BOF 6
Final Report

HDC

Horticultural Development Council

Working for Growers

Research Report

BOF/6 Final Report

Control of narcissus basal rot by antagonists.

FINAL REPORT

Control of narcissus basal rot by antagonists.

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Narcissus. Biological and integrated control of narcissus basal rot.

HDC project ref. B/6/87.

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Introduction

Basal rot of Narcissus, due to Fusarium oxysporum f.sp. narcissi, is difficult to control and causes major losses for the industry where it also threatens the export of dry bulbs from the U.K. Cultural and chemical methods have been largely palliative with fungicides remaining the most important means of controlling basal rot. Thiabendazole (TBZ) has both limited effectiveness and persistence as a pre-planting fungicide and replaced mercury which was environmentally undesirable. Currently available resistant cultivars are not generally favoured by growers, or the public, such that the most widely-grown cultivars, Golden Harvest and Carlton, are the most susceptible.

The main source of infection is infected bulbs, although soil-borne infections also occur, with the pathogen entering through the roots or the basal plate at points of root emergence. Infections can occur at soil temperatures as low as 5°C, which suggests crops are susceptible throughout the year (Price, 1981), but disease is most severe following unusually hot summers e.g. 1975-6; 1988-90 (Beale, 1987; Beale & Pitt, 1990).

Indications of a novel means of controlling the disease, by use of

biological agents, arose from work in Holland and at Exeter in the late 1970's, when certain soil-borne microorganisms were shown to inhibit (antagonise) growth of the pathogen. When such organisms were integrated with chemical treatments, some were able to suppress the pathogen at higher levels than was achieved with chemicals alone, even under conditions of high disease pressure (Beale & Pitt, 1990; copy enclosed).

Aim and approaches

The aim was to evaluate the potential of several soil- and bulb-borne microorganisms for the control of basal rot when used as biological control agents alone or integrated with TBZ.

The approaches were defined in the original agreements but modified in item iii) on approval of the ten-month extension set out in the letter from A.G. Scott (MAFF, Research and Development Requirements Division, Branch B) dated 6th July 1990, as follows:-

i) Use of existing fermentation equipment to optimise methods for bulking-up antagonists for subsequent use in a dry or wet form in the field.

ii) To examine shelf-life and longevity of the formulation(s) and agents selected.

iii) To monitor and evaluate the one- and two-year-down field trial planted at Kirton EHS in Autumn 1989, in which comparisons were made of the efficacy of the biological control products against, and in conjunction with, conventional fungicide control measures.

iv) Simultaneous observations and evaluation of the effect of antagonists on the occurrence of basal rot, neck rot and other relevant diseases in the trials.

v) To determine the effects of antagonists on the level and longevity of the population of the pathogen within the soil by use of selective media for Fusaria coupled with pathogenicity testing of the isolates.

vi) To monitor the levels and survival of the biological control agents at the bulb surface and in adjacent soil.

vii) Liaise with commercial organisations to produce and market a product.

Results

i) Bulk production of antagonists

Stabilization of antagonistic potentials against Fusarium oxysporum f.sp. narcissi. Since the five most promising antagonists isolated by Beale (1987), and described by Beale & Pitt (1990), had been stored in culture at 4°C, under oil, for 2 years in the interim period between the previous and the onset of the present work, their antagonistic potentials were re-established. Table 1 presents the antagonistic potentials of the original antagonists, at these key dates, determined by the original method (Beale & Pitt, 1990).

The substantial loss in antagonistic capacities of isolates 089, 128 and 134, which were originally the most effective organisms in achieving biological control, was a matter for concern. In subsequent work attempts were made to compensate for this reduced antagonistic capacity by increasing the loading of antagonists in the formulations. In addition a further screening programme was established to isolate new antagonists, especially amongst the Actinomycetes.

Thereafter the original strains of antagonists, and those subsequently isolated, were stabilized by growing them on a mixture of sterile bulb scales and Vermiculite moistened with Czapek-Dox liquid for 3 weeks, followed by lyophilization and storage in vacuo. For each subsequent experiment fresh

Table 1. Details of microorganisms used and their effective antagonism of *Fusarium oxysporum* f.sp. narcissi at various dates.

Antagonist and code number	Inhibition of *F. oxysporum* at dates		% change
	1983-1987	1988	
Penicillium rubrum Stoll 082A	56.90 ±2.71	68.0 ±3.6	+ 19.51
Trichoderma viride Pers. ex Gray 089	87.07 ±1.05	49.5 ±3.1	- 43.15
Minimedusa polyspora (Hotson) Weresub & Le Clair 128	91.06 ±0.24	66.6 ±1.3	- 27.87
Streptomyces sp. 131	53.98 ±0.79	63.0 ±1.5	+ 16.71
Trichoderma harzianum (Bonord.) Bain 134	84.06 ±0.72	60.2 ±2.0	- 28.39

cultures were re-established from the freeze-dried preparations and showed no significant decline in antagonistic potential thereafter.

Culture of antagonists. *Minimedusa polyspora* was cultured on a range of solid organic substrates, viz. hay, barley, straw, maize flakes, wheat bran, dry *Narcissus* bulb scales and loam. Vermiculite, Perlite or Cornish grit were used as carriers and Czapek-Dox salts were added to provide nutrients, with occasional addition of glucose. All of the organic substrates, except loam, supported growth of *M. polyspora* with maximum bulbil (propagule) production occurring on maize. Wheat bran also gave a satisfactory yield of bulbils and this material was generally easier to handle and was preferred.

Percentage germination of solid-substrate-derived propagules was initially

high but declined during the first year of storage in the laboratory to a mean of 81.4% (± 16.3) for the range of six organic substrates with maize (mean 98.0 ± 1.0) being highest after one year. After two years' storage, bulbils from both bran and bulb scales-based substrates regenerated colonies at a high frequency. However, there was some divergence in germination levels between batches which was thought to be due to differential drying-out of the medium which results in failure of propagules to mature and, therefore, survive.

This high level of germination under laboratory conditions was not necessarily maintained during freeze-drying and only propagules grown on bulb scales reliably regenerated colonies at high frequencies after lyophilisation.

It was found beneficial to include a diluent in cultures since without it the colonised bran became a solid mat which required further processing (grinding) prior to application to the soil. Of the three diluents tested, Vermiculite was preferred since it permitted separation of bulbils from the medium by centrifugation more readily than Perlite and could be crushed, if necessary. Growth was best when mineral salts were included in the presence of 0.25 - 0.5% glucose.

The ratio between water content and dry ingredients was critical with sufficient water necessary to ensure a friable consistency (9.5 ml liquid per 15g solid substrate). In the presence of Vermiculite this ratio may need to be varied depending on the dryness of the atmosphere. A tendency was noted for lower bulbil viability when bulkier volumes of solid medium were used such that the depth of the medium needs to be no greater than 3 cm.

The other four antagonists in regular use were also cultured successfully

in solid (bran) carriers. With the aim of developing a growth medium combined with a carrier (formulation), cultures were grown in enclosed aluminium foil trays.

Formulation. The observations of Beale (1987) were confirmed that aqueous suspensions of the propagules of all five antagonists showed a rapid decline in viability even after 2-3 days' storage at 4°C. Because such preparations were impracticable except in small-scale experiments, other methods of formulation were explored.

A method of formulating the antagonists as alginate pellets was standardised following modifications to the procedure of Lewis & Papavizas (1985). The pellets, based on 2% (w/v) aqueous alginic acid extruded into calcium chloride solution, included 1-5% wheat bran (comminuted by milling to pass through a 420 µm mesh sieve) and 112.5g powdered potter's clay. Pellets were harvested by filtration and air-dried in a sterile air flow for 24-48h before use. Propagules were present in the range 10^5 - 10^6 per bead for antagonists which spored asexually but for M. polyspora, where propagules are relatively massive, 10^1 per bead was used.

A solid substrate culture cum formulation, which could be used directly, without further formulation, would have definite advantages over e.g. alginate pellets since it would be simpler to produce, less labour intensive, and require little sophisticated laboratory equipment. In addition, the loss of viability of propagules during the wet phase of bead preparation could be avoided. Results indicated that the viability (regenerability) of the antagonist propagules (particularly M. polyspora) remained at the initially high level for up to a year (the maximum time tested) when produced as integrated culture: formulation in aluminium trays. A major disadvantage with this type of culture

is the dust hazard, particularly at the time of application to the soil. In contrast, alginate beads are far less hazardous since they contain the antagonist in an immobilised form. After the initial population crash in the wet phase of alginate bead preparation, which can be compensated for by increasing the numbers of propagules per bead, they are clean and convenient to handle and retained viability for at least two years when stored at room temperature (20-25°C).

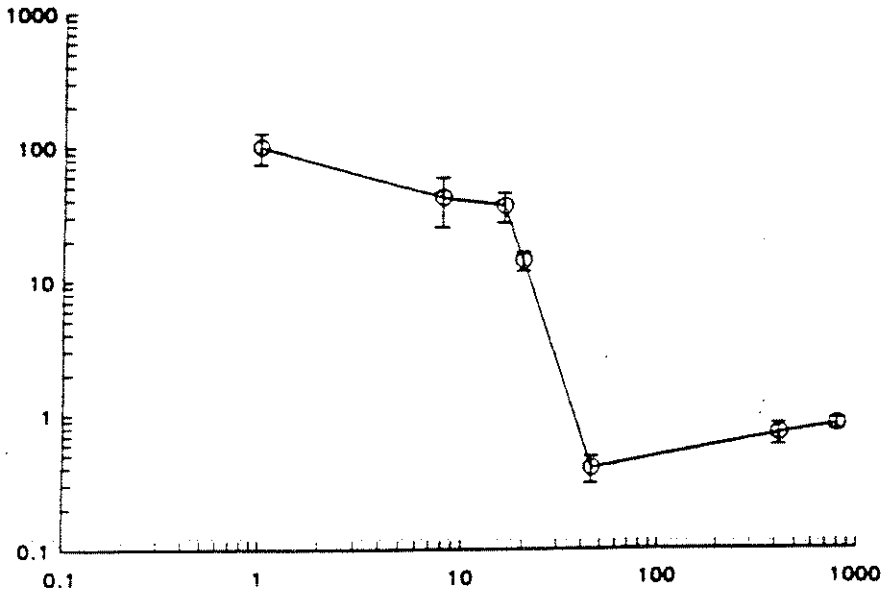
ii) Shelf-life of alginate formulations.

In the course of experiments on the shelf-life of alginate formulations, three types of storage conditions were compared: evacuated at room-temperature, evacuated at 0-4°C, storage at 0-4°C without evacuation. Evacuation had a beneficial effect on propagule survival in the long term, particularly with storage at 0-4°C. A sharp decline occurred in the number of colony-forming units arising from beads during the month subsequent to formulation. This was probably due to the induction of germination during the wet phase of bead preparation and the subsequent loss of viability of germling hyphae. However, sufficient viable propagules remained in the pellets such that there was no limitation to the effectiveness of the product (Fig. 1). A fully replicated glasshouse trial also confirmed that bead formulations of all five antagonists, varying in age from one week to one year, showed no decline in their capacity to control basal rot over the storage period.

iii) Field Trials.

Three replicated field trials were conducted on virgin sites within the grounds of the University of Exeter. Trial A repeated the experiment of Beale (1987), in which aqueous liquid formulations of the antagonists were used and

Figure 1.



Shelf-life of alginate bead preparation stored for three years under vacuum at room temperature.

The survival curve shows colony forming units (CFU) regenarated against days of storage of antagonist 082a and is typical of the various antagonists.

in addition the trial was extended for a second year. Trials B and C used the alginate bead formulations. Since preliminary glasshouse experiments had shown no clear correlation between the number of beads applied and freedom from disease over the range, 5-120 beads per bulb, 28 beads per bulb (17.5 kg/ha) were applied using a modified tea dispenser. This gave an antagonist application level equivalent to that arising in the liquid formulation in Trial A.

Exeter trial A, (1988-90). Results of this trial were equivocal, with both Trichoderma viride (089) and Streptomyces (131) fulfilling their earlier promise by reducing basal rot infection by 50% and 33%, respectively, whilst M. polyspora (128) failed to achieve significant control. It is likely that high prevailing winter temperatures (minimum average monthly soil temperature was never less than 5°C - appendix 1) and unprecedented high and prolonged summer soil temperatures (20-25°C) promoted a massive peak in the population of Fusarium, an artificially-high level of which had already been assured by the inclusion of 24% basal rot-infected bulbs. The favoured antagonist (128), applied at levels equivalent to and in the same liquid formulation as used by Beale during 1985-6, was unequal to the task of controlling the elevated level of basal rot. The imbalance was possibly accentuated by the fact that this antagonist, having been selected for its multi-faceted antagonistic qualities, may have reduced the indigenous population of natural antagonists. However, a more plausible explanation might be the effects of unprecedentedly high temperatures on the antagonists. These were screened for maximum antagonistic capacity within the normal temperature ranges experienced by the U.K. bulb crop. It has already been demonstrated (Beale, 1987) that at grossly elevated temperatures (25°C) both the bases and capacity of antagonism are adversely

affected (Fig. 2).

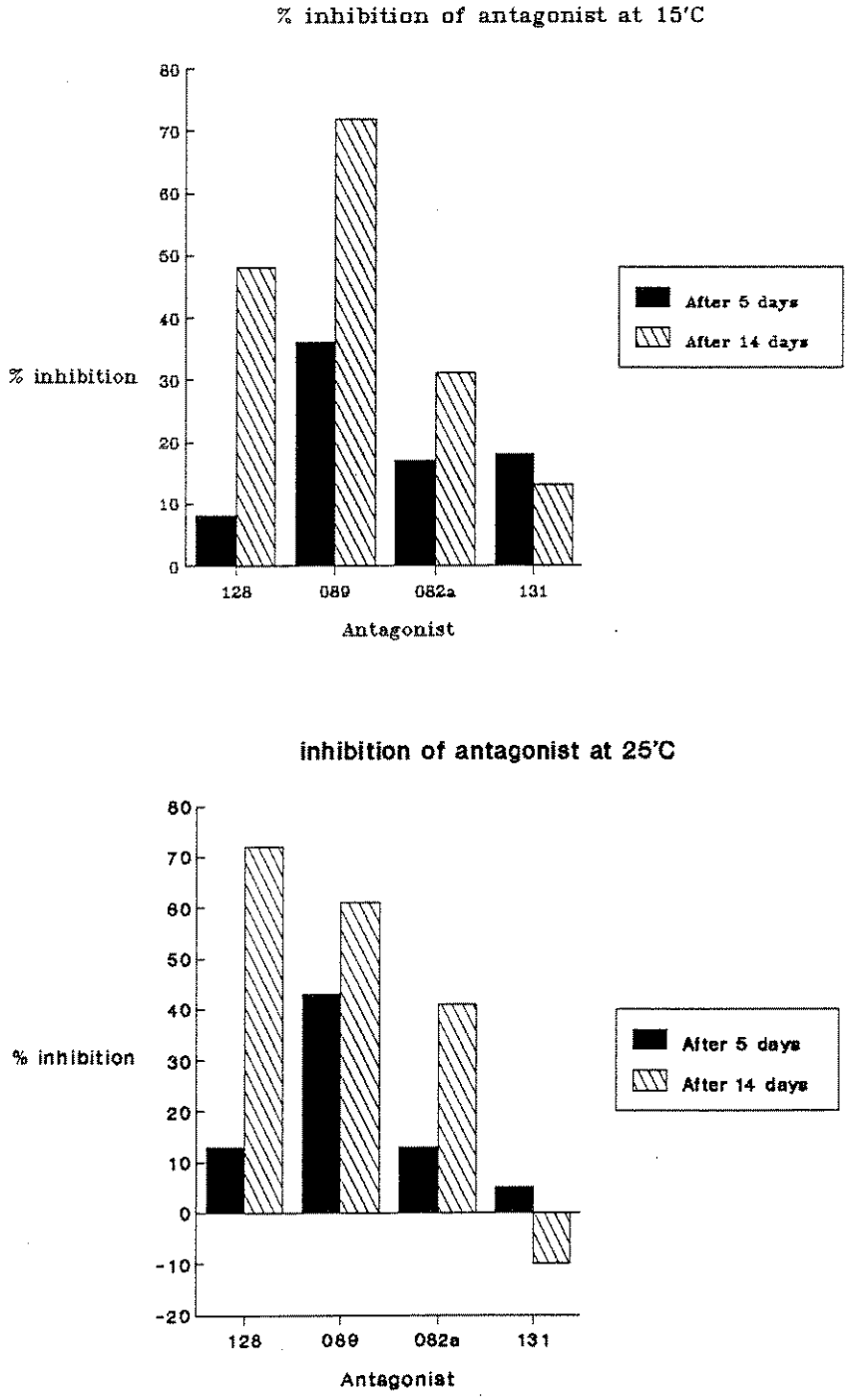
The evidence suggests that the extraordinary high temperatures during the trial promoted an exceptional disease pressure with which some antagonists were unable to cope.

Exeter trial B, (1990-1991). The aim of this one-year trial was to investigate the effect of various levels of basal rot inoculum, viz. 11, 16, 24 and 50% diseased bulbs, in conjunction with four antagonists, on disease incidence. Post-harvest rot due to F. oxysporum f.sp. narcissi was generally low but showed a trend towards increasing frequency at the higher inoculum levels. There was no evidence of any adverse effects of the antagonists on the bulb crop nor of any synergistic effects between these and the basal rot organism in promoting disease. The most successful treatments overall, on the basis of flower scores, bulb yield and the level of basal rot infection, were those for Streptomyces (131) at the 11% inoculum level and M. polyspora at the 11% and 24% levels. No antagonist gave significant control at 50% inoculum level.

Exeter trial C, (1990-1991). This one-year trial was to determine if mixtures of antagonists were more effective than antagonists applied singly, and to test twelve Actinomycetes, newly isolated from Kirton and Exeter soils, and which had been screened for antagonistic potential and tolerance towards Aldrin and thiabendazole. The data are shown in Fig. 3.

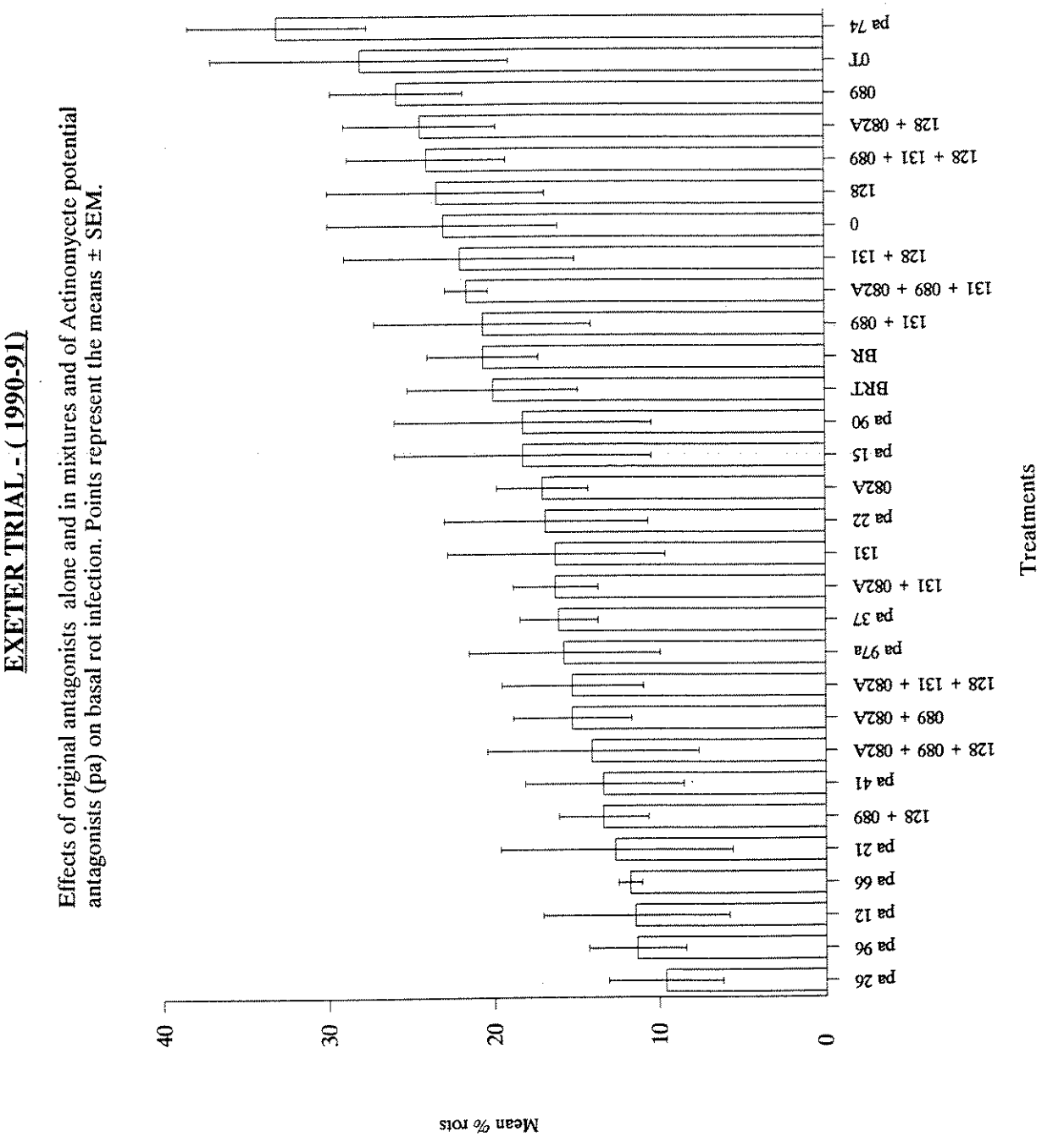
In terms of bulb yield alone, the four treatments containing mixtures of three antagonists, as opposed to two, or a single antagonist(s), all ranked among the top nine treatments. The most successful antagonist in this particular experiment was Penicillium rubrum (082A) which, either alone or in

Figure 2.



Effects of temperature on antagonistic potentials

Figure 3.


EXETER TRIAL - (1990-91)
Effects of original antagonists alone and in mixtures and of Actinomycete potential antagonists (pa) on basal rot infection. Points represent the means ± SEM.
Mean % rots
0
10
20
30
40
Treatments
pa 26
pa 96
pa 12
pa 66
pa 21
128 + 089
pa 41
128 + 089 + 082A
089 + 082A
128 + 131 + 082A
pa 97a
pa 37
131 + 082A
131
pa 22
082A
pa 15
pa 90
BRT
BR
131 + 089
131 + 089 + 082A
128 + 131
0
128
128 + 131 + 089
128 + 082A
089
0T
pa 74

combinations, reduced basal rot infections substantially. Six of the newly-screened Actinomycetes were among the seven treatments giving lowest disease incidence along with a mixture of M. polyspora (128) and T. viride (089) [Fig. 3].

Therefore, the use of mixtures of beads each containing single antagonists or of beads containing mixtures of antagonists is a possible means of extending the capacity of the method to cope with a broader range of environmental variables. However, the data illustrate the need for a judicious approach to mixing antagonists since they may adversely affect each other. This was also indicated by various glasshouse experiments conducted throughout this investigation.

It is worth mentioning that trials B and C were more encouraging and indicative of a useful degree of control of basal rot by the antagonists that is possible under the more normal weather conditions that prevailed over the period of these trials (appendix 2).

Kirton field trial (1989-1991). An independent one- and two-year-down field trial was conducted on our behalf by Dr. G. Hanks at Kirton EHS (trial ref. FN21/024). The data arising were analyzed by Ms. S. Hammond of HRI, Littlehampton, under trial identification ref. 20/ECG/C465
90048 HRI-L.

The trial comprised 30 treatments and investigated the effects of biological control agents on the occurrence of basal rot (and other bulb rots) in bulbs lifted after one and two years. The treatment structure, for each year of lifting, was a (4 agents + nil control) x 3 bulb types (including control) factorial. There were three replicates of a randomized block design. Treatments included single antagonists [089 (1); 128 (2);

131 (3); 082A (4), with a nil control (5)] in the presence and absence of the pathogen (20 diseased bulbs: 100 healthy bulbs per plot). Integrated treatments with thiabendazole (TBZ) were also included with each antagonist.

The protocol and methods for establishing the trial, as supplied by Dr. G. Hanks, are set out in Appendix 3.

The partial lift in the first year yielded insufficient disease to permit meaningful conclusions to be drawn from the data. Figure 4 gives the mean % bulb weight gain and the mean % basal rot in the first year with the summary of the analysis in Appendix 4a.

The two-year-down bulbs were lifted on 25th July 1991, dried, cleaned and graded before storage until December 3rd., when they were dissected lengthwise, and the numbers with whole bulb, basal or neck rot recorded. Table 2 shows the basal rots, neck rots and total rots per treatment for November 1990 and 1991, whilst Figure 5 illustrates the mean % bulb weight gain and the mean % basal rot in the second year. As in the first year, the amounts of basal rot and other rots were extremely low but even so, inoculation in the presence and absence of TBZ substantially reduced bulb yield.

An analysis of variance was performed on each of the following variates:-

Weights for individual gradings, < 8 cm, 8-10 cm, 10-12 cm, 12-14 cm, 14-16 cm and 16-18 cm.

Total weight of marketable bulbs

Total weight of bulbs allowing for planted weight

Total number of marketable bulbs

Flower numbers in 1990

Flower numbers in 1991

Figure 4.

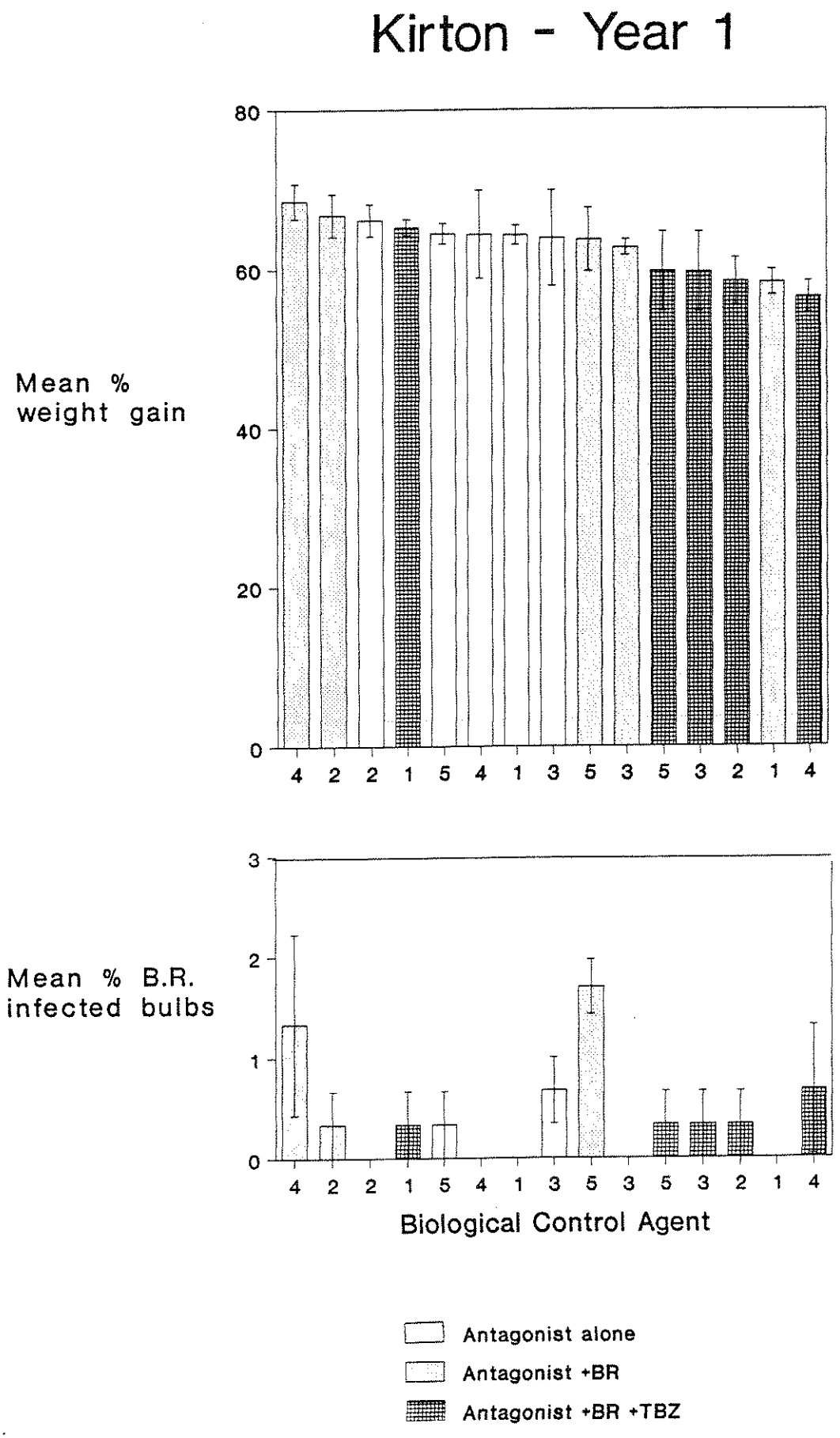
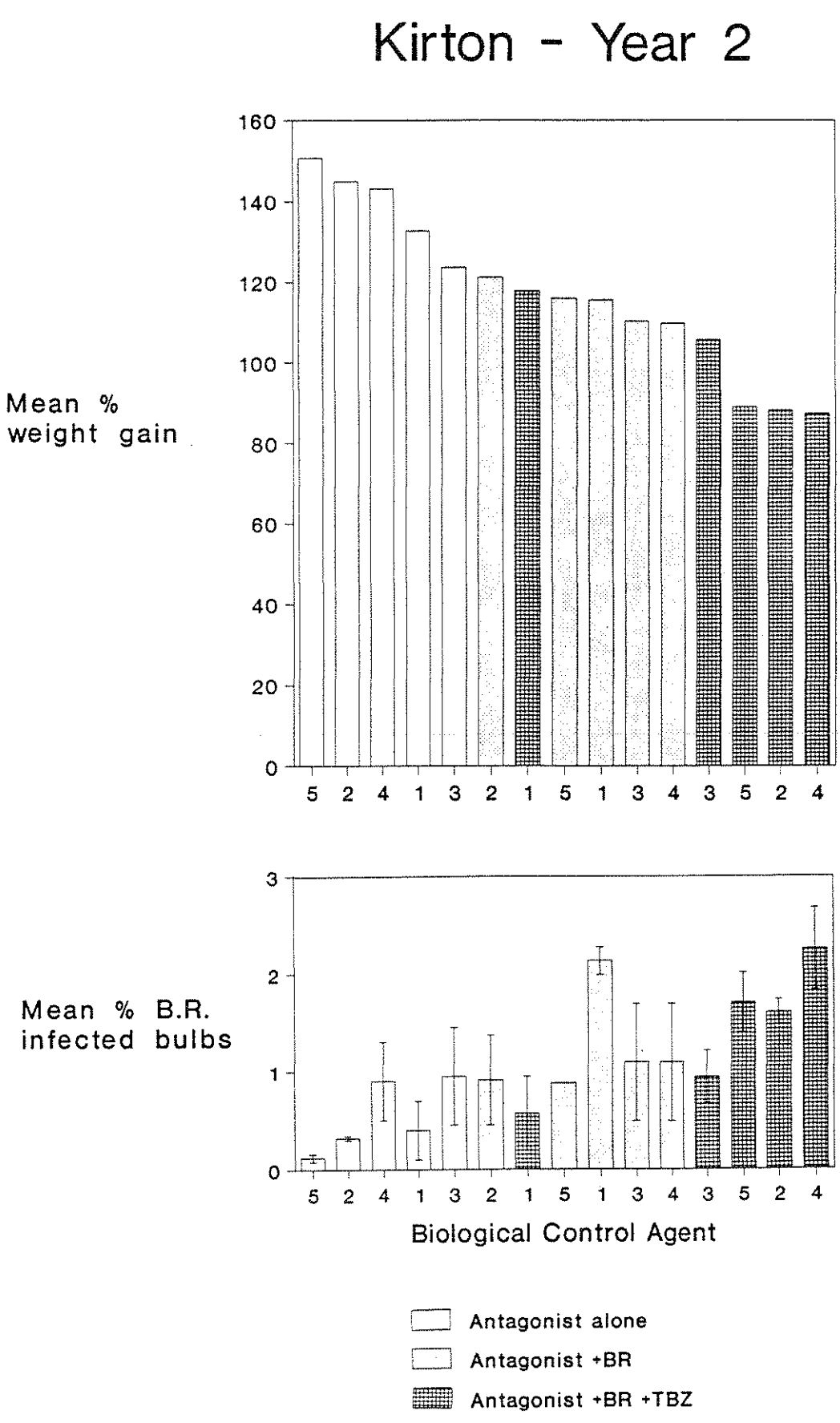

Kirton - Year 1
Mean % weight gain
80
60
40
20
0
4 2 2 1 5 4 1 3 5 3 5 3 2 1 4
Mean % B.R. infected bulbs
3
2
1
0
4 2 2 1 5 4 1 3 5 3 5 3 2 1 4
Biological Control Agent
Antagonist alone
Antagonist +BR
Antagonist +BR +TBZ

Table 2. Mean number of basal rots, neck rots, whole rots and total rots per treatment for November 1990 and 1991.

Time	Treatment	Agent	Basal rot	Neck rot	Whole rot	Total rots
Control Bulbs						
1 yr. down	1	089	0.7	0	1.3	2.0
	2	128	2.0	0	0.3	2.3
	3	131	0.7	0	1.3	2.0
	4	082A	0.7	0	0.7	1.3
	5	nil	0.3	0	0.7	1.0
2 yr. down	1	089	1.0	0.3	0	1.3
	2	128	0.7	0.3	0	1.0
	3	131	2.3	2.0	0	4.3
	4	082A	2.0	0	0	2.0
	5	nil	0.3	0.7	0	1.0
Inoculated Bulbs						
1 yr. down	1	089	0.7	0.3	6.3	7.3
	2	128	1.0	0	5.7	6.7
	3	131	1.0	0.3	7.7	9.0
	4	082A	1.3	0	6.0	7.3
	5	nil	1.0	0	3.7	4.7
2 yr. down	1	089	4.7	1.0	0.3	6.0
	2	128	2.0	1.3	0.3	3.7
	3	131	2.3	0.7	0	3.0
	4	082A	2.3	1.0	0.7	4.0
	5	nil	2.0	0.3	0.3	2.7
Inoculated + TBZ						
1 yr. down	1	089	0.3	0	4.7	5.0
	2	128	0.3	0	7.0	7.3
	3	131	0.3	0	4.3	4.7
	4	082A	0.7	0.3	6.7	7.7
	5	nil	0.7	0	8.0	8.7
2 yr. down	1	089	1.3	0.3	0.3	2.0
	2	128	3.3	0.7	1.3	5.3
	3	131	2.0	0.3	0	2.3
	4	082A	4.7	0	0.7	5.3
	5	nil	3.7	0.7	0	4.3

Figure 5.


Kirton - Year 2
Mean % weight gain
160
140
120
100
80
60
40
20
0
5 2 4 1 3 2 1 5 1 3 4 3 5 2 4
Mean % B.R. infected bulbs
3
2
1
0
5 2 4 1 3 2 1 5 1 3 4 3 5 2 4
Biological Control Agent
Antagonist alone
Antagonist +BR
Antagonist +BR +TBZ

Basal rots, all rots, % rotted in sample (July 1990)

Basal rots, all rots, % rotted in sample - November 1990 and 1991.

The grade outs of the bulb weight and numbers were tabulated by treatment as were the numbers of flowers and rots. For neck rots and whole bulb rots, there were insufficient data for a valid formal analysis. A separate analysis of the individual numbers of each grade was not performed due to the high correlation between these and the individual weights per grade. The results emerging from the analysis are set out in Appendix 4b, as provided by Ms. S. Hammond, HRI, Littlehampton but the salient points are as follows:-

1. Total rots and basal rot infections were extremely low in both years of the trial and the level of disease was largely unaffected by the agents either singly or in combination with TBZ.
2. Total rots after storage, though small in number, were greater in year 1 than in year 2. Controls (uninoculated) had fewer rots than the inoculated and inoculated + TBZ bulbs.
3. Basal rot occurrence was low but similar in control and inoculated bulbs in year 1 and increased in year 2. In year 2 there were fewer rots in the control series than in bulbs inoculated ± TBZ.
4. Flower numbers were not influenced by the treatments in year 1, but in year 2 the control treatment appeared to have more flowers than the inoculated series. In year 2 there were more flowers in the control series than with inoculated ± TBZ. Whilst inoculation reduced flowering, the agents did not ameliorate the effect.
5. Although disease levels were low, there was a suggestion of minor differential effects of inoculation on bulb grades between 1- and 2-year-down bulbs in respect of TBZ and the agents. In particular, agent 089 (1)

reduced bulb weight in the 10-12 cm category both singly and in combination with TBZ, whilst agents 128 (2) and 082A (4) increased both the weight of the uninoculated controls and the inoculated bulbs relative to those inoculated and treated with TBZ. Please see Appendix 4b, Figs 1, 2 & 3.

Because of the limited value of the independent trial at Kirton EHS we have been invited, outside the scope of the present contract, to participate in a further trial co-ordinated by Dr. G. Hanks at Kirton. The trial is a one- and two-year-down experiment on integrated control of basal rot in which the effects of reduced and full fungicide (TBZ) programmes are compared in conjunction with three Narcissus cultivars with varying basal rot susceptibility. We have provided alginate formulations of three of the current antagonists which will be included as separate treatments and incorporated with TBZ.

Integrated treatments with thiabendazole. In early glasshouse trials (1988-89) the phytotoxic effect of thiabendazole (TBZ) was noted when bulbs were treated rather late in the year with the fungicide. As early as the following January, normal treatment with TBZ resulted in plants that were notably taller and more vigorous than those which were untreated. Generally, the integrated treatments with TBZ showed considerably less post-harvest disease than those without TBZ. In the glasshouse TBZ tended to confer greater protection than with the antagonists alone.

Thiabendazole was used successfully in several of the Exeter field trials. In the one- and two-year-down trial, TBZ had an adverse effect on bulb weight and flower yield in the first year, but coupled with Streptomyces (131), gave a disease level as low as that in the uninoculated controls. However, this antagonist was adversely influenced by high temperatures in the second year of the experiment and control was poor.

iv) Occurrence of other diseases in trials.

Rhizopus rot

In the autumn of 1989 there was a large incidence of disease in bulbs harvested from various plots and trials at Exeter. There was some confusion of symptoms, therefore a total of 126 sites within bisected bulbs was sampled from 96 bulbs in order to identify any pathogens present. Of these sites 70 yielded Fusarium spp, 69 yielded Rhizopus spp. and 37 yielded both genera. Several bulbs showing classical reddish-purple colouration of Fusarium infection produced only Rhizopus on isolation plates. This observation has a bearing on our findings of competition between Rhizopus and Fusarium spp. in bulbs inoculated with Fusarium oxysporum f.sp. narcissi for a field trial. In many of these inoculated bulbs, spread of basal rot was limited to within 1 cm. of the inoculation site in the basal plate and purple-brown colouration was faint, whilst the rest of the bulb displayed characteristic symptoms of Rhizopus infection. In bulbs showing no evidence of the latter, F. oxysporum f.sp. narcissi had invaded at least half of the bulb, and colouration was characteristically intense.

When bulbs are dried under hot humid conditions, as prevailed in autumn 1989, development of Rhizopus rot may be favoured which could suppress the subsequent development of basal rot. This could have had implications in the Kirton trial where discarded rotted bulbs were used as a presumed source of basal rot inoculum.

Neck rot

When this investigation began, questions were asked within the industry regarding the frequency of entry of F. oxysporum f.sp. narcissi via the neck, as opposed to the basal plate, of the bulb. As a consequence field-grown

bulbs were scored for disease and the incidence of neck rot, in addition to other diseases, was recorded. In the second of the three field trials at Exeter (trial B), 167 bulbs (4.6% of those scored) were found to be only partially-infected with basal rot, as distinct from the 1.5% which were totally consumed by the disease. Of the partially-diseased bulbs only 12 had contracted the disease via the neck rather than the basal plate. There was no evidence that the antagonists influenced the level of neck rot in this trial.

Large Narcissus fly and the projected use of pesticides.

The incidence of large narcissus fly increased from near zero in 1989 to 1% (bulbs containing a visible, live grub) in 1991. A further 3.3% bore the traces of (presumed) attack by the fly in the form of an abortive tunnel or brown, horny areas in the basal plate.

Concern was felt over the projected use of insecticides against large narcissus fly, and of hitherto unused fungicides in the control of basal rot, which could affect antagonists used in biological control measures. An ad hoc investigation was conducted to investigate the possible toxicity of six of these projected control agents towards the basal rot antagonists.

Three candidate fungicides, prochloraz as 'Sportak', chlorothalonil as 'Bravo' and captan, all affected radial extension growth on agar plates at all concentrations used within the range 10^{-1} to 10^{-6} μmol ml^{-1} with e.g. Streptomyces unable to sustain any growth at a concentration greater than 1×10^{-6} μmol ml^{-1}. However, the antagonists were able to tolerate the presence of all three insecticides tested viz. chlorpyrifos as 'Spannit', carbofuran as 'Yaltox' and disulfoton as 'Disyston' at levels up to 1×10^{-1} μmol ml^{-1}.

The non-target effects of changes in pesticide use should be borne in

mind when microbial antagonists are contemplated as agents of biological control.

v) and vi) Soil population studies on Fusarium (v) and on the antagonists (vi).

Survival of Fusarium oxysporum f.sp. narcissi. Soil dilution plating on selective media was carried out on several occasions to check levels of the pathogen. In March, 1988, the level of Fusarium spp. colony-forming units in the plot formerly used by Beale (1987) during 1985-86 was $(3.4 \pm 1.1 \times 10^4)\ g^{-1}$ of soil. Four weeks later the population had increased three-fold and in originally non-inoculated soil was 25 times this level. This clearly suggested a pronounced capacity of the pathogen to spread between plots and show pronounced local variation in the soil and to persist for several years in the absence of a susceptible crop and in the presence of residual antagonists. These and other data clearly imply that basal rot could arise from infected soil in addition to being introduced on the bulbs.

Survival of the antagonists. Three of the five antagonists viz. T. viride (089), T. harzianum (134) and P. rubrum (082A) were recovered from the site of the Exeter trial A 4 months after planting. Characteristic antagonistic responses towards F. oxysporum f.sp. narcissi on dual culture plates were recorded in each case. An organism resembling the Streptomycete (131) was recovered only on one of the four isolation media adopted.

It was a matter of concern that M. polyspora was not recovered directly from the soil on any occasion despite the use of many different media and techniques. A special medium containing all the toxic metabolites (poly-acetylenes) generated by this organism also failed to select it from soil after suppression of its potential competitors.

Since by June, 1989, M. polyspora had still not been recovered, a radical change in approach was devised. Alginate beads, instead of being randomly distributed within the planting trench, were contained within nylon mesh bags and planted alongside bulbs in the soil. After 6 months' burial the beads retained their integrity but after four further months they became emaciated and tended to disintegrate on recovery. All five antagonists were recovered after 6 months, but only P. rubrum and the two species of Trichoderma were recovered after the later sampling. This technique was considered very satisfactory for studying experimentally the survival of these microorganisms and could be exploited in the future.

Six months after planting at the Kirton site T. viride was found in soils at $5 \pm 2.5 \times 10^{5}$ cfu g^{-1} which was three times its frequency in untreated soils. For Streptomyces sp. the ratio of recovery from treated vs. untreated soils was 2 : 1. Penicillium rubrum was found only in treated soil and M. polyspora was never recovered.

Pathogenicity tests on Fusarium isolates from Kirton trial site soil showed that the virulence of the pathogen was equivalent to that used in the Exeter trials.

vi) Liaison with commercial organisations.

Early contacts were established with ICI Agrochemicals, Jealott's Hill Research Station and funding was injected into two projects.

a. Biological and integrated control of soil-borne diseases of carrots.

Jointly funded by ICI Agrochemicals and British Fruit and Vegetable Canners' Association for work at NIAB, Cambridge, in conjunction with Dr. R.E. Beale and Dr. Pitt.

Considerable success was achieved in controlling, under experimental

conditions, Pythium violae on carrots using some of Dr. Beale's original antagonists of F. oxysporum f.sp. narcissi. After one year of this three-year project, work has been suspended following Dr. Beale's appointment to a post with Monsanto in Belgium. The future of the work will be discussed in a forthcoming visit to ICI in January, 1992.

b. Fungal antagonism. Funded by Science and Engineering Research Council studentship at Exeter University and supplemented by extra funding from ICI Agrochemicals. This project is in the second year and concentrates on the wider application of Dr. Beale's original antagonists to the control of Fusarium diseases on other crops in the glasshouse and field e.g. Fusarium wilt of pinks and Fusarium foot-rot complex of cereals. Good progress has been achieved in both areas and regular contacts with ICI are maintained in what is an excellent co-operation.

The outcome of these projects will indicate the potential and scope for biological control of Fusarium, and other soil borne diseases, by fungal antagonists and will determine the nature and extent of any action by ICI regarding commercial development of any product which would embrace the bulb industry, along with wider applications.

Conclusions

The alginate bead formulation of the original biological control agents has a useful shelf-life, is convenient to handle, and could be incorporated into normal practice in the bulb industry. It is not known if the product has biological properties which could present a safety hazard.

Initial high dose levels of the biological control agents were not

substantially maintained into the second year of the crop, whereas the causal organism of basal rot could persist in treated soils for several years.

Whilst some local field trials of the bead formulation confirmed the original potential of the majority of the antagonists, either singly or in various combinations and when integrated with thiabendazole, results with the best potential candidate, M. polyspora, were disappointing. The unprecedented high soil temperatures, coupled with summer droughts, prevailing throughout most of the project generally favoured development of basal rot, but disadvantaged the control agents which in a disabled state were unable to cope with the exceptional disease pressure encountered.

The inexplicably low level of disease in the independent trial at Kirton was a major setback and failed to provide any test of the agents' capacities to control basal rot. A further trial, separate from this project and incorporating some of the biological control agents, which is now in progress seeks to remedy this matter.

Future work.

1. Assuming a suitable screen is devised it is relatively easy to obtain candidate organisms for biological control purposes. With current knowledge and available techniques it is possible to generate and select desirable mutant strains. Very recent techniques e.g. protoplast fusion, should permit the introduction and combination of desirable features e.g. tolerance to microbial extremes, into organisms.
2. The massive microbial buffering capacity of soil usually tends to re-establish the status quo. To devise satisfactory methods of biological control using microbes, efforts and resources need to be directed towards

generating and selecting agents which can establish and compete in the environment.

3. To achieve 2, above, suitable stable genetic or molecular markers need to be introduced into candidate organisms in order to monitor population dynamics and ensure the original strain is being monitored amidst vast numbers of otherwise identical, but useless, biotypes.

4. Having established 1 to 3, above, particular attention should be paid to the responses of desirable control agents to non-target effects of pesticides introduced into disease management programmes. Indiscriminate use of agrochemicals can destroy natural and introduced predator species.

5. The Actinomycetes contain particularly useful candidates as biological control agents against Fusarium as shown in the present work, recent published data Tahvonen & Avikainen (1990) and the introduction of 'MYCOSTOP' by Kemira Co. of Finland for use in peat. However, in our hands representatives of this group of organisms were especially sensitive to environmental change.

6. Oil formulations of antagonists could be profitably explored.

7. It was increasingly apparent in the present project that bulk production and subsequent introduction of both common and exotic microbes into the environment present incalculable dangers. A Code of Practice is an urgent necessity for all aspects of this work.

References.

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Beale, R.E. & Pitt, D. (1990). Biological and integrated control of Fusarium basal rot of Narcissus using Minimedusa polyspora and other micro-organisms. Plant Pathology, 39, 477-488.

Lewis, J.A. & Papavizas, G.C. (1985). Characteristics of alginate pellets formulated with Trichoderma and Gliocladium and their effect on the proliferation of the fungi in soil. Plant Pathology, 34, 575-577.

Price, D. (1981). Bulb pathogens. Report of the Glasshouse Crops Research Institute 1979, pp. 142-143.

Tahvonen, R. & Avikainen, H. (1990). Effects of Streptomyces sp. on seed-borne foot rot disease of wheat and barley. I. Pot experiments. Ann. Agric. Penn., 29, 187-194.

APPENDICES.

Appendix 1. Monthly mean soil temperatures.

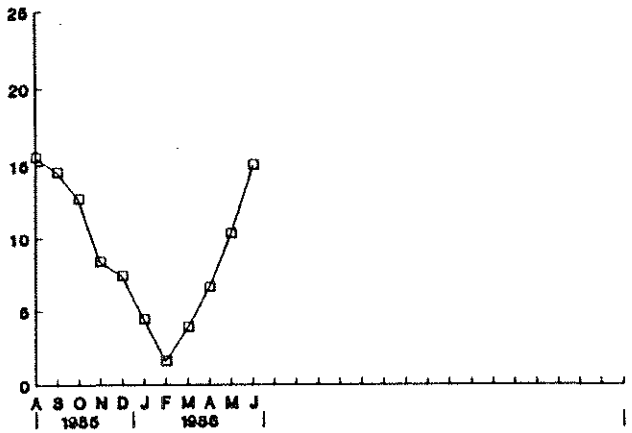
Appendix 2. Weekly soil temperatues, 1990-1.

Appendix 3. Materials and Methods, Kirton trial.

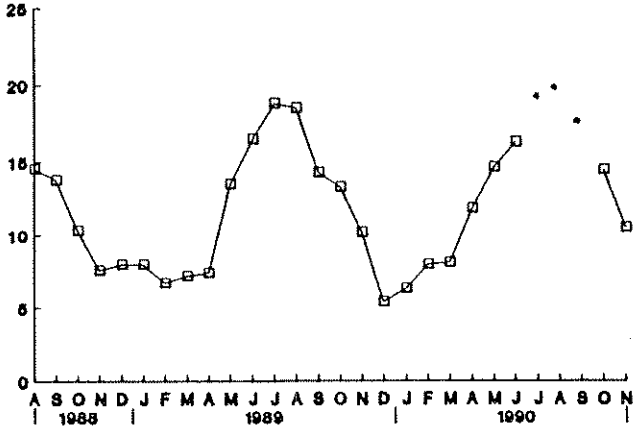
Appendix 4. Statistical report, Kirton trial year 1.
Statistical report, Kirton trial year 2.

Monthly mean soil temperature (°C) at 10 cm depth.

EXETER



EXETER



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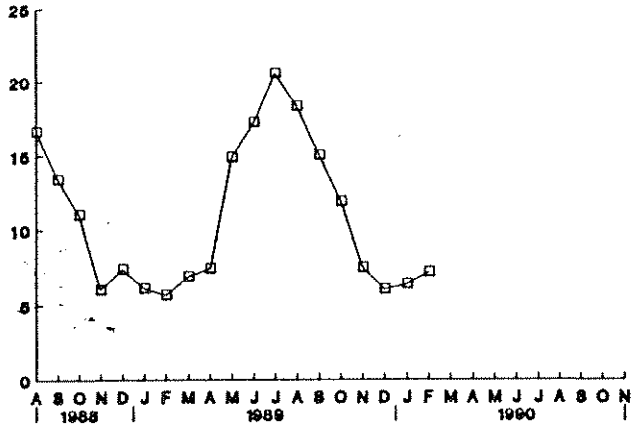
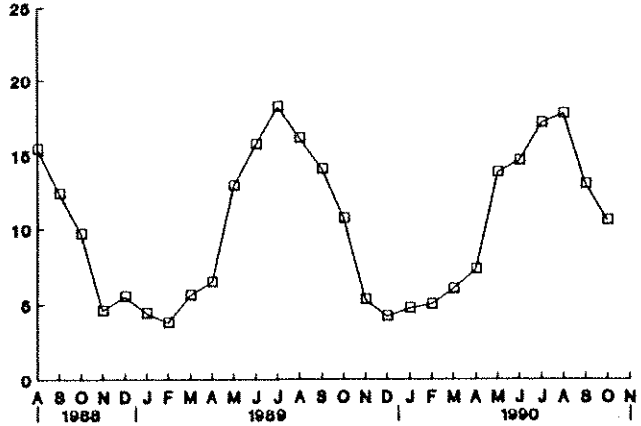
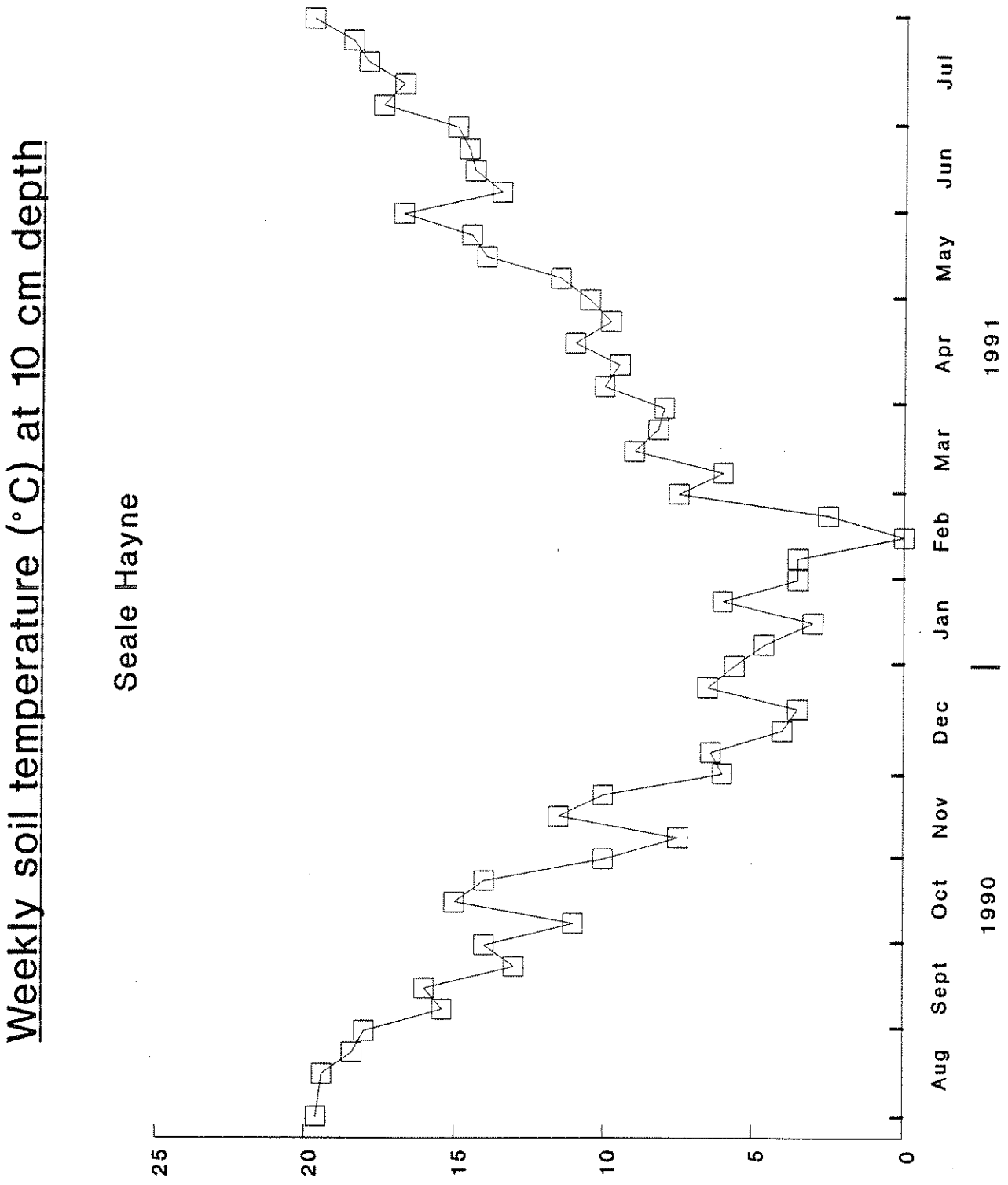


KIRTON



Appendix 1.

Appendix 2.


Weekly soil temperature (°C) at 10 cm depth
Seale Hayne
25
20
15
10
5
0
Aug
Sept
Oct
Nov
Dec
Jan
Feb
Mar
Apr
May
Jun
Jul
1990
1991

BASAL ROT ANTAGONISTS TRIAL FN21024 (1989/91)

Materials and methods

Plant material

Bulbs were taken from a stock of Narcissus cv. Golden Harvest grown at HRI Kirton, Lincs, under good commercial practice (eg, see ADAS, 1985), and lifted on 4 July 1989. Two days later the bulbs were graded and 0.75t of 11-13cm circumference bulbs selected. Half of these were dipped for 30 minutes at ambient temperatures in aqueous thiabendazole with formaldehyde and non-ionic wetter (as 500ml Storite Clear Liquid, 500ml commercial formalin and 62ml Agral/100 litres) ('thiabendazole-treated'), and the other half remained non-dipped ('untreated'). Bulbs were dried for 5 days at 35°C on a drying wall and then at 17°C under fans.

Bulbs to be used for preparing 'inoculator' bulbs were taken from a stock of cv Golden Harvest known to have a relatively high level of basal rot, which was maintained by using sub-optimum bulb treatments (eg, no post-lifting fungicide treatment, slow drying at ambient temperatures).

Inoculator bulb preparation

1200 'untreated' bulbs from the healthy stock were incised c. 1cm deep into the basal plate, using the blades of a bulb chipping machine (Machinefabriek Akerboom b.v.) to make eight radial cuts. Cut bulbs were immersed in a macerate of rotting bulbs taken from the infected stock. The bulbs were then stored in trays covered with polythene film, at 25°C for the first 4 weeks and then at ambient temperatures until required.

Bulb treatments

After drying, bulbs were counted into 100-bulb plots which were weighed. On 11 August 1989 bulbs were soaked for 3 hours at ambient temperatures in aqueous formaldehyde and wetter (as above) and then hot-water treated for 3 hours at 44.4°C in aqueous formaldehyde and wetter (as before). Bulbs were dried and stored under fans at 17°C.

Thirty 100-bulb plots from the 'untreated' (no thiabendazole) bulbs were placed in 10m-long lengths of tubular nylon netting (Netlon Oriented 1), using plastic clips to distribute bulbs evenly. 30 further plots from each of the 'untreated' and 'thiabendazole-treated' bulbs were netted in a similar fashion, except that an inoculator bulb was placed between every five healthy bulbs.

The bulbs were planted in ridges in the field on 21 August 1989. The trial area had been previously ridged and 5m-long plots marked in, and each net was laid in the ridge bottom to form a double row of bulbs. Plots were treated as appropriate with granular preparations of fungal antagonists, coded 089, 128, 031 or 082A, by sprinkling the granules over the bulbs, or were untreated. After granule application, ridges were split back to cover the bulbs.

Details of routine husbandry are given in Table 1.

Field observations

The numbers of flowers per plot were recorded on 7 March 1990 and 20 March 1991. Scape length was determined for 20 central blooms per plot on 13 March 1990 and 23 March 1991. The dates of first, 50 per cent and 100 per cent flowering were recorded. Percentage foliage senescence was recorded weekly at the end of the growing season.

Harvesting and yield recording

After one growing season, on 29 June 1990, bulbs were lifted from one half of the plots. The remaining plots were lifted after a second growing season, on 25 July 1991.

Bulbs were dried under fans at ambient temperatures, removed from the netting and cleaned by hand. Bulbs were graded, recording the number and weight of sound bulbs in each grade and the additional numbers of obviously rotted bulbs (which were then discarded).

Rot assessments

After grading, bulbs were stored in paper-lined trays at ambient temperatures in a well-ventilated store. In 1990, half the bulbs were examined on 13 July and the remainder on 27 November; in 1991, all bulbs were examined on 3 December. For this, bulbs were dissected lengthwise, and the numbers with whole bulb, basal or neck rot recorded.

Design and statistical analysis

The experiment was of a randomised block design, with three replicate blocks. There was a blank ridge or a 1m-long gap between experimental plots, and the whole trial was surrounded by equivalent guard plants. Trays of bulbs for rot assessments were stored in corresponding randomised blocks.

Data were subjected to the analysis of variance as appropriate.

Results

See Genstat output and reports.

The following data were not formally analysed as there was clearly no differences between treatments: mean scape length, flowering dates and rates of senescence.

Reference

ADAS (1985). Narcissus bulb production. Booklet 2150. MAFF (Publications): Alnwick.

Table 1: Outline and cultural and related details in the field

Soil texture:	Mixed fine silty and clayey marine alluvium ("40 Acres 4" field)
Previous cropping:	1987 Onion 1988 Wheat 1988/89 Short-term grass
Soil analysis:	pH 7.8 P_2O_5 index 4 K_2O index 2 Mg index 4
Fertilizers:	97kg K_2O/ha (as sulphate of potash, 50% K_2O) applied in base 4 July 1989 125kg N/ha (as Nitram, 34.5% N) applied as top-dressing 19 January 1990
Cultivations:	Sub-soiled 12 June 1989 Ploughed and rolled 13 June 1989 Cultivated (Lely Roterra) and rolled 4-5 July 1989 Cultivated (Lely Roterra) and ridged 13 July 1989 Re-ridged - September 1990
Insecticides:	None applied
Fungicides:	None applied
Herbicides:	Diquat + paraquat (as 5.5 litre Farmon PDQ/ha) applied 8 November 1989, (as 3 litre/ha) 7 December 1989, and (as 5.5 litre Parable/ha) 5 and 22 November and 17 December 1990. Chlorbufam + chloridazon (as 9kg Alicep/ha) applied 4 January 1990. Chlorpropham + linuron (as 11.2 litre Profalon/ha) applied 17 December 1990 Bentazone (as 3 litre Basagran/ha) applied 28 April 1990 and 14 May 1991.
Irrigation:	12mm irrigation applied 8-10 July 1989

GH129151

Narcissus: Use of antagonists for the control of basal rot (FN21/024)

References

Station: Kirton
Experimenter: Gordon Hanks
Trial Identification: 20/ECG/C465
90048 HRI-L

Experimental Design

The trial investigated the effect of 15 treatments on the control of basal rot. The treatment structure was a (4 agents + control) x 3 bulb types (including control) factorial. There were three replicates of a randomized block design.

The Analysis

An Analysis of Variance was performed on each of the following variates:-

Weights for individual gradings <8cm, 8-10cm, ... , 16-18cm
total weight of (marketable) bulbs
total weight of bulbs allowing for planted weight, % weight increase
total number of (marketable) bulbs
flower numbers in first year
total rots as a % of total number in sample - July, November, July and November

In addition, the details of the rotted bulbs, the flower numbers and the lifted rots per treatment have been tabulated. With the exception of flower numbers, there was insufficient details for formal analysis of these variates. A separate analysis of the individual numbers for each grade was not performed due to the high correlation between the results and the individual weights per grade.

Data Investigation

A graph of number of bulbs lifted against weight of bulbs lifted for each grading showed two possible outliers:-

(i) <8cm - treatment 6, rep B recorded as 40 bulbs @ 0.3 kg
changed to 14 bulbs @ 0.3 kg

(ii) 8-10cm - treatments 5, rep A recorded as 22 bulbs @ 0.26 kg
changed to 22 bulbs @ 0.62 kg

Results

Weights for individual gradings:-

<8cm : no evidence of differences between agents (including the control) nor between bulb types (including controls) nor interaction between the two factors.

8-10cm : the weight of control bulbs lifted appeared to be significantly less than the inoculated or inoculated + TBZ bulbs, the weight of bulbs with no agent appeared significantly less than those with agents A,B,C or D. No evidence of agent x bulb type interaction.

10-12cm, 12-14cm, 14-16cm, 16-18cm, total weight of bulbs : no evidence of differences between bulb types or formulations added.

Total number of (marketable) bulbs:-

Evidence of a bulb type effect, the inoculated and inoculated + TBZ appeared to have significantly more bulbs than the control bulbs.

Flower numbers in the first year:-

Evidence of a formulation x bulb type interaction. The sketch plot on the printout shows 'variable' responses! A square root transformation was used in the analysis.

Nil formulation → no difference between bulb types
Agent A → inoculated + TBZ had significantly fewer flower than control or inoculated alone.
Agent B → no difference between bulb type
Agent C → no difference between bulb type
Agent D → inoculated + TBZ had significantly fewer flowers than control or inoculated bulbs.

% weight increase, total weight allowing for planted weight:-
(the latter is the final analysis on the printout including the covariate)

Evidence of a difference between the bulb types, it appeared that the % weight increase (or total weight allowing for planted weight) was greater for the control and inoculated bulbs compared to the inoculated + TBZ bulbs. A logit transformation was used for % weight increase.

Total rots as a % of total number in sample :-

July, November, July and November - evidence of a difference between the bulb types, the control bulbs had significantly fewer rots than the inoculated or inoculated + TBZ bulbs. In November alone there was slight evidence of a bulb x formula effect (averaging across Agents A,B,C,D compared to nil) - it appeared that for control bulbs the nil formulation had significantly fewer % rots than the Agent formulations, for the inoculated and inoculated + TBZ there was no difference between nil or the agents. This effect did not feature in the total rots over the year (July and November).

Sarah Hammond 13.12.90

Narcissus : Use of antagonists for the control of basal rot (FN21/024)

References

Station	HRI Kirton
Experimenter	Gordon Hanks
Trial Identification	20/ECG/C465
	90048 HRI-L

Experimental Design

The trial comprised 30 treatments in a 2 time x 3 bulb type x (4 + 1) agent factorial structure
i.e.

1 yr down		control			Nil
2 yr down	x	+ inoc. bulbs		x	Agent A
		+ inoc. bulbs + TBZ			Agent B
					Agent C
					Agent D

and examined their efficacy in the control of basal rot. There were three replicates of a randomized block design.

Analysis

An Analysis of Variance was performed on each of the following variates

- Weights for individual gradings <8 cm, 8-10 cm,...,16-18 cm
- Total weight of marketable bulbs
- Total weight of bulbs allowing for planted weight
- Total number of maketable bulbs - square root transformed
- Flower numbers in 1990 - square root transformed
- Flower numbers in 1991 (2 yr down) - square root transformed
- Basal rots, all rots, %rotted in sample (1 yr down) - July 1990
- Basal rots, all rots, %rotted in sample - Nov 1990 and 1991

{basal rots and all rots - square root transformed}

The grade outs of the bulb weight and numbers were tabulated by treatment, similarly the numbers of flowers and rots. For neck rots and whole bulb rots, there were insufficient data for a valid formal analysis. A separate analysis of the individual numbers of each grade was not performed due to the high correlation between these and the individual weights per grade.

Data Investigation

A graph of number of bulbs lifted against weight lifted for each grading showed three possible outliers;-

(i)	<8 cm	Treatment 6	Rep B	recorded as	40 bulbs @ 0.3 kg
				changed to	14 bulbs @ 0.3 kg
(ii)	8-10 cm	Treatment 5	Rep A	recorded as	22 bulbs @ 0.26 kg
				changed to	22 bulbs @ 0.62 kg
(iii)	10-12 cm	Treatment 16	Rep B	recorded as	37 bulbs @ 2.96 kg
				changed to	73 bulbs @ 2.96 kg

The results described below were based on the altered data set though the changes had little effect on the overall significance of treatment comparisons. These give a summary of some of the main points in the data and should be used with refernce to the treatment means on the printed output.

Results

Individual weights:-
<8cm, 8-10cm, 10-12cm, 12-14cm - There was a suggestion of time x bulb type interaction (see Figure 1)

<8 cm - There was no evidence of a difference between the controls for 1yr and 2yr down bulbs, but for the 1yr down bulbs the inoc. and inoc.+TBZ appeared to have a greater yield compared to the control whereas for the 2yr down bulb the yield was less for inoc. and inoc.+TBZ.

8-10 cm - 1yr down control < inoc. and inoc.+TBZ
2yr down control > inoc. and inoc.+TBZ
For the control bulbs, the weight of 2yr down bulbs was greater than 1yr down in contrast to the inoc. for which the weight of 2yr down bulbs was less than 1yr down. There was slight evidence of a differential response to 1 or 2 yr down by the four agents.

10-12 cm - 1 yr down no 'bulb type' difference
2 yr down weight of controls > inoc. and inoc.+TBZ
The weights of 2yr down bulbs was greater than 1yr down for all three bulb types. There was evidence of differential response of bulb types by the four agents
Agent A weight of control and inoc.+TBZ > inoc.
Agent B and D weight of control and inoc. > inoc.+TBZ

12-14 cm - 1 yr down no 'bulb type' difference in weights
2 yr down weight of control and inoc. > inoc.+TBZ

14-16 cm - There appeared to be 'bulb type' differences, it would appear that the weight of control bulbs was greater than inoc. bulbs though the weight of inoc.+TBZ was in the middle of this range.

16-18 cm - There was evidence of an effect due to number of years down for which the weight of bulbs appeared greater for 2 yrs down.

Total weight - Evidence suggested a time x bulb type x formula effect, for this the response to nil agent was compared to the average of the ABCD agents, there was no evidence to indicate Agent differences that were not already described by this overall average comparison against the nil (see Figure 2). The difference between 2yr down and 1yr down was most apparent, especially in the control bulbs. With the exception of inoc.+TBZ bulb type, the response to nil agent was greater than the ABCD formula in the 2yr downs.

Total weight allowing for planted weight - There appeared to be time x bulb type interaction such that there seemed no difference between weights of bulb type for 1yr down bulbs whereas for 2yr down bulbs, the weight of control bulbs was greater than that of inoc. bulbs which,in turn, was greater than inoc.+TBZ bulbs.

Total number - The time x bulb type x agent effect are shown in Figure 3. The difference in number of bulbs lifted between the two year treatments was greatest for the control bulbs. The control bulbs also appeared to have the greater weight of 2yr down bulbs compared to the inoc. and inoc.+TBZ whereas there appeared no bulb type difference in

the 1yr down bulbs. The graph illustrates the responses of the individual agents.

Flower numbers 1990 - Evidence of time x bulb type interaction, there appeared to be no difference in flower numbers for the 1yr down bulbs, but for the 2yr down bulbs the control treatment appeared to have more flowers than inoc.+TBZ which, in turn, had more flowers than inoc.

Flower numbers 1991 - A bulb type effect suggested that there were fewer flowers on the inoc. and inoc.+TBZ bulbs compared to the control.

Basal rot, total storage rots, %rots of sample (July 1990) - A 'bulb type' effect indicated that the number of rots in control bulbs was less than the inoc. and inoc.+TBZ.

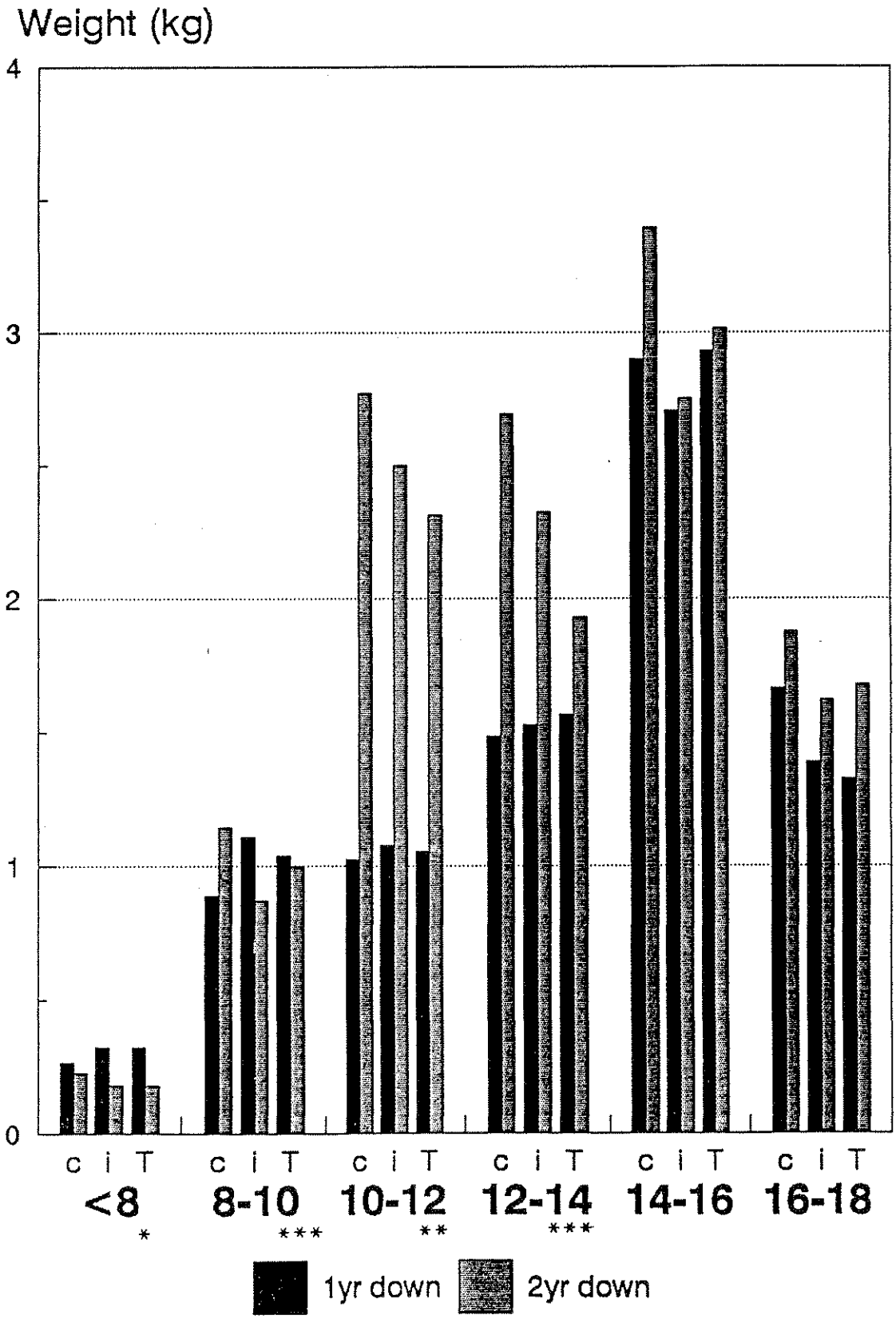
Basal rot (Nov 1990 and 1991) - There appeared to be a time x bulb type interaction, for 1yr down the bulb response appeared similar though for 2yr down the rots in the controls was fewer than the inoc. and inoc.+TBZ. For both inoc. and inoc.+TBZ bulbs the number of rots for the 1yr down bulbs was less than 2yr down bulbs.

Total rot (Nov 1990 and 1991) - A time effect indicated that the total number of rots was greater for 1yr down bulbs and a bulb type effect seemed to show the rots in control bulbs were fewer than the inoc. and inoc.+TBZ.

% rots of sample (Nov 1990 and 1991) - The time x bulb type interaction suggested that for inoc. and inoc.+TBZ bulbs the % rotted was greater in 1yr down bulbs compared to 2yr down, for the control bulbs there was no evidence to suggest a difference between 1yr and 2yr down bulbs. For 1yr down bulbs, the % rotted appeared fewer in control bulbs compared to inoc. and inoc.+TBZ.
$

Appendix 4b Fig. 1.

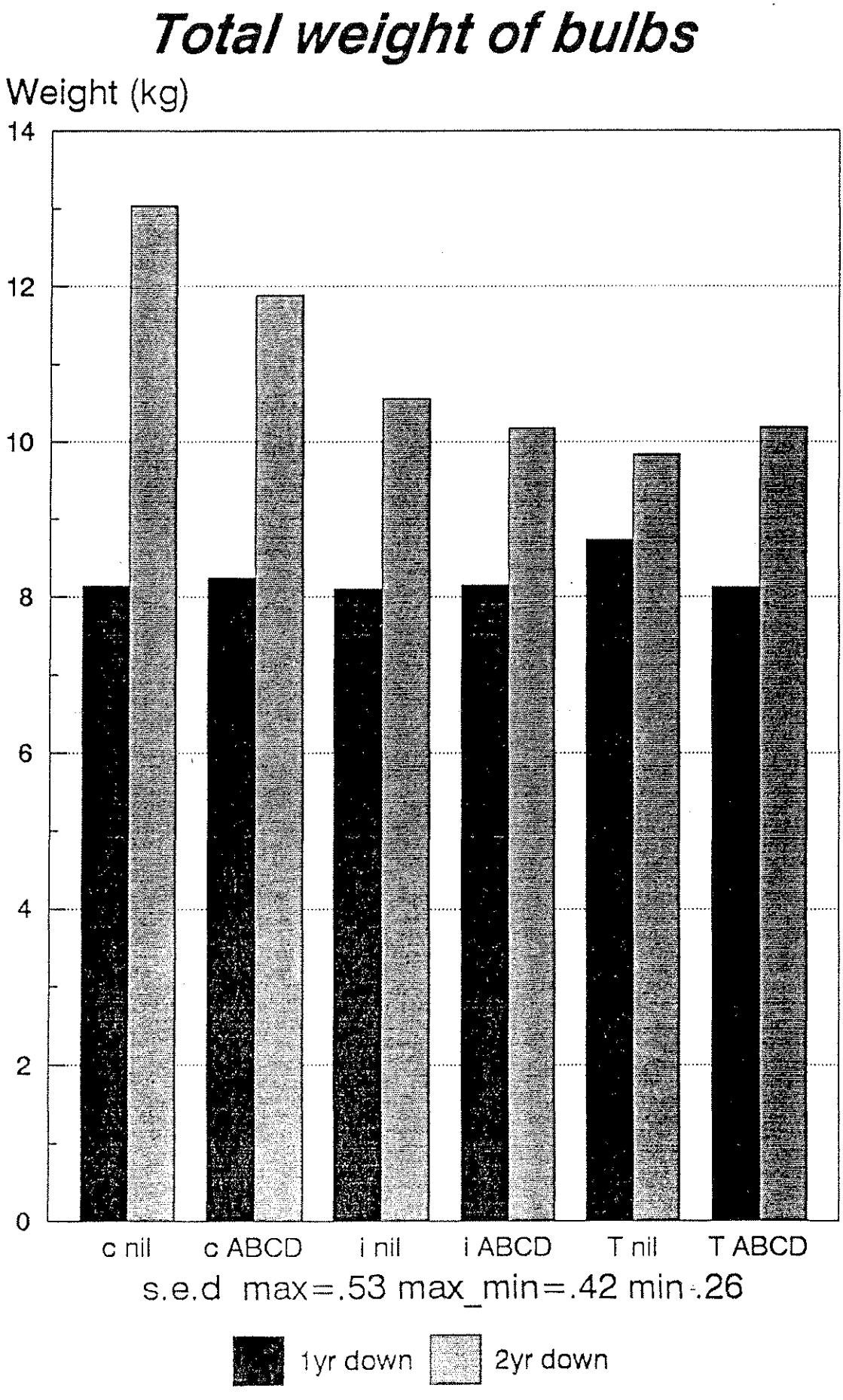
Weight of bulbs (Kg) time x bulb interaction



* sig.@ 5% level
** sig.@ 1% level
** sig.@ 0.1% level.

c = control
i = inoculated
T = inoc.+TBZ

Appendix 4b Fig. 2.

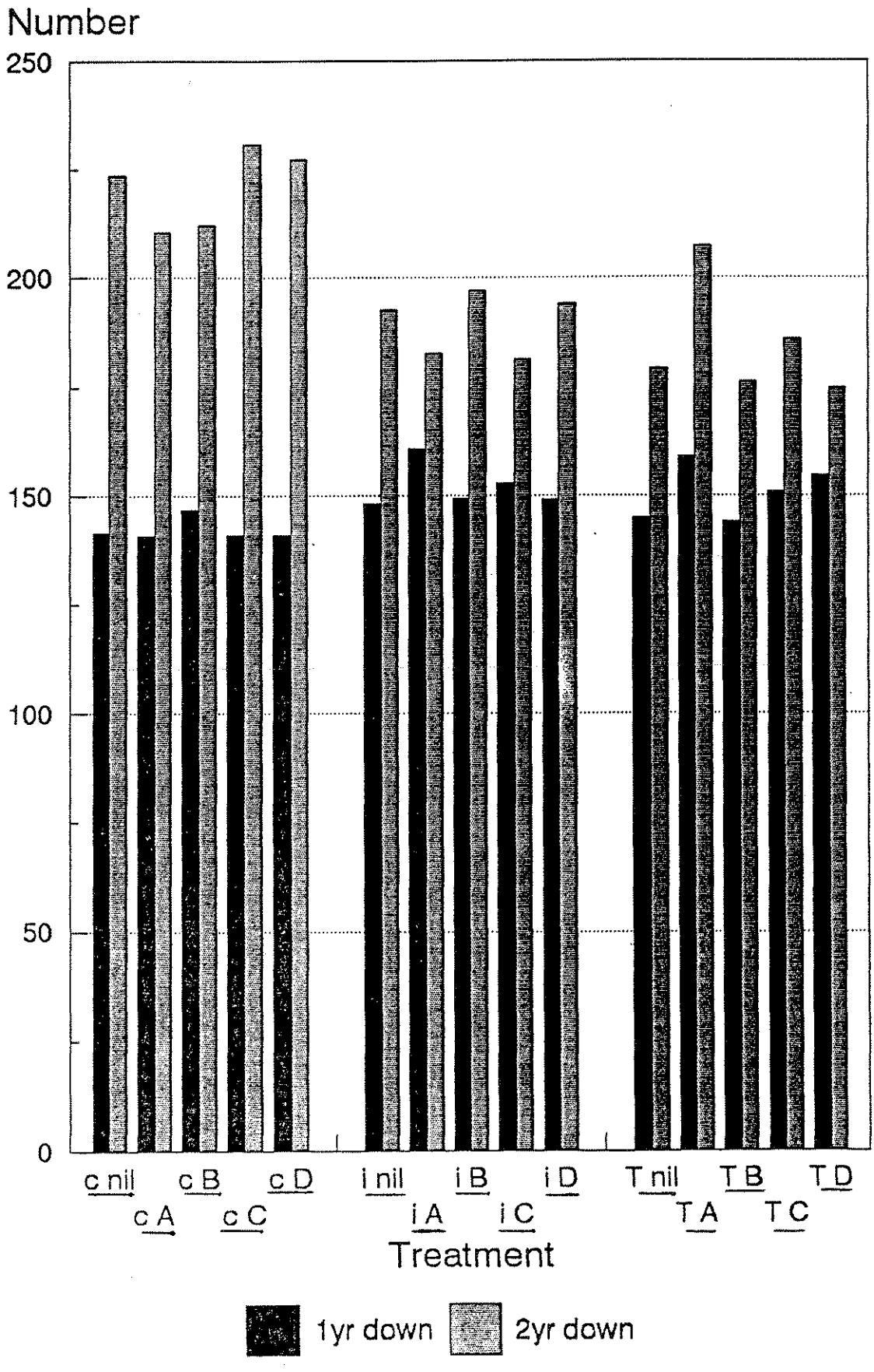


ABCD represents the average of 4 agents.

c = control i = inoculated T = inoculated+TBZ

Appendix 4b Fig. 3.

Total number of bulbs



c = control i = inoculated T = inoculated+TBZ