basal plate and frequently affected parts of the bulb scales across the centre of the bulb to give a mottle of white healthy and brown rotting tissue. Browning was seen in the neck region, but usually joined with a significant basal rot (Fig. 1 and Appendix 1) and so it was likely the rot originated at the base. In addition, much smaller areas (no more than 15% of the surface), principally on the outer scales at the widest part of the bulb, had a grey discolouration. A pale brown oxidation staining was rapidly produced across the whole cut surface of the bulbs, but this was clearly distinguishable from the dark dense areas of rot and not assessed. No fungal growth was visible on the rotting areas inside the bulb when first cut open.



**Figure 1.** Narcissus bulb cut in half after 2 week storage, showing dark brown Fusarium base and scale rot (with general brown speckling from oxidation after cutting)

**Table 6.** Effect after 7 weeks on bulbs of having been in FAM 30 treated water on the incidence and severity of internal discolouration, compared with undipped bulbs

<b>Treatment</b>	<b>Mean % of bulbs with internal browning or grey discolouration</b>	<b>Mean % internal bulb area brown or grey</b>
Undipped	86.5	25.2
FAM 30	71.0	5.70
s.e.d.	4.84	2.53
P	0.01	<0.001
d.f.	14	14

Following FAM 30 treatment 71% of bulbs were found to have internal browning and grey discolouration, while 86% of undipped bulbs were affected (Table 6), a significant difference. Subsequent incubation and re-recording of the same individual bulbs showed that bulbs which had been recorded with only grey discolouration along the sides of the bulb did not consistently produce *Fusarium* (they were rarely infected by pink *Fusarium* although sometimes they had a small area of white *Fusarium*) (Appendices 1 & 2). It was likely that these grey stained bulbs had been bruised during handling (rather than starting to show *Fusarium* rot) and that more bulbs were healthy than recorded.

When grey staining was ignored, by including as rotted only bulbs with over 15% surface area browning, then only 5.5% of bulbs which had been in the FAM 30 solution were seen to have been rotted by *Fusarium*, compared with 47% of undipped, a highly significant reduction in infection following treatment (Table 7). Over twice as many bulbs incubated without dipping in FAM 30 (47%) were seen to have developed internal surface area browning/rotting (Table 7) as had been detected by feeling for softness (Table 5), but the proportion of dipped bulbs with internal surface area browning (5.5%) was similar to the 4.5% determined to have internal rotting by feeling bulb softness. The undipped bulbs thus had a higher incidence of internal rotting (which had not yet caused the bulbs to soften) suggesting that the rot was progressing, as well as a higher incidence of soft rotting which probably resulted from earlier established infections.

**Table 7.** Effect after 7 weeks on bulbs of having been in FAM 30 treated water on the occurrence of over 15% internal area browning, and the proportion of bulbs with top and/or base discolouration, compared with undipped bulbs

Treatment	Mean % of total bulbs with more than 15% browning	Mean % of discoloured bulbs with upper and/or lower discolouration
Undipped	47.0	53.5
FAM 30	5.5	19.0
s.e.d.	4.44	3.13
P	<0.001	<0.001
d.f.	14	14

A total of 54% of the undipped bulbs with discolouration had the upper and or lower browning attributed to *Fusarium* (with or without discolouration on the sides of bulbs) and the remainder (46%) had side discolouration only (Table 7). Of the lower total number of dipped bulbs recorded as having discolouration, only 19% had upper and lower *Fusarium* browning (with or without side discolouration) and so most of those recorded with

discolouration (81%) had only side bruising. There were thus significantly fewer bulbs with *Fusarium* browning in the dipped than in the undipped, and most of the discolouration recorded in the bulbs from the FAM 30 treated water was likely to have been caused by bruising of the sides.

Bulbs with dark brown *Fusarium* rotting usually had at least 15% of the surface area rotted, with the inner surface area of some undipped bulbs being 95% rotted, and rot commonly covering 30% to 60% of the surface (Appendix 1). In contrast, although 71% of dipped bulbs had discolouration (Table 6), 94% of all dipped bulbs had less than 16% discolouration or no discolouration at all (Table 7) with many having only a very small area of discolouration of 1 to 5%, which was probably bruising (Appendix 1).

### After 9 weeks storage

After 9 weeks storage, with incubation of the cut bulbs for the last two weeks in warm, light and humid conditions, mycelium and sporulation of *Fusarium* was produced on the internal surfaces of the bulbs. There were traces of blue coloured *Penicillium* on some bulbs, but *Fusarium* was confirmed to be the cause of the dark brown rotting of the bulb scales.

The majority (84%) of undipped bulbs had *Fusarium* growth compared with 60% in the dipped, a significant 24% reduction in *Fusarium* incidence by FAM 30 (Table 8). The proportion of undipped bulbs with discolouration on sectioning 2 weeks earlier had been similar, but previously only 71% of dipped bulbs had discolouration (Table 6), confirming that not all the discolouration had been caused by *Fusarium*.

A minority (9%) of undipped (unwetted) bulbs had visible *Penicillium*. Significantly more FAM 30 cold dipped bulbs had specks of internal *Penicillium* growth, although it was easier to see than on undipped bulbs covered by *Fusarium* (Table 8).

**Table 8.** *Fusarium* and *Penicillium* incidence on cut bulbs following 2 weeks humid incubation. 9 weeks after half the bulbs were dipped in FAM 30 treated water

Treatment	Mean % of total bulbs with more than 15% browning	Mean % of discoloured bulbs with upper and/or lower discolouration
Undipped	84.5	9.0
FAM 30	60.0	40.0
s.e.d.	8.76	6.86
P	0.016	<0.001
d.f.	14	14

Both white and pink *Fusarium* mycelium grew on the bulbs, all bulbs having either one colour or the other. Salmon pink fungal growth was clearly visible and heavily sporulating, giving it a powdery appearance. This pink growth covered the cut surface of the basal plate and spread in a thick coat to cover the main area of most of the bulbs with this colour of *Fusarium* (Fig. 2). White mycelium was also present on bulbs, but less obvious, with the growth covering the outer faces of the bulb scales across the centre of the cut bulb surface and not obscuring the basal plate (Fig. 3). Both fungal coverings appeared to originate from the bottom half of the bulb, although when severe they occurred towards the neck. Both colours were recorded equally on the basal area of undipped and dipped bulbs, but twice as many basal areas were covered by pink as opposed to white *Fusarium* (Table 9).



**Figure 2.** Salmon pink thick *Fusarium* growth on a cut bulb 9 weeks after harvest



**Figure 3.** White thinly covering *Fusarium* growth on a cut bulb 9 weeks after harvest

**Table 9.** The % of bulbs with pink *Fusarium* on the basal area and the % with white *Fusarium* on the basal area out of the number of bulbs with *Fusarium* of each colour at any position, 9 weeks after dipping half the bulbs in FAM 30 treated water

Treatment	% of bulbs with pink <i>Fusarium</i> which had basal fungal coverage	% of bulbs with white <i>Fusarium</i> which had basal fungal coverage
Undipped	91.7	41.8
FAM 30	86.3	42.9
s.e.d.	8.19	13.94
P	ns	ns
d.f.	(12)	14
(12) d.f. because of 2 missing values		

The white fungal growth still appeared the same on the bulb scales for at least two further weeks after the final assessment. When white mycelium was transferred from bulbs to Potato Dextrose Agar the growth became pink on the underside of the agar plate. *Fusarium oxysporum* is known to have high variability in colour and for the pigmentation to change as the colony matures or with different nutrient sources. Variable sized single celled microconidia of *Fusarium* sp. (on average about 10µ long) and the structures (phialides) producing them were seen under the microscope for both coloured colonies, and bigger, crescent-shaped, spores (macroconidia) had developed in the more advanced salmon pink colony, assisting confirmation as *F. oxysporum*.

Bulbs had either pink or white *Fusarium* growth, or zero fungal growth, (Appendix 2) with 57% of the undipped bulbs covered by pink *Fusarium*. Significantly fewer of the dipped bulbs had pink fungal growth, with 12% affected. Twice as many dipped bulbs (88%), were thus free from pink *Fusarium* as undipped (44%) (Table 10 and Figs 4 & 5).

Of the total undipped bulbs, 28% had white *Fusarium* (Table 10). The majority of undipped bulbs had pink *Fusarium* instead, leaving only 15% of undipped bulbs without *Fusarium* (Table 8). More dipped than undipped bulbs had white *Fusarium* (49%), showing that this immature less dense *Fusarium* colonisation (usually covering 30% or less surface area) occurred more frequently where FAM 30 had been used (Table 10 and Appendix 2).



**Table 10.** The % of bulbs covered with either of the two *Fusarium* colony colours and the mean % internal surface area covered by each for all bulbs and in total, 9 weeks after dipping half the bulbs in FAM 30 treated water.

Treatment	% of bulbs		Mean % area		
	with pink <i>Fusarium</i>	with white <i>Fusarium</i>	of pink <i>Fusarium</i>	of white <i>Fusarium</i>	with pink and white <i>Fusarium</i>
Undipped	57.0	27.5	43.7	5.7	49.4
FAM 30	12.0	49.0	7.4	7.7	15.1
s.e.d.	6.52	6.25	4.01	2.55	5.38
P	<0.001	0.01	<0.001	ns	<0.001
d.f.	14	14	14	14	14



**Figure 4.** Undipped bulbs showing principally pink *Fusarium* growth on cut bulbs in the incubation tray 9 weeks after harvest



**Figure 5.** Bulbs after dipping in FAM 30 treated water showing few bulbs with pink *Fusarium* growth on cut bulbs in the incubation tray 9 weeks after harvest and treatment

The mean (of affected and unaffected bulbs) internal area of dipped bulbs covered by pink *Fusarium* was only 7.4%, compared with 44% in the undipped. The area of white *Fusarium* coverage was similar for both treatments, a mean 7% (Table 10). It is possible there could have been a greater incidence of white *Fusarium* in the undipped than recorded, because the pink *Fusarium* could have overgrown it. White was only recorded in both treatments where there was no pink *Fusarium*. In both treatments, individual bulb records for pink *Fusarium* were often 65% -100%, but the white *Fusarium* was more usually around 30% coverage (Appendix 2).

The mean % internal surface area covered by *Fusarium* of either colour was 49% for undipped, compared with 15% for dipped bulbs (Table 10). This was a highly significant reduction in % internal *Fusarium* followed treatment with FAM 30.

Overall, bulbs which had been in the FAM 30 treated water and had *Fusarium* had a low % internal surface area affected (Appendix 2) and this was predominately the weaker (initially white) growth with microconidia, whereas the undipped bulbs had thick salmon pink growth coating a high proportion of the internal surface and both micro and macroconidia.

There was no pattern of increased *Fusarium* incidence in bulbs from either treatment when bulbs had a daughter bulb, but there were insufficient for analysis (Appendices 1 & 2).

Bulbs were dipped in replicate order (using fresh batches of FAM 30 for each) and from Replicate 5 (dipped at 15.00h, 26 hours after harvest) to Replicate 8 (dipped 28 hours after harvest) there was a trend for a more bulbs to be affected by *Fusarium*, with a greater incidence of discolouration at 7 weeks and white *Fusarium* growth at 9 weeks than present in earlier dipped replicates (Appendices 1 & 2). The mud on the roots was starting to dry and although bulbs were selected with roots that had not started to shrivel they were not as fresh as the first batches.

## **Discussion**

### *In-vitro testing*

FAM 30 was shown to prevent the germination of *Fusarium oxysporum* f.sp. *narcissi* at both 1:125 and at the half rate of 1:250 following 25 minutes immersion at ambient temperature.

It had been thought possible that the effectiveness of FAM 30 might be reduced at 18°C, compared with the 44.4°C used in BOF 61a, where the higher temperature could have



helped to induce the germination of the chlamydospores and so given an opening for chemical entry through the thick walls of these resting spores.

BOF 61a used the 3 hour dip required for hot water treatment, but the shorter dip time used in the current work with cold water chemical treatment was able to achieve control in the majority of tests. However, in the current tests where the chlamydospores were trapped within lumps of inert material the FAM 30 was unable to penetrate in 15 minutes. Even a high FAM 30 concentration of 1:50 was shown to be no better than a lower concentration when seeking to penetrate into lumps holding spores.

The FAM 30 treatment should be effective for *F. oxysporum* spores loose within dust, or free in washing or dipping water. However this treatment may not be as effective against chlamydospores present in mud adhering to the bulb roots. Nor would control be expected of fungal mycelium already inside bulb tissue.

#### *Bulb testing*

The interpretation of the results was complicated initially by the presence of bruising around bulb sides. However, the dark brown discolouration caused by *F. oxysporum* rot was obvious on bulbs before humid incubation and present under both the white and the salmon pink *Fusarium* growth after incubation. It is not clear why only 12% of bulbs in FAM 30 treated water had pink *Fusarium* growth and those left undipped had significantly more bulbs with pink growth (57%). The effectiveness of FAM 30 in reducing the presence of some, but not all, growth of the pink *Fusarium* with high surface coverage and the dominance of white *Fusarium* growth could be explained by some of the following:

- Where 70% or more coverage by pink *Fusarium* growth was recorded in the dipped bulbs then it may have been inside the roots or basal plate before bulb treatment.
- Bulbs from treated water often had a smaller surface area affected than undipped, (mean 15% and 49% coverage, respectively). Where *Fusarium* infection was able to develop after bulb dipping its growth could have been delayed to produce a smaller area of thin coverage resulting in the white *Fusarium* growth.
- It was possible that the white *Fusarium* growth did not infect through the basal plate or roots, but through the neck or sides (particularly as the basal area was not always affected) and may have taken longer to grow to the bulb centre than the pink *Fusarium* and so its growth was less advanced. Delayed infection may have also caused the white *Fusarium* growth in the undipped bulbs.

- The FAM 30 could have been having an effect on the *Fusarium* within the bulb, stunting mycelium growth and not allowing the abundant pink growth to be formed as in the undipped.

The 84% incidence of *Fusarium* infected bulbs in the undipped batches was unusually high. This figure excludes bulbs with only partial root growth around the base plate which were not used in the experiment as this can be a symptom of *Fusarium* basal rot. Commercially, bulbs are inspected for *Fusarium* basal rot soon after harvest by cutting into the basal plate to look for black spots (of any size or number). A 20% *Fusarium* incidence would not be sold commercially, 10% would be just acceptable as planting stock for lifting after 2 years and 5% would be barely saleable for retail.

Several factors could have affected the performance of the FAM 30 and possibly contributed to the high natural infection levels recorded in the experiment:

- Moist soil remaining around undipped (undipped) bulbs probably both trapped *Fusarium* spores against the bulb and provided humid conditions favouring entry into the tissue. FAM 30 washed some, but not all, of the adhering soil from around roots. Bulbs lifted from sandier and drier soil with a similar *Fusarium* contamination level might have had a lower infection rate. Mechanical cleaning (using rollers, star cleaners and barrel cleaners) would have reduced the amount of adhering soil and facilitated drying of the bulb surface.
- The presence of soil and debris in the FAM 30 dip might have affected the performance of the disinfectant. However, half 1:125 rate was still effective *in vitro*.
- The bulbs dipped after 24 hours when the roots were starting to dry may have already been entered, and so infected, by *Fusarium* as these batches tended to have more bulbs affected. This seems to support the accepted knowledge that bulbs should be dipped as soon as possible after lifting while wounds and roots are still fresh.

The use of FAM 30 disinfectant may be of particular benefit to the few growers who still wash rather than mechanically clean their bulbs before storage. If FAM 30 could be used in the washing water (subject to health and safety implications) then this might especially help reduce the greater incidence of neck rot reported after washing in water flumes (possibly because *Fusarium* contaminated water enters the bulb neck (A. Jansen pers. comm.)).

There was a 24% reduction in the incidence of *Fusarium* with treatment. If this could be extrapolated to a more normal commercial incidence of 6%, then the reduction in infection

by FAM 30 would bring the incidence down to around 2%. This is the level of *Fusarium* basal rot that the retail industry sets and the PHSI limit for export to third countries.

## Conclusions

- FAM 30 at ambient temperature diluted 1:125 in water prevents the growth of *Fusarium oxysporum* f.sp. *narcissi* from resting spores;
- Dipping recently-lifted narcissus bulbs at ambient temperature for 25 minutes in washing water containing FAM 30 at 1:125 dilution significantly reduces the incidence and severity of *Fusarium* basal rot during storage, but does not give total control.

## Glossary

### ***Chlamydospores***

Thick walled, spherical resting spores produced from spores and mycelium in adverse conditions, such as the exhaustion of their food source. They can remain viable for several years, especially if kept dry, and will germinate when stimulated by a new nutrient source such as by the chemical solutions exuded from a new host. This spore type is likely to be found in the soil around old rotted bulbs and in bulb debris and dust.

### ***Macroconidia***

A crescent shaped spore with cross-walls, with various spore shapes and sizes typical of particular *Fusarium* species. Cultures can vary in their production of this spore type. They germinate to produce mycelium which can then produce more spores. They will be produced on rotted tissue on the outside of bulbs, particularly around the basal plate, especially in warm humid conditions.

### ***Microconidia***

A small oval spore produced abundantly by *Fusarium* species. They germinate to produce mycelium which may sporulate. Microconidia will be produced on rotted tissue on the outside of bulbs, particularly around the basal plate, especially in warm humid conditions.

### ***Mycelium***

Strands of fungus that grow on and inside plant tissue, feeding on it and producing spores.

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## APPENDICES

Two appendices have been provided with this report to show individual records for bulbs at 7 and 9 weeks after harvest, and the surface areas affected by *Fusarium*. Mean infection severity is given in the main text, together with infection incidence, and should be sufficient for the majority of those reading the report.

**Appendix 1:** Discolouration on inner faces of bulbs cut in half after 7 weeks storage after harvest

Plot	Treatment	Bulb Number	Softness (1=Yes)	External Fungal Growth (1=Yes)	% Total Internal discolouration	% Internal Discolouration Mother Bulb	% Internal Discolouration Daughter Bulb	% Internal Discolouration Daughter Bulb	Position of Internal Discolouration (1=Yes)		
									Upper	Lower	Side Only
1	Not dipped	1	0	0	0				0	0	0
		2	0	0	0				0	0	0
		3	0	0	40				1	1	0
		4	0	0	2	5	0		0	0	1
		5	0	0	65				1	1	0
		6	0	0	0				0	0	0
		7	0	0	3				0	0	1
		8	0	0	3				0	0	1
		9	0	0	45				1	1	0
		10	0	0	2				0	0	1
		11	0	0	60				1	1	0
		12	0	0	7				0	0	1
		13	0	0	0				0	0	0
		14	0	0	25				0	1	0
		15	0	0	15				0	0	1
		16	1	0	50				1	1	0
		17	1	0	95				1	1	0
		18	0	0	0				0	0	0
		19	1	0	75				1	1	0
		20	0	0	1				0	0	1
		21	0	0	2	1	7		0	0	1
		22	1	1	65	55	75		1	1	0
		23	0	0	20	35	1		1	1	0
		24	0	0	15	3	0	25	1	1	0
		25	1	0	15	1	40		1	1	0

Plot	Treatment	Bulb Number	Softness (1=Yes)	External Fungal Growth (1=Yes)	% Total Internal discolouration	% Internal Discolouration Mother Bulb	% Internal Discolouration Daughter Bulb	% Internal Discolouration Daughter Bulb	Position of Internal Discolouration (1=Yes)		
									Upper	Lower	Side Only
2	FAM 30	1	0	0	0				0	0	0
		2	0	0	1				1	0	0
		3	0	0	2				0	0	1
		4	0	0	4				0	0	1
		5	0	0	1				0	0	1
		6	0	0	7				0	0	1
		7	0	0	0				0	0	0
		8	0	0	5				0	1	0
		9	0	0	5				0	0	1
		10	0	0	0				0	0	0
		11	0	0	2				0	0	1
		12	0	0	5				0	0	1
		13	0	0	2				0	0	1
		14	0	0	9				0	0	1
		15	0	0	20				1	0	0
		16	0	0	0	0	0		0	0	0
		17	0	0	1				1	0	0
		18	0	0	10				0	0	1
		19	0	0	3				0	0	1
		20	0	0	15				0	0	1
		21	0	0	3	0	10	5	0	0	1
		22	0	0	6	10	3		0	1	0
		23	0	0	2	0	4		0	0	1
		24	0	0	2	3	1		0	0	1
		25	0	0	3	0	6		0	0	1

Plot	Treatment	Bulb Number	Softness (1=Yes)	External Fungal Growth (1=Yes)	% Total Internal discolouration	% Internal Discolouration Mother Bulb	% Internal Discolouration Daughter Bulb	% Internal Discolouration Daughter Bulb	Position of Internal Discolouration (1=Yes)		
									Upper	Lower	Side Only
3	Not dipped	1	0	0	7				0	0	1
		2	1	1	90				1	1	0
		3	0	0	35				1	1	0
		4	1	0	95				1	1	0
		5	0	0	5				0	0	1
		6	0	0	10				0	0	1
		7	0	0	35				1	1	0
		8	0	0	30				1	1	0
		9	1	1	96				1	1	0
		10	0	0	5				0	0	1
		11	0	0	2				0	0	1
		12	0	0	35				1	1	0
		13	0	0	20				0	0	1
		14	0	0	10				0	0	1
		15	1	0	60				1	1	0
		16	0	0	55				1	1	0
		17	0	0	5				0	0	1
		18	1	0	75				1	1	0
		19	0	0	30				1	1	0
		20	0	0	45				1	1	0
		21	0	1	30	20	40		1	1	0
		22	0	0	1	1	1		0	0	1
		23	0	0	4	0	7		0	0	1
		24	0	0	4	0	7		0	0	1
		25	0	0	2	2	3		0	0	1

Plot	Treatment	Bulb Number	Softness (1=Yes)	External Fungal Growth (1=Yes)	% Total Internal discolouration	% Internal Discolouration Mother Bulb	% Internal Discolouration Daughter Bulb	% Internal Discolouration Daughter Bulb	Position of Internal Discolouration (1=Yes)		
									Upper	Lower	Side Only
4	FAM 30	1	0	0	2				1	0	0
		2	1	1	65				1	1	0
		3	0	0	7				1	0	0
		4	0	0	0				0	0	0
		5	0	0	7				0	0	1
		6	0	0	1				0	0	1
		7	0	0	4				1	0	0
		8	0	0	1				0	0	1
		9	0	0	3				0	0	1
		10	0	0	5				1	0	0
		11	0	0	0				0	0	0
		12	0	0	2				0	0	1
		13	0	0	2				0	0	1
		14	0	0	3				0	0	1
		15	0	0	0				0	0	0
		16	0	0	4				0	0	1
		17	0	0	2				0	0	1
		18	0	0	50				0	1	0
		19	0	0	3				0	0	1
		20	0	0	3				0	0	1
		21	0	0	1	0	5		0	0	1
		22	0	0	1	0	0	5	0	0	1
		23	0	0	0	0	0		0	0	0
		24	0	0	0.2	0	0	1	0	0	1
		25	0	0	1	1	1		0	0	1



Plot	Treatment	Bulb Number	Softness (1=Yes)	External Fungal Growth (1=Yes)	% Total Internal discolouration	% Internal Discolouration Mother Bulb	% Internal Discolouration Daughter Bulb	% Internal Discolouration Daughter Bulb	Position of Internal Discolouration (1=Yes)		
									Upper	Lower	Side Only
5	Not dipped	1	0	0	2				0	0	1
		2	0	0	25				1	1	0
		3	0	0	20				0	1	0
		4	0	0	2				0	0	1
		5	0	0	1				0	0	1
		6	0	0	40				1	1	0
		7	0	0	0				0	0	0
		8	0	0	55				0	1	0
		9	0	0	2				0	0	1
		10	0	0	7				0	1	0
		11	0	0	0				0	0	0
		12	0	0	2				0	0	1
		13	0	0	2				0	0	1
		14	0	0	60				1	1	0
		15	0	0	4				0	0	1
		16	0	0	1				0	0	1
		17	0	0	3				0	0	1
		18	0	0	20				1	1	0
		19	1	0	90				1	1	0
		20	0	0	10				0	0	1
		21	0	0	2	1	4		1	0	0
		22	0	0	22	2	40		1	1	0
		23	1	0	12	15	2		1	1	0
		24	0	0	0.5	1	0.5		0	1	0
		25	0	0	0	0	0		0	0	0

Plot	Treatment	Bulb Number	Softness (1=Yes)	External Fungal Growth (1=Yes)	% Total Internal discolouration	% Internal Discolouration Mother Bulb	% Internal Discolouration Daughter Bulb	% Internal Discolouration Daughter Bulb	Position of Internal Discolouration (1=Yes)		
									Upper	Lower	Side Only
6	FAM 30	1	0	0	0				0	0	0
		2	0	0	0				0	0	0
		3	0	0	2				0	0	1
		4	0	0	4				0	0	1
		5	0	0	0				0	0	0
		6	0	0	0				0	0	0
		7	0	0	4				0	0	1
		8	0	0	6				0	0	1
		9	0	0	6				0	0	1
		10	0	0	4				0	0	1
		11	0	0	1				0	0	1
		12	0	0	3				0	0	1
		13	0	0	0				0	0	0
		14	0	0	10				0	0	1
		15	0	0	5				1	0	0
		16	0	0	7				0	1	0
		17	0	0	7				0	0	1
		18	0	0	1				0	0	1
		19	0	0	4				0	1	0
		20	0	0	0				0	0	0
		21	0	0	2	5	0		0	0	1
		22	0	0	3	3	3		0	0	1
		23	0	0	1	1	1		0	0	1
		24	0	0	2	2	1		0	0	1
		25	0	0	0.5	0	1		0	0	1

Plot	Treatment	Bulb Number	Softness (1=Yes)	External Fungal Growth (1=Yes)	% Total Internal discolouration	% Internal Discolouration Mother Bulb	% Internal Discolouration Daughter Bulb	% Internal Discolouration Daughter Bulb	Position of Internal Discolouration (1=Yes)		
									Upper	Lower	Side Only
7	Not dipped	1	0	0	1				0	0	1
		2	1	0	90				1	1	0
		3	0	0	1				0	0	1
		4	1	1	95				1	1	0
		5	1	1	90				1	1	0
		6	0	0	30				1	1	0
		7	0	0	12				0	0	1
		8	0	0	0				0	0	0
		9	0	0	30				0	1	0
		10	0	0	40				1	1	0
		11	0	0	25				1	1	0
		12	0	0	65				1	1	0
		13	0	0	30				1	1	0
		14	0	0	85				1	1	0
		15	0	0	0				0	0	0
		16	0	0	45				1	1	0
		17	0	0	0				0	0	0
		18	0	0	2				0	0	1
		19	0	0	50				1	1	0
		20	1	0	0				0	0	0
		21	0	0	50	50	50		1	1	0
		22	0	0	10	20	5		1	1	0
		23	0	0	20	30	2		1	1	0
		24	0	0	0	0	0		0	0	0
		25	0	0	35	20	60		1	1	0

Plot	Treatment	Bulb Number	Softness (1=Yes)	External Fungal Growth (1=Yes)	% Total Internal discolouration	% Internal Discolouration Mother Bulb	% Internal Discolouration Daughter Bulb	% Internal Discolouration Daughter Bulb	Position of Internal Discolouration (1=Yes)		
									Upper	Lower	Side Only
8	FAM 30	1	0	0	5				0	0	1
		2	0	0	4				0	0	1
		3	0	0	0				0	0	0
		4	0	0	5				1	0	0
		5	0	0	2				0	0	1
		6	0	0	0				0	0	0
		7	0	0	0				0	0	0
		8	1	0	0				0	0	0
		9	0	0	5				0	0	1
		10	0	0	0				0	0	0
		11	0	0	1				1	0	0
		12	0	0	2				1	0	0
		13	0	0	0				0	0	0
		14	0	0	0				0	0	0
		15	0	0	7				0	0	1
		16	0	0	1				0	0	1
		17	0	0	2				0	0	1
		18	0	0	1				0	0	1
		19	0	0	0				0	0	0
		20	0	0	8				0	0	1
		21	0	0	1	0	2		0	0	1
		22	0	0	1	0	2		0	0	1
		23	0	0	1	2	1		0	0	1
		24	0	0	0	0	0		0	0	0
		25	0	0	10	15	2		1	1	0

Plot	Treatment	Bulb Number	Softness (1=Yes)	External Fungal Growth (1=Yes)	% Total Internal discolouration	% Internal Discolouration Mother Bulb	% Internal Discolouration Daughter Bulb	% Internal Discolouration Daughter Bulb	Position of Internal Discolouration (1=Yes)		
									Upper	Lower	Side Only
9	Not dipped	1	0	0	0				0	0	0
		2	0	0	0				0	0	0
		3	0	0	2				0	0	1
		4	0	0	1				0	0	1
		5	0	0	1				0	0	1
		6	0	0	50				1	1	0
		7	0	0	50				1	1	0
		8	0	0	25				1	1	0
		9	1	0	90				1	1	0
		10	0	0	0				0	0	0
		11	0	0	2				0	0	1
		12	0	0	2				0	0	1
		13	1	0	85				1	1	0
		14	0	0	2				0	0	1
		15	1	1	65				1	1	0
		16	0	0	2				0	0	1
		17	0	1	40				1	1	0
		18	0	0	2				0	0	1
		19	0	0	20				1	1	0
		20	0	0	10				0	0	1
		21	0	0	15	0	35		1	1	0
		22	0	0	30	40	10		1	1	0
		23	0	0	1	2	0		0	0	1
		24	1	0	25	15	90		1	1	0
		25	0	0	2	3	2		0	0	1

Plot	Treatment	Bulb Number	Softness (1=Yes)	External Fungal Growth (1=Yes)	% Total Internal discolouration	% Internal Discolouration Mother Bulb	% Internal Discolouration Daughter Bulb	% Internal Discolouration Daughter Bulb	Position of Internal Discolouration (1=Yes)		
									Upper	Lower	Side Only
10	FAM 30	1	0	0	2				0	0	1
		2	0	0	5				0	0	1
		3	0	0	3				0	0	1
		4	0	0	0				0	0	0
		5	0	0	2				0	0	1
		6	0	0	0				0	0	0
		7	0	0	0				0	0	0
		8	0	0	2				0	0	1
		9	0	0	10				0	0	1
		10	0	0	0				0	0	0
		11	0	0	1				0	0	1
		12	0	0	7				1	0	0
		13	0	0	9				0	0	1
		14	0	0	2				0	1	0
		15	0	0	0				0	0	0
		16	0	0	2				0	0	1
		17	0	0	0				0	0	0
		18	1	0	80				1	1	0
		19	0	0	3				0	0	1
		20	0	0	0				0	0	0
		21	0	0	7	0	15		0	0	1
		22	1	1	55	50	60		1	1	0
		23	0	0	0	0	0		0	0	0
		24	0	0	1	0	3		0	0	1
		25	0	0	1	0	2		0	0	1

Plot	Treatment	Bulb Number	Softness (1=Yes)	External Fungal Growth (1=Yes)	% Total Internal discolouration	% Internal Discolouration Mother Bulb	% Internal Discolouration Daughter Bulb	% Internal Discolouration Daughter Bulb	Position of Internal Discolouration (1=Yes)		
									Upper	Lower	Side Only
11	Not dipped	1	1	0	95				1	1	0
		2	0	0	95				1	1	0
		3	0	0	2				0	0	1
		4	0	0	30				0	1	0
		5	0	0	2				0	0	1
		6	0	0	90				1	1	0
		7	0	0	20				1	1	0
		8	0	0	20				1	1	0
		9	0	0	5				0	0	1
		10	0	0	0				0	0	0
		11	0	0	7				0	0	1
		12	0	0	3				0	0	1
		13	0	0	3				0	0	1
		14	0	0	0				0	0	0
		15	0	0	25				1	1	0
		16	0	0	65				1	1	0
		17	1	0	50				1	1	0
		18	0	0	10				0	1	0
		19	0	0	4				0	0	1
		20	0	0	15				1	1	0
		21	1	1	80	90	50		1	1	0
		22	0	1	20	5	40		1	1	0
		23	0	0	12	20	0		1	1	0
		24	0	0	15	20	7		1	1	0
		25	1	1	60	65	95		1	1	0

Plot	Treatment	Bulb Number	Softness (1=Yes)	External Fungal Growth (1=Yes)	% Total Internal discolouration	% Internal Discolouration Mother Bulb	% Internal Discolouration Daughter Bulb	% Internal Discolouration Daughter Bulb	Position of Internal Discolouration (1=Yes)		
									Upper	Lower	Side Only
12	FAM 30	1	0	0	0				0	0	0
		2	0	0	0				0	0	0
		3	0	0	0				0	0	0
		4	0	0	0				0	0	0
		5	0	0	5				0	0	1
		6	0	0	15				1	1	0
		7	0	0	1				1	0	0
		8	0	0	7				0	0	1
		9	0	0	2				1	1	0
		10	0	0	5				0	0	1
		11	0	0	5				0	0	1
		12	0	0	7				1	1	0
		13	0	0	1				0	0	1
		14	0	0	3				1	0	0
		15	0	0	2				0	0	1
		16	0	0	1				0	0	1
		17	0	0	4				1	0	0
		18	0	0	0				0	0	0
		19	0	0	5				0	0	1
		20	0	0	2				0	0	1
		21	0	0	0	0	0		0	0	0
		22	0	0	0	0	0		0	0	0
		23	0	0	1	2	0		0	0	1
		24	0	0	8	10	1		1	0	0
		25	0	0	0.5	0	0	1	0	0	1



Plot	Treatment	Bulb Number	Softness (1=Yes)	External Fungal Growth (1=Yes)	% Total Internal discolouration	% Internal Discolouration Mother Bulb	% Internal Discolouration Daughter Bulb	% Internal Discolouration Daughter Bulb	Position of Internal Discolouration (1=Yes)		
									Upper	Lower	Side Only
13	Not dipped	1	0	0	0				0	0	0
		2	1	0	55				1	1	0
		3	0	0	35				1	1	0
		4	0	0	0				0	0	0
		5	0	0	30				1	1	0
		6	1	1	80				1	1	0
		7	0	0	0				0	0	0
		8	0	0	1				0	0	1
		9	0	0	0				0	0	0
		10	0	0	7				0	0	1
		11	0	0	0				0	0	0
		12	0	0	3				0	0	1
		13	1	0	65				1	1	0
		14	1	0	85				1	1	0
		15	1	0	90				1	1	0
		16	1	0	70				1	1	0
		17	1	0	50				0	1	0
		18	1	1	70				1	1	0
		19	0	0	1				0	0	1
		20	0	0	30				1	1	0
		21	1	0	45	45	45		1	1	0
		22	0	0	2	4	1		0	0	1
		23	0	0	1	2	0		0	0	1
		24	0	0	2	0	3		0	0	1
		25	1	1	25	0	95		1	1	0

Plot	Treatment	Bulb Number	Softness (1=Yes)	External Fungal Growth (1=Yes)	% Total Internal discolouration	% Internal Discolouration Mother Bulb	% Internal Discolouration Daughter Bulb	% Internal Discolouration Daughter Bulb	Position of Internal Discolouration (1=Yes)		
									Upper	Lower	Side Only
14	FAM 30	1	0	0	2				0	0	1
		2	0	0	4				0	0	1
		3	0	0	2				0	0	1
		4	0	0	1				0	0	1
		5	0	0	0				0	0	0
		6	0	0	0				0	0	0
		7	0	0	0				0	0	0
		8	0	0	0				0	0	0
		9	0	0	1				0	0	1
		10	0	0	4				0	0	1
		11	0	0	2				0	0	1
		12	0	0	50				1	0	0
		13	0	0	0				0	0	0
		14	0	0	0				0	0	0
		15	0	0	1				0	0	1
		16	0	0	1				0	1	0
		17	0	0	25				0	1	0
		18	0	0	0				0	0	0
		19	1	0	95				1	1	0
		20	1	0	3				0	0	1
		21	0	0	1	1	2		0	0	1
		22	0	0	1	0	1		0	0	1
		23	0	0	1	2	1		0	0	1
		24	0	0	0	0	0		0	0	0
		25	0	0	1	2	0		0	0	1

Plot	Treatment	Bulb Number	Softness (1=Yes)	External Fungal Growth (1=Yes)	% Total Internal discolouration	% Internal Discolouration Mother Bulb	% Internal Discolouration Daughter Bulb	% Internal Discolouration Daughter Bulb	Position of Internal Discolouration (1=Yes)		
									Upper	Lower	Side Only
15	Not dipped	1	1	0	0				0	0	0
		2	1	0	50				1	1	0
		3	0	0	0				0	0	0
		4	0	0	5				0	0	1
		5	1	0	35				1	1	0
		6	0	0	3				0	0	1
		7	0	0	30				0	1	0
		8	0	0	7				0	0	1
		9	0	0	5				0	0	1
		10	0	0	20				1	1	0
		11	0	0	25				1	1	0
		12	0	0	25				1	1	0
		13	0	0	20				0	1	0
		14	0	0	7				0	0	1
		15	0	0	0				0	0	0
		16	0	0	10				0	1	0
		17	0	0	20				1	1	0
		18	1	0	95				1	1	0
		19	0	0	20				1	1	0
		20	0	0	30				1	1	0
		21	0	0	2	0	5		0	0	1
		22	0	0	0	0	0		0	0	0
		23	0	0	15	20	3		1	1	0
		24	0	0	2	0	5		0	0	1
		25	0	0	30	45	0		1	1	0

Plot	Treatment	Bulb Number	Softness (1=Yes)	External Fungal Growth (1=Yes)	% Total Internal discolouration	% Internal Discolouration Mother Bulb	% Internal Discolouration Daughter Bulb	% Internal Discolouration Daughter Bulb	Position of Internal Discolouration (1=Yes)		
									Upper	Lower	Side Only
16	FAM 30	1	0	0	0				0	0	0
		2	0	0	0				0	0	0
		3	0	0	10				1	0	0
		4	0	0	0				0	0	0
		5	0	0	0				0	0	0
		6	0	0	0				0	0	0
		7	0	0	5				0	0	1
		8	1	1	90				1	1	0
		9	0	0	0				0	0	0
		10	0	0	5				0	0	1
		11	1	0	55				1	1	0
		12	0	0	0				0	0	0
		13	0	0	5				0	0	1
		14	0	0	2				0	0	1
		15	0	0	2				0	0	1
		16	0	0	2				0	0	1
		17	0	0	0				0	0	0
		18	0	0	7				0	0	1
		19	0	0	0				0	0	0
		20	1	0	80				1	1	0
		21	0	0	2	0	5		0	0	1
		22	0	0	0	0	0		0	0	0
		23	0	0	0	0	0		0	0	0
		24	0	0	0	0	0		0	0	0
		25	0	0	7	10	5		1	0	0

**Appendix 2:** Fusarium coverage inside incubated bulbs after 9 weeks from harvest, and presence (=) or absence (0) fungal growth

Plot	Treatment	Bulb	Internal Pink Fusarium					Internal White Fusarium					Penicillium	Botrytis	Other
			Total % Cover	Neck	Mid	Base	On Daughter	Total % Cover	Neck	Mid	Base	On Daughter			
1	Not dipped	1	0	0	0	0	0	5	0	1	0	0	0	0	0
		2	0	0	0	0	0	0	0	0	0	0	0	0	0
		3	0	0	0	0	0	55	1	1	1	0	0	0	0
		4	0	0	0	0	0	10	0	1	1	1	0	0	0
		5	80	0	1	1	0	0	0	0	0	0	0	0	0
		6	0	0	0	0	0	0	0	0	0	0	1	0	0
		7	0	0	0	0	0	5	0	1	0	0	0	0	0
		8	10	0	1	0	0	0	0	0	0	0	0	0	0
		9	75	1	1	1	0	0	0	0	0	0	0	0	0
		10	0	0	0	0	0	20	0	1	1	0	0	0	0
		11	90	1	1	1	0	0	0	0	0	0	0	0	0
		12	0	0	0	0	0	5	0	1	0	0	0	0	0
		13	0	0	0	0	0	2	0	1	0	0	0	0	0
		14	70	0	1	1	0	0	0	0	0	0	0	0	0
		15	40	1	1	1	0	0	0	0	0	0	0	0	0
		16	80	1	1	1	0	0	0	0	0	0	0	0	0
		17	90	1	1	1	0	0	0	0	0	0	0	0	0
		18	0	0	0	0	0	20	0	1	0	0	0	0	0
		19	100	1	1	1	0	0	0	0	0	0	0	0	0
		20	0	0	0	0	0	20	0	1	1	0	0	0	0
		21	0	0	0	0	0	0	0	0	0	0	0	0	0
		22	95	1	1	1	1	0	0	0	0	0	0	0	0
		23	65	1	1	1	1	0	0	0	0	0	0	0	0
		24	45	1	1	1	1	0	0	0	0	0	0	0	0
		25	25	0	0	0	1	0	0	0	0	0	0	0	0

Plot	Treatment	Bulb	Internal Pink Fusarium					Internal White Fusarium					Penicillium	Botrytis	Other
			Total % Cover	Neck	Mid	Base	On Daughter	Total % Cover	Neck	Mid	Base	On Daughter			
2	FAM 30	1	0	0	0	0	0	2	1	0	0	0	0	0	0
		2	0	0	0	0	0	5	1	1	0	0	0	0	0
		3	0	0	0	0	0	5	1	1	0	0	0	0	0
		4	0	0	0	0	0	0	0	0	0	0	0	0	0
		5	0	0	0	0	0	2	1	0	0	0	1	0	0
		6	0	0	0	0	0	5	0	1	0	0	0	0	0
		7	0	0	0	0	0	7	0	0	1	0	0	0	0
		8	0	0	0	0	0	7	0	1	1	0	0	0	0
		9	0	0	0	0	0	70	1	1	1	0	0	0	0
		10	0	0	0	0	0	0	0	0	0	0	0	0	0
		11	0	0	0	0	0	0	0	0	0	0	1	0	0
		12	0	0	0	0	0	0	0	0	0	0	0	1	0
		13	0	0	0	0	0	0	0	0	0	0	1	0	0
		14	0	0	0	0	0	1	1	0	0	0	0	0	0
		15	0	0	0	0	0	0	0	0	0	0	1	0	0
		16	0	0	0	0	0	0	0	0	0	0	1	0	0
		17	0	0	0	0	0	7	0	1	0	0	1	0	0
		18	0	0	0	0	0	0	0	0	0	0	1	0	0
		19	0	0	0	0	0	2	0	0	1	0	1	0	0
		20	0	0	0	0	0	0	0	0	0	0	1	0	0
		21	0	0	0	0	0	0	0	0	0	0	1	0	0
		22	0	0	0	0	0	30	0	1	1	1	0	0	0
		23	0	0	0	0	0	5	0	0	0	1	1	0	0
		24	0	0	0	0	0	0	0	0	0	0	0	0	0
		25	0	0	0	0	0	2	0	0	0	1	0	0	0

Plot	Treat- ment	Bulb	Internal Pink Fusarium					Internal White Fusarium					Penicil- lium	Botrytis	Other
			Total % Cover	Neck	Mid	Base	On Daugh- -ter	Total % Cover	Neck	Mid	Base	On Daugh- -ter			
3	Not dipped	1	0	0	0	0	0	20	0	1	0	0	0	0	0
		2	55	0	1	1	0	0	0	0	0	0	0	0	0
		3	100	1	1	1	0	0	0	0	0	0	0	0	0
		4	100	1	1	1	0	0	0	0	0	0	0	0	0
		5	20	0	1	1	0	0	0	0	0	0	0	0	0
		6	0	0	0	0	0	0	0	0	0	0	0	0	0
		7	95	1	1	1	0	0	0	0	0	0	0	0	0
		8	60	1	0	1	1	0	0	0	0	0	0	0	0
		9	100	1	1	1	0	0	0	0	0	0	0	0	0
		10	40	0	1	1	0	0	0	0	0	0	0	0	0
		11	15	0	0	1	0	0	0	0	0	0	0	0	0
		12	80	1	1	1	0	0	0	0	0	0	0	0	0
		13	45	0	1	1	0	0	0	0	0	0	0	0	0
		14	0	0	0	0	0	15	0	1	0	0	0	0	0
		15	75	1	1	1	0	0	0	0	0	0	0	0	0
		16	70	1	1	1	0	0	0	0	0	0	0	0	0
		17	15	0	1	0	0	0	0	0	0	0	0	0	0
		18	90	1	1	1	0	0	0	0	0	0	0	0	0
		19	100	1	1	1	0	0	0	0	0	0	0	0	0
		20	80	1	1	1	0	0	0	0	0	0	0	0	0
		21	80	1	1	1	1	0	0	0	0	0	0	0	0
		22	0	0	0	0	0	20	0	1	1	1	0	0	0
		23	0	0	0	0	0	15	0	1	1	0	0	0	0
		24	0	0	0	0	0	10	0	1	0	0	1	0	0
		25	0	0	0	0	0	30	0	1	0	0	0	0	0

Plot	Treat- ment	Bulb	Internal Pink Fusarium					Internal White Fusarium					Penicil- lium	Botrytis	Other
			Total % Cover	Neck	Mid	Base	On Daugh- -ter	Total % Cover	Neck	Mid	Base	On Daugh- -ter			
4	FAM 30	1	0	0	0	0	0	15	1	0	1	0	0	0	0
		2	100	1	1	1	0	0	0	0	0	0	0	0	0
		3	0	0	0	0	0	40	0	1	0	0	1	0	0
		4	0	0	0	0	0	0	0	0	0	0	0	0	0
		5	0	0	0	0	0	10	0	1	0	0	1	0	0
		6	0	0	0	0	0	0	0	0	0	0	1	0	0
		7	0	0	0	0	0	5	0	1	0	0	1	0	0
		8	0	0	0	0	0	5	0	0	1	0	1	0	0
		9	0	0	0	0	0	10	0	0	1	0	1	0	0
		10	0	0	0	0	0	10	0	0	1	0	1	0	0
		11	0	0	0	0	0	20	0	1	0	0	0	0	0
		12	0	0	0	0	0	0	0	0	0	0	1	0	0
		13	0	0	0	0	0	0	0	0	0	0	1	0	0
		14	0	0	0	0	0	5	0	0	1	0	1	0	0
		15	0	0	0	0	0	0	0	0	0	0	0	0	0
		16	0	0	0	0	0	0	0	0	1	0	0	0	0
		17	0	0	0	0	0	7	0	0	1	0	0	0	0
		18	85	1	1	1	0	0	0	0	0	0	0	0	0
		19	0	0	0	0	0	60	1	1	1	0	0	0	0
		20	0	0	0	0	0	20	0	1	0	0	0	0	0
		21	0	0	0	0	0	0	0	0	0	0	0	0	0
		22	0	0	0	0	0	5	0	0	1	0	0	0	0
		23	0	0	0	0	0	0	0	0	0	0	1	0	0
		24	0	0	0	0	0	30	0	1	1	0	1	0	0
		25	0	0	0	0	0	7	0	0	1	0	0	0	0



Plot	Treat- ment	Bulb	Internal Pink Fusarium					Internal White Fusarium					Penicil- lium	Botrytis	Other
			Total % Cover	Neck	Mid	Base	On Daugh- -ter	Total % Cover	Neck	Mid	Base	On Daugh- -ter			
5	Not dipped	1	0	0	0	0	0	0	0	0	0	0	0	0	0
		2	65	0	1	1	0	0	0	0	0	0	0	0	0
		3	70	0	1	1	0	0	0	0	0	0	0	0	0
		4	0	0	0	0	0	15	0	1	1	0	0	0	0
		5	0	0	0	0	0	0	0	0	0	0	0	0	0
		6	95	1	1	1	0	0	0	0	0	0	0	0	0
		7	0	0	0	0	0	10	0	1	0	0	0	0	0
		8	90	1	1	1	0	0	0	0	0	0	0	0	0
		9	0	0	0	0	0	0	0	0	0	0	0	0	0
		10	60	0	1	1	0	0	0	0	0	0	0	0	0
		11	0	0	0	0	0	55	0	1	0	0	0	0	0
		12	0	0	0	0	0	0	0	0	0	0	0	0	0
		13	0	0	0	0	0	10	0	1	0	0	0	0	0
		14	100	1	1	1	0	0	0	0	0	0	0	0	0
		15	0	0	0	0	0	25	1	1	0	0	0	0	0
		16	0	0	0	0	0	10	0	0	1	0	0	0	0
		17	0	0	0	0	0	2	0	0	1	0	0	0	0
		18	100	1	1	1	0	0	0	0	0	0	0	0	0
		19	95	1	1	1	0	0	0	0	0	0	0	0	0
		20	0	0	0	0	0	30	0	1	0	0	0	0	0
		21	0	0	0	0	0	20	0	0	0	1	0	0	0
		22	35	0	0	0	1	0	0	0	0	0	1	0	0
		23	30	1	1	1	0	0	0	0	0	0	0	0	0
		24	0	0	0	0	0	0	0	0	0	0	1	0	0
		25	0	0	0	0	0	5	0	1	0	1	0	0	0

Plot	Treat- ment	Bulb	Internal Pink Fusarium					Internal White Fusarium					Penicil- lium	Botrytis	Other
			Total % Cover	Neck	Mid	Base	On Daugh- -ter	Total % Cover	Neck	Mid	Base	On Daugh- -ter			
6	FAM 30	1	0	0	0	0	0	0	0	0	0	0	0	0	0
		2	0	0	0	0	0	0	0	0	0	0	1	0	0
		3	0	0	0	0	0	0	0	0	0	0	1	0	0
		4	0	0	0	0	0	0	0	0	0	0	1	0	0
		5	0	0	0	0	0	0	0	0	0	0	1	0	0
		6	0	0	0	0	0	20	0	0	1	0	1	0	0
		7	0	0	0	0	0	10	0	1	0	0	0	0	0
		8	0	0	0	0	0	2	0	1	0	0	0	0	0
		9	0	0	0	0	0	0	0	0	0	0	1	0	0
		10	0	0	0	0	0	0	0	0	0	0	0	0	0
		11	0	0	0	0	0	10	0	0	1	0	1	0	0
		12	0	0	0	0	0	15	0	1	0	0	1	0	0
		13	0	0	0	0	0	0	0	0	0	0	1	0	0
		14	0	0	0	0	0	25	0	1	1	0	0	0	0
		15	0	0	0	0	0	0	0	0	0	0	1	0	0
		16	0	0	0	0	0	2	0	0	1	0	1	0	0
		17	0	0	0	0	0	5	0	0	1	0	0	0	0
		18	0	0	0	0	0	0	0	0	0	0	1	0	0
		19	0	0	0	0	0	0	0	0	0	0	1	0	0
		20	0	0	0	0	0	25	0	0	1	0	0	0	0
		21	0	0	0	0	0	5	0	0	0	1	0	0	0
		22	0	0	0	0	0	0	0	0	0	0	1	0	0
		23	0	0	0	0	0	0	0	0	0	0	0	0	0
		24	0	0	0	0	0	2	0	1	0	0	1	0	0
		25	0	0	0	0	0	20	0	1	0	0	1	0	0

Plot	Treat- ment	Bulb	Internal Pink Fusarium					Internal White Fusarium					Penicil- lium	Botrytis	Other
			Total % Cover	Neck	Mid	Base	On Daugh- -ter	Total % Cover	Neck	Mid	Base	On Daugh- -ter			
7	Not dipped	1	0	0	0	0	0	10	0	1	0	0	0	0	0
		2	100	1	1	1	0	0	0	0	0	0	0	0	0
		3	0	0	0	0	0	35	0	1	1	0	0	0	0
		4	95	1	1	1	0	0	0	0	0	0	0	0	0
		5	100	1	1	1	0	0	0	0	0	0	0	0	0
		6	75	1	1	1	0	0	0	0	0	0	0	0	0
		7	0	0	0	0	0	60	0	1	1	0	0	0	0
		8	0	0	0	0	0	10	0	0	1	0	0	0	0
		9	70	0	1	1	0	0	0	0	0	0	0	0	0
		10	100	1	1	1	0	0	0	0	0	0	0	0	0
		11	95	1	1	1	0	0	0	0	0	0	0	0	0
		12	100	1	1	1	0	0	0	0	0	0	0	0	0
		13	100	1	1	1	0	0	0	0	0	0	0	0	0
		14	95	1	1	1	0	0	0	0	0	0	0	0	0
		15	0	0	0	0	0	25	0	1	0	0	0	0	0
		16	100	1	1	1	0	0	0	0	0	0	0	0	0
		17	0	0	0	0	0	10	0	1	0	0	0	0	0
		18	0	0	0	0	0	5	0	0	1	0	0	0	0
		19	100	1	1	1	0	0	0	0	0	0	0	0	0
		20	0	0	0	0	0	30	0	1	1	0	0	0	0
		21	95	1	1	1	1	0	0	0	0	0	0	0	0
		22	75	1	1	1	0	0	0	0	0	0	0	0	0
		23	80	1	1	1	1	0	0	0	0	0	0	0	0
		24	60	1	1	1	0	0	0	0	0	0	0	0	0
		25	100	1	1	1	1	0	0	0	0	0	0	0	0

Plot	Treatment	Bulb	Internal Pink Fusarium					Internal White Fusarium					Penicillium	Botrytis	Other
			Total % Cover	Neck	Mid	Base	On Daughter	Total % Cover	Neck	Mid	Base	On Daughter			
8	FAM 30	1	0	0	0	0	0	0	0	0	0	0	1	0	0
		2	0	0	0	0	0	0	0	0	0	0	1	0	0
		0	0	0	0	0	0	0	0	0	0	0	0	0	0
		4	0	0	0	0	0	0	0	0	0	0	1	0	0
		5	0	0	0	0	0	5	1	0	0	0	0	0	0
		6	0	0	0	0	0	0	0	0	0	0	1	0	0
		7	0	0	0	0	0	0	0	0	0	0	0	1	0
		8	0	0	0	0	0	0	0	0	0	0	1	0	0
		9	0	0	0	0	0	15	0	0	1	0	0	0	0
		10	0	0	0	0	0	15	0	0	1	0	1	0	0
		11	0	0	0	0	0	0	0	0	0	0	1	1	0
		12	0	0	0	0	0	2	1	0	0	0	0	0	0
		13	0	0	0	0	0	0	0	0	0	0	1	0	0
		14	0	0	0	0	0	15	0	0	1	0	1	0	0
		15	0	0	0	0	0	30	0	1	1	0	0	0	0
		16	0	0	0	0	0	0	0	0	0	0	1	0	0
		17	0	0	0	0	0	0	0	0	0	0	1	0	0
		18	0	0	0	0	0	0	0	0	0	0	1	0	0
		19	0	0	0	0	0	10	0	0	1	0	0	0	0
		20	0	0	0	0	0	30	0	1	1	0	1	0	0
		21	0	0	0	0	0	0	0	0	0	0	1	1	0
		22	0	0	0	0	0	25	0	1	1	0	0	0	0
		23	0	0	0	0	0	0	0	0	0	0	1	0	0
		24	0	0	0	0	0	0	0	0	0	0	1	0	0
		25	70	1	1	1	0	0	0	0	0	0	1	0	0

Plot	Treat- ment	Bulb	Internal Pink Fusarium					Internal White Fusarium					Penicil- lium	Botrytis	Other
			Total % Cover	Neck	Mid	Base	On Daugh- -ter	Total % Cover	Neck	Mid	Base	On Daugh- -ter			
9	Not dipped	1	0	0	0	0	0	0	0	0	0	0	1	0	0
		2	0	0	0	0	0	0	0	0	0	0	1	0	0
		3	0	0	0	0	0	5	0	0	1	0	0	0	0
		4	0	0	0	0	0	2	0	0	1	0	0	0	0
		5	0	0	0	0	0	0	0	0	0	0	0	0	0
		6	100	1	1	1	0	0	0	0	0	0	0	0	0
		7	95	1	1	1	0	0	0	0	0	0	0	0	0
		8	100	1	1	1	0	0	0	0	0	0	0	0	0
		9	100	1	1	1	0	0	0	0	0	0	0	0	0
		10	0	0	0	0	0	40	0	1	1	0	0	0	0
		11	0	0	0	0	0	0	0	0	0	0	1	0	0
		12	0	0	0	0	0	0	0	0	0	0	0	1	0
		13	95	1	1	1	0	0	0	0	0	0	0	0	0
		14	0	0	0	0	0	0	0	0	0	0	0	0	0
		15	100	1	1	1	0	0	0	0	0	0	0	0	0
		16	45	0	1	0	0	0	0	0	0	0	0	0	0
		17	100	1	1	1	0	0	0	0	0	0	0	0	0
		18	0	0	0	0	0	0	0	0	0	0	0	1	0
		19	65	0	1	1	0	0	0	0	0	0	0	0	0
		20	20	0	1	1	0	0	0	0	0	0	0	0	0
		21	80	1	1	1	1	0	0	0	0	0	0	0	0
		22	85	1	1	1	1	0	0	0	0	0	0	1	0
		23	20	0	0	0	1	0	0	0	0	0	0	1	0
		24	60	1	1	1	1	0	0	0	0	0	0	0	0
		25	20	0	1	0	1	0	0	0	0	0	1	0	0

Plot	Treat- ment	Bulb	Internal Pink Fusarium					Internal White Fusarium					Penicil- lium	Botrytis	Other
			Total % Cover	Neck	Mid	Base	On Daugh- -ter	Total % Cover	Neck	Mid	Base	On Daugh- -ter			
10	FAM 30	1	0	0	0	0	0	5	0	1	0	0	0	0	0
		2	0	0	0	0	0	2	0	0	1	0	0	0	0
		3	0	0	0	0	0	20	0	1	0	0	0	0	0
		4	0	0	0	0	0	15	0	1	0	0	0	0	0
		5	0	0	0	0	0	0	0	0	0	0	1	0	0
		6	0	0	0	0	0	2	0	1	0	0	1	0	0
		7	0	0	0	0	0	5	0	0	1	0	0	0	0
		8	0	0	0	0	0	0	0	0	0	0	0	0	0
		9	0	0	0	0	0	7	0	0	1	0	1	0	0
		10	0	0	0	0	0	10	0	1	0	0	0	0	0
		11	0	0	0	0	0	0	0	0	0	0	0	0	0
		12	0	0	0	0	0	0	0	0	0	0	1	0	0
		13	0	0	0	0	0	25	0	1	0	0	1	0	0
		14	0	0	0	0	0	10	0	1	0	0	0	0	0
		15	0	0	0	0	0	0	0	0	0	0	1	0	0
		16	0	0	0	0	0	0	0	0	0	0	0	0	0
		17	95	1	1	1	0	0	0	0	0	0	0	0	0
		18	0	0	0	0	0	35	0	1	1	0	0	0	0
		19	0	0	0	0	0	5	0	1	0	0	0	0	0
		20	0	0	0	0	0	0	0	0	0	0	0	0	0
		21	0	0	0	0	0	0	0	0	0	0	0	0	0
		22	90	1	1	1	1	0	0	0	0	0	0	0	0
		23	70	1	1	1	1	0	0	0	0	0	0	0	0
		24	0	0	0	0	0	10	0	0	0	1	0	0	0
		25	0	0	0	0	0	0	0	0	0	0	1	0	0

Plot	Treat- ment	Bulb	Internal Pink Fusarium					Internal White Fusarium					Penicil- lium	Botrytis	Other
			Total % Cover	Neck	Mid	Base	On Daugh- -ter	Total % Cover	Neck	Mid	Base	On Daugh- -ter			
11	Not dipped	1	100	1	1	1	0	0	0	0	0	0	0	0	0
		2	100	1	1	1	0	0	0	0	0	0	0	0	0
		3	0	0	0	0	0	80	0	0	0	0	0	0	0
		4	70	0	1	1	0	0	0	0	0	0	0	0	0
		5	90	0	1	1	0	0	0	0	0	0	0	0	0
		6	90	0	1	1	0	0	0	0	0	0	0	0	0
		7	100	1	1	1	0	0	0	0	0	0	0	0	0
		8	0	0	0	0	0	30	0	1	0	0	0	0	0
		9	0	0	0	0	0	0	0	0	0	0	0	1	0
		10	0	0	0	0	0	0	0	0	0	0	0	0	0
		11	0	0	0	0	0	5	0	1	0	0	0	0	0
		12	0	0	0	0	0	10	1	0	0	0	1	0	0
		13	0	0	0	0	0	0	0	0	0	0	0	0	0
		14	0	0	0	0	0	5	0	1	0	0	0	0	0
		15	50	1	1	1	0	0	0	0	0	0	0	0	0
		16	85	1	1	1	0	0	0	0	0	0	0	0	0
		17	0	0	0	0	0	30	0	1	1	0	0	0	0
		18	0	0	0	0	0	10	0	1	0	0	0	0	0
		19	45	1	1	1	0	0	0	0	0	0	1	0	0
		20	0	0	0	0	0	0	0	0	0	0	0	0	0
		21	95	1	1	1	1	0	0	0	0	0	0	0	0
		22	60	1	1	1	1	0	0	0	0	0	0	0	0
		23	45	1	1	1	0	0	0	0	0	0	0	0	0
		24	75	1	1	1	1	0	0	0	0	0	0	0	0
		25	95	1	1	1	1	0	0	0	0	0	0	0	0

Plot	Treat- ment	Bulb	Internal Pink Fusarium					Internal White Fusarium					Penicil- lium	Botrytis	Other
			Total % Cover	Neck	Mid	Base	On Daugh- -ter	Total % Cover	Neck	Mid	Base	On Daugh- -ter			
12	FAM 30	1	0	0	0	0	0	15	0	1	0	0	0	0	0
		2	0	0	0	0	0	30	0	1	0	0	0	0	0
		3	0	0	0	0	0	25	0	0	0	0	0	0	0
		4	0	0	0	0	0	0	0	0	0	0	0	0	0
		5	0	0	0	0	0	0	0	0	0	0	1	0	0
		6	80	1	1	1	0	0	0	0	0	0	0	0	0
		7	40	0	1	1	0	0	0	0	0	0	0	0	0
		8	5	0	0	0	0	0	0	0	0	0	0	0	0
		9	50	0	1	0	0	0	0	0	0	0	0	0	0
		10	5	0	1	0	0	0	0	0	0	0	0	0	0
		11	0	0	0	0	0	0	0	0	0	0	0	0	0
		12	0	0	0	0	0	2	0	1	0	0	0	0	0
		13	0	0	0	0	0	10	0	1	0	0	0	0	0
		14	0	0	0	0	0	2	0	1	0	0	0	0	0
		15	0	0	0	0	0	5	0	1	0	0	0	0	0
		16	0	0	0	0	0	0	0	0	0	0	0	0	0
		17	0	0	0	0	0	15	0	1	0	0	0	0	0
		18	0	0	0	0	0	0	0	0	0	0	1	0	0
		19	0	0	0	0	0	0	0	0	0	0	1	0	0
		20	0	0	0	0	0	0	0	0	0	0	1	0	0
		21	0	0	0	0	0	0	0	0	0	0	0	0	0
		22	0	0	0	0	0	5	0	1	0	0	1	0	0
		23	0	0	0	0	0	2	0	0	1	1	0	0	0
		24	75	1	1	1	0	5	0	0	0	1	0	0	0
		25	15	0	0	0	1	0	0	0	0	0	0	0	0



Plot	Treat- ment	Bulb	Internal Pink Fusarium					Internal White Fusarium					Penicil- lium	Botrytis	Other
			Total % Cover	Neck	Mid	Base	On Daugh- -ter	Total % Cover	Neck	Mid	Base	On Daugh- -ter			
13	Not dipped	1	0	0	0	0	0	0	0	0	0	0	0	0	0
		2	100	1	1	1	0	0	0	0	0	0	0	0	0
		3	100	1	1	1	0	0	0	0	0	0	0	0	0
		4	0	0	0	0	0	0	0	0	0	0	0	0	0
		5	85	1	1	1	0	0	0	0	0	0	0	0	0
		6	95	1	1	1	0	0	0	0	0	0	0	0	0
		7	0	0	0	0	0	10	0	1	0	0	0	0	0
		8	0	0	0	0	0	0	0	0	0	0	1	0	0
		9	0	0	0	0	0	10	0	1	0	0	0	0	0
		10	0	0	0	0	0	15	0	1	0	0	0	0	0
		11	0	0	0	0	0	0	0	0	0	0	1	0	0
		12	100	1	1	1	0	0	0	0	0	0	0	0	0
		13	0	0	0	0	0	0	0	0	0	0	0	0	0
		14	100	1	1	1	0	0	0	0	0	0	0	0	0
		15	95	0	1	1	0	0	0	0	0	0	0	0	0
		16	100	1	1	1	0	0	0	0	0	0	0	0	0
		17	90	1	1	1	0	0	0	0	0	0	0	0	0
		18	95	0	1	1	0	0	0	0	0	0	0	0	0
		19	0	0	0	0	0	5	1	0	0	0	0	0	0
		20	75	0	1	1	0	0	0	0	0	0	0	0	0
		21	0	0	0	0	0	0	0	0	0	0	1	0	0
		22	10	0	0	0	1	0	0	0	0	0	0	0	0
		23	0	0	0	0	0	0	0	0	0	0	1	0	0
		24	30	0	1	0	1	0	0	0	0	0	0	0	0
		25	0	0	0	0	0	0	0	0	0	0	0	0	0

Plot	Treatment	Bulb	Internal Pink Fusarium					Internal White Fusarium					Penicillium	Botrytis	Other
			Total % Cover	Neck	Mid	Base	On Daughter	Total % Cover	Neck	Mid	Base	On Daughter			
14	FAM 30	1	0	0	0	0	0	0	0	0	0	0	1	0	0
		2	0	0	0	0	0	0	0	0	0	0	1	0	0
		3	0	0	0	0	0	2	1	0	0	0	0	0	0
		4	0	0	0	0	0	2	1	0	0	0	1	0	0
		5	0	0	0	0	0	0	0	0	0	0	0	0	0
		6	0	0	0	0	0	0	0	0	0	0	1	0	0
		7	0	0	0	0	0	0	0	0	0	0	0	0	0
		8	0	0	0	0	0	1	0	1	0	0	0	0	0
		9	0	0	0	0	0	0	0	0	0	0	0	0	0
		10	0	0	0	0	0	5	0	0	1	0	0	0	0
		11	0	0	0	0	0	0	0	0	0	0	0	0	0
		12	55	0	1	1	0	0	0	0	0	0	0	0	0
		13	0	0	0	0	0	0	0	0	0	0	0	0	0
		14	0	0	0	0	0	0	0	0	0	0	0	0	0
		15	0	0	0	0	0	0	0	0	0	0	1	0	0
		16	0	0	0	0	0	0	0	0	0	0	1	0	0
		17	85	1	1	1	0	0	0	0	0	0	0	0	0
		18	0	0	0	0	0	1	1	0	0	0	0	0	0
		19	100	1	1	1	0	0	0	0	0	0	0	0	0
		20	0	0	0	0	0	0	0	0	0	0	0	0	0
		21	0	0	0	0	0	0	0	0	0	0	0	0	0
		22	0	0	0	0	0	5	1	0	0	0	1	0	0
		23	0	0	0	0	0	0	0	0	0	0	0	0	0
		24	0	0	0	0	0	0	0	0	0	0	1	0	0
		25	0	0	0	0	0	0	0	0	0	0	1	0	0

Plot	Treat- ment	Bulb	Internal Pink Fusarium					Internal White Fusarium					Penicil- lium	Botrytis	Other
			Total % Cover	Neck	Mid	Base	On Daugh- -ter	Total % Cover	Neck	Mid	Base	On Daugh- -ter			
15	Not dipped	1	0	0	0	0	0	0	0	0	0	0	1	0	0
		2	100	1	1	1	0	0	0	0	0	0	0	0	0
		3	0	0	0	0	0	60	0	1	1	0	0	0	0
		4	0	0	0	0	0	30	0	1	0	0	1	0	0
		5	97	1	1	1	0	0	0	0	0	0	0	0	0
		6	0	0	0	0	0	35	0	1	1	0	0	0	0
		7	75	0	1	1	0	0	0	0	0	0	0	0	0
		8	0	0	0	0	0	85	1	1	1	0	0	0	0
		9	0	0	0	0	0	0	0	0	0	0	0	0	0
		10	95	1	1	1	0	0	0	0	0	0	0	0	0
		11	65	0	1	1	0	0	0	0	0	0	0	0	0
		12	75	1	1	1	0	0	0	0	0	0	0	0	0
		13	60	0	1	1	0	0	0	0	0	0	0	0	0
		14	0	0	0	0	0	5	0	1	0	0	1	0	0
		15	0	0	0	0	0	10	0	1	0	0	0	0	0
		16	70	0	1	1	0	0	0	0	0	0	0	0	0
		17	90	1	1	1	0	0	0	0	0	0	0	0	0
		18	95	1	1	1	0	0	0	0	0	0	0	0	0
		19	70	1	1	1	0	0	0	0	0	0	1	0	0
		20	80	1	1	1	0	0	0	0	0	0	0	0	0
		21	0	0	0	0	0	15	0	1	0	1	0	0	0
		22	0	0	0	0	0	0	0	0	0	0	0	0	0
		23	60	1	1	1	1	0	0	0	0	0	0	0	0
		24	0	0	0	0	0	20	1	1	1	1	0	0	0
		25	80	1	1	1	1	0	0	0	0	0	0	0	0

Plot	Treat- ment	Bulb	Internal Pink Fusarium					Internal White Fusarium					Penicil- lium	Botrytis	Other
			Total % Cover	Neck	Mid	Base	On Daugh- -ter	Total % Cover	Neck	Mid	Base	On Daugh- -ter			
16	FAM 30	1	0	0	0	0	0	30	0	1	1	0	0	0	0
		2	0	0	0	0	0	15	0	1	0	0	0	0	0
		3	40	1	1	1	0	0	0	0	0	0	0	0	0
		4	0	0	0	0	0	20	0	1	0	0	0	0	0
		5	0	0	0	0	0	20	0	1	1	0	0	0	0
		6	0	0	0	0	0	5	0	1	0	0	0	0	0
		7	0	0	0	0	0	10	0	1	0	0	0	0	0
		8	95	1	1	1	0	0	0	0	0	0	0	0	0
		9	0	0	0	0	0	80	1	1	1	0	0	0	0
		10	0	0	0	0	0	35	0	1	1	0	0	0	0
		11	95	1	1	1	0	0	0	0	0	0	0	0	0
		12	0	0	0	0	0	90	1	1	1	0	0	0	0
		13	0	0	0	0	0	50	0	1	1	0	1	0	0
		14	0	0	0	0	0	40	0	1	1	0	0	0	0
		15	0	0	0	0	0	55	0	1	1	0	1	0	0
		16	25	0	1	0	0	0	0	0	0	0	0	0	0
		17	20	0	1	1	0	5	0	1	0	0	0	0	0
		18	20	0	1	0	0	0	0	0	0	0	0	0	0
		19	0	0	0	0	0	15	0	1	0	0	0	0	0
		20	90	1	1	1	0	0	0	0	0	0	0	0	0
		21	0	0	0	0	0	70	1	1	1	1	0	0	0
		22	0	0	0	0	0	10	0	1	0	1	0	0	0
		23	0	0	0	0	0	7	0	0	0	1	0	0	0
		24	0	0	0	0	0	10	0	1	0	1	0	0	0
		25	70	1	1	1	0	0	0	0	0	0	1	0	0