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Project number:	FV 392
Project leader:	Dr S J Roberts (Plant Health Solutions Ltd) and Dr J P Clarkson (Warwick Crop Centre) (Joint Leaders)
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Previous report:	None
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AUTHENTICATION

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

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CONTENTS

GROWER SUMMARY

Headline

This project is identifying the main causes of rots in stored onion bulbs grown from sets and investigates the where the infection could be coming from.

Background

There is a perception that the risk of certain onion diseases may be increased when crops are grown from sets. The major diseases of concern to the industry are: bacterial rots thought to be caused mainly by *Burkholderia gladioli* pv. *alliicola* (*Bga*; particularly in heat-treated red Rijnsburger type onions), neck rot caused primarily by *Botrytis allii* and Fusarium basal rot caused by *Fusarium oxysporum* fsp *cepae*. One possibility is that the necessary heat treatment of some cultivars may lead to increased risk of disease. This project represented a first step to address these issues by determining the incidence of the major bacterial and fungal onion pathogens thought to be associated with sets of different types and establish if there is a relationship between disease incidence in sets and subsequent problems in the harvested bulb onion crop. It is anticipated that, based on these initial results, a follow-on project will investigate way of reducing the impact of any disease problems associated with sets.

Summary

- The primary causes of rots in the stored bulbs grown from sets was the bacteria *Burkholderia gladioli* pv. *alliicola* (*Bga*) and the fungus *Fusarium oxysporum.* The neck rot pathogen, *Botrytis aclada*, was not detected.
- There was an indication that the primary source of *Bga* may be the sets and that heat treatment of sets may predispose them to or exacerbate infection/disease in the harvested bulbs.
- Bulk testing of sets for *Bga* may give an indication of the risk of disease in the harvested crop.
- The primary source of *Fusarium* in these trials appeared to be the field sites themselves, with a different Fusarium 'type' responsible for disease at each site.

Twelve samples of onion set lots with different expected disease levels were obtained from three suppliers just before planting time. These included nine red onion samples, and three

browns. The samples were allocated random code letters, sub-samples were retained for laboratory testing/analysis, and the remainder planted at two field sites, one in Lincolnshire and one in Suffolk. Plots were 3 beds wide by 10 m long, and were surrounded by a commercial red onion set crop. At each site the trial plots received the same fertiliser, irrigation and spray program as the surrounding crop. Bulbs were topped and harvested at the same time as the surrounding crops, then bulbs from both trial sites were cured and stored following standard practice at a single location.

Disease assessments and isolations from sets

Sub-samples of 200 sets from each lot were cut in half and examined for disease symptoms, and the incidence of different symptom types (bacterial, fungal, basal rots) recorded. None of the lots had clear symptoms of basal rot, although many had some degree of discolouration of the base plate. In most cases internal rots were not obviously bacterial or fungal, so assignment to internal bacterial or fungal was to some extent arbitrary, unless there was obvious fungal sporulation. Direct isolations of potential pathogens were attempted from the different symptom types. A range of bacteria, and fungi were obtained. The only pathogenic bacterium obtained by direct isolation from symptoms was *Bga* (from two lots). Although a number of different Fusarium 'types' were isolated, these were were not detected in the harvested bulbs and in preliminary tests were non-pathogenic or only very weakly pathogenic. The neck rot pathogen *Botrytis aclada* was not isolated.

In addition a 'bulk soak test' (irrespective of symptoms) for *Bga* was also done on a further 2 sub-samples of 200 bulbs from each lot. *Bga* was detected in three lots by this method.

Disease assessment and isolations from harvested bulbs

Table 1. Summary of symptoms, isolations and tests on the twelve set lots planted in the field trials.

Lot	General appearance	% Basal	% Shriv. + int. rots	% Bga (direct)[1]	% Bga (bulk)[2]	% Fusarium[3]
A	Shrivelled obvious, occasional sprouting	0.5	11.5	0	<0.8	100
B	Good, but many slightly soft	1.0	4.5	0	<0.8	0
C	Good, but most slightly soft	8.5	5.5	0	<0.8	59
D	Generally look okay	0.5	5.5	0	0.3	0
E	Generally okay, some soft, some surface mould.	2.0	2.0	0	<0.8	17
F	V. good, firm	5.5	4.0	0	>0.1	35
G	V. good, firm	6.0	3.0	0	<0.8	53
H	Damp, external mould growth on most, soft	8.0	9.0	0	<0.8	13
J	Generally good, several shrivelled.	2.0	9.5	22	<0.8	55
K	Generally. okay, some surface mould.	6.5	3.5	0	<0.8	6
L	V. good, v. firm.	0.0	10.0	11	>0.1	27
M	Good, firm. Difficult to decide about basal symptoms.	3.0	1.5	0	<0.8	14

Abbreviations: Shriv. - shrivelled, int- intenal
[1] The % of direct isolation attempts from up to nine bulbs with symptoms yielding *Burkholderia gladioli* pv. *alliicola* (*Bga*).
[2] The estimated % of bulbs carrying *Bga* based on bulb testing.
[3] The % of direct isolation attempts from up to ten bulbs with symtoms yielding *Fusarium* spp.

Seven weeks after harvest, samples of stored bulbs were cut in half and visually assessed for disease symptoms (rots). Diseased bulbs were assigned to one of four categories: neck, basal, bacterial, other/mushy. Bulbs from site 1 were much larger than those from site 2, and were harvested two weeks earlier when foliage was greener. Following storage, the overall quality of bulbs from site 1 was much poorer than site 2, with a higher proportion of total rots (30%) compared to site 2 (13%). Except for one obvious outlier (lot J), the overall proportion of total rots was highly correlated between sites, i.e. the ranking of lots was the same at each site, although the overall levels were higher at site 1 (Fig 1).

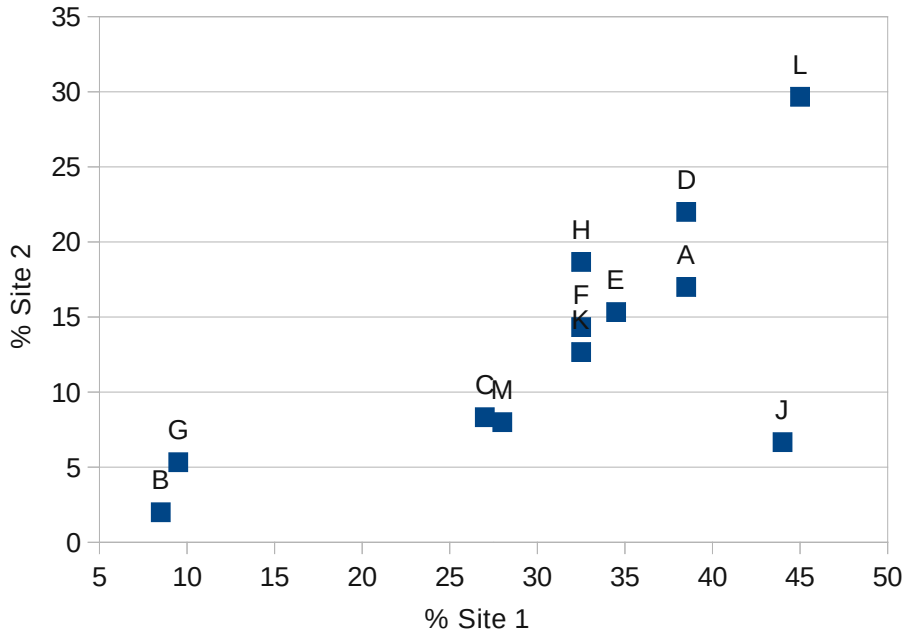

Figure 1. Percentage total rots in the bulbs harvested from the two trial sites.

Bacterial and 'neck rots'

Assessing bulbs with bacterial or neck rots was more difficult than basal rots. Except where bulbs were completely mushy/rotten, the two types of symptoms were easy to differentiate from bulbs with basal rots, but it was difficult to distinguish between them. The main criterion for discriminating between 'bacterial' and 'neck' rots was whether the rot was affecting a group of scales with the disease progressing down all scales at the same time (neck rot), or affecting individual scales (bacterial rot). However, it proved difficult to apply this criterion in many cases. In addition we consistently failed to isolate *Botrytis allii/aclada* (the neck rot pathogen) from any bulbs assigned to the 'neck rot' category, and *Bga* was isolated from close to the neck in some bulbs which could have been assigned to the neck rot category. We therefore consider that the majority of bulbs initially assigned to the neck rot category (mainly at site 1) were mis-diagnosed and were included in the bacterial rot category for the purposes of analysis.

Bga was the only pathogenic bacterium isolated from bulbs with bacterial rots and was isolated from a range of different symptoms:

- partially and completely rotten single internal scales
- completely rotten/mushy entire bulbs
- completely rotten central scales
- partially and completely rotten outer scales

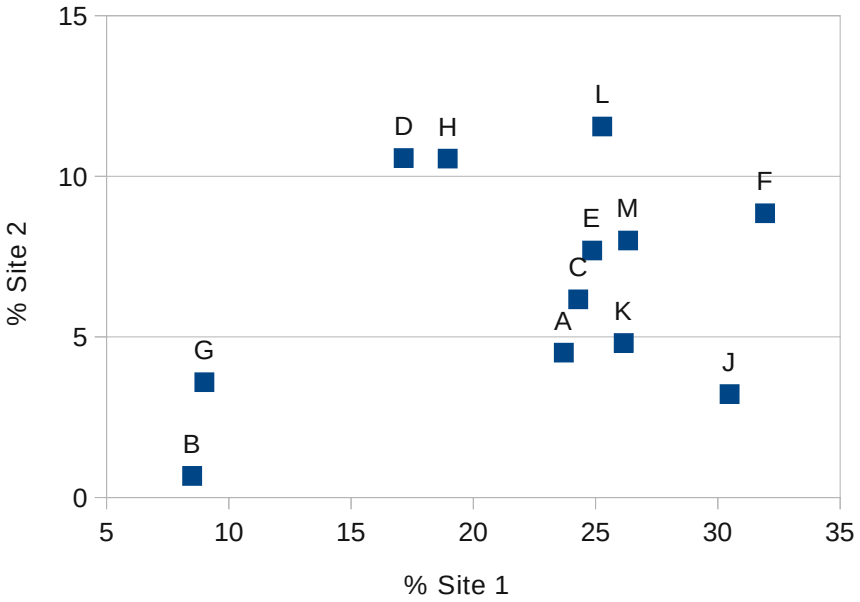

Figure 1: Percentage of bulbs with bacterial rots at each field trial site

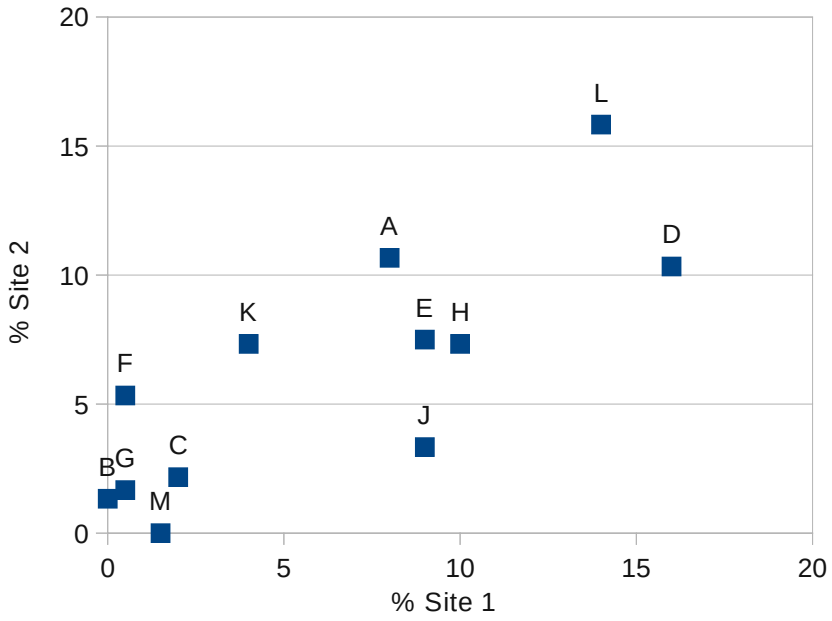

Figure 2. Percentage of bulbs with Fusarium basal rots at each field trial site

Conclusions

Given the limitations of this initial experiment and approaches, all conclusions should be considered with some caution and to be of a preliminary nature:

- The external visual appearance of sets was a poor indicator of health status or storage rots.
- *Burkholderia gladioli* pv. *alliicola* (*Bga*) was the main cause of bacterial and 'neck' rots and was the only bacterium isolated from both sets and bulbs that was consistently pathogenic.
- *Bga* was detected in some set lots and these lots had a tendency to give the highest levels of bacterial rots in the harvested bulbs.
- Bacterial storage rots may be under-reported due to confusion with Botrytis neck rot symptoms.
- There was an indication of a significant effect of heat treatment of sets on bacterial rots in storage.
- There was strong effect of site on the levels of bacterial rots – mainly associated with harvest date.
- A range of at least nine *Fusarium* 'types' was isolated from sets but there appeared to be no relationship between the levels or type isolated from sets and the levels of basal rots in bulbs.
- Basal rot in the stored bulbs was associated with two different *Fusarium* 'types' which appeared to be specific to each field site, and were not detected in the sets.
- There was no effect of site on the levels of Fusarium basal rot.
- The different set lots appear to differ in susceptibility to Fusarium basal rot.
- There was no evidence of an association between heat treatment of sets and Fusarium basal rot based on a limited data set.

Financial benefits

The UK onion area is around 9,000 ha producing 400,000 tonnes. Sets are grown on up to a third of the area. Up to 40% losses have been reported for bacterial storage rots, and based on an average yield of 40 t/ha this could amount to losses equivalent to 48,000 tonnes for set crops. It is clear that, in the conditions of the trials in the project that losses from rots may exceed the values previously reported (e.g. 45% identified in one lot in this study), and that losses may result from infection with either *Fusarium* or *Burkholderia.* This

was a preliminary project intending to identify targets for further work, therefore there are no direct financial benefits at this stage.

Action points for growers

- Send samples for laboratory diagnosis to discriminate Botrytis neck rot and bacterial rot symptoms.
- Consider having sets bulk tested for the presence of Bga before planting.

SCIENCE SECTION

Introduction

There is a perception that production of onions from sets is associated with an increased risk of disease in the crop and in harvested bulbs. The major diseases of concern to the industry are: bacterial rots thought to be caused mainly by *Burkholderia gladioli* pv. *alliicola* (*Bga;* formerly called *Pseudomonas gladioli* pv. *alliicola;* particularly in red onions), neck rot caused by *Botrytis aclada/allii* and Fusarium basal rot caused by *Fusarium oxysporum* fsp *cepae*.

After harvesting, onion sets are dried / cured at 25-28°C and 50-65% RH. Stuttgarter types are then stored cool over winter (7-10°C) whereas Rijnsburger types require heat treatment to de-vernalise (prevent bolting when planted) at 26-28°C and 70-95% RH. Commercially, problems are significantly greater in heat-treated Rijnsburger types and it is thought that this may be due to the prolonged heat/humidity treatment.

Previous work at HRI-Wellesbourne indicated that bacterial rots in storage were caused mainly by *Bga* and that set-raised crops were more at risk. A 'hot box' test on a pre-harvest sample of bulbs was devised to indicate the likely risk of disease in storage (see HDC FV 111 and Davies et al 1996). Following the discovery of opportunisitic human infections caused by *Burkholderia* spp., there have recently been several publications on the detection and isolation of *Burkholderia* spp. in the environment, and from the soil/rhizosphere. Although related bacteria (*Burkholderia* spp.) have been isolated from soil, there is no clear evidence that the specific onion pathogen, Bga itself, is soil-borne, and suggestions from elsewhere that *Bga* infection may result from soil-splash may be erroneous.

The overall aim of the project was to determine the incidence and identify current disease problems associated with onion sets and relate these to storage rots in the harvested bulbs.

The specific objectives were to:

- Determine the incidence of the major bacterial and fungal onion pathogens associated with sets
- Isolate and confirm identity of pathogens (particularly bacteria).
- Determine if there is a relationship between the incidence of different set diseases at planting with those causing problems subsequently in the stored bulb onion crop.

This projected was intended to be a preliminary project that will set the scene for a future follow-up project which will investigate ways of reducing the impact of onion set diseases such as:

- understanding the impact of ‘first year’ set drying/curing/heat-treatments on onion diseases and potentially optimising these regimes
- understanding the relative importance of diseased onion sets in transmission / spread in different field environments and their impact on subsequent problems of bulb onions in store
    - developing appropriate tests for sets to assess disease levels and determine the likelihood of subsequent problems in field and store
    - testing onion set and in-field treatments which may reduce onion bulb storage diseases.

Materials and methods

Set samples

Twelve samples of onion sets were obtained from three suppliers, shortly before planting. Suppliers were asked to provide samples from batches with different expected disease levels, and included nine red onion samples (all heat-treated), and three browns (one heat-treated and two non-heat-treated). Sets were not treated with fungicides.

Samples were delivered by suppliers under code-numbers/letters to a central location (PGRO) where they were sub-divided and re-packed in such a way as to avoid any sampling bias: the content of the bags, as received, were divided amongst two bags and the samples for the trials and assessments drawn alternately from each bag. Samples were re-allocated random code letters (to avoid any potential for bias) and then collected for planting.

Set disease assessments (PHS)

A random sample of two hundred sets from each lot was assessed. The general appearance of each lot and the number of sprouting bulbs were recorded. Bulbs were then cut in half with a knife and visually assessed for disease symptoms. Bulbs were allocated to one of four different symptom categories: 'basal' where the base-plate was discoloured; 'shrivelled' where the bulbs were dry and shrivelled; 'internal fungal' where the appearance of the internal rot was suggestive of fungal infection; 'internal bacterial' where the appearance of the internal rot was suggestive of bacterial infection. Sub-samples of bulbs in each category from each lot were retained for attempted isolation of potential pathogens.

Direct isolation of bacteria from sets (PHS)

Isolation of bacteria was attempted from up to nine individual shrivelled sets or sets with internal 'bacterial' rots from each lot. Individual sets or halves were dissected and small (approx 1.5 mm square, i.e. ~4 mm^3) tissue pieces were aseptically excised either from the freshly exposed margin of rotten tissues, or, where there was no margin, as far as possible from previously-cut edge. Tissue pieces were placed in the bottom of a sterile micro-centrifuge tube, covered with 100 µl of sterile saline, and left to stand for up to 30 min. Tissues were then gently macerated with a bacteriological loop and loopfuls (~20 µl) of the resulting suspensions streaked out on plates of two agar media: Difco Pseudomonas Agar F (PAF) and PAF plus streptomycin, polymixin B, novobiocin, cycloheximide (SPNC). Plates were incubated at 35°C for 2-3 d. The appearance of growth on the plates was recorded and representative single colonies of the predominant colony type or types sub-cultured to fresh plates of PAF. Representatives of each 'type' were tested for pathogenicity (ability to rot) on onion scales (sectors or discs) at low (20 or 25°C) and high (35°C) temperatures.

Bulk soak testing of sets for Bga (PHS)

In addition to the originally planned direct isolations above, two sub-samples of up to 200 sets from each lot were bulk tested for the presence of Bga. The method is based on an approach developed during previous work at HRI. Briefly, sub-samples of up to 200 bulbs were soaked overnight at 4°C in sterile buffer plus Tween. The resultant soakate was then serially diluted and spread onto plates of PAF+C and SPNC media. Plates were incubated at 35°C for 2-4 d, and the number of 'suspect' Bga colonies counted. 'Suspect' colonies were then sub-cultured, and their identity confirmed based on colony morphology, physiological tests, pathogenicity on onion sectors/discs and/or PCR using species-specific primers.

Isolation of Fusarium species and other fungi from sets (WCC)

Fungal isolations were carried out from onion sets from each lot (up to 10 per lot) which had been classified as having discoloured base plates or showing symptoms of internal rot. Pieces of tissue (two per set – one from the surface of each side of one of the cut bulb halves, approx. 5mm^2) were surface sterilised in 70% ethanol for 5 min, placed on PDA plates amended with chlortetracycline to prevent bacterial contamination and incubated at 20°C for 7 days. The resulting fungal colonies were then sub-cultured, and after incubation at 20°C for 14 days were identified on the basis of gross colony appearance/morphology and microscopic examination of conidia as being Fusarium, Penicillin, Aspergillus or Botrytis

species. Fusarium species were then subdivided into nine different types based on colony characteristics.

Field trials

The coded samples were planted in two field sites: Lincolnshire and Suffolk. A single plot (3 x 1.8 m beds x 10 m) of each lot was planted at each site. Plots were randomly allocated to lots, with a different randomisation at each site. The aim of having a single large plot rather than several smaller replicate plots was to minimise the risk of interference between plots (due to pathogen spread between plots).

The plots were embedded within a normal commercial crop, with a 3-bed (5.4 m) x 6 m spacing between plots. There were four rows per bed with a target population of 44 plants per m^2.

At each site, the crop (and embedded plots) was managed/treated according to standard practice by the growers and their advisors, and summarised in Table 2. In particular, at both sites the crops received a spray programme that included a number of fungicides and the bactericide/fungicide copper oxychloride.

Plots were machine topped and windrowed at the same time as the surrounding crop. A sample of at least 300 bulbs was harvested from the centre of each plot, taken into store and cured according to normal practice. Bulbs from both sites were stored/cured at the same location.

Bulb disease assessments (PHS)

Seven weeks after harvest, samples of bulbs were cut in half and visually assessed for disease symptoms (rots). Diseased bulbs were assigned to one of four categories: neck, basal, bacterial, other/mushy. Bulbs assigned to 'neck' had a rot developing downwards from the neck across all scales. Bulbs assigned to ‘basal’ had a rot progressing upwards from the base-plate across all scales and in the case of reds often had a 'blueish' colour. Bulbs assigned to 'bacterial' had rots generally confined to one or more individual or groups of scales, or the other scales and the base-plate remained in-tact. Bulbs assigned to 'other/mushy' were generally completely rotten and mushy making it impossible to assign them to one of the other categories.

Bacterial isolations from bulbs (PHS)

Isolation of bacteria was attempted from up to ten individual bulbs assigned to the 'bacterial' rots category. Individual bulb halves were dissected and small (approx 1.5 mm square, i.e. ~4 mm^3) tissue pieces were aseptically excised and isolations attempted in a

similar way to that described for sets. The resulting isolates were identified and pathogenicity confirmed as described for sets.

Botrytis aclada/allii isolations from bulbs (PHS)

Tissue pieces from the margins of up to four bulbs with 'neck rot' symptoms were plated on PLYSE agar (sem-selective/diagnostic for Botrytis). Plates were sealed with parafilm, incubated at 21°C in the dark, and examined for the presence of typical *Botrytis* colonies after 5-7 d.

Isolation of Fusarium species and other fungi from bulbs (WCC)

Fungal isolations (up to 10 per plot) were made from onion bulbs from each plot which had been classified as having symptoms of Fusarium basal rot. The methods were similar to those used for the sets except that the pieces of tissue used were cut from each side of the back of an infected bulb scale to minimise contamination. Resulting fungal colonies were identified as before and *Fusarium* species assigned to the nine types based on morphology.

Statistical analysis (PHS)

The effects of set lot, site, and their interaction on the proportions of harvested/stored bulbs with different symptoms were examined by fitting a series of generalised linear models with a binomial error distribution and logit link function using Genstat statistical analysis software (Payne *et al.*, 2005)

Results and Discussion

Table 2. Field sites summary details

	Site 1	Site 2
Plot size:	5.4 x 10 m	5.4 x 10 m
Layout:	6 x 2	4 x 3
Location:	New York, Lincs	Sizewell, Suffolk
Soil type:	Sandy clay loam	Sandy loam
Previous crop:	Winter wheat	Winter barley
Surrounding crop:	Red Garnet	Red Garnet
Planting date:	23 March	23 March
Irrigation:	May-June, 3 x 25 mm	June-July, 5 x 18 mm
Topped:	29 July	12 August
Windrowed:	29 July	12 August
Harvested:	01 August	15 August
Bulbs assessed:	20 September	03 October
Fungicides:	Cu oxychloride mancozeb fluoxastrobin prothioconazole benthiavalicarb-isopropyl dimethomorph chlorothalonil	Cu oxychloride mancozeb fluoxastrobin prothioconazole benthiavalicarb-isopropyl dimethomorph cyprodinil fludioxinil bosaclid pyraclostrobin

The details of the field sites and summary of field operations are shown Table 2.

Disease assessment and isolations from sets

A summary of the disease assessments for each set lot are shown in Table 3. Assignment of the sets to the defined symptom categories was difficult. None had clear symptoms of basal rot, although many had some degree of discolouration of the base plate. In most cases internal rots were not obviously bacterial or fungal, so assignment to internal bacterial or fungal was to some extent arbitrary, unless there was obvious fungal sporulation.

Table 3. Initial assessment of disease symptoms in the 12 onion set lots planted in the field trial

Lot	N	General appearance	Sprouting	No with symptoms[1]:				% Shriv. + Int.
				Basal discol.	Shriv.	Int.? bact.	Int.? fungal	
A	200	Shrivelled obvious, occasional sprouting	8	1	18	2	3	12
B	200	Good, but many slightly soft	11	2	4	1	4	5
C	200	Good, but most slightly soft	1	17	2	4	5	6
D	200	Generally look okay	4	1	7	2	2	6
E	200	Generally okay, some soft, some surface mould.	8	4	0	2	2	2
F	200	V. good, firm	3	11	1	1	6	4
G	200	V. good, firm	2	12	1	2	3	3
H	200	Damp, external mould growth on most, soft	27	16	4	6	8	9
J	200	Generally good, several shrivelled.	9	4	5	7	7	9
K	200	Generally. okay, some surface mould.	19	13	1	3	3	4
L	200	V. good, v. firm.	0	0	1	8	11	10
M	200	Good, firm. Difficult to decide about basal symptoms.	2	6	1	1	1	2

Abbreviations:
dicol. - discolouration; shriv. - shrivelled; int. - internal; bact. - bacterial;

Bacterial isolations from sets

Attempts at isolation of bacteria were made from shrivelled bulbs and from those with internal rots. Fifty out of 60 attempted isolations yielded growth on the non-selective medium (PAF), in many cases these were mixed cultures of bacteria and yeasts, but less frequently, apparently pure cultures of bacteria or yeasts. Most plates of the selective medium were sterile (44 out of the 60). Representative isolates were sub-cultured and tested for pathogenicity on onions. Pathogenic bacteria, identified as *Bga,* were isolated in only 3 out of the 60 attempts and from two lots (2 from J, and 1 from L). An additional 36 isolates (bacteria and yeasts) were also tested for pathogenicity and were either non-pathogenic or gave only a slight reaction at the highest temperature (35°C).

Yeasts were frequently isolated and sometimes in pure culture, including on the selective medium containing the anti-fungal compound cycloheximide. Pathogenicity tests suggest that they were unlikely to be the primary cause of symptoms. Some yeasts are known to produce anti-bacterial compounds, therefore their presence as secondary invaders of diseased tissues may have masked the presence of the primary pathogen.

In addition to the originally planned isolations from set with symptoms a 'bulk soak test' was also done on a initial sample of 200 bulbs from each lot. A further test was also done on a second sub-sample of up to 200 bulbs for some lots (where sufficient bulbs remained). Pathogenic *Bga* was detected in 3 lots (D, F and L) with numbers per set from 1.2×10^2 to 2.8×10^4 (Table 4). Most lots had high background counts of bacteria even on the selective medium (SPNC) thus it is likely that, even if present, *Bga* may have been undetected.

Combining the 'soak test' results with the results for direct isolations, *Bga* was detected in four lots (D, F, J and L). Only in lot L was *Bga* detected by both direct isolation from symptoms and by the bulk soak method. It should also be noted that all the four lots from which *Bga* was obtained were recorded as being either very good and firm (F and L) or generally good or okay (D, J) in appearance. This suggests that the external visual appearance of the sets provides no indication of the health status with respect to *Bga.*

Table 4. Summary of bacterial isolations from the 12 onion set lots planted in the field trial.

Lot	Direct isolation[a]	Bulk test[b] 1 (CFU/bulb)	Bulk test[b] 2 (CFU/bulb)	%
A	0/9	<45	<54	<0.75
B	0/3	<45	<45	<0.75
C	0/5	<51	NT[c]	<1.5
D	0/5	<45	9×10^2	0.3
E	0/2	<45	<60	<0.75
F	0/1	1.3×10^3	1.2×10^2	>0.1
G	0/3	<53	<53	<0.75
H	0/9	<45	<56	<0.75
J	2/9	<45	<48	<0.75
K	0/3	<45	<60	<0.75
L	1/9	2.8×10^4	6.3×10^2	>0.1
M	0/2	<53	<61	<0.75

[a] No. of direct isolations from symptomatic bulbs yielding pathogenic *Bga* out of the number of attempts.
[b] Bulk test on 200 bulbs, indicating the number of *Bga* (CFU) detected or the theoretical detection limit.
[c] NT – not tested

Alternatively it may be that, in these lots, detection of *Bga* was easier due to lower numbers of background saprophytes.

There were indications that recovery of *Bga* on the selective medium (SPNC) used for both direct isolation from symptoms and in the soak test was less than 100% (possibly around

50%), this may also have contributed to poor detection, if numbers of *Bga* were relatively low and background populations high.

Given the difficulties in assessment/assignment of sets to particular symptom categories and the high background populations it seems likely that our detection of *Bga* in 4 out of the 12 lots represents an underestimate of the prevalence of the pathogen.

Fungal isolations from sets

Attempts at isolation of fungi were done from sets with discoloured base plates and with internal rots. *Penicillium* spp. were the most commonly isolated fungi. *Fusarium* spp. were isolated from 32% of attempts (37% basal attempts; 25% internal attempts). *Fusarium* isolates could be divided into eight different 'types' based on colony characteristics. However, no single 'type' was consistently isolated from either type of symptom or from any particular lot and up to 5 types were isolated within any single set lot (Fig 4., Lot C). Preliminary pathogenicity tests on representatives of each type indicated that they were either non-pathogenic or only weakly pathogenic.

Given that there were no obvious basal rot symptoms in the sets, it is likely that the *Fusarium* 'types' isolated from sets represent the natural background population associated with soil contamination adhering to the sets.

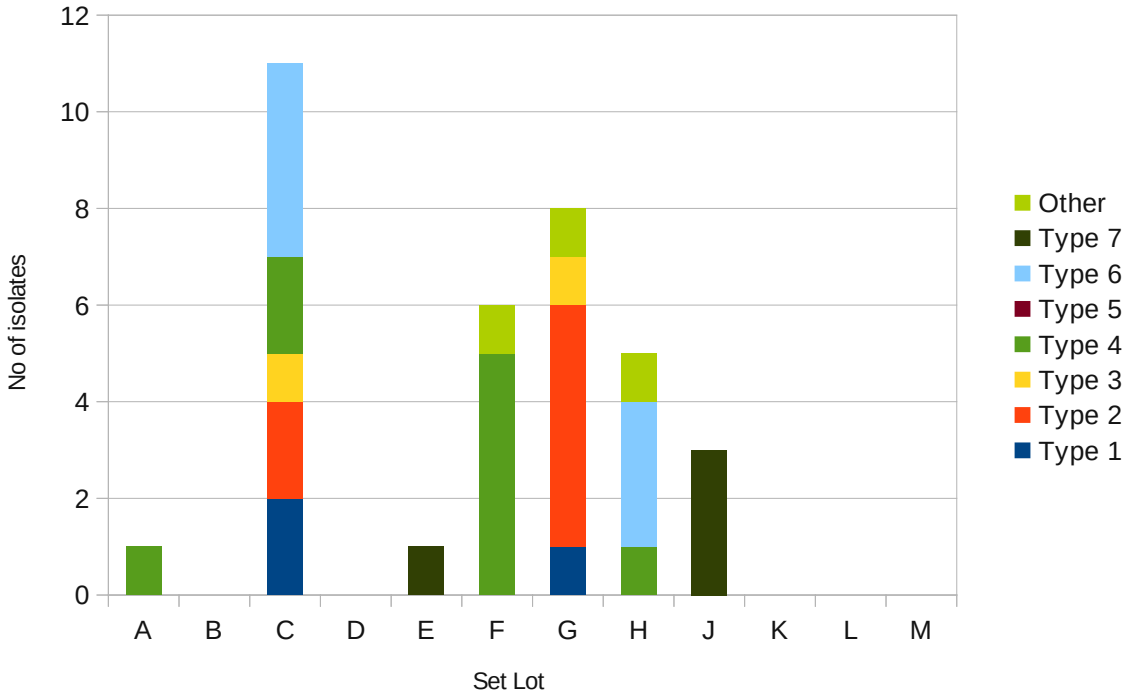

Figure 3. The different Fusarium 'types' isolated from each of the twelve onion set lots planted in the field trial

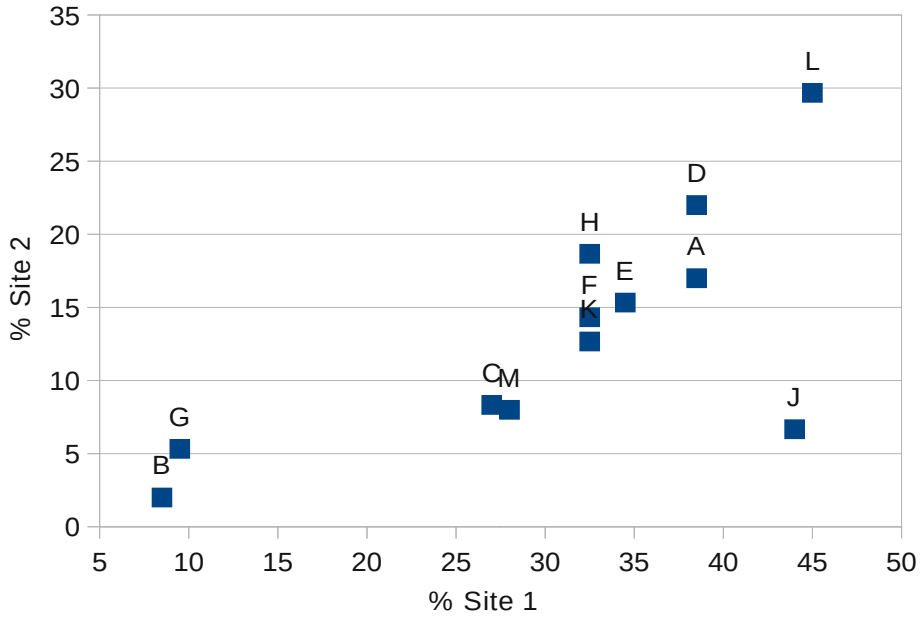

Figure 4. Percentage total rots in the bulbs harvested from the two trial sites.

discs/sectors were identified as *Bga*. At site 1 56% of isolation attempts yielded pathogenic *Bga*, and at site 2 15% yielded *Bga*. *Bga* was isolated from a range of different symptoms:

- partially and completely rotten single internal scales
- completely rotten/mushy entire bulbs
- completely rotten central scales
- partially and completely rotten outer scales
- groups of rotten rotten scales near the neck (i.e. neck rot symptoms)

There were indications of the presence of bacteriophage on some isolation plates, and if widely present could have been responsible for some isolation failures.

There was a significant difference in the mean level of bacterial rots between sites (21% at site 1 vs. 5% at site 2) and there were significant differences amongst the lots, but there was no evidence of a site x lot interaction. Heat treatment of sets had a very significant effect on the level of bacterial rots, and explained most of the differences between lots. Nevertheless, there was a tendency for two of the four lots in which *Bga* was detected in the sets (F and L) to have the highest levels of bacterial rots, the other two lots in which *Bga* was detected in the sets (lots D and J) had amongst the highest levels at site 2 (lot D) or Site 1 (Lot J) (Fig. 6).

The significantly higher levels of bacterial rots at site 1 may be a result of agronomic differences between the sites, particularly the much larger bulb size and the earlier harvest when there was much more green foliage. It may also have been a reflection of higher inoculum levels in the surrounding commercial crop, particularly as topping was done at the same time with the same machinery at the crop. It is unfortunate that we were not able to test a sample of the sets used for the surrounding crops or examine samples of bulbs harvested from the surrounding crops.

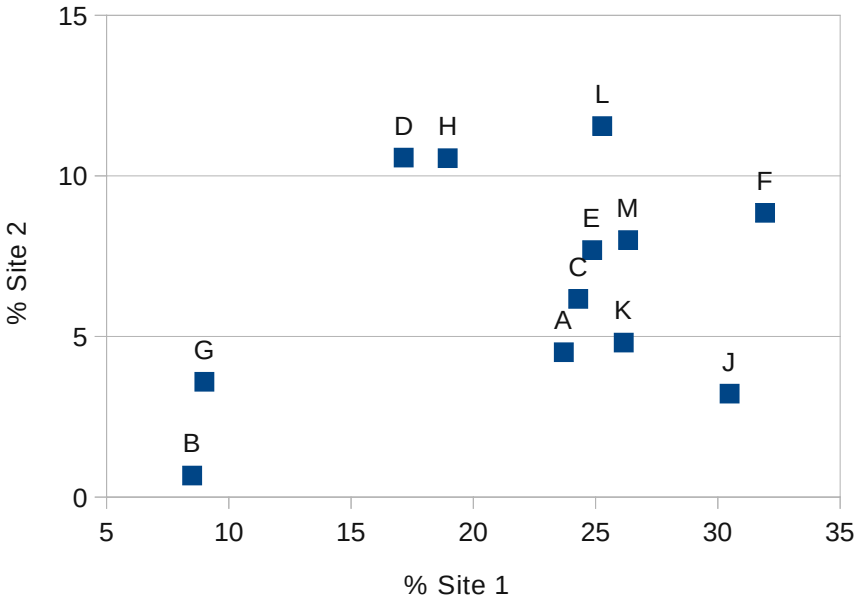

Figure 5: Percentage of bulbs with bacterial rots at each field trial site.

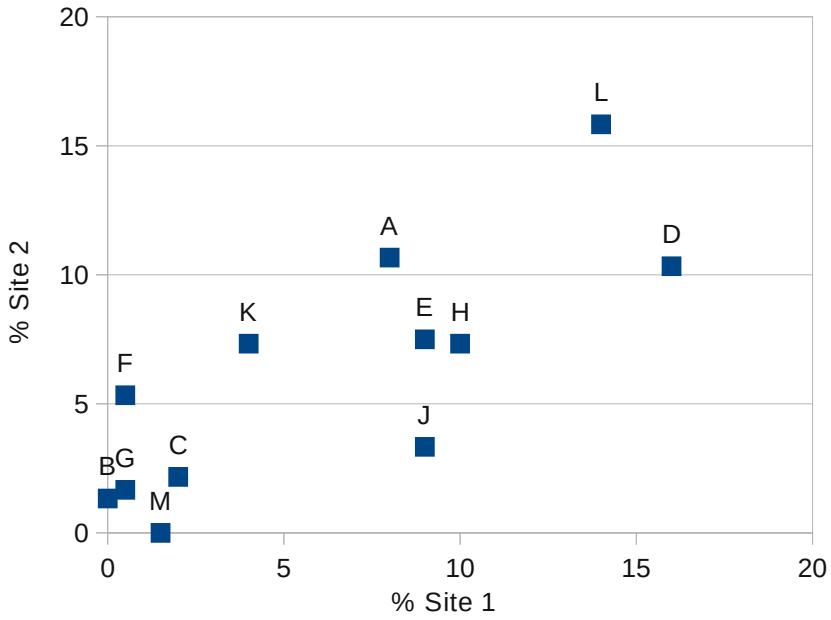

Figure 6. Percentage of bulbs with Fusarium basal rots at each field trial site

from the field rather than being carried with the sets. This is further supported by a lack of a relationship between the frequency or type of *Fusarium* isolated from the sets and the level or type observed in the harvested bulbs: the two types isolated from bulbs were not detected in the sets; Lot D had some of the highest basal rot in the bulbs, but no *Fusarium* isolated from the sets; Lot C had the highest frequency of *Fusarium* in the sets, but the lowest basal rot level in the harvested bulbs. Hence it would appear that differing levels of *Fusarium* rots seen in the harvested bulbs are a result of differences in susceptibility amongst lots to the *Fusarium* inoculum present in the fields.

Preliminary data on the pathogenicity tests with representative isolates of each *Fusarium* type from each set lot and from diseased bulbs from each plot indicated that all of the isolates from sets were non-pathogenic with a few weakly pathogenic; whereas the majority of isolates from bulbs were highly pathogenic.

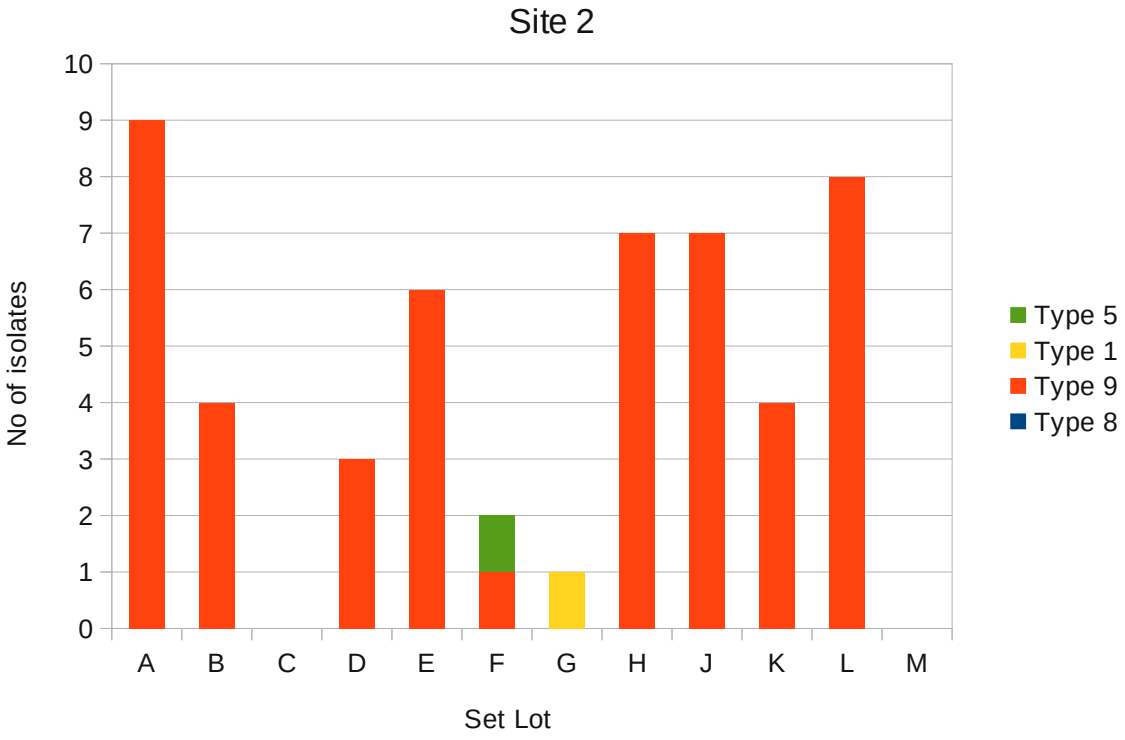

Figure 7. Numbers of each Fusarium type isolated from bulbs with basal rot symptoms at each field trial site.

Main conclusions

Given the limitations of this initial experiment and approaches, all conclusions should be considered with some caution and to be of a preliminary nature.

- The external visual appearance of sets is a poor indicator of health status or storage rots.
- *Burkholderia gladioli* pv. *alliicola* (*Bga*) was the main cause of bacterial and 'neck' rots and was the only bacterium isolated from both sets and bulbs that was consistently pathogenic on onions.
- *Bga* was detected in some set lots and these lots had a tendency to give the highest levels of bacterial storage rots.
- Bacterial storage rots may be under-reported due to confusion with Botrytis neck rot symptoms.
- A range of at least nine *Fusarium* 'types' was isolated from sets but there appeared to be no relationship between the levels or type isolated from sets and the levels of basal rots in bulbs.
- Basal rot in the stored bulbs was associated with only two different *Fusarium* 'types' which appeared to be specific to each field site, and were not detected in the sets.
- There was no effect of site on the levels of Fusarium basal rot.
- There was strong effect of site on the levels of bacterial rots – mainly associated with harvest date.
- The different set lots appear to differ in susceptibility to Fusarium basal rot.
- There was no evidence of an association between heat treatment of sets and Fusarium basal rot based on a limited data set.
- There was an indication of a significant effect of heat treatment of sets on bacterial rots in storage.

References

Davies, J M LL; Taylor, J D and Conway J (1996) Forecasting of bacterial (*Pseudomonas gladioli* pv. *alliicola*) storage rots of bulb onion based on a pre-harvest rapid serological test. In: *BCPC Symposium Proceedings No. 65: Diagnostics in Crop Production* pp. 275-80.

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Appendix I: Bacterial rots – Analyses of deviance

Genstat output, generalised linear model, binomial error distribution, logit link function:

Change	d.f.	deviance	mean deviance	deviance ratio	approx F pr.
+ Lot	11	100.097	9.100	4.44	<.001
+ site	1	308.879	308.879	150.57	<.001
+ Lot.site	11	44.900	4.082	1.99	0.059
Residual	36	73.851	2.051		
Total	59	527.726	8.945		

Means, lower and upper 95% confidence limits - output from predict (full model):

Lot	p	LCL	UCL
A	9.12	5.710	14.24
B	1.88	0.559	6.15
C	11.03	7.360	16.20
D	12.89	9.187	17.78
E	12.64	8.771	17.88
F	15.42	11.061	21.09
G	5.22	2.957	9.05
H	13.42	9.622	18.42
J	8.54	4.982	14.26
K	9.92	6.326	15.22
L	16.04	11.814	21.41
M	13.27	9.295	18.61

site	p	LCL	UCL
1	21.05	18.70	23.61
2	5.54	4.37	7.01

Fitting lot followed by heat treatment:

Change	d.f.	deviance	mean deviance	deviance ratio	approx F pr.
+ lot2	10	82.726	8.273	4.06	<.001
+ ht	1	17.370	17.370	8.53	0.006
+ site	1	308.879	308.879	151.65	<.001
+ lot2.site	10	43.387	4.339	2.13	0.047
Residual	37	75.363	2.037		
Total	59	527.726	8.945		

Appendix II: Fusarium basal rots – Analyses of deviance

Genstat output, generalised linear model, binomial error distribution, logit link function:

Change	d.f.	deviance	mean deviance	deviance ratio	approx F pr.
+ Lot	11	234.379	21.307	13.56	<.001
+ site	1	0.050	0.050	0.03	0.859
+ Lot.site	11	37.815	3.438	2.19	0.038
Residual	36	56.582	1.572		
Total	59	328.827	5.573		

Means, lower and upper 95% confidence limits - output from predict (full model):

site	p	LCL	UCL
1	1.814	0.001990	94.50
2	2.244	0.008695	85.84

Means, lower and upper 95% confidence limits - output from predict (only Lot fitted due to collinearity issues):

Lot	p	LCL	UCL
A	9.600	6.538	13.882
B	0.800	0.203	3.106
C	2.100	0.902	4.813
D	12.600	9.052	17.275
E	8.100	5.318	12.151
F	3.400	1.755	6.486
G	1.200	0.391	3.618
H	8.400	5.560	12.500
J	5.600	3.358	9.195
K	6.000	3.664	9.675
L	15.100	11.203	20.047
M	0.600	0.124	2.861

Fitting lot followed by heat treatment:

Change	d.f.	deviance	mean deviance	deviance ratio	approx F pr.
+ lot2	10	233.116	23.312	15.01	<.001
+ ht	1	1.264	1.264	0.81	0.373
+ site	1	0.050	0.050	0.03	0.858
+ lot2.site	10	36.930	3.693	2.38	0.027
Residual	37	57.468	1.553		
Total	59	328.827	5.573		

