GREATSOILS

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Use of organic and biological amendments in horticultural production systems and the monitoring for any effects on soil and plant health.

Narcissus

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This is one part of the final report of a 48-month project (Project 7 of the Soil Biology and Soil Health Partnership) which started in September 2017. The work was funded by AHDB and BBRO for £79,942.

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1. Abstract

As part of the work within the Soil Biology and Soil Health Research and Knowledge Exchange Partnership, this project explored the effects on soil and plant health of amendments in horticultural crop production; particularly their direct or indirect potential suppressive effects on soil-borne pathogens. In all the trials within Project 7 (onion, *Narcissus*, raspberry), organic material was incorporated pre-planting to determine any benefits or otherwise to crop health. In this trial, *Narcissus* was planted in August 2018 following winter wheat. No *Fusarium oxysporum* had been detected by qPCR in the soil. However, Fusarium mycelium was visible after incubation in 38% of the cv. Carlton bulbs sampled at planting and PCR of the isolates identified *F. oxysporum.* Pig manure (FYM) at 35 t/ha or PAS 110 certified green compost at 30 t/ha, was applied prior to cultivation and planting. The third treatment was a granular Mycorrhiza product applied onto the bulbs during machine planting.

In the first year of growth (2019) no Fusarium-related foliage yellowing developed. In January 2020, leaf emergence was significantly least advanced in the Untreated control. In February 2020, significantly more flowerbud stems grew in the Mycorrhiza treatment at 66 flower buds/m of row, with 16 and 21 fewer buds in the FYM and Untreated. Covariate analysis indicated an influence of plot position; due to the inoculation process the Mycorrhiza plots were all at the top of the field. A mean 7.6% of leaf area was yellowed by Fusarium in April 2020, with no significant treatment differences.

After harvest in June 2020 a mean 8.8% of bulbs had externally visible Fusarium (5.6 bulbs/m of row), with a mean 62 healthy bulbs/m of row, without significant treatment differences. The Mycorrhiza treatment had significantly more 2-nose healthy bulbs/m than the Untreated or FYM treatments (30/m from Mycorrhiza plots, but only a mean 20 from untreated and FYM), however, the former were significantly lighter in weight. 6.7% of Mycorrhiza treated bulbs from the plot sampled had *Fusarium* necrosis visible outside or inside, compared with 23.3% from an Untreated plot. Most of the 30 bulbs sampled from the untreated and Mycorrhiza treated plot had mycorrhiza, but more root area was colonised in Mycorrhiza treated bulbs than in Untreated (30.4% v 6.5%, respectively).

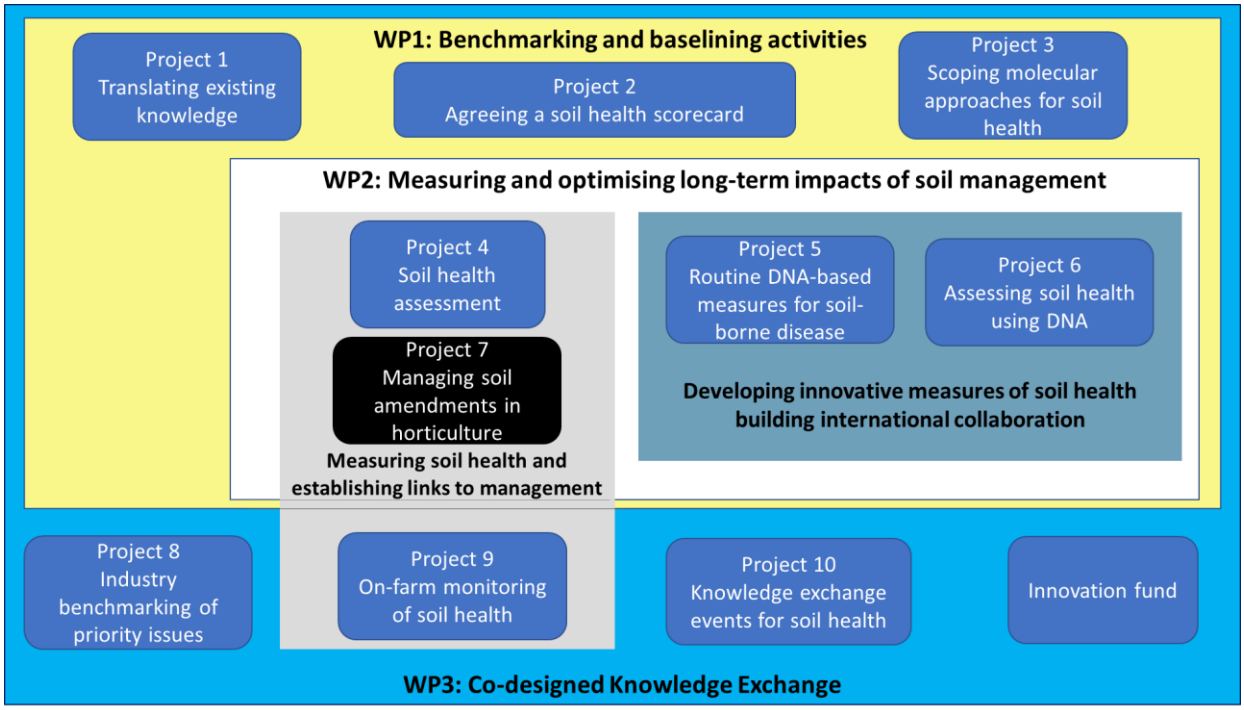
In Spring 2020, fewer *Verticillium dahliae* microsclerotia were present in the Mycorrhiza treatment, with 11.2/g of soil, compared with 17.2/g for the other treatments. No treatment differences were shown by the populations of free-living nematode species. Soil health scorecards before and after organic matter incorporation showed little change as a result of the treatments. *F. oxysporum* was detected in the soil by qPCR in both 2019 and 2020, but plot records showed no relationship with treatment or bulb rot incidence.

In conclusion, a single application of pig FYM or green compost had very little effect on topsoil health and no impact on crop performance and Fusarium infection; it is likely that multiple annual applications would be required. The successful inoculation of bulbs with mycorrhiza was achieved using farm-scale, commercial equipment, and there was some evidence that this improved crop performance, disease levels and reduced *V. dahliae* levels in the soil. However, these effects could not be reliably concluded, due to possible confounding effects of treatment position in the field.

2. Introduction

This project is part of a suite of integrated projects within the Soil Biology and Soil Health Research and Knowledge Exchange Partnership (see Diagram below of how this project fits into the wider organisation of projects). This project (Project 7 of the Soil Biology and Soil Health Partnership – SBSH, together with Project 5) aimed to gain an understanding of any benefits gained from non-chemical inputs in horticultural cropping systems in the management of intractable soil diseases via potential changes to the soil microbial population and other biological, physical, and chemical aspects impacting on soil and crop health. This was focussed on a greater understanding of the effects on soil and plant health of amendments in horticultural crop production; particularly their direct or indirect potential suppressive effects on soil-borne pathogens.

Project 07 shown within the integrated project delivery of the Soil Biology and Soil Health Research and Knowledge Exchange Partnership



Inter-related objectives in Project 7 aimed to gain a better understanding of the soil biology and key soil health metrics that should be recorded by growers in order to be able to manage soils to be good for plant health and development:

1. To identify three fields with a history of fungal and/or oomycete soil-borne diseases and quantify the presence of up to six intractable soil pathogens by qPCR.
2. To carry out physical, chemical and biological assessments of the field soils in tandem with sampling for molecular assay and seek to determine any relationship between these.
3. To record changes in the soil microbiome following the use of soil amendments. To determine any relationship between the microbial population composition and levels of disease in the crop.

Work on soil-borne diseases was carried out as part of the wider Project 07 within the Soil Biology Soil Health Partnership; each of the three crops studied (onion, *Narcissus* and raspberry) have been covered in separate reports (91140002-07a, -07b and -07c). In all three crops, organic material was incorporated pre-planting to determine any benefits or otherwise to crop health. In *Narcissus*, reported here, a mycorrhizal product was also applied at planting. The other two crops examined were raspberry (where a plant protection product containing a beneficial fungus was applied) and onion (where no products were applied).

Soil health measures related to physical and chemical indicators and earthworm numbers were recorded. Information on biological indicators, such as the quantity and type of microbes present in the soil as the trial progressed, was obtained from molecular assessment carried out in the trials as part of Project 5 and full details of these will be found in that report (91140002-05 Elphinstone *et al*., 2022). Molecular techniques have developed rapidly in the 21st Century and quickly allow the identification and quantification of fungi and bacteria (qPCR). A key step forward has been the development of affordable methods for routine DNA extraction from larger soil samples (up to 1kg), so increasing reliability and the chance to relate these soil DNA levels to the risk of a crop variety grown in that soil developing symptoms that would reduce crop marketability.

Soilborne plant pathogens, such as *Fusarium oxysporum*, are among the most important limiting factors for UK horticultural crop production and build up with repeat cropping of susceptible hosts. Most have resting spores whose thick walls allow them to survive a minimum two years in the soil, so short rotations with non-hosts have little benefit. These structures include oospores (as in *Phytophthora* spp.), chlamydospores (as in *Fusarium* spp.) and sclerotia or microsclerotia (as in *Sclerotinia* spp. and *Verticillium dahliae*). Soil disinfestation pre-cropping used to be done by chemical treatment, but chloropicrin and dazomet products were not permitted in the UK from 2020. Significant yield and/or quality losses occur most years to soil-borne diseases in numerous crops including: Brassicas (club root and Rhizoctonia); carrot (Pythium cavity spot); lettuce (Botrytis, Sclerotinia and Rhizoctonia); field-grown nursery stock (replant disease; Verticillium wilt); onion/leek (Fusarium basal rot; Sclerotium white rot); spinach/leafy salads (Pythium damping-off); raspberry (Phytophthora root rot; Verticillium wilt); strawberry (Phytophthora crown rot and red core; Verticillium wilt) as well as the focus of this project - *Narcissus* (Fusarium basal rot).

In this work, *Verticillium dahliae* microsclerotia were counted before and after treatments as, although *Narcissus* crops are not affected by this soil-borne pathogen, subsequent crops in the rotation such as potatoes or linseed do succumb to Verticillium wilt and information on any reduction in levels by incorporations able to be used in a preceding crop would be useful. Similarly, the presence of various species of free-living nematode in the soil is important to know, not only for

Narcissus where some nematode species cause damage either directly or by the transmission of viruses, but for other crops in the rotation.

Soil features which have been associated with disease suppression are variable, interacting and complex; they include physical, chemical and biological components. Changes to nutrient concentrations in plant tissues can make them more resilient to pathogen attack and can increase the microbial activity and effect changes in soil physico-chemical properties or structure. The inhibition of germination and growth of soilborne pathogenic fungi by fungistasis may arise via the depletion of labile organic compounds and nutrients due to intense competition among soil microorganisms (Steiner & Lockwood, 1970), and / or the presence of inhibitory compounds, including volatiles with antifungal activity (Gerbeva *et al.,* 2011; Berendsen *et al*., 2012). Microbial plant protection products which utilise such modes of action are available. Products registered in the UK include Trianum G (*Trichoderma harzianum* strain T22), T34 Biocontrol (*Trichoderma asperellum* strain T34), Serenade ASO (*Bacillus subtilis* strain QT 713) and Prestop (*Gliocladium catenulatum* strain J1446). These bioprotectants were not selected for use in the current project because *Narcissus* is not grown with the trickle irrigation that can be used to facilitate the fungicide drenching of fruit and ornamental crops. Instead, arbuscular mycorrhizal fungi (AMF) in a commercial granular product, (not requiring registration as a plant protection product), were tested using a novel application method for this crop. Mycorrhiza products can provide a similar health promoting role to the indigenous rhizosphere microbes (Berendsen *et al.,* 2012). Through their symbiotic relationship with the plant roots, nutrient and water uptake can be improved (Rouphael *et al*., 2015; Begum, *et al.* 2019) and some research has shown them to effectively reduce root disease caused by a number of soilborne pathogens (Cordier *et al*.,1998).

In *Narcissus*, the effects of three amendments (farmyard manure (FYM), green compost and mycorrhiza inoculation) on crop yield, quality and disease burden were compared over the two years of the crop before the bulbs were harvested for sale. *Narcissus* lack an extensive root system, consequently, nutrients can only be utilised from a relatively small area of soil surrounding each bulb. Thus, the use of either organic amendments to improve soil structure, moisture retention and supply nutrients (Stockdale, 2018; Guo, 2021), or the addition of AMF, were used as treatments with the aim of achieving a good marketable yield of bulbs. This report focuses on the impact that these soil treatments may have had on bulb health (in particular on *Fusarium oxysporum* causing basal rot), flower bud development and bulb quality. Some organic amendments such as composts and crop residues have potential for controlling soilborne pathogens (Gamliel *et al*., 2000; Hoitink & Boehm, 1999; Noble & Coventry, 2005; Bonanomi *et al*., 2007; 2010; O'Neill, 2010). Any control could extend to pathogens of future crops in the rotation, such as *Verticillium dahliae* which does not affect *Narcissus* but causes wilt in crops such as potatoes, linseed, peas and sugar beet (Hanks, 2013).

The microsclerotia produced by *V. dahliae* remain viable in soil for up to 14 years (Fradin & Thomma, 2006; Subbarao, 2020).

In Fusarium basal rot of *Narcissus* caused by *F. oxysporum f.sp. narcissi* (Hanks, 2013), the pathogen enters young roots to cause a dark brown rot of the basal plate which can then progress up through the bulb scales. Field infection is encouraged by high soil temperatures and will also progress in infected lifted bulbs stored in warm conditions. Chlamydospores in the soil cause infection (surviving in this resting stage for up to 10 years), but the pathogen can also be brought into the field on bulbs with no external rot symptoms at planting. The first symptoms in the growing crop are pale, prematurely senescing foliage and sometimes short-lived crooked shoots. Roots can fail to emerge or emerge and then be attacked, thus reducing the ability to take up water. Bulbs can rot totally, or the later infected bulbs can be harvested with spots of rot only visible if the bulb were to be cut open. When infection has not progressed to be visible externally around the basal plate or bulb neck then batches of bulbs can be accepted for planting that subsequently succumb to basal rot. Chlamydospores will form in the infested bulbs and be left in the rotted roots and debris remaining in the soil at bulb harvest.

3. Materials and methods

3.1. Treatment application and bulb planting

3.1.1. Treatments and plot layout

On 29 August 2018, a two-year project was set-up in Orange Field (16 ha), near Terrington St Clement, Norfolk, (Grid Reference TF 54227 20587). This field was due to be planted with a commercial crop of *Narcissus* directly following wheat sown in Autumn 2017. The trial area contained 24 plots, each 3.0 m wide and 10 m long (**Figure 1**).

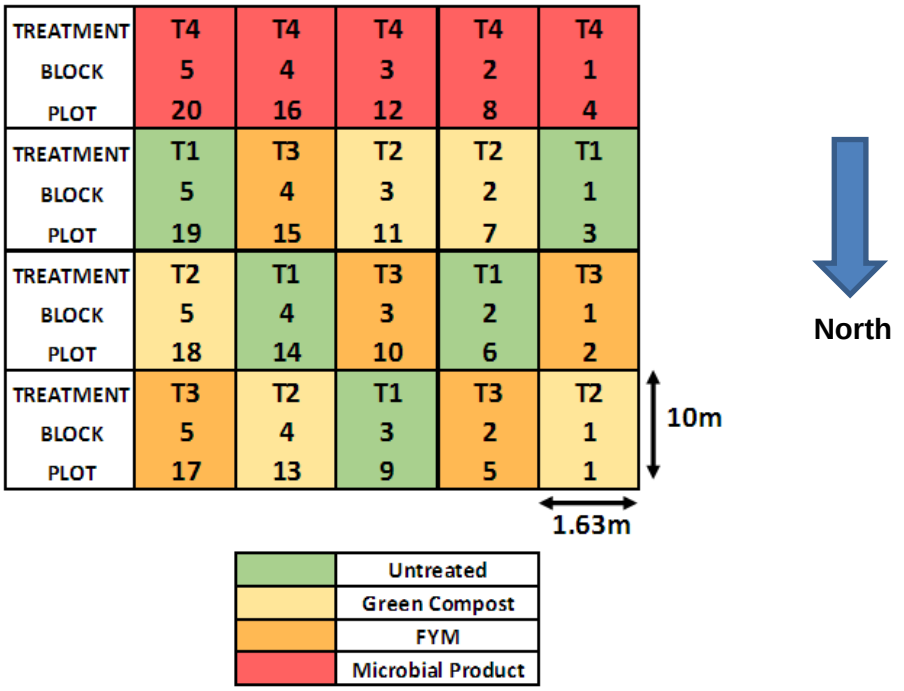


Figure 1: Trial layout for 20 plots of *Narcissus* cv. Carlton (with assessed area of two central rows 1.63 m x 10 m). Five replicate Blocks with Treatments T1, T2 & T3 randomized, but Treatment T4 (Mycorrhiza, microbial product) plots along the South side of the trial area. *Narcissus* cv. California Reclaim was planted in a 6th Block but not crop recorded.

The four treatments are given in **Table 1**. Six replicate blocks were arranged between tractor sprayer tramlines in the cereal stubble. The tramlines were re-used in the *Narcissus* crop. The tramline to the West of the 1.63 m wide two-ridge discard plot (beside Block 1) had been used as a wider access track through the field. The plots were marked out in the cereal stubble for pre-trial soil sampling and for application of the organic amendments. The position of the plots was measured and related to marker flags in the boundary so that they could be relocated once the field had been cultivated prior to bulb planting. Once the *Narcissus* were planted, each replicate Block was composed of four planting ridges.

Table 1: Treatments, rate and timing of applications to Orange Field, Terrington.

Code	Treatment type	Rate	Application timing
T1	Untreated	-	-
T2	PAS 110 certified Green compost of 0 - 30 mm grade	30 t/ha FW = 90 kg per 30m^2 plot	29.08.2018 onto cereal stubble the day before planting
T3	Pig manure (FYM)	35 t/ha FW = 105 kg per 30m^2 plot	29.08.2018 onto cereal stubble the day before planting
T4	Mycorrhiza granules (six fungal species)	approx. 0.8g / bulb	30.08.2019 as bulbs were planted at 85 bulbs / m of row

Green Compost and FYM were sent for chemical analysis (see Results section). The components of the Mycorrhiza product are detailed further below in the Methods.

3.1.1. Organic material applications and soil preparation pre-planting

Application of organic materials

On 29 August 2018, organic matter treatments (**Figure 2**) green compost (T2) and pig FYM (T3), were applied by hand-fork and spread as evenly as possible into the cereal stubble across the 3.0 m plot width. This spread was 0.68 m either side of the soil destined to become the central two planting ridges within which the crop would later be assessed. This spreading width was to allow for potential sideways soil movement by post-application ploughing. The full 10 m length of each plot was covered although one metre at the start and end was not included in the 8 m length of the plot assessed for crop growth. Treatments T1-T3 were fully randomised in the Blocks. Treatment T4 (Mycorrhiza product) plots were placed side by side at the southern end of the trial area (**Figure 1**), as explained below.

a) Green compost (T2) as darker patches. b) Pig manure (T3) showing a dark lump.

Figure 2: Orange Field on 30 August 2018 after chisel ploughing the cereal stubble, with the Green compost and FYM applied on 29 August still visible on the soil surface. These were further incorporated by ploughing and other cultivations the next day to prepare the soil to allow ridges to be formed at *Narcissus* bulb planting immediately afterwards.

Analysis of organic materials

Analysis of samples of the FYM and Green Compost applied in August 2018 showed in particular the greater dry matter content of the Green Compost compared with pig manure. The FYM had a higher proportion of organic carbon than the Green Compost (**Table 2**). The loadings to Treatment 2 (Green compost) and Treatment 3 (FYM) plots based on this analysis is given in **Table 3**.

Table 2: Fresh weight (FW), dry matter and organic carbon content for pig FYM and Green Compost applied as treatments on Orange Field in August 2018.

Property	**Pig FYM**	**Green Compost**
Dry matter (%)	43.5	76.8
Total N (kg/t FW)	7.0	12.5
Readily available N (kg/t FW)*	n.d.	0.69
P_2O_5 (kg/t FW)	10.5	5.79
K_2O (kg/t FW)	10.9	8.6
MgO (kg/t FW)	4.1	3.2
SO_3 (kg/t FW)	n.d.	4.28
Organic C % DM	32.0	14.2

*Readily available Nitrogen = Ammonium-N + Nitrate-N

Table 3: Loadings of FYM and Green compost applied and then well incorporated by ploughing, harrowing and bed-forming before planting *Narcissus* in ridges in Orange Field in 2018.

	Pig FYM	**Green compost**
Application rate (t/ha)	35	30
Dry solids (t/ha)	15	23
Total N (kg/ha)	245	375
Organic matter (t/ha)	8.4	5.6

Cultivation and bed-forming operations

On 30 August 2018, the farmer carried out multiple cultivation operations to create the soil consistency required for the *Narcissus* (**Figure 3**). The sandy silt loam soil was dry at the surface, but moist below. A three-blade subsoiler (chisel plough) with a rotaspike roll was driven diagonally across the trial area which partially buried the applied treatments. The was followed by a five-blade mould-board plough and furrow-press in line with the tramlines. A power harrow crumbler roller was then used to create a flat bed of fine tilth between each wheeling. The ridges for the bulbs were created by discs behind the coulters on the planting machine.

a) Chisel ploughed stubble (foreground).

b) Ploughing (left) and power harrowing.

Figure 3: Cultivations of Orange Field on 30 August 2018 just before planting. Chisel ploughing was followed by a mould-board plough and furrow press rings. This was immediately followed by a power harrow with soil crumbler cylinder that flattened the soil.

3.1.2. Bulb planting procedure

The bulbs within the trial area were planted on 30 August 2018 as part of the operation to plant the commercial crop of *Narcissus* in the field. The machine had two coulters which each dropped a band of bulbs onto the bed and a ridge was formed up over the bulbs to create two rows per bed. The machine worked alternately up and down the field to plant adjacent beds. The same variety of bulb, cv. Carlton, was planted outside the trial area alongside Replicate 1 and on through the plots of Replicates 1 to 5. When the sixth replicate was planted there were no further crates of cv. Carlton left and cv. California Reclaim was instead planted in this and subsequent rows of the commercial crop. In the first year all six replicates were assessed, but the sixth replicate was omitted from the analysis as it was an earlier flowering variety. The 20 plots of cv. Carlton assessed and analysed in the second year were laid out as shown in **Figure 1**. Subsequently, the plants within 1 m of each plot end were excluded from assessments (8 m assessed) in case there was any movement of the incorporated products by the machinery working up and down the field.

A sample of 29 cv. Carlton bulbs were collected at planting on 30 August 2018. They were cut open from nose to base and spaced cut surface uppermost on damp paper towel in a tray. The tray was enclosed in a transparent polythene bag to raise humidity and incubated in the warmth of an unheated glasshouse, under natural daylight, for 14 days. The number of bulbs which developed white or pale-pink mycelium were counted and a sample of spores checked to confirm the presence of *Fusarium oxysporum*.

3.1.3. Mycorrhiza product contents and application

Mycorrhiza product contents

There were six arbuscular mycorrhizal fungal species (AMF) in the Mycorrhiza product supplied by PlantWorks (**Figure 4a**); these were on a mineral carrier comprising an equal ratio of pumice and zeolite (with a bulk density of 0.95 g/ml). Each of the fungal species comprised 16.64% of the total. The species were *Claroideoglomus claroideum*, *Funneliformis mosseae*, *Funneliformis geosporum*, *Rhizophagus irregularis*, *Glomus microaggregatum* and *Diversispora* sp.

The PlantWorks product is usually sold with "bioadditives", but for this project (in order to only investigate any effect of the AMF) the granules were supplied with just the mycorrhizal fungi species, in the same proportions as the standard product. Changes made to the classification of mycorrhizal fungi in 2011 mean that in older publications some of the contents will be named differently e.g., *Glomus mosseae* is now *Funneliformis mosseae*, and *Glomus intraradices* is split into *Rhizophagus intraradices* and *Rhizophagus irregularis* (pers. comm. Natallia Gulbis, PlantWorks UK Ltd.).

a) 500 ml beaker of Mycorrhiza granules of Treatment 4 (1.4 kg used for 2x 10 m rows).

b) Scattering Mycorrhiza granules on bulbs before they dropped down into two coulters.

Figure 4: Mycorrhiza granule use at *Narcissus* bulb planting on 30 August 2018.

Mycorrhiza product application

Mycorrhiza product of 7 kg was received, sufficient to treat the 1120 bulbs within two rows of 10 m based on applying 1 g per bulb. However, on the day, the grower had set the planter to 1700 bulbs (85 bulbs/m of row) and so it was necessary to allow 0.8 g of granules per bulb. A weight of 1.4 kg was used per two-row length (determined to be the same as a volume of 1.4 L). Six bags of 1.4 L were made up in the field for spreading over the T4 plots.

Bulbs from the hopper were continually dropped onto a vibrating plate which moved the bulbs in a single layer towards the back of the machine to fall down a pair of coulters (**Figure 4b**). On approaching the front of a T4 plot a bag of granules was opened and gradually scattered side to side over the vibrating plate. Scattering was adjusted to be completed after about 8 m so that the last of

the treated bulbs fell within the plot. Granule scattering was done while the machine moved forwards at 2 km/h over the 10 m of the plot and the discs then immediately covered over the bulbs with fine soil across each of the two 0.3 m wide bands so leaving a pair of ridges.

The Mycorrhiza product was applied to the bulbs of T4 plots on 30 August 2018 while they were being planted in two central rows spanning 1.63 m of the plot width. The central pair of rows per plot were from the same two-coulter pass of the planter, whereas the outer row either side belonging to that plot and the adjacent plot was planted by the planter returning in the opposite (northerly) direction. Each plot was thus planted with four rows of *Narcissus* bulbs, the rows running continuously up the field. There were no additional rows between the four rows of each Block. The Mycorrhiza product plots were positioned at the southern margin of the trial area (**Figure 1**). This was so that the central rows being treated were the last treatment in a line before the machine carried on southwards up the field where it created row ridges and planted bulbs for the commercial crop. By doing this, the bulbs subsequently planted in the commercial crop were expected to carry off any residue deposits of Mycorrhiza granules from the planter platform and coulters before the machine returned to plant the trial area. No Mycorrhiza product was applied when the planter returned northwards down the field into the trial area planting the outer rows of plots. Planting was carried out from west to east, from replicate Block 1 to 6 (**Figure 5**).

a) Driving south before bulb inoculation.

b) Planted ridges in trial area, looking north.

Figure 5: *Narcissus* bulb planting on 30 August 2018 at Orange Farm Field, Terrington.

3.2. Soil sampling

Baseline soil sampling for soil-borne pathogen and free-living nematode (FLN) presence was carried out in the field to a depth of 150 mm using a “cheese corer” tool at 50 points on 14 November 2017 with a sugar beet crop present (this was followed by winter wheat, prior to planting the *Narcissus* in August 2018). Samples of 2 kg were sent both for Harris testing (extraction method) for *Verticillium dahliae* and free-living nematode counting by the ADAS laboratory at High Mowthorpe and for

quantitative molecular testing (qPCR) for *Sclerotium cepivorum* and *Fusarium oxysporum* at Fera, York as part of SBSH Project 5. *V. dahliae* microsclerotia content in the soil was assessed because, although the fungus does not cause a problem for *Narcissus*, its long persistence in the soil means that susceptible crops later in the rotation might benefit from any reduction in levels caused by treatments during *Narcissus* cropping. *V. dahliae* was not assessed using qPCR because this analysis was being used in Project 5 to seek any correlation between the incidence or severity of disease in the *Narcissus* crop to the levels of DNA of the associated pathogen in the soil.

Soil health scorecard parameters were evaluated as at other sites within the SBSH Partnership, with assessments made before planting, and in the final (second) year of the crop. These assessments included: Visual Evaluation of Soil Structure (VESS) earthworm numbers and penetrometer resistance, with a topsoil sample analysed for pH, extractable P, K and Mg, organic matter (loss on ignition and Dumas methodologies), total N, respiration (CO_2-burst), and potentially mineralisable N (PMN).

a) Soil pit dug into stubble. b) Dug soil being assessed.

Figure 6: Soil visual assessment and earthworm count following the digging of pits, a week before cultivations and *Narcissus* bulb planting. Orange Field, 22 August 2018.

On 22 August 2018, soil health was assessed prior to cultivation of the wheat stubble from the recently harvested crop which had followed lifting of the sugar beet. The area was marked out for the plots and samples taken. Penetrometer readings were taken at 10 positions in each of the six replicate blocks, measuring the maximum resistance to 30cm and the depth of the maximum. Soil structure (VESS) and earthworm counts were made based on pits dug in replicate Blocks 1, 3 and 5 (**Figure 6**). Soils were dry at this time, so not in an ideal condition for these assessments.

On 22 August 2019, Fera grid sampled across all 24 plots for qPCR for *F. oxysporum* only, but no other soil samples were taken within the first crop year.

On 29 April 2020, the soil from replicate Blocks 1, 3 and 5 (within the plots of cv. Carlton) was sampled from within the central two rows of each plot. Samples were taken at 150 mm depth to obtain 6 kg of soil which was then mixed and split and divided equally between samples for a) extraction of free-living nematodes and *V. dahliae* microsclerotia by ADAS, b) molecular testing for the quantification of *F. oxysporum* DNA by Fera and c) reference sample cold storage. The plot boundary co-ordinates were recorded using GPS.

On 2 June 2020, two days before bulb harvest, soil samples were taken to 150 mm depth from each of the two central rows of the 24 *Narcissus* plots (i.e., the five replicates of cv. Carlton and the one replicate of cv. California Reclaim). These were couriered in cool boxes to Fera for molecular diagnostics (qPCR).

It had been intended to carry out the final end of year soil samples in the next crop, however directly after the bulbs were lifted in the rented field the management reverted to the owner and he arranged for a delivery of biosolids. Therefore, the sampling for nutrients was instead done not long after all the *Narcissus* bulbs had been lifted from across the field by the grower (to avoid confounding of the results by the biosolids treatment). On 8 July 2020, the top 0-150 mm of soil was sampled from replicates 1, 3 and 5 of each treatment. Approximately 1 kg of soil was collected per plot then mixed and split to send half to the NRM laboratory for analysis of pH, extractable P, K, Mg, organic matter loss on ignition, texture and CO_2 respiration burst. The other half of each plot sample was sent to the Hillcourt laboratory for potentially mineralisable nitrogen analysis (PMN). The remaining soil health tests were left for a later date.

By 26 August 2020, the biosolids ordered by the farmer had been incorporated (and the remains of the post-harvest *Narcissus* ridges flattened). The soil sampling commenced in July was completed on 2 September when each plot area was assessed for soil structure (VESS), penetration resistance and earthworm numbers, recording the total number of earthworms using a single 200 x 200 x 200 mm pit in each plot. The numbers of earthworm juveniles, adult epigeics, endogeics and anecics present were counted.

The soil assessment results were compared statistically by analysis of variance and compared using Duncan's multiple range test. Soil health scorecards were created based on the mean results for the five replicates with cv. Carlton bulbs.

3.3. Husbandry after *Narcissus* planting

After planting in August 2018, nitrogen, phosphate and potassium were applied by the grower at 300 kg/ha using a fertiliser with N:P:K of 9:9:24. Sulphate of potash was applied in spring 2019 at 200 kg/ha and the same amount again in spring 2020. The crop was not irrigated. The grower harvested flower heads for sale in spring 2020 and the bulbs lifted for sale in July 2020.

3.4. Photographic and meteorological records

Photographs were taken across all 20 plots of cv. Carlton throughout the trial to illustrate differences such as plot density or yellowing. Emergence stage photographs were taken on 13 February 2020 and crop density photographs were taken on the 1 April 2020.

Meteorological data was obtained for the period of the trial from “Irriguide” and mean daily air temperature and rainfall calculated. In 2020 data was also available from the ADAS Terrington MET station. A soil logger was buried at 10 cm in plot 10 on 13 February 2020.

3.5. Leaf emergence and yellowing assessments

On emergence of the leaves after Winter in 2019 and 2020 the crop was examined for yellowing or other symptoms that might be caused by *F. oxysporum* damage to the roots or basal plate. A second assessment was made after flowering before the leaves started to senesce. The proportion of the leaf area that was yellow was recorded per plot.

In 2020, the crop rows emerged unevenly, and leaf extension was variable and so a record of this was made using a 0 to 9 Index with the following descriptors; 0 = no shoots visible, 2 = very poor, 3 = poor / backward with shoots short and row gaps, 4 = emergence a bit tardy, 5 = satisfactory emergence but leaves below 10 cm height, 6 = more advanced leaf production i.e. more leaves per neck and these are tall / and few row gaps, 7 = gaps rare and good leaf height, 30% have flower buds, 8 = leaves quite uniform in height, 50% have flower buds, and 9 = uniform height, good thick row cover most necks have a flower bud.

3.6. Flowerbud stem counts and bulb size grade yield

On 13 February 2020, four 1 m lengths were marked out in the two central rows, zig-zagging across the two central rows down the 8 m assessed length of each plot. The positions were named as “a to d” with subsequent records kept separately for each length (although treatment means only are presented in this report).

On 20 February 2020, when flower stalks had all extended, the total number of flower buds visible within each of the one metre row sections per plot were counted. It was not possible to determine the number of heads per bulb as the bulbs were planted so that they were touching and sometimes on top of each other. The flowers buds were then picked by the grower.

On 4 June 2020, with all the foliage dead, all the *Narcissus* bulbs within each marked 1 m length were hand harvested, keeping each of the four lengths separate in a paper sack. The bulbs from each of the four positions in one of the plots (plot 6, untreated) were weighed on arrival from the field to be able to have an indication of bulb weight loss during storage. All collected bulbs were transferred into trays for each row length and stacked in a ventilated barn to dry for 19 days prior to analysis (establishing an interval usually left commercially between lifting and bulb-grading).

An indication was sought at harvest of how successful the root colonisation and survival of the mycorrhiza had been since the granules were scattered over the bulbs at planting the previous year. Therefore, on 4 June, 30 additional bulbs were collected at random from outside the assessed lengths of row in plot 9 (Untreated) and another 30 from plot 12 (Mycorrhiza product). These plots were in the central replicate block, Block 3. The bulbs were sent to Plantworks Ltd (the source of the test product) for mycorrhiza testing (performed by Joanne O' Regan).

On 17 June 2020, 13 days after harvest, all the healthy bulbs from each of the four row positions in the previously weighed plot (plot 6, untreated) had their diameter recorded against their nose category. This was to be able to cross-reference this to the alternative method of bulb grading, by bulb circumference. The combined weight of all the nose categories for this row length post-storage was recorded to be able to compare with the record made pre-storage.

3.7. Fusarium basal rot assessment

On 23 June 2020, after storage of the five replicates of cv. Carlton bulbs from each of the four metre row lengths per plot, the bulbs were cleaned by removing the outer flaky layers. Each bulb was then visually categorised into size grades by counting the nose number (the number of attached bulbils and any smaller "daughters" plus the originally planted "mother bulb"). During harvest, some of the daughter bulbs that would otherwise have been counted as a nose on a mother bulb had separated and so these were recorded as a separate category and included as part of the total number of bulbs.

Each bulb was examined to record whether they had obvious *F. oxysporum* external basal rot or neck rot symptoms. Symptoms diagnostic of *F. oxysporum* were browning around the basal plate, potentially coupled with reduced numbers of roots or brown roots, and / or browning visible extending up the bulb sides from the base usually resulting in softening of the bulb scales. Severity was not recorded, only incidence as a measure of unmarketability. Some bulbs were cut open to confirm that

the symptoms externally were matched by dark brown staining of the scales, but overall, the bulbs were not assessed for internal rotting. This was to match commercial grading for marketability, which can only be done on external symptoms (as the bulbs would be unsaleable cut in half). A sample of externally symptomatic bulbs were taken for warm, damp incubation to confirm the presence of *F. oxysporum* by the development of pale pink, mauve tinged or white mycelium.

On 23 June, once the bulbs from each metre length had been separated by nose number, the number of healthy and the number with externally visible Fusarium basal rot were counted per nose category. All the bulbs of the same nose category per metre length were then weighed together, keeping separate records for the healthy and diseased. A digital balance was used (Brecknell, Model: 405, Precision: 0.001 Kg). The total mass (g) of each nose category was then divided by the total number of bulbs within that category to produce a calculated mean weight of an individual bulb for that category.

3.8. Colonisation of *Narcissus* roots by mycorrhiza

At PlantWorks, samples of bulb roots were cut off each bulb sampled on 4 June 2020, mounted in cassettes and placed in Quinks stain (Vierheilig *et al*., 2005). Stained sections were then arranged on microscope slides for assessment under a light microscope. This “RLC assessment” determined the colonisation of roots by arbuscular mycorrhizal fungi (AMF) of any species and an estimation was made of the % of root area with AMF for each of 30 bulbs from the untreated and the mycorrhiza treated plot. Some of the bulbs had few roots because of severe Fusarium basal rot, so the % AMF colonisation referred only to the roots remaining.

Whether (1) or not (0) there was necrosis associated with Fusarium either externally or internally in the bulb from which the roots were sampled was recorded after each bulb was cut in half from nose to base. Photographs of a sample of sectioned bulbs with brown staining were sent to E. Wedgwood at ADAS by J. O’ Regan to confirm the type of symptoms expected internally in bulbs with Fusarium neck or basal rot before completing the records. Only tissue with dark chocolate colouration was counted as Fusarium damage, not any greying of the bulb scales. Earlier research by E. Wedgwood had shown that internal grey *Narcissus* tissue when incubated does not produce Fusarium mycelium and this discolouration probably follows bruising of the bulb. The record produced by the PlantWorks laboratory provided an indication of the incidence of Fusarium internally in the samples submitted, but it was not intended to be a fully replicated sample of the trial as only one plot of T1 and T4 had been sampled.

4. Results

The farmer had anticipated that there would be sufficient cv. Carlton bulbs to plant 26 rows running north-south the length of the field to encompass the trial area. However, at planting, the final replicate block of plots had to be planted with cv. California Reclaim as well as further rows beyond the trial area to the east. Although records were made in 2019 for all the plots, only the cv. Carlton was recorded in 2020 as the cv. California Reclaim was seen to be an earlier flowering variety.

The sample of cv. Carlton bulbs, collected at planting on 30 August 2018 and cut open and incubated for 14 days, initially showed areas of dark brown necrosis on 11 of the 29 bulbs. The internal necrosis then developed pale pink mycelial growth typical of *Fusarium* spp. and this was isolated onto agar. The isolates were identified at Fera using PCR and confirmed to be *F. oxysporum* (the identification did not seek to determine the *formae speciales*).

4.1. Leaf emergence and leaf yellowing in the first crop year

Observations of plot emergence/vigour and yellowing were made until the leaves senesced in May after the crop had flowered in early Spring. No symptoms of phytotoxicity were seen throughout the two years of the crop.

Foliage yellowing compared between treatments

Foliage of cv. Carlton assessed post-flowering on 24 April 2019 was still mainly green (**Figure 7**), after a month without rain and a period of unusually hot sunny weather (over 20°C), however some foliage showed thickening and distortion (**Figure 8**). Microscope examination found this to be associated with roughening of the epidermis with impacted soil associated with epidermis loss, particularly on leaf undersides. Golden brown thickened depressions held fine sand particles. In addition, there was streaking of yellow and sometimes brown particularly towards the leaf tips (which could have been virus symptoms) not necessarily on distorted leaves, the rest of the leaf being green. Symptoms did not resemble the leaf yellowing associated Fusarium basal rot, i.e., from either poor water uptake because of root loss, or internal rotting of leaf bases. No fungal pathogens developed after incubation of the leaves in the laboratory.

Crop assessment across all six replicates (including cv. California Reclaim) post-flowering on 24 April 2019 showed no significant ($P>0.05$) treatment differences in yellowing (**Table 4**). Leaves arising in a cluster were assessed as being “a plant” growing from the planted bulb, although leaf overlap between bulbs meant this could not be precise. There was a wide range of 25% to 100% of plants per plot affected by distortion, yellowing or browning or a combination of these, with a mean 71.2% of plants affected. In addition to recording incidence, the severity of damage was assessed on the proportion of the leaf area affected. A mean 6.3% of the leaf area per plot was affected by

distortion/yellowing for both varieties. In cv. Carlton there was a plot range from 2% to 17% of leaf area affected.

Figure 7: *Narcissus* in Orange Field trial area on 24 April 2019 after flowering, with leaves still green but with some distortion and yellowing concentrated on the tips of the leaves. Views looking Southwards up the slight slope from a position in plot 1 in replicate Block 1.

a) Leaf thickening and distortion with sand particles lodged in the underside lesions.

b) Brown and yellow streaking probably caused by virus infection.

Figure 8: *Narcissus* cv. Carlton leaves with thickening and distortion and/or streaking. Examples from Plot 17, Orange Field 24 April 2019. No differences between treatments.

Table 4: The proportion of *Narcissus* plants of both cultivars with yellowing, browning and/or leaf distortion and the % leaf area affected/plot. Post-flowering on 24 April 2019.

Distortion, yellowing and/or browning	Treatments						15 df
	Untreated	Green Compost	FYM	Mycorrhiza Product	Overall mean	L.s.d.	F value
% of plants affected	84.17	86.67	59.17	55.00	71.20	36.440	0.180
% of leaf area affected	6.33	8.17	5.92	4.87	6.32	5.165	0.598

At re-examination of the six replicates on 3 May 2019, more yellowing potentially resulting from Fusarium was present with no treatment differences obvious. Plot yellowing ranged between 10% to 30% of the canopy cover, with means of 20.0%, 20.83%, 20.0% and 19.2% of the canopy yellowing for Untreated, Green Compost, FYM and Mycorrhiza, respectively.

By 19 June 2019, no Fusarium wilting had developed, the small amount of flowering had finished, and the leaves were dying back with seasonal senescence. No further records were made until new leaves emerged in Spring 2020.

4.2. Leaf emergence, crop density and leaf yellowing in the second crop year

Leaf emergence and crop density compared between treatments

Emergence of the cv. Carlton was incomplete when the trial site was visited with the grower on 30 January 2020. The record made of the emergence extent using the index 0 (none visible) to 9 (excellent, complete row lengths emerged and tall leaves) to encompass both the proportion of leaves emerged and how tall the leaves had grown resulted in a mean emergence index of 3.8 for Untreated and around 5.0 for the other treatments (**Table 5**). Individual plot emergence extent index records spanned Index 3 (poor), 4, 5 and to Index 6 (good leaf numbers and length).

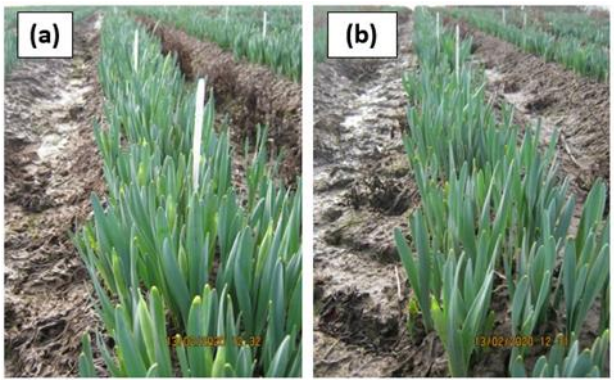
Table 5: Mean 0 - 9 index of emergence per treatment (0 = zero emerged to 9 = excellent establishment) for cv. Carlton *Narcissus* plots on 30 January 2020. Orange Field.

	Treatments						12 df
Leaf emergence extent	**Un-treated**	**Green Compost**	**FYM**	**Mycorrhiza Product**	**Overall mean**	**L.s.d.**	**F value**
Index per plot	3.80 a	4.80 b	5.00 b	5.00 b	4.65	0.723	0.010

Different letters show significant differences using Duncan's multiple range test.

All the treatments had significantly (P<0.01) better emergence than the Untreated. More bulb noses showed several emerged leaves, and these leaves were longer. There were also fewer gaps in the row caused by absent leaves still to push through. The plot records (not given here) used to derive the means in **Table 5** showed that the FYM and Mycorrhiza treatments had more plots than Untreated and Green Compost in Index 5 (middling emergence). Untreated plots were principally Index 3 and 4 (poorer emergence progress). There was no virus mottling or other symptoms seen at this time in 2020, all leaves being green.

On both the 20 February 2020 (when flowerbud stalks had fully extended) and 3 April 2020 (at the end of flowering) the Mycorrhiza treated plots had more leaves resulting in a denser canopy (**Figure 9 a & b**) and leaf height was taller within these Mycorrhiza treated plots (**Figure 9 c & d**).



Mycorrhiza Feb 2020 **Untreated**

Mycorrhiza April 2020 **Untreated**

Figure 9 : Plot 16 Mycorrhiza dense, taller canopy & Plot 9 Untreated in February and April 2020.

Leaf emergence and density compared between replicates

Emergence extent in Block 3 on 30 January 2020 was significantly ($P<0.01$) greater than all other Blocks (**Table 6**).

On 30 January 2020, looking into the trial from the southern end, it was noted that foliage cover (a combination of leaf density and leaf height) increased across the rows with distance from the main tramline / field track near Block 1. When each of the central two rows of each replicate were scored (ignoring the treatments) Block 1 had a mean 55.0% cover whereas by Block 5 this was 75% and so significantly greater ($P<0.001$) when analysed taking each plot per replicate as having the same cover (**Table 6**).

Table 6: Mean 0 - 9 index of emergence per replicate block (0 zero emerged to 9 = excellent establishment) for cv. Carlton *Narcissus* and the overall mean % of foliage cover per replicate Block across all treatments. 30 January 2020, Orange Field.

	Replicate Blocks							
Leaves	**BLOCK 1**	**BLOCK 2**	**BLOCK 3**	**BLOCK 4**	**BLOCK 5**	**L.s.d.**	**F value**	**Df**
Emergence extent Index per plot.	4.25	4.25	5.75	4.50	4.50	0.808	0.008	12
% foliage cover per replicate.	55.0 ab	50.0 a	60.0 b	70.0 c	75.0 c	8.10	<0.001	34

Different letters show significant differences using Duncan's multiple range test.

It was additionally noted on 30 January 2020 that there was delayed emergence where plot ridges had been compressed by the planter tractor wheel as it returned alongside an already planted row in the opposite direction. Similarly, the leaf cover in the return (discard) pair of rows was usually half or less that in the assessed central rows (data not presented) and this was attributed by the grower (M. Eves pers. comm.) to deeper planting of the bulbs as the tractor drove northwards down the slope than had been the case when planting southwards.

Leaf yellowing compared between treatments

The assessment of the proportion of leaf area yellowing was carried out on 3 April 2020 at the end of flowering, when any stresses due to inadequate root uptake from Fusarium basal root should have produced symptoms before the leaves started to senesce. Plants with fewer roots due to Fusarium would be less able to take up water and this, as well as rotting of the leaf bases at the infected basal plate could cause yellowing.

All treatments had a similar range of yellowing symptom severity across the affected leaves, with some cv. Carlton plants fully yellowed and nearly dead and others with just the tips yellowing or some smaller leaves yellowing lower down in the canopy (**Figure 10**). The cv. California Reclaim had been more advanced in growth and were starting to die back.

A greater proportion of the leaf canopy in the Mycorrhiza treatment appeared to be yellowing or necrotic than in the other treatments. However, leaf yellowing did not differ statistically between treatments using ANOVA (P>0.05) for cv. Carlton (**Table 7**), with an overall mean 7.6% of leaf area yellowed. However, using Duncan's multiple range test a significant difference was shown between the 11.4% area yellowing in the Mycorrhiza plots and the 4.6% in the Untreated.

In February 2020 the Mycorrhiza plots' foliage had appeared more abundant than the Untreated (**Figure 9**). This was still visible by 3 April 2020; the Mycorrhiza treated plots looked to have a dense

canopy, and the untreated plots appeared to have sparser leaves. It was possible that competition for light, water and nutrients in the denser Mycorrhiza treatment could have been contributing to their higher yellowing incidence than the Untreated. The soil, however, appeared moister under the denser canopy of the Mycorrhiza treatment even though these were all in the uppermost line up a slight slope.

a) Leaves from one nose yellowing.

b) Dead leaves from a single nose.

Figure 10: Yellowing of shoots coming from single bulb noses leading to leaf necrosis, probably caused by Fusarium rotting of the roots restricting water uptake and nutrient supply. Orange Field 3 April 2020.

Table 7: The proportion of the cv. Carlton *Narcissus* leaf area yellowing on 3 April 2020 after flowering in Orange Field in each of the treatments.

	Mean % Leaf yellowing per Treatment						12 df
	Un-treated	**Green Compost**	**FYM**	**Mycorrhiza Product**	**Overall mean**	**L.s.d.**	**F value**
% yellowing.	4.60 a	6.20 ab	8.00 ab	11.40 b	7.55	5.292	0.080
% yellowing adjusted for covariate	4.52	6.12	7.76	11.81	7.55	6.048	0.121
% yellowing omitting Mycorrhiza.	4.60 a	6.20 a	8.00 a	omitted	6.30	6.270	0.489

Different letters indicate significant difference using Duncan's multiple range test.

Leaf yellowing compared between plot lines

A covariate was put into the Analysis of Variance to take account of the positional effect of plot lines up the field to determine whether or not the yellowing on 3 April 2020 was statistically worse in the upper (southernmost) two lines of plots. The trend was not significant (Covariate F probability P = 0.722 1 df). A further analysis excluding the Mycorrhiza treatments did not show any significant difference between the remaining treatments (**Table 7**).

When a covariate for lines was used in analysis in order to account for any difference in leaf density which might have resulted from the position of treatments up the slope (data not shown), the proportion of leaf area yellowed per plot on 3 April 2020 showed no significant difference between replicate Blocks irrespective of treatment, with (P = 0.521) or without (P = 0.485) insertion of a positional covariate for plot lines in the Analysis of Variance. Block means ranged from 5.5% to 10.0% yellowing (L.s.d. 5.917) (table of data not shown).

Samples of plants with healthy green leaves and yellowing *Narcissus* plants were taken from across the trial on 3 April 2020 and the bulbs cut open. Some plants that had green leaves had dark brown internal necrosis typical of Fusarium symptoms and so it was determined that the true incidence of Fusarium infection was not able to be shown by assessing leaf yellowing alone at this stage of disease development, with further symptoms likely to be seen as the crop started bulb filling and nose production.

4.3. Flowerstem development

Flowerbud production compared across replicate blocks

On 20 February 2020, flowerbud stems of cv. Carlton were counted in the four one metre marked lengths per plot when the stems had fully extended. It was clear in the field that the upslope (Southern) plots in a line that were of all T4 (Mycorrhiza product) had more flowerbud stems per metre (lines being across the rows, at right angles to the replicate Blocks). Significantly (P<0.05) more flowerbud stems were present in the Mycorrhiza treatment than the FYM or Untreated, with 66 flower buds / m of row in the Mycorrhiza treatment, but 16 and 22 fewer buds in the FYM and Untreated, respectively. Neither the Green Compost, nor the FYM treatments differed from the Untreated (**Table 8**).

When plot means were mapped onto the trial plan, as well as more stems in the T4 line, there were more flowerbud stems in the next line of plots down the slope from the Mycorrhiza treatment line. This suggested that position in the field could have affected flower bud production treatment, therefore the Analysis of Variance was repeated with a covariate to take account of the potential positional effect. This resulted in an F-value for the covariate of P = 0.002 (1 df) indicating a positional effect. Taking out the positional covariate showed there was no significant difference (P>0.05)

between any of the four treatments, with a mean 54 flower bud stems per metre (**Table 8**, second row). As there was quite a wide range in flowerbud stem numbers between the Untreated at 44 / m and the Green Compost at 54 / m, analysis was repeated without the Mycorrhiza treatment, but still no significant difference (P>0.05) was shown between the other treatments (**Table 8**, third row).

Table 8: Comparison of the mean number of cv. Carlton *Narcissus* flowerbud heads per metre of row between treatments in Orange Field on 20 February 2020, before and after removal of a positional covariate due to a trend of more buds in the upper half of the field.

	Treatments						12 df
Flowerbud stems	**Untreated**	**Green Compost**	**FYM**	**Mycorrhiza Product**	**Overall mean**	**L.s.d.**	**F value**
Mean stems/m.	44.00 a	53.75 ab	50.20 a	65.85 b	53.50	14.81	0.044
Mean stems/m adjusted for covariate.	45.59	55.34	54.96	57.91	53.50	11.01	0.105
Mean stems/m for treatments other than Mycorrhiza.	44.00	53.75	50.2	-	49.3	17.87	0.478

Different letters show significant differences using Duncan's multiple range test.

Flowerbud production compared across replicate blocks

On 20 February 2020, as well as a greater number of flowerbud stems within the rows in the top two lines of the field, it was seen that these increased in density across the trial area moving away from the tramline near replicate Block 1 (mean 61 flowerbud stalks / m row) across to Block 5 (mean 39 flowerbud stalks / m row) (**Table 9**, first row), with significant difference (P<0.05) between blocks.

Table 9: Comparison of the mean number of cv. Carlton *Narcissus* flowerbud heads per metre of row between replicate Blocks in Orange Field on 20 February 2020, before and after removal of a positional covariate due to a trend of more buds in the upper half of the field.

	Replicates						12 df
Flowerbud stems	**BLOCK 1**	**BLOCK 2**	**BLOCK 3**	**BLOCK 4**	**BLOCK 5**	**L.s.d.**	**F value**
Mean stems/m.	39.1	47.3	51.9	68.0	61.0	16.56	0.019
Mean stems/m adjusted for covariate.	39.1	47.3	51.9	68.0	61.0	11.29	0.001
Mean stems/m without Mycorrhiza.	37.0	44.1	48.5	60.8	56.2	23.07	0.223

The difference between replicate blocks increased in significance (P<0.001) when the positional covariate (for the lines, as described above) was taken out (**Table 9**, second row). The significance of difference was lost (P>0.05), however, when the high number of flowerbud stems in the Mycorrhiza plots were excluded from the Block means (**Table 9**, third row).

4.4. Foliage diseases

On 2 April 2020, a low incidence of smoulder (*Botrytis narcissicola,* which develops from mycelium carried in the bulb neck) was recorded on the leaves across some plots, without any obvious visible treatment differences. The Untreated had a mean 0.06%, Green compost 0.08%, FYM 0.1% and the Mycorrhiza 0.02% of leaf area affected. Regression analysis was performed on the basis of presence or absence of smoulder symptoms in the plots (**Table 10**). There was no significant difference between treatments (as plots of the same treatment varied in disease presence).

Table 10: The proportion of cv. Carlton *Narcissus* plots per treatment in Orange Field with traces of smoulder on 3 April 2020 compared using regression analysis.

	Treatments				12 df
	Un-treated	**Green Compost**	**FYM**	**Mycorrhiza Product**	**Approx Chi pr**
% of plots with Smoulder.	40.00	80.00	80.00	20.00	0.106
Standard error of means.	20.82	17.57	17.57	16.98	-

Some streaking that resembled the soil-borne disease yellow stripe virus was seen on 35% to 40% of the leaves across the trial area on 3 April (plot scores were not made), without any obvious difference across the treatments.

4.5. Fusarium incidence on harvested bulbs

Fusarium basal rot compared between treatments

On the 23 June 2020, following storage after bulb harvest on 4 June, the total number of healthy bulbs and Fusarium affected bulbs within each nose category was recorded for each of the four one metre row lengths per plot, and mean results per plot compared between treatments.

There were no significant differences between treatments in external Fusarium symptom incidence (**Table 11** & **Figure 11**). On average 8.8% of the bulbs were affected per treatment, with a mean 5.6 Fusarium affected bulbs per metre of row, resulting in a mean of 129 g of the harvest per metre of row being unmarketable (**Table 11**). On average 2.54 kg of healthy bulbs were harvested per metre (**Table 24**). The greatest number of Fusarium affected bulbs in any nose category was for single-nosed bulbs, with few bulbs having grown to produce more noses when affected (**Table 12**).

Table 11: The mean number of *Narcissus* bulbs per treatment that had external symptoms of Fusarium basal rot per metre, the mean total weight of all the Fusarium affected bulbs per metre and the % of the total bulbs that had externally visible Fusarium. Assessed on cv. Carlton after storage for 19 days following hand-lifting on 4 June 2020 from Orange Field.

	Treatments						12 df
	Un-treated	Green Compost	FYM	Mycorrhiza Product	Overall mean	L.s.d.	F value
Number Fusarium affected bulbs/m.	5.80	4.40	5.85	6.25	5.58	3.694	0.718
% of total bulbs with Fusarium.	10.13	6.93	9.66	8.48	8.80	5.750	0.635
Weight (g) / m of row of bulbs with Fusarium.	147.0	97.0	145.0	126.0	129.0	110.7	0.738

Table 12: The number of cv. Carlton bulbs per metre of row that had externally visible Fusarium per nose category for each of the four treatments. Assessed after storage for 19 days following hand-lifting on 4 June 2020 from Orange Field.

	Mean bulbs per metre with externally visible Fusarium basal rot per treatment			
Size grade	**Untreated**	**Green Compost**	**FYM**	**Mycorrhiza Product**
1-nose	2.8	2.2	2.2	3.1
2-nose	1.4	1.4	2.1	1.3
3-nose	1.0	0.3	0.5	0.6
4-nose	0.0	0.1	0.1	0.0
5-nose	0.0	0.0	0.0	0.0
Daughter	0.6	0.6	1.1	1.4
Total	**5.8**	**4.4**	**5.9**	**6.3**

Fusarium basal rot compared across replicate blocks

There was no difference between the replicate blocks in the incidence of Fusarium, with a range from 5.2% to 12.6% of bulbs with externally visible symptoms (**Table 13**).

Table 13: The mean number/metre of cv. Carlton bulbs/replicate with external symptoms of Fusarium basal rot, the mean total weight of all Fusarium affected bulbs/metre and the % of the total bulbs with externally visible Fusarium. Assessed after 19 days storage after lifting on 4 June 2020.

	Replicates						12 df
	BLOCK 1	**BLOCK 2**	**BLOCK 3**	**BLOCK 4**	**BLOCK 5**	**L.s.d.**	**F value**
Number of Fusarium affected bulbs/ m.	6.38	4.56	8.19	3.75	5.00	4.130	0.216
Weight (g) / m with Fusarium.	134.0	97.0	192.0	77.0	143.0	123.7	0.347
% total bulbs with Fusarium.	12.15	6.45	12.58	5.19	7.63	6.428	0.089

Healthy bulb yield

4.5.1. Total healthy bulb yield

Comparison of healthy bulb numbers between treatments

The majority of harvested bulbs had no externally visible Fusarium basal rot, with no significant treatment differences in healthy bulbs per metre (**Figure 11** and **Table 14**), with a mean 54.4 bulbs.

Across all the nose categories when including the daughters, there was no significant difference in the total number of healthy bulbs for any treatment, with a mean 61.7 bulbs per metre of row (**Table 14**). There was a total count in the Mycorrhiza treatment of 77.4 bulbs/m across all bulb categories, or 67.8 bulbs/m for full-sized bulbs (when excluding detached daughters), ranking the highest count, but this was not significantly more than in the other treatments (mean 54.4 bulbs/m) (**Table 14**).

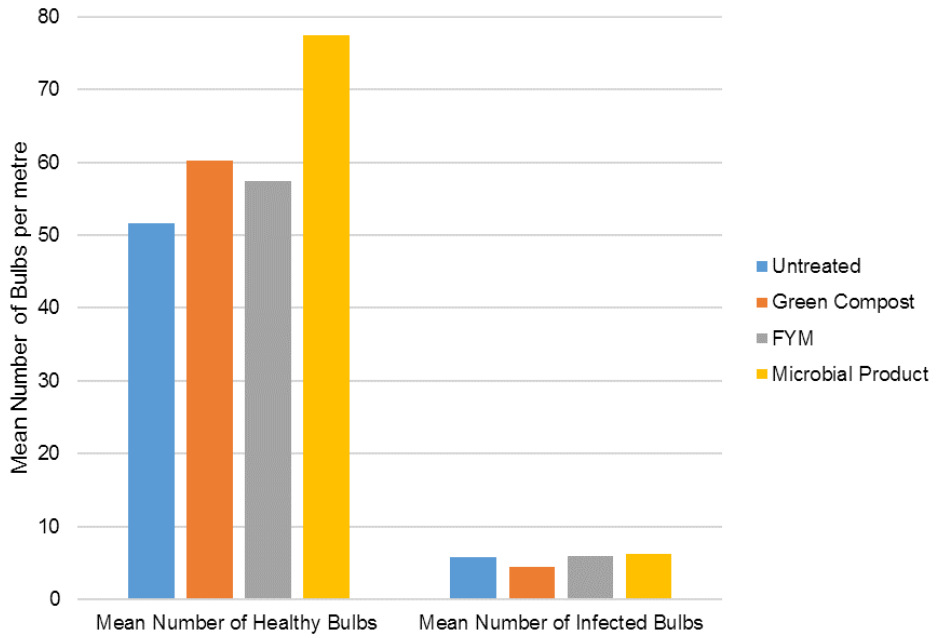


Figure 11: Mean number of *Narcissus* bulbs cv. Carlton after harvest in June 2020 in each of four treatments either with exterior symptoms of Fusarium or appeared healthy. There were no significant differences between treatments, with 51.7 to 77.4 healthy bulbs (L.s.d. 22.09) and 4.4 to 6.3 Fusarium basal rot bulbs (L.s.d. 3.69) per metre of row. Four metres sampled/plot.

Table 14: The mean number of externally healthy bulbs / metre of row per treatment for cv. Carlton (four lengths/plot) within each nose category. Assessed 19 days after harvest of Orange Field on 4 June 2020. Significantly (P<0.05) fewer healthy 2-nose bulbs in the Untreated and FYM than for Mycorrhiza treated.

	Mean number of healthy bulbs per metre						12 df
Nose Category	**Untreated**	**Green Compost**	**FYM**	**Mycorrhiza Product**	**Overall mean**	**L.s.d.**	**F value**
1-nose	12.05	15.35	12.05	21.50	15.24	9.635	0.163
2-nose	19.00a	23.55ab	21.60a	30.25b	23.60	7.784	0.045
3-nose	12.40	14.90	15.65	15.65	14.65	5.335	0.521
4-nose	1.35	0.65	0.70	0.45	0.79	0.877	0.187
5-nose	0.20	0.10	0.05	0.00	0.09	0.322	0.588
Noses Total	**45.00**	**54.55**	**50.05**	**67.85**	**54.36**	**20.345**	**0.140**
Daughters	6.70	5.70	7.45	9.55	7.35	4.128	0.268
Overall Total	**51.70**	**60.25**	**57.50**	**77.40**	**61.71**	**22.09**	**0.121**

Different letters show significant differences using Duncan's multiple range test.

Comparison of healthy bulb numbers between plot lines

The absence of a significant difference between treatments in the number of healthy bulbs was because of wide variation in the plot means for the same treatment, with a bias towards higher bulb counts in the Southern half of the field. The means for the Untreated were very consistent across replicate blocks with a range of 40 to 50, whereas the Green Compost varied from 37 in Block 5 up to 90 in Block 2. FYM varied from 32 to 83 and the Mycorrhizal Product from 40 to 83 bulbs per metre (**Table 15**). All ten plots with less than the mean 58.99 healthy bulbs per metre were in the lower, northern, field half (**Table 15**). Fusarium affected bulbs were too few/plot to compare densities.

Table 15 : Plot arrangement for Untreated, Green compost, FYM and Mycorrhiza to show the greater mean number of healthy bulbs of 1 to 5 noses lifted per metre in southernmost lines three and four.

Block 5		**Block 4**		**Block 3**		**Block 2**		**Block 1**		**Line**
Plot & treatment	Bulbs/m	Plot & treatment	Bulbs/m	Plot & treatment	Bulbs/m	Plot & treatment	Bulbs/m	Plot & treatment	Bulbs/m	
20M	70.8	16M	83.5	12M	61.2	8M	83.8	4M	40.0	**Four**
19U	45.0	15F	83.2	11G	56.8	7G	90.0	3U	46.8	**Three**
18G	37.5	14U	50.5	10F	50.5	6U	43.2	2F	31.8	**Two**
17F	42.5	13G	51.8	9U	39.5	5F	45.2	1G	36.8	**One**

Key of letters next to plot numbers: M = Mycorrhiza, U = Untreated, G = Green compost, F = FYM

4.5.2. Healthy bulb nose number increase and nose grade frequency

Bulbs planted were single nosed, with favourable growing conditions over two years increasing bulb size and usually the number of noses. The only difference between the treatments in the proportion of healthy bulbs in each of the nose categories was for the two-nose category (**Table 14**)). More bulbs were two-nosed than any other category (mean 23.6 two-nose bulbs/m) across the treatments. Significantly more ($P<0.05$) healthy two-nosed healthy bulbs per metre were counted in the Mycorrhiza treatment (with 30.2 bulbs / m) than in either the FYM (21.6) or Untreated treatments (19.0). The additional two-nose bulbs in the Mycorrhiza treatment were not because of a reduction in the number of one-nosed or three-nosed bulbs as the numbers were statistically similar in the other treatments. The next most frequent bulb sizes in all treatments were the one and three nosed.

There were no significant differences in the percentage of healthy bulbs within each of the nose categories, indicating that no particular treatment increased the nose number, with a mean 38.2% of bulbs across the four treatments having two noses, and 23.9% and 24.0% being one and three-nosed, respectively (**Table 16**).

Table 16 : The % of total cv. Carlton *Narcissus* bulbs per treatment that were externally visibly healthy per nose category. After storage for 19 days following hand-lifting on 4 June 2020 from Orange Field.

Nose Category	% of total bulbs within each treatment that were healthy within each nose category						12 df
	Untreated	Green Compost	FYM	Mycorrhiza Product	Overall mean	L.s.d.	F
1-nose	23.18	22.68	22.16	27.49	23.88	5.599	0.199
2-nose	37.14	38.68	37.56	39.59	38.24	5.833	0.794
3-nose	23.90	26.90	25.50	19.80	24.00	7.550	0.250
4-nose	2.57	1.28	1.55	0.60	1.50	1.776	0.165
5-nose	0.36	0.20	0.14	0.00	0.18	0.610	0.647
Daughter	12.87	10.28	13.10	12.50	12.19	5.121	0.622

5-nose bulbs were absent in the row lengths assessed for the Mycorrhiza treatment.

4.5.3. Total bulb numbers harvested (healthy and unmarketable)

Adding together the number of one to five-nose healthy bulbs that would have originated from the planted bulbs (i.e. excluding the daughters that detached from the original bulbs) (**Table 14**) and the number of one to five nosed bulbs with visible Fusarium (**Table 12**) gave the total mean number of bulbs per metre of row, ranging from 50 /m in the Untreated to 73 /m in the Mycorrhizal treatment (**Table 17**), with no significant difference between treatments although the range was wide.

Table 17: The total mean number of *Narcissus* bulbs per metre of row per treatment (with or without externally visible Fusarium) harvested from Orange Field on 4 June 2020.

1 to 5 nose categories	Mean number of bulbs/metre per treatment				12 df	
	Untreated	Green compost	FYM	Mycorrhizal Product	L.s.d.	F value
Healthy + Fusarium	50.10	59.35	54.80	72.70	21.096	0.164

There was also a wide variation across the five replicate blocks in the number of bulbs retrieved per treatment (**Table 18**). The planter was set to drop 85 bulbs per metre of row, whereas the mean number of bulbs harvested was 59. It was possible that this resulted from bulbs rotting away from the Fusarium that they had carried with them into the field.

Table 18: The total mean number of *Narcissus* bulbs per metre of row per replicate block (with or without externally visible Fusarium) harvested from Orange Field on 4 June 2020.

	Mean number of bulbs/metre per replicate						12 df
1 to 5 nose categories	**BLOCK 1**	**BLOCK 2**	**BLOCK 3**	**BLOCK 4**	**BLOCK 5**	**L.s.d.**	**F value**
Healthy + Fusarium	44.12	68.81	59.00	70.00	53.00	23.587	0.155

Bulb counts and nose number compared across replicate blocks

Position in the field (already found to potentially cause differences up the field between plot lines) was also found to have some effect across the replicate blocks (**Table 19** & **Table 20**).

Table 19: The mean number of external visibly healthy cv. Carlton *Narcissus* bulbs / metre of row within each nose category per replicate block after harvest on 4 June 2020 at Orange Field. Comparing across five replicate blocks and showing that 3-nosed bulbs became more frequent sampling from Block 1 to 5, 1-nosed predominated in Block 2, and 2-nosed were commonest in the Blocks 2, 3 and 4.

Nose Category	Mean number of healthy bulbs per metre						12 df
	BLOCK 1	**BLOCK 2**	**BLOCK 3**	**BLOCK 4**	**BLOCK 5**	**L.s.d.**	**F value**
1-nose	14.12	29.50	10.12	15.81	6.62	10.772	0.006
2-nose	17.06	25.69	26.75	30.12	18.38	8.703	0.028
3-nose	6.75	9.00	14.56	20.69	22.25	5.964	<0.001
4-nose	0.81	0.62	0.56	0.56	1.38	0.980	0.372
5-nose	0.06	0.00	0.00	0.06	0.31	0.360	0.350
Noses total	38.81	64.81	52.00	67.25	48.94	22.746	0.095
Daughter	5.50	5.56	6.81	7.75	11.12	4.615	0.110
Total	**44.30**	**70.40**	**58.80**	**75.00**	**60.10**	**24.69**	**0.130**

Combining across the treatments, there were differences in the number of bulbs of particular categories in each of the replicates (plots in a replicate being in the same row ridges) (**Table 19**). Block 2 plots had a mean 29.5 healthy bulbs / metre with one nose and the other four replicates significantly ($P<0.01$) fewer. For two-nosed bulbs the central three blocks held the greatest numbers ($P<0.05$). A highly significant difference ($P<0.001$) was found for three-nosed bulbs, with Blocks 4 and 5 having a greater number of healthy three-nosed than the other replicates. This block effect was of greater statistical significance than any treatment effects. Across all the nose categories, however, no particular replicates had more or less bulbs than the others, indicating that more bulbs in a particular nose category resulted in less in others as bulbs grew more noses.

The differences in bulb numbers in particular categories across the replicates was reflected in the % of total bulbs that were healthy within each nose category, with the bulbs that had grown the most being in Block 5 (reducing the number of single and double-nosed), and the higher proportion of single nose bulbs being in Blocks 1 and 2 (**Table 20**).

Table 20: The **%** of total cv. Carlton *Narcissus* bulbs that were externally visibly healthy per nose category out of the total recorded per replicate block across all four treatments. Assessed after storage for 19 days following hand-lifting on 4 June 2020 from Orange Field. Showing that Block 2 had more 1-nose than the other blocks. Block 3 had more 2-nose than Blocks 2 and 5. Block 5 had more 3-nose than the other blocks.

Nose Category	% of total bulbs in each Replicate Block that were healthy within each nose category						12 df
	BLOCK 1	BLOCK 2	BLOCK 3	BLOCK 4	BLOCK 5	L.s.d.	F
1-nose	31.65	40.64	16.96	20.44	9.70	6.260	<0.001
2-nose	38.66	36.68	45.48	41.02	29.36	6.522	0.002
3-nose	15.30	12.50	24.80	27.90	39.50	8.450	<0.001
4-nose	2.03	1.02	1.07	0.94	2.43	1.985	0.389
5-nose	0.18	0.00	0.00	0.11	0.59	0.682	0.355
Daughter	12.15	9.14	11.65	9.58	18.41	5.725	0.028

4.5.4. Individual bulb weights

Comparison of individual bulb weights between treatments

The mean individual bulb weights per nose, calculated from the numbers in the four one metre rows lifted on 4 June 2020, increased with increasing numbers of noses (**Figure 12** & **Table 21**).

Whereas the Untreated, Green Compost and FYM had similar weights in each category, the two and three nosed Mycorrhiza treated bulbs were significantly ($P<0.05$) lighter in weight than the other treatments causing a significantly ($P<0.05$) lower total mean weight of Mycorrhiza treated bulbs (**Table 21**). Although not significantly lower (**Table 21**) the Mycorrhiza treated bulbs in the one and four nose categories also ranked the lowest weights. Significantly more double-nosed Mycorrhiza treated lighter bulbs were lifted (**Table 14**) and so there could have been more competition for resources, but bulb counts were similar across the treatments for the other nose categories.

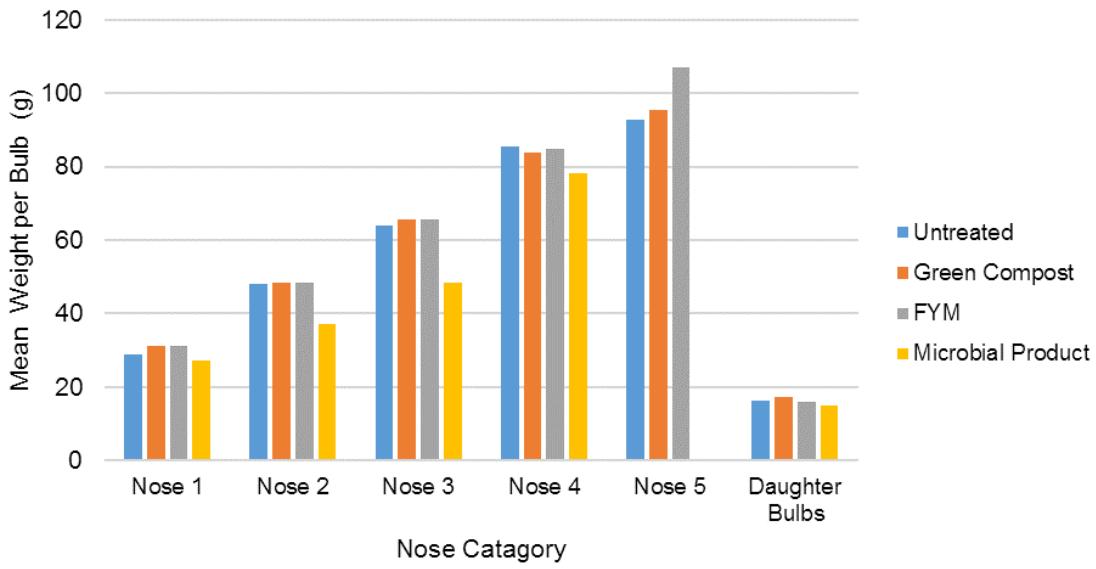


Figure 12: The mean weight per healthy cv. Carlton *Narcissus* bulb (g) within each nose category for each of the four treatments. Bulbs from Mycorrhiza plots with two or three noses were significantly (P<0.05) lighter than from the other treatments.

Table 21: The mean individual weight per externally visibly healthy cv. Carlton *Narcissus* bulb (g) within each of five nose categories for Untreated, Green compost, FYM and the Mycorrhiza product weighed after storage for 19 days following lifting on 4 June 2020 from Orange Field.

Nose Category	Mean individual weights of healthy bulbs (g)						12 df
	Untreated	Green Compost	FYM	Mycorrhiza Product	Overall mean	L.s.d.	F value
1-nose	28.86	31.29	31.19	27.12	29.62	5.296	0.301
2-nose	48.00	48.50	48.40	37.10	45.50	7.500	0.014
3-nose	64.10	65.70	65.70	48.50	61.00	14.060	0.050
4-nose	85.50	83.80	84.80	78.30	83.10	23.020	0.895
5-nose	92.80	95.40	107.20	x			
Noses mean	63.90	64.90	67.50	47.80			
Daughter	16.27	17.21	15.85	15.06	16.10	2.665	0.400
Overall Mean	**45.20**	**47.30**	**45.70**	**34.00**	**43.10**	**8.59**	**0.020**

X = 5-nose bulbs were absent in the row lengths assessed for the mycorrhiza treatment and so analysis was not done where shown by blanks in the table.

Comparison of individual bulb weights between replicates

Examination of the results across the treatments for the replicate blocks (**Table 22**) did not show any reduction in mean bulb weight where the one, two and three nosed healthy bulbs were lifted in significantly greater number in particular replicates (**Table 19**). Daughter (detached) bulbs were significantly (P<0.05) heavier in Block 5 than in Blocks 1 and 4 (**Table 22**).

Table 22: The mean individual weight per externally visibly healthy cv. Carlton *Narcissus* bulb (g) per nose category within each of five replicate blocks. Weighed after storage for 19 days following hand-lifting from Orange Field on 4 June 2020.

Nose Category	Mean individual weights of healthy bulbs (g)						12 df
	BLOCK 1	BLOCK 2	BLOCK 3	BLOCK 4	BLOCK 5	L.s.d.	F value
1-nose	34.07	29.91	28.32	29.10	26.67	5.921	0.148
2-nose	46.60	42.60	46.80	42.60	49.00	8.380	0.409
3-nose	66.10	55.70	60.80	56.30	66.10	15.710	0.455
4-nose	92.20	64.20	88.20	78.70	92.20	25.740	0.151
Daughter	14.39	16.49	16.78	13.64	19.19	2.980	0.012
Total mean	**42.70**	**36.80**	**44.10**	**41.40**	**50.40**	**9.61**	**0.099**

Five-nose data omitted as few were present, with none in the Mycorrhiza treatment.

4.5.5. Bulb weight related to size grade

The metre row length samples of cv. Carlton bulbs taken in June at four positions outside the main assessment lengths of Untreated plot 6 (to measure bulb diameters in relation to bulb weight) contained from 44 to 66 bulbs (**Table 23**) with two noses being the commonest category. All except the daughters were over size 10 and so suitable for forcing in trays or retail sale. These row lengths were weighed at lifting and then reweighed after a fortnight in storage when a mean 12.36% weight reduction had occurred (data not shown). Therefore, weights for the whole trial would have been greater at lifting on 4 June 2020 than when the records were taken after the commercial standard storage interval before grading.

Table 23: 4 June 2020 sample of untreated bulbs recording the mean bulb diameter for the nose categories and corresponding grading size based on circumference at broadest girth.

Nose Category	Mean bulb diameter (cm)	Circumference (cm)	Size grade	Mean bulb weight (g)
1-nose	3.60	11.29	11	30.60
2-nose	4.86	15.26	15	49.49
3-nose	6.01	18.89	18	62.90
4-nose	7.16	22.48	22	78.75
Daughter	3.09	9.71	9	17.64

4.5.6. Total weights per treatment of bulbs harvested

Total weights of healthy bulbs compared between treatments

The total weight of healthy bulbs within each of the nose categories and daughters after storage did not differ significantly between the treatments (**Table 24**). There was consequently no significant difference in the mean total weight of healthy bulbs harvested for each treatment, with a mean 2.54 kg of bulbs per metre of row (**Table 24**). This was produced by a mean 61.7 healthy bulbs of all nose categories plus daughters per metre of row (**Table 14**).

Table 24: Mean results from four metre lengths per plot for four treatments and five replicates of cv. Carlton *Narcissus* for total weight of externally visibly healthy bulbs within a metre of row. Assessed after storage for 19 days following hand-lifting from Orange Field on 4 June 2020.

Nose Category	Total weight (g) of healthy bulbs / metre						12 df
	Untreated	Green Compost	FYM	Mycorrhiza Product	Overall mean	L.s.d.	F value
1-nose	374.0	444.0	383.0	571.0	443.0	220.9	0.238
2-nose	917.0	1085.0	1014.0	1121.0	1034.0	289.5	0.460
3-nose	803.0	1001.0	975.0	724.0	876.0	303.1	0.190
4-nose	107.0	52.0	62.0	35.0	64.0	66.0	0.162
5-nose	18.6	9.5	5.4	0.0	8.4	29.6	0.589
Daughter	110.8	100.6	119.7	143.3	118.6	57.5	0.445
Total	**2330.0**	**2693.0**	**2559.0**	**2495.0**	**2544.0**	**465.5**	**0.412**

The bulbs in the assessed two rows of each of the four treatments per replicate block were planted in the same pair of ridges up the field. A significant difference ($P<0.01$) was shown between replicate blocks in the total weight of healthy bulbs harvested (**Table 25**). Highly significant differences between replicates ($P<0.001$) were shown for one and three nosed bulbs, with two nosed also differing ($P<0.05$) between replicates.

Total weights of healthy bulbs compared between replicate blocks

The replicate blocks with significantly higher numbers of healthy bulbs per metre in the one, two and three nose categories (**Table 19**) corresponded to those with a higher weight of healthy bulbs per replicate block (**Table 25**). Greater total weights were recorded for one nosed in Block 2, two nosed in Blocks 2, 3 and 4 and three nosed in Blocks 4 and 5 (**Table 25**). The total weight of daughters in Block 5 was also greater than in all other replicates ($P<0.01$) (**Table 25**) and this replicate had had a higher ranking, but not significantly higher, number of bulbs than the other replicates (**Table 19**). Block 1 yielded significantly less total weight of healthy bulbs ($P<0.01$) than the other blocks, with only 1.86 kg of bulbs/m of row, whereas the other four replicates were similar (mean 2.72 kg/m).

Block 1 did not have significantly more bulbs with Fusarium, nor a significantly greater incidence of visibly affected bulbs (**Table 13**) that might account for the lower yield of healthy bulbs. Block 1 was closest to the main thoroughfare tramline (separated by two discard rows).

Table 25: Mean results from four metre row lengths lifted per plot for the five replicates of cv. Carlton *Narcissus* for total weight of externally visibly healthy bulbs per metre. Assessed after storage for 19 days following hand-lifting from Orange Field on 4 June 2020.

Nose Category	Total weight (g) of healthy bulbs / metre						12 df
	BLOCK 1	BLOCK 2	BLOCK 3	BLOCK 4	BLOCK 5	L.s.d.	F value
1-nose	478.0	843.0	290.0	441.0	164.0	247.0	<0.001
2-nose	782.0	1056.0	1232.0	1239.0	861.0	323.7	0.028
3-nose	440.0	468.0	871.0	1121.0	1478.0	139.1	<0.001
4-nose	71.0	42.0	51.0	43.0	116.0	30.3	0.220
5-nose	6.7	0.0	0.0	3.8	31.3	33.1	0.264
Daughters	78.9	93.4	112.8	101.7	206.4	64.2	0.007
Total	**1858.0**	**2502.0**	**2556.0**	**2950.0**	**2856.0**	**520.4**	**0.005**

4.5.7. Comparison between flowerbud stem counts and harvested bulb numbers

As harvested bulb counts were made within each plot from the same four one metre lengths as used for flowerbud stem counting the two can be directly compared to determine any relationship. Significantly more flowerbud stems were produced in the Mycorrhiza treatment (**Table 8**) and this could be related to how many noses were present and producing a flowerbud stem. The Mycorrhiza treatment had a mean of 66 flowerbud stems per metre and the Untreated had 44 flowerbud stems / m (**Table 8** & **Table 26**). When the healthy bulbs including daughters were counted, the Mycorrhiza treatment had 77 bulbs / m, whereas the untreated had 52 bulbs / m (**Table 14** & **Table 26**). The number of noses present in each treatment were estimated by multiplying the number of healthy bulbs within each of the one to five nose categories plus daughters by the nose number of each category (as given in **Table 14**). This gave 140 noses / m for Mycorrhiza treatment compared with 100 noses / m in the untreated plots (**Table 26**). Extrapolating further from this; 47% of the noses in the Mycorrhiza treatments may have produced flowerbuds compared with 44% in the Untreated (**Table 26**). The additional estimated 3% of noses of Mycorrhiza treated bulbs that produced a flowerbud stem was not assessed statistically but suggests the additional bulb numbers present at harvest (that were likely to have been present earlier during flowering) were influential in the additional flowerstem numbers rather than that the product stimulated increased flowerstem production of nodes.

Table 26: Comparison of flowerbud numbers in February 2020 and bulb nose numbers at June harvest (daughters counted as one nose) from the same lengths of row in Orange Field.

	Mean records per metre of row			
Treatment	**Number of healthy bulbs (counted)**	**Number of flower buds (counted)**	**Number of noses (calculated)**	**% of noses producing flower buds (estimated)**
Untreated	51.70	44.00	100.35	43.8
Green compost	60.25	53.75	115.95	45.7
FYM	57.50	50.20	112.70	44.5
Mycorrhiza product	77.40	65.85	140.30	46.9

4.6. Mycorrhizal colonisation of roots and Fusarium basal root symptoms

Percentage of bulbs colonised and percentage of root area colonised by mycorrhiza

Staining and microscope assessment of the roots from the 30 bulbs from a range of sizes collected in June 2020 from plot 9 (Untreated) and plot 12 (Mycorrhiza) (**Figure 13**) showed that all but one of the bulbs from Mycorrhiza treatment had mycorrhizal colonisation (i.e., 97% of bulbs) compared with six of the 29 Untreated bulbs with roots present (86% of bulbs colonised) (**Table 27**).

Bulbs from Untreated plot 9

Bulbs from Untreated plot 9

Bulbs from Mycorrhiza treatment plot 12

Bulbs from Mycorrhiza treatment plot 12

Figure 13: Bulbs examined for the extent of root colonisation by mycorrhiza. Two trays of 15 *Narcissus* bulbs from an Untreated and a Mycorrhiza treated plot harvested from Orange Field on 4 June 2020 showing the range of sizes in the random samples.

The most noticeable difference between the treatments was the proportion of the root area colonised; with a mean of 30.4% root area per Mycorrhiza treated bulb showing mycorrhiza presence, whereas colonisation was 6.5% in the bulbs which were not planted with Mycorrhiza granules (**Table 27**). The maximum root area colonised in the Mycorrhiza treated plots was 63%, whereas the Untreated bulbs' roots were at most 26% colonised (**Table 27**). Statistical comparison of the plots was not intended, as the examination was carried out as a spot check without replication.

Table 27: The percentage of cv. Carlton *Narcissus* root area colonised by arbuscular mycorrhizal fungi (AMF) of any species from 30 bulbs from each of an Untreated and a Mycorrhiza treated plot. Whether (1) or not (0) Fusarium necrosis was present either externally or internally in each bulb. Bulbs harvested from Orange Field on 4 June 2020 and examined by PlantWorks Ltd.

Individual results for 30 bulbs Plot 9 Untreated				**Individual results for 30 bulbs Plot 12 Mycorrhiza Product**			
Fusarium rot present	**% of root area with AMF**	**Fusarium rot present**	**% of root area with AMF**	**Fusarium rot present**	**% of root area with AMF**	**Fusarium rot present**	**% of root area with AMF**
1	*	0	8.5	1	51.0	0	34.0
1	10.0	0	8.0	1	14.0	0	31.0
1	10.0	0	7.5	0	63.0	0	26.0
1	6.0	0	6.5	0	58.0	0	25.0
1	2.0	0	5.0	0	58.0	0	24.0
1	0.0	0	5.0	0	54.5	0	24.0
1	0.0	0	4.5	0	52.0	0	13.5
0	26.0	0	4.0	0	47.0	0	12.5
0	15.0	0	3.0	0	45.0	0	8.0
0	13.0	0	3.0	0	44.5	0	7.0
0	11.0	0	1.0	0	42.0	0	6.0
0	10.5	0	0.0	0	41.5	0	5.5
0	10.0	0	0.0	0	41.0	0	4.0
0	10.0	0	0.0	0	40.0	0	3.0
0	9.0	0	0.0	0	37.0	0	0.0

***** This Fusarium infested bulb had no roots left as they had rotted away.

Internal and external Fusarium rot in bulbs sampled for mycorrhiza

There was only a small number of bulbs visibly infested by Fusarium. The symptom was a dark brown rot (**Figure 14**b & d). In the Untreated samples, of the seven bulbs with visible Fusarium, one bulb had totally rotted away roots, with indigenous mycorrhizal colonisation of 0% to 10% in the remainder (**Table 27**). All but five of the 23 visibly healthy Untreated bulbs were also within this

colonisation range. The two bulbs with visible Fusarium in the Mycorrhiza inoculated plots were above this colonisation range, and so bulbs with less mycorrhizal root colonisation at harvest were not necessarily those showing Fusarium (**Table 27**).

Mycorrhiza treated bulbs in the 30-bulb sample from plot 12 had a lower incidence of Fusarium rot visible either externally or on cutting the bulb open; (6.7% of the sample) compared with Untreated bulbs (23.3% of the sample) from plot 9 (**Table 27** & **Table 28**). However, statistical significance of this difference is not proven because the sampling was designed to see if there had been successful mycorrhizal colonisation, not to relate this to Fusarium presence, and so sampling was not replicated across the field.

Fusarium had been recorded in 38% of bulbs sampled at the time of planting, but this had followed incubation to encourage any Fusarium mycelium present to develop through the bulb tissue. An incidence of infestation of 23% with Fusarium rot (**Table 28**) was shown in the similar Untreated sample size checked for mycorrhiza at harvest. The latter would have excluded any browning on tissue not visible on cut bulb faces, and these bulbs were also not incubated.

The small sample of cut-open bulbs showed only 6.7% of Mycorrhiza treated bulbs had Fusarium compared to 23% on the untreated control (**Table 28**). However, no significant differences had been shown (**Table 11**) between the Untreated and Mycorrhiza treated bulbs from the bigger sample size of four metre samples of row per replicate across the full harvest lift of five replicates of cv. Carlton in the proportion of Fusarium infested bulbs (10.1% and 8.5%, respectively).

Table 28: Summary of assessment by PlantWorks laboratory of 30 bulbs lifted on 4 June 2020 from each of plot 9 (Untreated) and plot 12 (Mycorrhiza), Orange Field.
Mean % of root area per bulb colonised by mycorrhiza. % of bulbs with mycorrhiza on their roots and the % of bulbs with either internal or external symptoms of Fusarium.

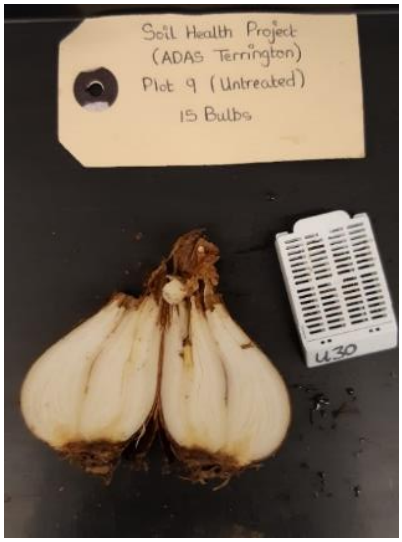
	Treatment	
Measurement	**Untreated**	**Mycorrhiza product**
% of bulbs with mycorrhiza (of those bulbs with roots)	79.3	96.7
Mean % root area per bulb colonised by mycorrhiza whether or not Fusarium was present	6.5	30.4
% of bulbs with Fusarium rot visible externally or on cutting	23.3	6.7

In both treatments mycorrhiza includes that indigenous to the soil or on the bulbs pre-inoculation.

a) Mycorrhiza treated.
No Fusarium rot.
58% AMF root colonisation

b) Mycorrhiza treated
Fusarium basal rot.
51% AMF root colonisation

c) Untreated.
No Fusarium rot.
0% AMF root colonisation



d) Untreated.
Fusarium basal rot.
2% AMF root colonisation

Figure 14: Two of the *Narcissus* bulbs harvested in June 2020 from plots 9 (Untreated) and 12 (Mycorrhiza) cut open showing the dark brown internal necrosis produced by *F. oxysporum*. The roots have been cut off and placed in the white cartridges for staining to aid microscope assessment of the proportion of the root area colonised by arbuscular mycorrhizal fungi (AMF). Pictures provided by J. O'Reagan / PlantWorks Ltd..

4.7. *Verticillium dahliae* microsclerotia in soil samples

Harris tests carried out by the ADAS laboratory at High Mowthorpe, following soil sampling in Orange Field on 14 November 2017, gave 14.2 viable propagules of *Verticillium dahliae* / g of soil. Neither *Sclerotium cepivorum* nor *Fusarium oxysporum* were detected in the soil by qPCR. No qPCR results were sought for *Verticillium longisporum* and *Verticillium dahliae* (they are not pathogens of *Narcissus* and so determining any correlation of levels with disease in the crop was not applicable). The *V. dahliae* viable propagule count, due to the persistence of microsclerotia in soil for up to 14 years, was relevant to potential susceptible future crops in the rotation and any changes to the levels brought about by treatments associated with the *Narcissus* crop would be relevant to them.

Comparison of V. dahliae counts between treatments

Soil samples were taken in April 2020 from within individual plots in the trial area (**Table 29**).

Table 29: Harris test results for viable *V. dahliae* microsclerotia counts in soil samples taken on 29 April 2020 from the 20 plots of cv. Carlton in Orange Field comparing treatments.

	Treatments						12 df
Viable *V. dahliae*	**Un-treated**	**Green Compost**	**FYM**	**Mycorrhiza Product**	**Overall mean**	**L.s.d.**	**F value**
Micro-sclerotia / g of soil	17.98 b	17.12 b	16.76 b	11.24 a	15.80	4.77	0.037

Duncan's multiple range test letters a & b show where treatments differ.

Samples of soil taken from the Mycorrhiza treated plots had significantly (P<0.05) fewer viable microsclerotia (**Table 29**). The maximum and mean (11.24) number of microsclerotia in the Mycorrhiza plots was below that of the field in 2017.

The highest individual plot record across the trial was in an Untreated plot, with 25.5 viable *V. dahliae* microsclerotia per gramme of soil (**Figure 15**). Although Mycorrhiza plots were all at the top of the trial (in Line 4, as shown on **Figure 15**) the adjacent Line 3 plots mapped onto the trial layout did not show a trend to lower propagule numbers, thus indicating the lower counts in the Mycorrhiza plots were less likely to have been related to their position.

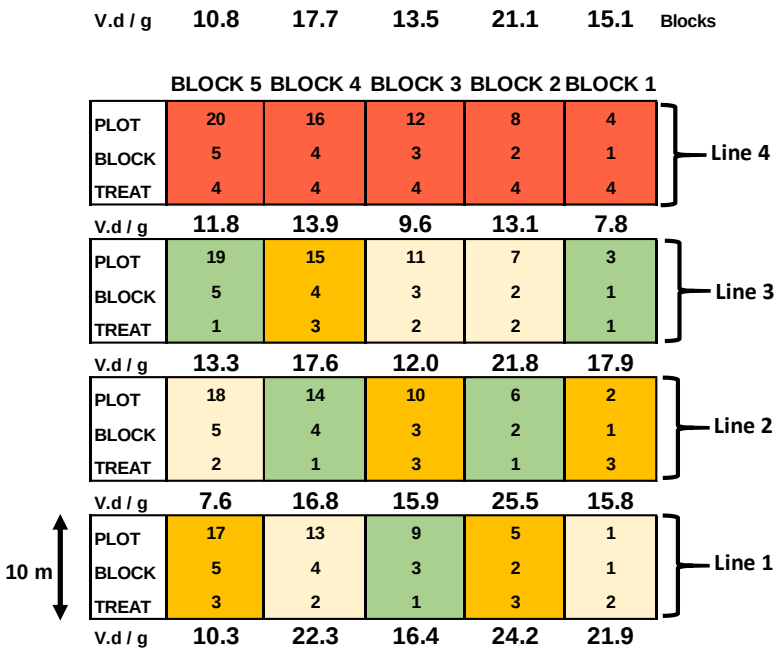


Figure 15: Viable *V. dahliae* (V.d.) propagules / g of soil given below each plot from samples in April 2020 to show the distribution across the Blocks and Lines of the trial area.

Comparison of V. dahliae counts between replicate blocks

There was a significant statistical difference (P <0.05) in mean propagule counts between the replicate Blocks (**Table 30**), with an alternation of lower means, between 11 to 16, in Blocks 1, 3 and 5 and higher means, between 18 and 21, in Blocks 2 and 4 (**Figure 15**).

Table 30: Harris test results for viable *V. dahliae* microsclerotia counts in soil samples taken on 29 April 2020 from the 20 plots of cv. Carlton in Orange Field, analysed by Block.

	Replicates						12 df
Viable *V. dahliae*	**BLOCK 1**	**BLOCK 2**	**BLOCK 3**	**BLOCK 4**	**BLOCK 5**	**L.s.d.**	**F value**
Microsclerotia / g of soil	15.8	21.1	13.5	17.7	10.8	5.33	0.011

Although, no significant difference between Block means had been found in the number of healthy bulbs / m harvested, it was recorded (**Table 19**) that Blocks 2 and 4 (which had the higher *V. dahliae* counts) had over 70 bulbs / m, and the others had 60 bulbs / m or less.

4.8. Free-living nematode samples in soil

Sampling of Orange Farm Field on 14 November 2017 while planted with sugar beet gave;

50 juvenile cyst nematodes (*Heterodera* sp.) per litre of soil

50 root lesion nematodes (*Pratylenchus* sp.) per litre of soil

475 stunt / spiral nematodes (*Tylenchorynchus* sp.) per litre of soil

On 29 April 2020, juvenile cyst nematodes (*Heterodera* sp.) were found in all plots at a similar (P>0.05) density, with a mean 201 per litre of soil. Plots also did not differ significantly in their numbers of root lesion nematodes, with a mean 199 / L, and stunt / spiral nematodes, with a mean 134 / L of soil. There were no stubby root (*Trichodorus* sp.), needle (*Longidorus* sp.), dagger (*Xiphinema* sp.) or stem (*Ditylenchus* sp.) nematodes. Only 25 root knot (*Melodogyne* sp.) nematodes were found and these were all in plot 4 at the top of the field. Details of individual plot counts and the analysis of variance for each species are given in the Appendix in **Table 33**. Any presence of stem, stubby root or needle nematodes (the latter two transmit virus) would have exceeded the "threshold" for *Narcissus*, based on advice provided to growers by the ADAS laboratory.

In the three and a half years since the sample taken across the whole field, there was a noticeable increase in the cyst nematode juveniles, by up to five times, and the root lesion nematodes had nearly similarly increased. However, stunt nematodes were at most a third of their original numbers.

As the Verticillium samples were quantified by gramme of soil and the nematodes by litres of soil, the fresh and the dry weight of 200 ml of soil for each plot are given in the Appendix (**Table 37**).

4.9. Soil sample results

Pre-trial soil sampling for soil health measures

Measurements from soil under the wheat stubble on 22 August 2018 are given in Appendix **Table 38** and **Table 39**. Within each of the replicate blocks similar records were obtained across the field from the soil penetrometer and VESS/VSA and earthworm soil pit records. However, nutrient analysis from the same date showed a trend of reducing extractable Phosphorus, Potassium and Magnesium levels with distance from Block 1 and should be borne in mind when comparing replicates planted with *Narcissus* bulbs.

Comparison of soil health before and after amendments and* Narcissus *cropping

The 2020 topsoil analysis within crop, or immediately post-crop, plot results, treatment means and statistical comparisons are given in the Appendix **Table 40** and **Table 41** for July 2020. Comparisons with background soil results, and any potential treatment effects are given below and summarised in soil health scorecards (**Table 31** and **Table 32**). In the scorecards the mean results are colour-coded according to the scorecard protocol, Red = Investigate, Amber = review, and Green = continue rotational monitoring.

Table 31: Soil health scorecard for pre-trial soil sampling in Orange Field on 22 August 2018 in cereal stubble

Attribute	Site mean	Notes
pH	8.3	Potential for nutrient interaction
Ext P (mg/l) [Index]	10 [1]	see RB209 for guidance
Ext K (mg/l) [Index]	78 [1]	see RB209 for guidance
Ext Mg (mg/l) [Index]	84 [2]	see RB209 for guidance
SOM (% LOI)	3.1	Light textured (<18% clay), >2% above average
VESS score (limiting layer)	2.3	Good, no structural problem
CO_2-C (mg/kg)	58	Low microbial activity
Earthworms (No./pit)	1.3	Depleted

Table 32: Soil health scorecard for post-harvest soil sampling in Orange Field 2020. Extractable P, K and Mg, pH, soil organic matter (SOM), potentially mineralisable nitrogen (PMN) and CO_2-burst for respiration was obtained for soil sampled on 8 July soon after *Narcissus* bulb lifting. Soil structure (VESS) and earthworms were assessed on 2 September a week after the farmer cultivated-in biosolids.

Attribute	Control	Green compost	FYM	Mycorrhiza
pH	8.2	8.1	8.2	8.1
Ext P (mg/l) [Index]	13 [1]	14 [1]	20 [2]	13 [1]
Ext K (mg/l) [Index]	99 [1]	89 [1]	120 [1]	106 [1]
Ext Mg (mg/l) [Index]	83 [2]	82 [2]	86 [2]	82 [2]
SOM (% LOI)	2.9	2.9	3.0	3.0
VESS score (limiting layer)	1.3	1.2	1.3	1.3
PMN (mg/kg)	21	23	29	23
CO_2-C (mg/kg)	50	39	46	36
Earthworms (No./pit)	2.7	1.3	4.3	1.3

Soil type, pH and organic matter

The topsoil texture in Orange Field was a sandy silt loam with up to 16% clay. The pH after the *Narcissus* crop was unaffected by the treatments; however, at pH 8 there is the potential for nutrient interactions. The soil organic matter (SOM%), in comparison with "typical" levels for the soil type and climate, was not of concern at the start and there were no differences following the treatments.

Earthworms & VESS

Before the *Narcissus* was planted few earthworms were found in the dry sandy soil in August, but in September two years after the application of FYM there had been a small increase in worm numbers in the plots which received pig manure, with the Untreated finally having 2.7 worms and FYM 4.3. Epigeics, endogeics and juveniles were present in all treatments, with no anecics. Overall, the worm count was low, probably reflecting the light texture, low SOM content and tillage operations used to establish the crop.

There was no concern about the soil structure from the VESS pit samples in either the cereal stubble or after cultivations following *Narcissus* bulb harvest, with soils classed as having a friable or intact soil structure.

NPK

After two years of bulb growth the level of extractable Phosphorus where FYM had been incorporated before planting had improved the status from “review” to “continue rotational monitoring” on the soil health scorecard (**Table 31** and **Table 32**), but treatments were not statistically significant different in 2020.

The extractable Potassium status was not improved by any treatment, with no statistical difference shown by ANOVA (P=0.094), although levels were numerically higher on the FYM treatment.

Potentially mineralisable nitrogen (PMN) results was not measured in the cereal stubble, but when recorded in 2020 after the *Narcissus* crop the plots that had received FYM had a mean 29 mg/kg and the Untreated a mean 21 mg/kg, with the other means intermediate, but with no significant differences, and all below average for UK arable soils.

PCR of *Fusarium oxysporum*

Molecular analysis within Project 5 of soil from the 20 plots of cv Carlton on 2 June 2020 prior to harvest of the trial on 4 June 2020 and detected *F. oxysporum* DNA. Differences in the amounts of DNA detected could not be related to the incidence of basal rot recorded on 23 June 2020 after the post-harvest storage of the bulbs (R^2 = 0.52). There had been no significant difference in the incidence of bulb rot between treatments. Although 13 of the cv. Carlton plots ranged between 3% and 16% incidence of rotted bulbs the plots’ soil DNA content ranged randomly from 0.002 to 0.063 fg DNA per kg soil. The other six plots with a similar bulb rot incidence range of between 1% and 12% had from 0.104 to an outlying 0.686 fg DNA per kg soil. No *F. oxysporum* had been detected by qPCR of the soil of the previous crop in November 2017, but *F. oxysporum* was confirmed by visual examination to be present in 38% of 29 bulbs collected from the trial at planting in 2018 after incubation to develop necrosis, mycelium and sporulation.

The results of the quantity of *F. oxysporum* DNA in soil were compared between treatments from samples taken in the first year (22 August 2019) and second year (2 June 2020) of the crop across all six replicates (five replicates of cv. Carlton and one of cv. California Reclaim). The soil samples from the untreated plots (of both varieties) in 2019 showed a greater variation in DNA content than the other three treatments, but there was no significant difference in the levels between the four treatments, all with means falling below 0.1 fg DNA per kg soil. In the second year, at harvest time,

there was again no significant difference between treatments, although it was noted that the mycorrhizal treatment had zero detection of *F. oxysporum*. The mean DNA levels detected were lower than in the previous year for the other three treatments (all below 0.01 fg DNA per kg soil, with the variation about the mean falling to below zero.

A qPCR assay for specific detection of *F. oxysporum* f. sp. *narcissi* did not become available to be used within the life of the project, meaning that some other *F. oxysporum* variants (*formae speciales*) that might not have contributed to the bulb rot (even if carried on the bulbs) could have been included in the soil sample detections and quantification of the species *F. oxysporum*.

Soil sampled in August 2019 and June 2020 was to be used to quantify the populations of mycorrhizal fungi of the species present in the applied granules using qPCR, as part of Project 5. However, due to a reduction in laboratory access and the disrupted supply of certain materials brought about by the covid-19 pandemic, quantification was not able to be achieved within the period of PhD research associated with this project. However, *Gliocladium catenulatum* (*Clonostachys rosea*), *Funneliformis mosseae* and *Rhizophagus irregularis* assays were optimised and their detection in inoculated field and glasshouse soils demonstrated, thus paving the way for any future DNA quantification in soils.

4.10. Weather data

Throughout the two years, conditions differed from the seasonal averages, with UK Meteorological office reviews reporting temperature peaks, extremes of rainfall and milder winters (https://www.metoffice.gov.uk/about-us/press-office/news/weather-and-climate/2019/weather-overview-2019 https://www.metoffice.gov.uk/about-us/press-office/news/weather-and-climate/facet/Year/2020). In both 2019 and 2020 a warm and particularly dry period in April hastened the end of flowering and impacted on bulb fill (**Figure 16**). An exceptional amount of rain fell on 10 June 2019. The 25 July 2019 was exceptionally hot. Consecutive days of rain at the end of August 2020 delayed the final soil sampling (**Figure 16**).

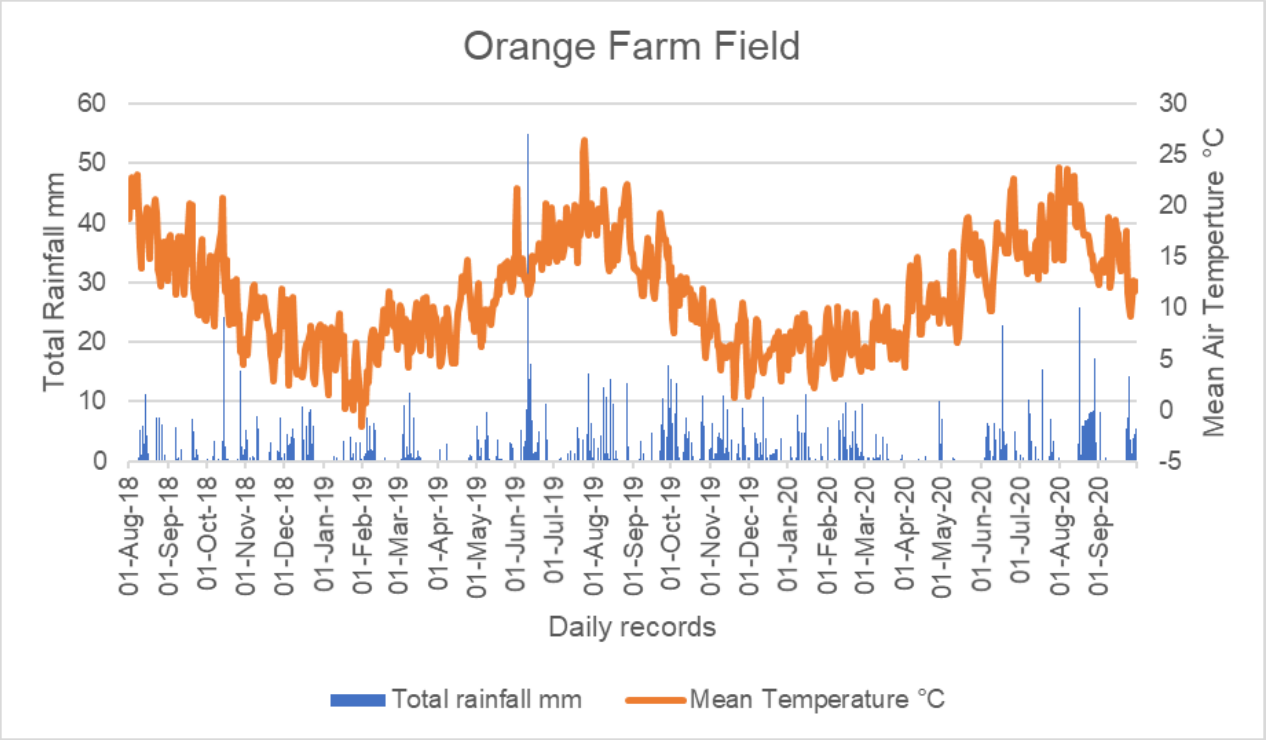


Figure 16: Total daily rainfall and mean daily air temperature for Orange Field near Terrington St Clements, Norfolk obtained from METMAKER for the two years 2018 to 2020 between the dates of first and last soil sampling in the *Narcissus* trial area

5. Discussion

5.1. Potential effects of Fusarium basal rot on crop growth and yield

Infested planting material and soil

The Fusarium basal rot of harvested cv. Carlton *Narcissus* bulbs was assessed as would be done by commercial grading, i.e., by examining the bulbs externally. This showed that on average 8.8% were visibly infected, without significant difference between the four treatments. However, it is probable that some infection had not developed sufficiently to cause softening and browning of the bulb scales that was visible externally; rotting may have developed with longer storage. Given that from a sample of 29 bulbs of cv. Carlton taken from the field at planting, and then incubated to aid diagnosis by advancing rotting, 38% were found to infested by Fusarium, it seems likely that infested bulbs are missed during commercial grading. Once infested bulbs are planted in a field then the land will become contaminated by the resting spores (chlamydospores) of *F. oxysporum formae speciales* narcissi (which will only cause wilt in *Narcissus*). The fungicide products thiabendazole (Storite) and chlorothalonil (Bravo) were used to manage the disease for many years as part of the hot water treatment of bulbs for the control of stem nematode.

Reduction in photosynthesis and bulb fill

The leaf yellowing recorded similarly across all treatments in early 2019 was unlikely to have been caused by Fusarium infection of the roots, but by physical damage from wind-driven soil in the dry conditions and potentially a systemic virus in the bulbs. In 2020, yellowing was more likely to have been caused by Fusarium basal rot, but it was shown from cutting some bulbs open that it had progressed insufficiently to cause leaf yellowing. Yellowing was, however, more prevalent across the Mycorrhiza treatment plots in the top line of plots. Subsequently the data showed a trend for more bulbs per metre to be harvested in the upper half of the trial and so greater crowding could have increased physiological yellowing due to competition for nutrients.

Loss of flowerstem production and bulb yield

Fusarium brought in on the bulbs could have caused the death of bulbs. Indeed, it is likely that the difference in bulb numbers between what was calibrated on the planter (85 /m) and the mean 59 /m healthy plus visibly Fusarium affected bulbs / m (excluding separated daughter noses) counted at harvest (of which a mean 8.8% had visible Fusarium), resulted from loss to Fusarium. This was a loss of 30.6% of the number of bulbs that had been planted, which was only partially compensated for by the individual weight/size grade gain of the remaining bulbs. Plots at the top of the field with a greater total number of bulbs, and consequently bulb noses, also produced more flowerbud stems for harvest than less dense plots.

Consultation with the grower about the much lower harvest recovery of bulbs than the number planted, confirmed that the counter on the planter was unlikely to have been miscounting other than a leeway of one or two bulbs / m. He and other growers had noted that they had very poor lifts in 2020. He thought that the large reduction in the bulb numbers in our trial was most likely because many had rotted away (Mark Eves pers.comm.). The majority of bulbs with externally visible Fusarium were in the categories with one or two noses and so it is likely that their further growth was restricted by the pathogen. Healthy bulbs more frequently had up to three noses.

The reason for the greater number of bulbs harvested in the upper half of the trial (which included the line of Mycorrhiza-treated bulbs), further up a slight slope is unclear. The same crate of bulbs was used for each pair or rows running up the length of the field, so variation in the severity of bulb infection initially is unlikely. This number in the upper field half was closer to what was calibrated to have been planted. This confuses the situation because it implies that more bulbs were lost in the lower half of the field, perhaps because of the growing conditions there. An alternative explanation might be that more bulbs were planted in the upper half as a result of the tractor driving slower while the bulbs dropped down the coulters at the same rate and speed reduction could have unconsciously happened when another staff member mounted the planter to scatter the mycorrhiza granules over the bulbs and then dismounted the planter again before the tractor driver carried on up the field.

5.2. Variation of plots between replicate blocks and similarity within plot lines

Significant differences were shown between the replicate Blocks, with both poorer leaf emergence extent in 2020 and fewer bulbs harvested in the plots of Block 1 closest to the tramline used as a main access route into the field. The emergence extent increased quite steadily from Block 1 to Block 5. This was possibly because of compaction, although no particular differences across the replicates were seen in the visual soil assessment, penetrometer resistance and topsoil nutrient results taken just before planting. Both the dominance of particular nose numbers/bulb and the total healthy bulb yield weight differed significantly between replicate blocks.

There were more flowerbud stems and more healthy bulbs lifted in the top two lines of plots (at right angles to the replicate Block rows and with all the Mycorrhiza plots in the top line). This illustrates the benefit of the usual practice of randomising treatments within replicate Blocks, but it had not been possible to do this with the Mycorrhiza granules with the bulbs being planted as part of a commercial operation. Further work will be needed to determine if there was a true benefit to marketable crop yields from the mycorrhiza use.

Fewer leaves emerged early in 2020 in rows planted in 2019 by the tractor driving down, rather than up, the field and this probably indicates a difference in bulb planting depth. Fortunately, all the central assessed two rows of each plot were planted in the same tractor pass up the field.

5.3. Effect of organic matter

No significant benefit or detriment from the application of either green compost or FYM before planting was shown on leaf yellowing, Fusarium incidence at harvest, or other measures of yield including bulb size and weight or numbers of cv. Carlton bulbs per metre. There was also no difference between these incorporations and the untreated plots in the numbers of free-living nematodes or *V. dahliae* microsclerotia. No correlation was shown in either the first or second year of the crop between the concentration of *F. oxysporum* in the soil and whether or not organic matter was applied. However, it is probable that the *F. oxysporum* was being shed from infested bulbs that were planted and the variation in *F. oxysporum* levels seen between plots could depend on how often the individual soil core samples (taken to make each plot bulk) were randomly taken in the vicinity of infested bulbs (even though the proportions of infested bulbs / plot were similar).

Composts typically have a higher lignin content, which is more resistant to microbial breakdown, than farmyard manures and therefore tend to increase organic matter content more quickly (relative to the same amount of organic matter added). Farmyard manures tend to contain more fresh organic matter and are better at stimulating biological activity and increasing microbial biomass (AHDB, 2018). The addition of the FYM and green compost could have assisted in the moisture-holding capacity of the soil and provided some nutrients (Sinclair and Measures, 2016; Stockdale, 2018). Indeed, although soil organic matter levels remained unchanged following the application of FYM and green compost, topsoil P and K levels were increased as a result of the FYM additions. Organic matter can also favour the development of communities of beneficial fungi and bacteria (Njira and Nabwami, 2013) which can compete with soil-borne pathogens for resources and are also reported to stimulated plant host defence responses (Berendsen *et al.,* 2012). Such an effect would be enhanced by further application in subsequent years, as the amount applied per year is limited by regulations linked to nutrient loading and resulted in relatively low organic matter loading in the current trial.

This experiment investigated the effect of the treatments on bulbs that were already infested by Fusarium, rather than the crop being planted into an infested field. However, treatments that increase the effectiveness or efficiency of crop water and nutrient uptake and so allow the bulbs to grow more strongly could reduce the susceptibility of bulbs to infestation from mycelium or spores shed by their neighbours. *Narcissus* bulbs are planted in a band into a deep trough so that they are touched all around by other bulbs and this facilitates the spread of pathogen mycelium and spores between them and causes competition for resources thus weakening the plants.

According to the host grower (Mark Eves, pers. comm.) there is a limited window for *Narcissus* growers to use either green compost or FYM on their fields before planting bulbs because they need to plant in August directly after the winter cereal is harvested. The income from bulb sales is low and

fertiliser and other inputs are kept low. When the land is rented, any longer-term benefits of organic matter incorporation will not be seen by the tenant. Although assessment of soil structure and penetration resistance was carried in the cereal stubble, within the space of a few hours this had been cultivated to a fine tilth so that it was able to be ridged up around the bulbs at planting.

A review by Bonanomi *et al.* (2010) seeking to identify the characteristics of organic soil amendments that suppress soilborne plant diseases found that the response of pathogen populations to organic matter amendments was a reliable feature only for some organic matter types (e.g., crop residues and organic wastes with C-to-N ratio lower than around 15) and for pathogens with a limited saprophytic ability (e.g., *Thielaviopsis basicola* and *Verticillium dahliae*). Instead, population responses of the pathogenic fungi *Phytophthora* spp., *Rhizoctonia solani* and *Pythium* spp. appeared unrelated to disease suppression. Fluorescein diacetate (FDA) hydrolysis assay has been used to measure non-specific enzyme activity (e.g., esterases, proteases, lipases, etc.) and has been correlated with organic matter decomposition but is also positively correlated with peat and compost suppressiveness. Overall, enzymatic and microbiological parameters, rather than chemical ones, were much more informative for predicting suppressiveness. The most useful features were FDA activity, substrate respiration, microbial biomass, total culturable bacteria, fluorescent pseudomonads and *Trichoderma* populations. They concluded that the integration of different parameters (e.g., FDA hydrolysis and chemical composition by 13C NMR) may be a promising approach for identification of suppressive amendments. Here CO_2 respiration burst and PMN were measured to indicated non-specific microbial activity, but no differences were found between the treatments.

5.4. Effect of Mycorrhiza supplementation on plant growth

Mycorrhiza products can provide a similar health promoting role to the indigenous rhizosphere microbes (which will include mycorrhiza) described by Berendsen *et al.,* (2012). There is evidence that through their symbiotic relationship with the plant roots, nutrient and water uptake to the plant can be improved (Rouphael *et al*., 2015, Begum, *et al.* 2019). Gholamhoseini *et al.* (2013) showed that *G. mosseae* enhanced sunflower growth under drought conditions by reducing drought stress and enhanced Nitrogen and Phosphorus percentages of tissues. There is also evidence of increased benefits to plant growth from co-inoculation of plants with arbuscular mycorrhizal fungi and plant growth promoting bacteria (PGPRs) (Emmanuel & Babalola, 2020).

In 2020 there was initially shown to be a statistically greater number of flowerbud stems per metre in the Mycorrhiza treated plots (65.8/m) than in the Untreated (44.0/m), but this was queried because further analysis indicated a trend of increase up the field that applied to the other treatments not just the Mycorrhiza plots. At harvest, although ranking as having the highest density of bulbs, the bulb

count in the Mycorrhiza plots was not significantly greater than the other treatments whether or not daughters were included or whether or not both healthy and Fusarium affected were totalled. Therefore, it cannot be concluded that Mycorrhiza addition benefited the crop. It is noteworthy that the Mycorrhiza treated plots had significantly lighter weight two- and three-nosed bulbs than the other three treatments and the reason for this weight loss requires further research. However, it is essential that such work ensures that treatments start with the same bulb planting densities.

Nose numbers were recorded because bulb size is relevant to the destination market for the bulbs as well as the number harvested, and bigger bulbs should be able to develop if growing conditions and plant health are good. There was an increased proportion of 2-nosed bulbs in the Mycorrhiza treated plots compared with the Untreated (which was similar to in the other treatments), but this did not significantly affect the proportion in the other categories. All the nose categories were suitable for retail, but above the size 10 specification ideal for bulb bowls, so the nose numbers may be less important. For bulb forcing, the optimum bulbs are those that are smaller and heavy so that more bulbs can be planted in a tray for producing flowers and the bulbs have lots of energy to grow quality flowers (Mark Eves pers. comm.). Only the separation of daughter bulbs (a size suitable for planting) from mothers could increase the number of bulbs lifted and this number did not differ between treatments, nor did total healthy bulb count differ significantly between treatments.

Treatments also had no effect on the number or percentage of bulbs that had externally visible Fusarium and thus marketable yield. Here, unreplicated exploratory sampling found Fusarium symptoms in 23% of the bulbs from an untreated plot and 6% of bulbs from a Mycorrhiza treated plot at harvest. Therefore, further investigation would be worthwhile to give a full evaluation based on internal browning. Affected bulbs without external symptoms could be marketed, however, and those kept by the grower for re-planting either in the field or in trays could show reduced yields.

Although there was no statistical difference in the levels of *F. oxysporum* measured by qPCR present in the soil of different treatments (in either August 2019 or just before the bulb harvest in June 2020), the zero detection of this fungus in soil in 2020 in any of the Mycorrhiza treated plots requires further evaluation. Levels of *F. oxysporum* were much lower in the second than the first year for the other three treatments and it is possible that there was enhanced *F. oxysporum* DNA degradation where the bulbs had been treated with the mycorrhiza, or it could have been related to the position of these plots in the field; we also observed a greater number of flower-heads produced there. It is possible the mycorrhiza reduced the growth of fusarium mycelium and spores, so less DNA was detected in the soil. Inoculation of asparagus seedlings with either *Glomus* sp., *Gigaspora marginata* or *Glomus fasciculatum* reduced the incidence and severity of Fusarium crown and root rot caused by *F. oxysporum* f.sp. *asparagi* (especially so using *Glomus* sp.) probably as their presence in the host pre-infection suppressed the invasion of the pathogen (Matsubara *et al*., 2001). Infection between

neighbouring roots on the basal plate of *Narcissus* might be reduced producing fewer rotted roots to shed the pathogen into the soil. Any further work should be done using a molecular assay able to distinguish the different variants of *F. oxysporum*, although there is no reason to suppose that soil populations of this species would respond differently to any of the treatments used.

Results obtained by Plantworks Ltd from a non-replicated sample of bulbs from an Untreated and a Mycorrhiza treated plot demonstrated that bulbs treated with the mycorrhiza product established higher levels of mycorrhizal colonisation (up to 63% of roots colonised) compared with untreated bulbs (up to 26%). The majority of untreated bulbs had some mycorrhizal association, and mycorrhizal root colonisation of an average 6.5% was said to not be uncommon from indigenous mycorrhiza (pers. comm. Joanne O'Reagan PlantWorks Ltd.). Further investigation to follow the establishment of mycorrhiza within the root system throughout the trial would have provided information as to whether Mycorrhiza treated plots formed these association earlier than did Untreated bulbs and whether there were changes in colonisation over the period. Some of the bulbs from the Mycorrhiza product treated plot which had internal Fusarium symptoms had a high level of mycorrhizal root colonisation (up to 51%) and so this could be interpreted as the product having no benefit in reducing *F. oxysporum* infection incidence or infection severity even at high levels of mycorrhiza presence. However, this should be tested in future in Fusarium-infested soil to determine if the pathogen might be less successful in entering the *Narcissus* roots where mycorrhiza had colonised, because in the current experiment there was already a high incidence of Fusarium inside the bulbs planted. The Mycorrhiza product would not be expected to give curative action, but a benefit to an infested bulb could be from help with water and nutrient uptake by the roots to mitigate against reduced root function caused by the pathogen.

5.5. Verticillium levels in the soil before and after treatment application

The significantly lower number of viable *V. dahliae* microsclerotia (as assessed by the Harris test) in the Mycorrhiza treated plots in April 2020 than in the other three treatments is noteworthy. This pathogen does not affect *Narcissus* but would be important for any future crops such as linseed, peas, potatoes, strawberries, cane fruit and nursery trees. For strawberry cultivars with good Verticillium wilt resistance, more than five propagules / g soil would be of medium risk (and very high risk for a moderately susceptible cultivar) (Scott and O'Neill, 2006). However, microsclerotia thresholds are not available for other crops. The mean of 11.2 propagules / g of soil in these plots was from a plot range between 7.8 and 13.9 propagules and, as this is below the benchmarking pre-trial record of 14.2 propagules /g of soil from across the field, a reduction during the trial could have occurred. Although the Mycorrhiza treated plots were all at the upper, southern end of the trial area (in line 4), there was no trend in the results for the other plots to indicate that the microsclerotia levels decreased from line 1 to 4 and this reduces the chance of a positional influence in the distribution of the propagules. Whether an increased dosage of granules at planting would have increased the

reduction should be investigated, as although most of the bulbs sampled from the treated plots had mycorrhiza present, with a mean 30.4% of root area colonised, there was potential for further colonisation.

Potential activity of mycorrhiza against soil-borne pathogens

How the mycorrhiza might have reduced the Verticillium propagules in the soil is unclear and requires investigation although there are indications in the scientific literature (Cordier *et al.* 1998, Mol, 1995, Garmendia *et al*. 2006). It is possible that chemicals are either produced by the mycorrhiza or they stimulate their production by the *Narcissus* roots and these reduce the viability of nearby propagules (which then do not germinate in the Harris tests). *Narcissus* is not noted as being affected by *Verticillium* spp. (Hanks, 2013). However, Verticillium may still colonise the roots, but the mycorrhiza then may act to reduce the production of *V. dahliae* microsclerotia. Some hosts, such as linseed, increase microsclerotia in soil and the rhizosphere associations may play a part in this.

The arbuscular mycorrhizal fungus *Glomus mosseae* confers bioprotection against *Phytophthora parasitica* in tomato roots (Cordier *et al*., 1998). Cordier *et al*. (1998), referred to various research showing that arbuscular mycorrhizal fungi, which form symbiotic associations with a wide range of plant species, effectively reduce root disease caused by a number of soilborne pathogens. They state that different hypotheses have been proposed to explain bioprotection by arbuscular mycorrhizal fungi. These include (i) improvement of plant nutrition and root biomass in mycorrhizal plants, which could contribute to an increased plant tolerance and compensate for root damage caused by a pathogen, (ii) changes in root system morphology, (iii) modification of antagonistic Mycorrhiza populations in the mycorhizosphere, and (iv) competition between arbuscular mycorrhizal and pathogenic fungi to colonize root tissues, with the possible induction of resistance mechanisms. They stated that very little was known of the physiological, cellular, or molecular mechanisms that are really active.

That stimulation of germination of microsclerotia by exudates from plant roots may be important for the control of *V. dahliae* was shown by experiments (Mol, 1995). All crops tested stimulated germination, but the roots of some potato cultivars had a larger stimulation effect on microsclerotia than that of another potato cultivar and of, pea, flax, sugar beet or onion. The number of hyphae per microsclerotium (indicative of germination) decreased with distance from the root surface regardless of the crop species or cultivar. Differences in root densities, and in the stimulation effect on germination of microsclerotia caused large differences among crops in the effect on the population of microsclerotia in the soil (Mol, 1995). In the current *Narcissus* experiment the higher (but not significantly) final density of bulbs in the mycorrhiza plots could have similarly acted to stimulate more germination, but then, as *Narcissus* is not noted as being affected by *Verticillium* spp. (Hanks, 2013), once germinated then host colonisation could fail or be poor and produce no microsclerotia.

In mycorrhizal plants, the bioprotection against soil-borne pathogens can result from the preactivation of defence responses that include some structural modifications and the accumulation of Pathogenesis-Related proteins. *Glomus deserticola* colonisation induced chemical changes in the pepper roots and only in such roots were chemicals associated with a defense response produced following subsequent *V. dahliae* inoculation (Garmendia *et al*., 2006). Similarly, inoculation of tomato with AMF induced a stronger and quicker chemical defense response (more superoxide dismutase and peroxidase in the leaves) in tomato plants inoculated with *Alternaria solani* (Song *et al*., 2011).

The Mycorrhiza product supplied by PlantWorks Ltd was a special order for the project as the commercial product normally contains additives that are stated to be biostimulants, but for the project it was decided to just investigate mycorrhizae. PlantWorks Ltd (Qu Lin pers.comm) have found that inclusion of biostimulants greatly enhances mycorrhizal (AMF) colonisation of plants, however, in the current project noticeably higher root colonisation by mycorrhiza occurred in plots with the Mycorrhiza product compared with those with only indigenous mycorrhiza fungi in the soil. A commercial equivalent to the material used in this project that is sold by PlantWorks Ltd would be their RGPRO HORTI 2 Young Plant and Potting-on Mix for Horticulture with an application rate similar to the 1 g of granules per bulb that we aimed to use in the current project. It would contain 10% biostimulant and 90% arbuscular mycorrhizal fungi of five species at a minimum of 500K propagules per litre. Bioadditives included would be rock phosphate, hoof and horn, seaweed meal and urea. The price in January 2021 was £110 + VAT for 10 kg (a 10 L tub), and if planting 1120 bulbs in two rows per 10 m this amount could treat around 90 m of these two rows. This AMF product might also be suitable for scattering within bulb forcing trays.

Overall, this project has shown that a single application of pig FYM or green compost had very little effect on topsoil health and no impact on crop performance and Fusarium infection; it is likely that multiple annual applications would be required. The successful inoculation of bulbs with mycorrhiza was achieved using farm-scale, commercial equipment, and there was some limited evidence that this improved crop performance, disease levels and reduced *V. dahliae* levels in the soil. However, these effects could not be reliably concluded, due to possible confounding effects of treatment position in the field.

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7. Appendix

Table 33: Free-living nematodes (FLN) per litre of soil collected in April 2020 from Orange Field *Narcissus* plots using visual identification and counting (ADAS High Mowthorpe). Plots have been ranked by treatment number. See next three tables for analysis of plot and block differences.

Plot No.	Treat-ment	Stubby root	Stunt / spiral	Cyst juveniles	Root lesion	Needle	Dagger	Stem	Root knot
3	1	0	125	400	225	0	0	0	0
6	1	0	75	50	275	0	0	0	0
9	1	0	100	125	175	0	0	0	0
14	1	0	75	150	175	0	0	0	0
19	1	0	400	275	150	0	0	0	0
1	2	0	100	225	200	0	0	0	0
7	2	0	200	125	250	0	0	0	0
11	2	0	100	100	125	0	0	0	0
13	2	0	125	225	125	0	0	0	0
18	2	0	75	125	125	0	0	0	0
2	3	0	150	500	175	0	0	0	0
5	3	0	50	75	275	0	0	0	0
10	3	0	75	100	150	0	0	0	0
15	3	0	200	175	150	0	0	0	0
17	3	0	175	175	275	0	0	0	0
4	4	0	50	150	175	0	0	0	25
8	4	0	150	50	250	0	0	0	0
12	4	0	150	175	200	0	0	0	0
16	4	0	175	525	350	0	0	0	0
20	4	0	125	350	150	0	0	0	0
Mean			134	204	199				

Table 34: Mean counts of juvenile cyst nematodes (*Heterodera* sp.) in 1 litre of soil per treatment and per replicate block from individual cv. Carlton *Narcissus* plot samples taken on 29 April 2020 from Orange Field and Analysis of Variance showing no significant differences between treatments or replicate blocks. Overall mean 204 cyst juveniles / L soil.

Numbers of nematodes / L of soil						L.s.d.	F pr.	d.f.
Treatment	**Untreated**	**Green Compost**	**FYM**	**Mycorrhiza Product**				
Mean Count	200	160	205	250		171.0	0.728	12
Block	**1**	**2**	**3**	**4**	**5**			
Mean Count	319	75	125	269	231	191.2	0.085	12

Table 35: Mean counts of root lesion nematodes (*Pratylenchus* sp.) in 1 litre of soil per treatment and per replicate block from individual cv. Carlton *Narcissus* plot samples taken in April 2020 from Orange Field and Analysis of Variance showing no significant differences between treatments or replicate blocks. Overall mean 199 root lesion nematodes / L soil.

Numbers of nematodes / L of soil						L.s.d.	F pr.	d.f.
Treatment	**Untreated**	**Green Compost**	**FYM**	**Mycorrhiza Product**				
Mean Count	200	165	205	225		80.7	0.466	12
Block	**1**	**2**	**3**	**4**	**5**			
Mean Count	194	262	162	200	175	90.2	0.206	12

Table 36: Mean counts of spiral stunt nematodes (*Tylenchorynchus* sp.) in 1 litre of soil per treatment and per replicate block from individual cv. Carlton *Narcissus* plot samples taken in April 2020 from Orange Field and Analysis of Variance showing no significant differences between treatments or replicate blocks. Overall mean 134 spiral stunt nematodes / L soil.

Numbers of nematodes / L of soil						L.s.d.	F pr.	d.f.
Treatment	**Untreated**	**Green Compost**	**FYM**	**Mycorrhiza Product**				
Mean Count	155	120	130	130		119.5	0.929	12
Block	**1**	**2**	**3**	**4**	**5**			
Mean Count	106	119	106	144	194	133.6	0.593	12

Table 37: Fresh weight (g) of 200 ml soil by displacement on arrival and after drying to be able to relate FLN / litre to the weight of 200 ml (5 x 200 ml = 1 litre). Orange Field April 2020.

Plot number	Block	Treatment	Fresh weight	Dry weight
3	**1**	**1**	408.1	356.0
6	**2**	**1**	384.6	334.1
9	**3**	**1**	380.8	331.6
14	**4**	**1**	406.2	352.3
19	**5**	**1**	400.8	351.8
1	**1**	**2**	430.2	371.3
7	**2**	**2**	395.0	341.1
11	**3**	**2**	405.1	356.6
13	**4**	**2**	420.1	364.7
18	**5**	**2**	403.6	349.4
2	**1**	**3**	400.8	347.0
5	**2**	**3**	411.6	358.1
10	**3**	**3**	409.4	356.4
15	**4**	**3**	408.4	361.9
17	**5**	**3**	432.8	375.9
4	**1**	**4**	400.7	352.7
8	**2**	**4**	402.2	354.1
12	**3**	**4**	413.6	363.5
16	**4**	**4**	417.6	371.2
20	**5**	**4**	401.3	355.4

Table 38: Soil measurements taken 22 August 2018 just before the beds were prepared by the farmer for planting *Narcissus* in Orange Field.

Replicate	VESS score	VSA score	Maximum penetrometer resistance (Mpa)	Depth of resistance (cm)	Earthworms No./ 20 cm cube of soil
Block 1	1.8	27	1.8	32.6	4
Block 2	-	-	1.7	33.6	-
Block 3	1.9	26	1.8	33	0
Block 4	-	-	1.9	29	-
Block 5	1.9	26	1.8	34.4	0
Block 6	-	-	1.8	29.4	-

Table 39: Results of soil analysis of samples taken in August 2018 with the area marked into the six replicates in cereal stubble the day before *Narcissus* planting in Orange Field.

Replicate	Texture	% sand	% silt	% clay	Organic matter (LOI) %	P (mg/l)	K (mg/l)	Mg (mg/l)	pH	CO2 burst (mg/kg)
Block 1	Sandy Silt Loam	21	64	15	3.4	13.2	84.2	88.5	8.3	59
Block 2	Sandy Silt Loam	21	64	15	3.2	10.2	79.8	86.4	8.6	44
Block 3	Silt Loam	20	64	16	3.1	11.4	77.8	95.4	8.2	50
Block 4	Sandy Silt Loam	21	64	15	3.0	9.4	74.1	85.9	8.0	76
Block 5	Sandy Silt Loam	23	63	14	3.0	8.8	70.9	74.7	8.5	53
Block 6	Sandy Silt Loam	23	63	14	2.8	7.6	81.0	71.8	8.3	66

VESS scores of 1.8, 1.9 and 1.9 for Rep 1,3 & 5 respectively, with VSA scores 27, 26, 26

Table 40: Laboratory results for three replicates of topsoil sampled on 8 July 2020 from *Narcissus* plots in Orange Farm Field. Treatment means shown and ANOVA below the table of plot results.

Plot	I.d.	Treat-ment	Texture	% sand	% silt	% clay	pH	Ext P (mg/l)	Ext K (mg/l)	Ext Mg (mg/l)	Ext. Na (mg/l)	Ext. Ca (mg/l)
1	2	**Green compost**	szl	21	63	16	7.8	17.8	93.9	104	12.5	1945
2	3	**FYM**	zl	20	64	16	8.2	18	105	84.8	12.4	1990
3	1	**Control**	szl	21	63	16	8.1	15	88.6	74.6	12.6	2010
4	4	**Myco-rrhiza**	szl	23	62	15	8.1	14.4	116	86.8	14.7	2200
9	1	**Control**	zl	20	63	17	8.2	15.8	109	101	12.3	2140
10	3	**FYM**	zl	20	64	16	8.4	17.6	118	84.2	15.5	1995
11	2	**Green compost**	zl	19	65	16	8.1	13	80.6	71.3	12.6	1967
12	4	**Myco-rrhiza**	szl	22	63	15	7.9	12.2	92.3	78.9	14.9	1944
17	3	**FYM**	zl	20	64	16	7.9	23.4	136	88.5	11.4	1965
18	2	**Green compost**	zl	20	65	15	8.3	10	91.5	71.5	10.8	1935
19	1	**Control**	szl	21	64	15	8.2	9.2	99.9	72.8	13.1	2024
20	4	**Myco-rrhiza**	szl	23	62	15	8.2	13.2	109	81.2	15.6	2017

I.d.	Treat-ment Means	Texture	% sand	% silt	% clay	pH	Ext P (mg/l)	Ext K (mg/l)	Ext Mg (mg/l)	Ext. Na (mg/l)	Ext. Ca (mg/l)
1	**Control**	szl	20.7	63.3	16.0	8.2	13.3	99.2ab	82.8	12.7ab	2058.0
2	**Green compost**	szl	20.0	64.3	15.7	8.1	13.6	88.7a	82.3	12.0a	1949.0
3	**FYM**	szl	20.0	64.0	16.0	8.2	19.7	119.7a	85.8	13.1ab	1983.3
4	**Myco-rrhiza**	szl	22.7	62.3	15.0	8.1	13.3	105.8ab	82.3	15.1b	2053.7
	Mean		20.83	64.5	15.67	8.11	14.97	103.3	83.3	13.2	2011.0
	F-value		<0.001	0.036	0.142	0.883	0.149	0.094	0.985	0.095	0.366
	d.f.		6	6	6	6	6	6	6	6	6
	s.e.d.		0.36	0.527	0.408	0.1764	2.762	9.93	10.98	1.022	67.3
	l.s.d		0.881	1.29	0.999	0.4316	6.759	24.31	26.87	2.501	164.8

Different letters beside means show significant differences using Duncan's multiple range test

Table 41: Laboratory results for three replicates of topsoil sampled on 8 July 2020 from *Narcissus* plots in Orange Farm Field. Treatment means shown and ANOVA below the table of plot results.

Plot	I.d.	Treatment	SOM (%LOI)	Total N (%)	CaCO3 (%)	SOC (%)	SOM (%) - calc	CO2-C (mg/kg)	Colour index	PMN
1	2	**Green compost**	3.3	0.157	<1	1.8	3.1	46	3.55	22.4
2	3	**FYM**	3.1	0.14	<1	1.5	2.6	48	3.6	34.4
3	1	**Control**	2.9	0.126	<1	1.3	2.2	44	3.51	18.6
4	4	**Mycorrhiza**	2.9	0.13	<1	1.4	2.4	38	3.34	26.1
9	1	**Control**	3.2	0.147	<1	1.5	2.6	55	3.76	29.6
10	3	**FYM**	2.9	0.126	2	1.3	2.2	43	3.47	23.8
11	2	**Green compost**	2.7	0.115	2	1.3	2.2	37	3.3	25.0
12	4	**Mycorrhiza**	3	0.132	1	1.4	2.4	48	3.6	18.3
17	3	**FYM**	3	0.134	1	1.3	2.2	48	3.6	29.2
18	2	**Green compost**	2.7	0.111	3	1.1	1.9	34	3.22	22.0
19	1	**Control**	2.7	0.105	3	1.2	2.1	51	3.68	13.9
20	4	**Mycorrhiza**	3	0.123	<1	1.3	2.2	21	2.68	23.4

I.d.	Treatment Means	SOM (%LOI)	Total N (%)	CaCO3 (%)	SOC (%)	SOM (%) - calc	CO2-C (mg/kg)	Colour index	PMN
1	**Control**	2.9	0.1	1.0	1.3	2.3	50.0	3.7	20.7
2	**Green compost**	2.9	0.1	1.7	1.4	2.4	39.0	3.4	23.1
3	**FYM**	3.0	0.1	1.0	1.4	2.4	46.3	3.6	29.1
4	**Mycorrhiza**	3.0	0.1	0.3	1.4	2.4	35.7	3.2	22.6
	Mean	2.95	0.1288	1.25	1.367	2.356	42.8	3.443	23.9
	F-value	0.953	0.946	0.507	0.973	0.973	0.235	0.265	0.388
	d.f.	6	6	6	6	6	6	6	6
	s.e.d.	0.1866	0.01298	0.73	0.1447	0.2494	6.79	0.2161	4.73
	l.s.d.	0.4566	0.0376	1.785	0.354	0.6102	16.61	0.5288	11.56

Table 42: Results for 2 and 3 September 2020 for penetrometer and earthworms for three replicates of *Narcissus* plots in Orange Farm Field. Recorded after cultivation to incorporate biosolids. Treatment means shown and ANOVA below the table of plot results.

Plot	I.d.	Treatment	Epigeic	Endogeic	Anecic	Juveniles	Total	Penetration resistance (Mpa)	Depth of resistance (cm)
1	2	**Green compost**	1	0	0	1	2	0.89	22.0
2	3	**FYM**	2	2	0	1	5	0.95	16.0
3	1	**Control**	2	0	0	0	2	0.89	15.0
4	4	**Mycorrhiza**	0	1	0	0	1	1.27	29.0
9	1	**Control**	2	1	0	2	5	0.94	24.0
10	3	**FYM**	4	1	0	0	5	0.98	14.0
11	2	**Green compost**	0	2	0	0	2	1.26	21.0
12	4	**Mycorrhiza**	0	1	0	0	1	0.95	11.0
17	3	**FYM**	1	1	0	1	3	0.98	17.0
18	2	**Green compost**	0	0	0	0	0	0.67	14.0
19	1	**Control**	0	1	0	0	1	0.66	10.0
20	4	**Mycorrhiza**	2	0	0	0	2	0.89	12.0

I.d.	Treatment Means	Epigeic	Endogeic	Anecic	Juveniles	Total	Penetration resistance (Mpa)	Depth of resistance (cm)
1	**Control**	1.3	0.7	0.0	0.7	2.7ab	0.8	16.3
2	**Green compost**	0.3	1.0	0.0	0.3	1.3a	0.9	19.0
3	**FYM**	2.3	1.3	0.0	0.7	4.3b	1.0	15.7
4	**Mycorrhiza**	0.7	1.0	0.0	0.0	1.3a	1.0	17.3
	Mean	1.17	1.0	n.a	0.42	2.42	0.945	17.1
	F-value	0.313	0.816		0.709	0.061	0.564	0.922
	d.f.	6	6		6	6	6	6
	s.e.d.	1.027	0.687		0.653	0.972	0.1402	5.18
	l.s.d.	2.514	1.908		0.597	2.378	0.3431	12.68

Different letters beside means show significant differences using Duncan's multiple range test