FINAL REPORT
HORTICULTURAL DEVELOPMENT
COUNCIL

Calabrese: Factors controlling symptom development in bacterial spear rot (FV/104)

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report prepared 27 July 1995

Project no: FV/104

Project title: Calabrese: Factors controlling infection and symptom development in bacterial spear rot

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Location: SAC Edinburgh

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Date project commenced: 1 Oct 1991

Date project completed: 30 Sep 1994

Key words: calabrese, broccoli, spear rot, head rot, disease control, environment, *Pseudomonas*, *Erwinia*

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1.1 KEY RESULTS & APPLICATION

Calabrese: Factors controlling symptom development in bacterial spear rot

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Spear rot is an important disease of calabrese: the average loss to spear rot in the UK in 1993 (the latest figures available) was estimated at 29%, representing a loss of 13,950 tonnes of marketed output and a loss to the industry of £9.5 million. If we wish to maintain the competitive edge of the UK industry against imports, we need to know more about the disease and how to control it.

The aim of the project was to determine the cause of spear rot and the effects of crop environment on disease. The possibility of using this information to help growers by predicting disease was assessed.

We have shown that spear rot is caused by bacteria from the groups *Pseudomonas* and *Erwinia*. In a trial in which a major source of these bacteria from the soil was reduced by using a plastic mulch, disease was reduced by 50%.

Under conditions of alternating high and low temperatures, with continuous wetness of the head, spear rot can occur in three days following infection. Because of this very quick speed of development, and for other reasons discussed in this report, a disease forecasting scheme would be very difficult to devise and implement.

However, a simple test has been developed, which may be used by growers, to detect the presence of the bacteria on immature heads before disease occurs. Although this test cannot predict that disease will occur, it alerts growers to the presence of the bacteria and therefore to the risk of disease, and will help growers decide whether to spray and/or to harvest as soon as practicable.

1.2 SUMMARY OF PROJECT

Spear rot is a disease of major importance in calabrese in the UK but despite this, little was known about the biology of the disease in this country at the start of this work.

As part of this project, 257 questionnaires were mailed individually to calabrese growers during 1993, asking for information on their cropping practices and spear rot levels in the 1992 crop. We received 21 replies from Scotland and 24 from England. Marathon was the most popular variety, followed by Shogun, Green Belt and Caravelle. Several other varieties accounted for smaller areas grown. Growers reported fewer losses to spear rot in Marathon and Green Belt than in Shogun. Caravelle was more susceptible than these three varieties. Spear rot incidence in 1992 was highest in September and October (some crops suffered 60% loss during these months) with the average loss across the season to disease in England and Scotland being around 29%. This represented a loss of 13,950 tonnes marketed output, with an average value to the industry of £9.5m (Basic Horticultural Statistics, 1993, HMSO; these are the latest figures available). Spear rot is thus a major constraint to profitability.

Research at SAC in the 1980s, funded by the HDC (project FV/8), led to an Off - label approval for the use of copper oxychloride (Cuprokylt) to control spear rot; this approval is widely used by growers (OLA 0993/92). We have tested many alternative and novel products for their potential to control this disease, without much success, and copper oxychloride remains the only approved chemical control.

We believe that improved control of spear rot will come from a greater understanding of the disease itself. For this reason, HDC commissioned SAC in 1991 to carry out research into the cause of spear rot in the UK and the effects of crop environment on disease. This basic information was not known for the UK although studies in Australia and Canada identified bacteria of the genus *Pseudomonas* as the cause. We would use this information to assess if disease forecasting might be feasible to assist growers in predicting disease.

Our specific objectives were:

1. to determine the causal organism(s) by isolating and testing bacteria from diseased crops. To facilitate this, diagnostic tests were developed in conjunction with HRI Wellesbourne (HDC project FV 104a).

2. the effect of environment on disease was studied in two ways: firstly, by observing natural disease occurrence in the field in relation to records of temperature, relative humidity, surface wetness and rainfall taken at the site; secondly, to examine the effect of temperature and duration of wetness of the head, or spear surface, on disease in controlled environment experiments.

3. to examine the possibility of using information from 1. and 2. above to predict disease occurrence. If growers can identify particular crops at risk, they can take preventative action by applying copper oxychloride *at the optimum times* and/or plan to harvest these crops as soon as practicable.

4. small scale pilot studies were conducted on new products, and on the use of a mulch for disease control.

To determine the cause of the disease in the UK, we isolated bacteria from diseased calabrese collected from various sites in Scotland. To test whether the bacteria we had isolated were able to cause disease, we developed a laboratory method which allowed us to rapidly screen the isolates we had collected. We also tested bacteria for their ability to produce pectic enzymes (which break down cells and cause rotting) and surfactants (natural wetting agents which allow bacteria to spread over the waxy calabrese surface).

Over 300 isolations were made from diseased crops during a two year period. The causal agents of spear rot proved to be bacteria of two types - *Pseudomonas* and *Erwinia* with the former being most numerous. Pectic enzymes and surfactants produced by bacteria are important components of disease caused by *Pseudomonas* isolates, but *Erwinia* could cause disease without producing surfactants. Both types of bacteria naturally occur in soil and water and are predominately spread in crops by rain splash and aerosols (fine water droplets carrying bacteria in air currents).

In laboratory experiments using artificially - inoculated heads, spear rot could appear in 3 days under ideal environmental conditions: continuous wetness of the head surface and alternating day/night temperatures of $20^0C/5^0C$. These are the kind of conditions seen during autumn when spear rot is most prevalent - warm days and cool nights, often with rapid drops between the two extremes of temperature, leading to persistent dew formation on the heads. The wetness caused by the dew, coupled with the alternating temperatures (which not only lead to dew formation, but have a direct effect on the bacteria, or in weakening plant resistance, or both), causes spear rot to occur. The popular belief is that rainfall is the prime cause of spear rot. Rainfall, if persistent over several days, will certainly lead to spear rot, but our experiments in 1992 and 1993 showed that dew formation was more important than rainfall as a cause of spear rot outbreaks.

Once the causal agents of the disease had been identified, the next step was to devise a method which could detect them in growing crops. Diagnostic tests based on antibodies were unfortunately not suitable for the detection of bacteria which cause spear rot. This is because of the highly variable nature of the bacteria, some of which can escape detection by this method.

We examined instead a simple diagnostic test, which could be used by growers. Very young calabrese heads are incubated in polythene bags in conditions of alternating high and low

temperatures, such as found in a kitchen and refrigerator. Alternating temperatures are necessary to induce rapid rot development and under these conditions, the young heads will rot within a few days if sufficient numbers of pathogenic bacteria are present. This test gave good agreement with subsequent spear rot development in the crops from which the young heads were taken and proved to be a simple, rapid and inexpensive method to demonstrate the presence of spear rot organisms in the growing crop.

This is not a test which can predict whether or not disease will occur in a crop: this depends upon the weather conditions between the time of sampling and spear maturation. It has not been possible within this project to provide this kind of forecasting scheme. With spear rot, this expectation is unrealistic and probably unattainable for several reasons:

Calabrese crops are not uniform. Growers make use of different cultivars with different growth habits and, as we have shown, different susceptibilities; successional plantings are made, meaning different stages of crop maturity; plant spacings, irrigation and nitrogen inputs will differ. Many of these factors apply to vegetable Brassica crops in general, and present problems in developing predictive schemes.

Regarding the cause of spear rot, it is a bacterial disease and one which is moreover an opportunistic disease: it is caused by more than one pathogen, and these are weak pathogens which normally reside harmlessly in the soil and on the leaves and heads of the calabrese plant. They multiply and cause disease given the right temperatures and if the heads are persistently wet, or if the plants are stressed or damaged (by frost, or rapid changes in temperature). Each of these pathogens varies in its aggressiveness and probably multiplication rate. These are crucial considerations in any predictive scheme. Under ideal conditions, disease can occur in only 3 days from the time of infection. Unlike fungal pathogens, bacteria depend more heavily upon optimal conditions of temperature and free water availability for their multiplication almost up to the time of symptom appearance. There will of course be a threshold reached beyond which symptoms will inevitably appear, but the intervening time is likely to be so short (hours) for this disease that no timely action would be possible.

The test described above alerts the grower to the presence of pathogenic organisms in the crop; this information can be used to help decide whether treating the crop with bactericide, or preparing to harvest as soon as possible, would be justified.

To carry out the tests, 10 to 20 clean, freshly cut spears should be taken at random, one spear per sampling point through the crop. Random sampling is essential because field observations show that spear rot spreads from small discrete foci often spread throughout the crop. Spears can be sampled any time from the stage of 1.5 cm diameter. They are placed in polythene bags, 5 spears to a bag approximately 30 by 45 cm, the bag inflated slightly by mouth and tied at the neck. During the day,

the bag should be kept in a warm place such as a kitchen, and then transferred to a refrigerator overnight. Additional bags may also be left out in the shade, preferably off the ground near the crop, thereby subjecting the spears to similar temperature changes as in the field. Symptoms become evident after 3 to 4 days if pathogenic bacteria are present.

If pathogenic bacteria were detected on the young spears, as indicated by rotting symptoms, and the weather following sampling shows, or is predicted to show, large and sharp drops in day/night temperatures leading to persistent dew, or there is persistent rainfall, the inexpensive two - spray programme with copper oxychloride (Cuprokylt) should be commenced immediately. Plans can also be made for harvesting as soon as practicable. The spray programme consists of treating the developing spears at about 2.0 - 2.5cm diameter, and a second spray 7 days later; both sprays should be of 5 kg product/ha in 600 l water. From previous work at SAC, these are the *optimum times* for applying this product. Applying copper when the crop is maturing and wet weather or dewfall prevail is too late to be effective: bacterial numbers will already be too high.

As a predictive tool on its own, the test showed too many false positives (i.e. pathogens were detected as present but disease did not subsequently develop in the crop) for growers to rely on it as an indicator of disease. However, more importantly, we did not detect any false negatives, i.e. pathogens were not demonstrated in the test but disease subsequently developed in the crop. Because of the difficulties of devising a forecasting scheme, and because these are data from one season and a limited number of crops, we cannot be confident about recommending *no* action be taken if *no* pathogens are detected. Growers may wish to sample and test again as the crop matures; as noted above, disease can occur in only 3 days from the time of arrival of the bacteria under conditions of ideal temperature and humidity, so a close eye should be kept on the weather as the crop matures. We would like to see growers evaluating the method for themselves and accumulating more experience with it before we can make a recommendation for no action.

Two field experiments were conducted on disease control: 1. an experiment where calabrese was grown through plastic mulch. The aim was to reduce the soil - borne source of bacteria which cause spear rot; 2. chemical control with kasugamycin, a product registered in other parts of the world, but not the UK, for controlling various bacterial and fungal diseases. The mulch successfully reduced disease by 50%, confirming a report from Canada where a straw mulch also reduced spear rot. At the present time, the use of a mulch is probably not practical in conventional production, but its effect on disease is worth noting. Kasugamycin by itself had no effect on disease, but if combined with copper oxychloride, reduced disease by 40%; however this was no better than copper oxychloride alone, although this latter treatment was applied at a higher dose.

Long - term effort should be made towards the development of resistant cultivars for disease control. To this end, with our current state of knowledge of this disease, further research is required to

understand and exploit the findings already gained in this project on the basis for host resistance and pathogen virulence. We are actively pursuing research into these aspects at SAC.

2. EXPERIMENTAL SECTION

2.1 IDENTIFICATION OF CAUSAL ORGANISMS

Introduction

Spear rot, also known as soft, or head rot, has been described from various parts of the world (Wimalajeewa et al., 1987; Hildebrand, 1989) (Fig 1). The first reports came from England; Dowson and Dillon-Weston (1937) isolated *Pseudomonas fluorescens* and *Bacterium carotovorum* (=*Erwinia carotovora*) from diseased heads, but claimed that only the latter species was capable of causing symptoms when inoculated on to *wounded* heads. *Erwinia carotovora, Pseudomonas marginalis, P viridiflava* and other fluorescent pseudomonads were isolated from diseased heads in Australia by Wimalajeewa et al. (1987), but only *P marginalis* was reported as being capable of causing disease in unwounded heads. Hildebrand (1989) in Canada, identified *P marginalis* and *P fluorescens* as causal organisms of spear rot in unwounded heads; other species, including an *Erwinia* sp, which were non-pathogenic by themselves may also be involved, creating a disease complex. In a brief report, Brokenshire and Robertson (1986) determined the presence, but not the pathogenicity of of *P marginalis*, *E carotovora* ssp *carotovora* and *E carotovora* ssp *atroseptica* on diseased calabrese in Scotland.

In this work we aimed to isolate bacteria from diseased heads, and develop a reliable method for testing pathogenicity of isolates on unwounded heads and thereby determine the causal organisms.

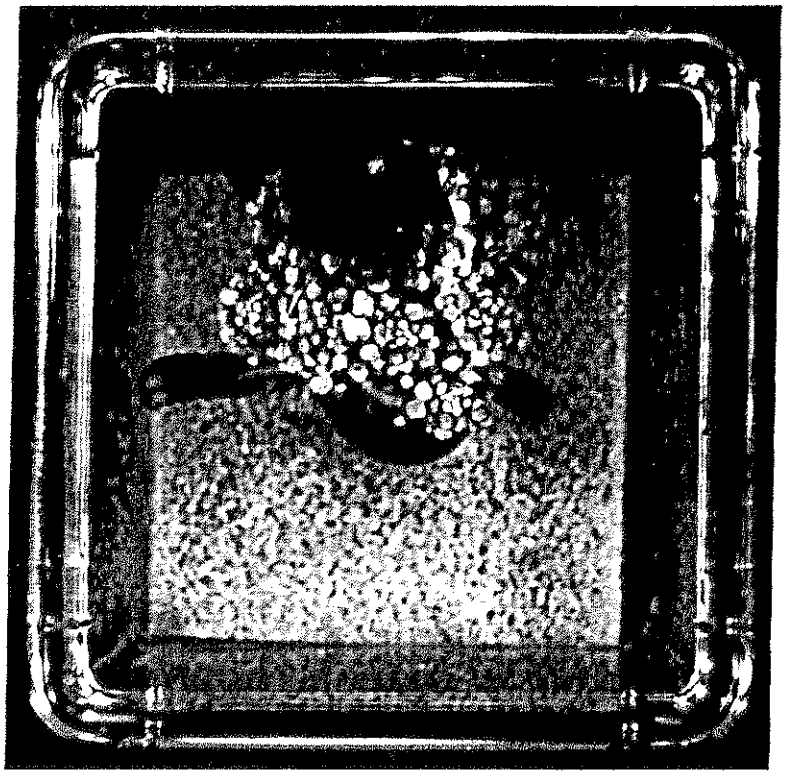
Materials and methods

Bacterial isolation, identification and response to conjugated antisera

Diseased heads were collected from locations in SE Scotland during summer /autumn 1992. Some isolates were also collected in 1991. Pieces of tissue, approximately $1cm^2$, were excised, sonicated for 5 min in sterile Ringer's solution (1 part tissue, 9 parts Ringer's) and 1ml aliquots removed for serial dilution to 10^{-8} in Ringer's solution. Dilutions (0.1ml) were plated on to King's medium B agar (to detect fluorescent colonies) and crystal violet pectate agar (to detect pectic enzyme production) and incubated at 25^0C for 2 days (media recipes in Lelliot and Stead 1987) . Bacterial colonies were purified and identified according to procedures in Lelliot and Stead (1987).

The ability of individual isolates to produce surfactant was tested by touching the surface of a droplet of water placed on a plastic petri dish; presence of surfactant was indicated by immediate spread of the droplet, indicating reduction in surface tension.

Fig 1: Spear rot of calabrese



Figs 2 and 3: Laboratory test to identify bacteria which can cause spear rot.
Fig 2 (top): Calabrese heads are placed in plastic boxes (Magenta vessels) and held upright with a sponge collar. Pieces of lint soaked in bacteria are placed on top of the head. **Fig 3 (bottom):** After 5 days, the lint is removed to show presence or absence of rot.

Polyclonal antisera were raised to five pathogenic isolates: 1015 (*Pseudomonas putida*, Group IVb (biotype B), isolated in 1991), 1065 (*Erwinia carotovora* subsp *carotovora*, isolated in 1991), 5038 (*P. fluorescens* Group IVb (biotypeA), isolated in 1992), 5049 (*P.fluorescens* Group IVb (biotype A), isolated in 1992) and 5067 (*Erwinia carotovora* subsp *atroseptica*, isolated in 1992). Identity of the *Pseudomonas* isolates was confirmed by fatty acid profiling at the International Mycological Institute, Kew, Surrey. Antisera were conjugated to *Staphylococcus aureus* to produce a rapid diagnostic test for the identification of these pathogenic isolates (Lyons and Taylor 1990); conjugated antisera were produced by JD Taylor and NF Lyons, HRI Wellesbourne (HDC contract FV 104A). Conjugated antisera from 1015, 1065 and 5067 were tested for homology and cross reactivity against 72 bacterial isolates collected in 1992 as described above from diseased calabrese. Conjugated antisera to 5038 and 5049 were tested against 15 pathogenic isolates from 1992.

In addition, antisera were tested against sap (5ul sap + 5ul conjugate) expressed from healthy and diseased heads from the trial at Bush Estate in 1993 and from commercial crops (see 2.2).

Pathogenicity test

Following identification, isolates were tested for their ability to cause disease on healthy excised calabrese heads incubated *in vitro*. Pieces of sterile cotton lint soaked in a bacterial suspension of 10^8 cells per ml or sterile distilled water (negative controls) were applied to the surface of glasshouse-grown spears cv Shogun in sterilised 'Magenta GA7' vessels (Sigma Chemicals) (Figs 2 and 3). The sealed vessels, containing sponge collars to support each head, were incubated at 20°C day/ 10°C night with a 16h photoperiod. Positive controls (a known pathogenic *Erwinia carotovora* subsp. *carotovora* isolate, no. 1065) were also incorporated into each assay. Four replicate heads were used to test each isolate. Symptoms on the heads were scored at 5 days after incubation using the following criteria: 0 = healthy; 1 = watersoaked; 2 = watersoaked and soft; 3 = brown soft rot; 4 = black soft rot.

Results

Isolate identification and characterisation

Of 99 representative isolates, 70 were fluorescent *Pseudomonas* spp., 2 were *Erwinia* sp(p) (Tables 1a and 1b) and 27 were unidentified Gram-negative (19) or Gram-positive (8) non-fluorescent bacteria (results not shown for these unidentified organisms). The fluorescent pseudomonads belonged to groups IVa (19), IVb (13), Vb (15),Va (8), Ia (1), with the remainder (14) giving atypical responses to LOPAT tests (Lelliot and Stead 1987). Most isolates produced surfactant.

Antisera reactions

Conjugated antisera from *Erwinia* isolates 1065 and 5067 cross-reacted with some pathogenic and non-pathogenic *Pseudomonas* isolates (Tables 1a and 1b). Sometimes a "stringy" reaction was seen on the slide, where the conjugated antibodies appeared to agglutinate in long strings; this was considered negative but it was not always possible to distinguish this from a true positive response. This was a problem with all the conjugated antisera in the slide agglutination test. Conjugated antisera raised to *Pseudomonas putida* 1015 was highly specific to its homologous isolate and failed to identify other pathogenic IVb isolates. Conjugated antisera raised to *Pseudomonas* IVb isolates 5038 and 5049 reacted with most of the other pathogenic *Pseudomonas* isolates; they were not tested with non-pathogenic isolates. We failed to see positive reactions with any of the conjugated antisera tested against sap from healthy or diseased heads.

Pathogenicity tests

From the 70 fluorescent *Pseudomonas* isolates characterised above , 13 were pathogenic giving a brown or a black rot, ie they gave average scores of at least 3.0 in the excised spear test; isolates giving scores lower than 3.0 were considered non-pathogenic. Eight of these pathogenic isolates belonged to the *fluorescens-putida* complex (IVb group), three were from the IVa group, one isolate was from Group Vb and one gave atypical LOPAT reactions. The two *Erwinia* sp(p) were both pathogenic, in fact highly aggressive. The 27 unidentified non fluorescent isolates were all non pathogenic.

Most of the pathogenic *Pseudomonas* isolates were pectolytic and produced surfactant. The *Erwinia* isolates and two *Pseudomonas* isolates (5098 and 5080) were pectolytic but surfactant-negative and one *Pseudomonas* isolate was negative for both criteria (5017, which gave atypical LOPAT characterisation).

Discussion

Diseased calabrese heads carried a mixed bacterial population, comprising fluorescent pseudomonads, erwinias and other Gram-negative or -positive non-fluorescent bacteria. Both fluorescent pseudomonads and erwinias were capable of causing spear rot symptoms on non wounded heads in an *in vitro* test. We isolated a similar spectrum of pathogenic and non pathogenic bacteria from crops in 1991 (Robertson et al. 1993). Hildebrand (1989) found that only fluorescent pseudomonads which produced *both* pectic enzymes and surfactants could cause disease on non wounded heads; he isolated *Erwinia* spp. but these were not able to cause disease on unwounded heads. Wimalajeewa et al. (1987) attributed the cause of disease only to *Pseudomonas marginalis*. Whilst we agree that most isolates from Scottish calabrese possess the attributes both of pectic

Table 1(a) Characterisation of non-pathogenic fluorescent *Pseudomonas* isolates from selected 1992 commercial crops

ISOLATE NUMBER	SOURCE	ID. (LOPAT)*	ANTISERUM 1065 1015 5067	SURFACTANT ABILITY	PATHOGENICITY
5002	Montrose	+ + - + +	S S -	+	2
5003	Montrose	+ - + - +	- - S	+	1.25
5004	Montrose	IVb	+ - +	-	1
5006	Montrose	IVb	- S -		2.25
5008	Montrose	IVa	+/S - -	-	1
5009	Montrose	IVb	+/S - -	+	2
5012	Montrose	+ - - - -	- - -	+	1.75
5013	Montrose	- - - + -	S - -	-	1.25
5014	Montrose	- + + - +	- S -	-	1
5015	Blairgowrie	Vb	+/S S S	+	1.25
5018	Blairgowrie	IVb	S S S	-	2.25
5019	Blairgowrie	Va	- S S	++	1
5020	Blairgowrie	Va	- S -	-	1.5
5021	Blairgowrie	Ia	S S -	-	1
5022	Blairgowrie	IVa	S - -	+	2.5
5023	Blairgowrie	IVa	- S S	++	1.5
5024	Blairgowrie	Vb	S +/S S	++	1
5026	Blairgowrie	IVa	S S -	++	2.25
5027	Carmyllie	Vb	- + -	++	1
5028	Carmyllie	- - - - -	+ S +/S	-	1.25
5030	Carmyllie	- + - - -	S S S	-	2.25
5031	C. Huntly	IVa	- S S	++	1
5034	C. Huntly	IVa	- S -	++	2.75
5036	C. Huntly	Vb	+ S +	-	2
5037	C. Huntly	IVa	+ S +	++	2.25
5039	Alyth	Va	+/S S S	++	2
5041	Alyth	+ + + - -	S - S	+	1.25
5042	Alyth	IVa	- - S	+	0.75
5045	Alyth	IVa	- - S	+	2.5
5046	Alyth	+ - + - +	S S -	-	2
5047	C. Huntly	Va	- - S	-	1
5048	C. Huntly	Vb	S - -	++	1
5050	C. Huntly	Vb	S S +/S	++	1.25
5051	C. Huntly	IVa	- - S	++	2.25
5056	C. Huntly	IVb	+/S S S	++	1.25
5057	C. Huntly	Vb	+/S S S	++	1
5061	C. Huntly	Vb	+/S S S	-	2.5
5062	C. Huntly	- + + + +	+ + S	+	2.5
5063	C. Huntly	Vb	+ S +/S	+	2.5
5066	C. Huntly	+ - - - -	S S -	-	1.75
5069	C. Huntly	Vb	S - -	-	1
5072	C. Huntly	IVa	- S -	++	2.25
5073	C. Huntly	Vb	+/S - S	++	2.25
5076	Carmyllie	Va	- - +/S	+	0.75
5077	Carmyllie	Va	S - S	+	1.5
5078	Carmyllie	Va	S S -	+	2
5079	Carmyllie	IVa	S - S	+	1.75
5082	Errol	IVa	+/S - S	+	2.25
5083	Errol	IVa	S - -	+	2.25
5086	Errol	Vb	S - -	+	2.25
5089	Errol	Va	S - -	+	1
5090	Errol	+ - - - +	S - S	-	0.75
5091	Errol	Vb	S - -	-	1.5
5093	Bush	IVa	- - -	+	1
5096	Bush	- + + + +	S - -	++	1
5100	Bush	Vb	- S S	+	1
5101	Bush	IVa	- - -	-	1.25

Table 1(b) **Characterisation of pathogenic isolates from selected 1992 commercial crops.**

ISOLATE NUMBER	SOURCE	ID. (LOPAT) *	ANTISERUM ˜ 1065 1015 5067 5038 5049	SURFACTANT ABILITY '	PATHOGENICITY "
5040	Alyth	IVa	- - - s/+ s/+	+	3
5075	C. Huntly	IVa	s/+ - - s/+ s/+	+	3
5098	Bush	IVa	- s s s/- -	-	3.5
5000	Montrose	IVb	s - s - s/+	+	3
5035	C. Huntly	IVb	+ + s/+ + +	+	3.75
5049	C. Huntly	IVb	s s + + +	++	4
5055	C. Huntly	IVb	s/+ s s + s/+	+	4
5064	C. Huntly	IVb	s - s/+ s/+ s/+	++	3.5
5038	Alyth	IVb	+ - s + +	+	4
5080	Errol	IVb	s - s - -	-	3.25
5085	Errol	IVb	s - s + +	+	3.5
5007	Montrose	Vb	s/+ s s s +	+	3.5
5017	Blairgowrie	- + - - -	s s s s -	-	3
5066	C. Huntly	Erwinia sp.	- s - s/+ -	-	4
5067	C. Huntly	Erwinia c. a.	+ s + s/- -	-	4

* Lelliot & Stead 1987

˜ + = agglutination
- = no agglutination
s = stringy: considered negative.

' + reduction of surface tension of water; - = no reduction.

" 0 = healthy
4 = black spreading rot.

enzyme and surfactant production, some isolates did not, notably the erwinias (these lacked surfactant production), and these were still capable of causing disease on non wounded heads. The most likely explanation of this is that bacteria already resident on the heads can supply the missing factors, although we take steps to reduce unwanted bacterial contamination as far as possible by using glasshouse - grown heads and including appropriate controls. The roles of surfactants and pectic enzymes are being investigated further at SAC.

It was envisaged that the antisera raised at HRI would help the identification of pathogenic organisms on diseased heads. For this, a rapid test was appropriate for dealing with large numbers of samples. The slide agglutination test, as used at HRI, was a suitable format, but unfortunately in this format for this disease it is less sensitive and is open to error in interpretation when compared to using antibodies in an ELISA (see results reported in project FV104a). We are not disputing the value of this format for diagnosis of other diseases. The evidence from our isolations and identifications, and from testing the antisera, shows that spear rot is not caused by one single bacterial species; we have isolated, and demonstrated pathogenicity on unwounded heads, of *Pseudomonas fluorescens, P putida, P marginalis* and *Erwinia carotovora*. With the exception of *Erwinia carotovora*, these are weak, opportunist pathogens. In addition, we have confirmed pathogenicity of these species in a seperate seedling test (results not shown). Within the fluorescent *Pseudomonas* species, especially Group IV, the different responses we obtained to the conjugated antisera (viz. cross-reactivity or extreme specificity) would suggest considerable strain (serotype) variation. Given the cross - reactivity of some of the antisera, it is surprising that we observed no positive reactions to sap from healthy or diseased heads. It may be that insufficient numbers of bacteria with the appropriate antigenic sites were present to generate a response in the conjugated antisera, but this seems unlikely for rotted heads; we can offer no real explanation for this. These reasons render the development of a diagnostic test for spear rot pathogens a difficult prospect. We are currently attempting to assess strain diversity using PCR profiling.

References

Brokenshire, T and Robertson, NH (1986). Control of calabrese spear rot with copper fungicides. Tests of Agrochemicals and cultivars No 7 (Annals of Applied Biology 108, Supplement) 52 - 53.

Dowson, WJ and Dillon - Weston, WAR (1937). Soft rot of broccoli. The Gardener's Chronicle, July 1937.

Hildebrand, PD (1989). Surfactant - like characteristics and identity of bacteria associated with broccoli head rot in Atlantic Canada. Canadian Journal of Plant Pathology 11, 205- 214.

Lelliot, RA and Stead, DE (1987). Methods for the Diagnosis of Bacterial Diseases of Plants. Oxford: Blackwell Scientific Publications.

Lyons, NF and Taylor, JD (1990). Serological detection and identification of bacteria from plants by the conjugated *Staphylococcus aureus* slide agglutination test. Plant Pathology 39, 584 - 590.

Robertson, S, Brokenshire, T, Kellock, LJ, Sutton, M, Chard, J and Harling, R (1993). Bacterial spear (head) rot of calabrese in Scotland: causal organisms, cultivar susceptibility and disease control. Proceedings Crop Protection in Northern Britain, Dundee, Scotland 1993, 265 - 270.

Wimalajeewa, DLS, Hallam, ND, Hayward, AC and Price, TV (1987). The etiology of head rot disease of broccoli. Australian Journal of Agricultural Research 38, 735 - 742.

2.2 EFFECT OF ENVIRONMENT ON DISEASE OCCURRENCE

Introduction

Environment can have a large and direct effect on disease and can determine whether an encounter between host and pathogen fails or succeeds. Of particular importance with bacterial plant diseases are temperature and duration of free water presence (surface wetness) on the plant surface; both are necessary for infection and multiplication of bacterial cells.

To our knowledge, there are no published scientific data on the effect of environment on spear rot; however growers know only too well that the disease is of particular importance in the autumn. This is usually believed to be due to increased rainfall at this time of year, but this is only partly true, as this section will show.

To determine how environment can affect spear rot occurrence, we placed weather stations in several growers' crops and in a calabrese crop at our experimental site at Bush Estate, Penicuik, and made regular observations on natural disease occurrence. Weather conditions leading to spear rot occurrence could then be identified and compared between sites. In addition, we carried out infection experiments in controlled environment cabinets to examine the interaction between temperature and surface wetness.

Materials and methods

SAC/University of Edinburgh Field station at Bush Estate, Penicuik, Midlothian

Module - raised seedlings of cvs Shogun and Skiff were made under cage-netting in May, June, July and August, 1993. The design was of a split-plot type and included a treatment for disease control where seedlings were planted through black polythene mulch to prevent splash infection from soil-borne inoculum (this is described further in 2.4). Each split-plot contained 210 plants at 18cm spacings and 34cm between rows. Soil samples were taken from the trial site before planting and at the end of the trial and analysed for nutrients (see 2.4). Delta T environmental monitoring equipment was set up in the crop to record surface wetness (via an artificial sensor placed at crop height), air temperature, % relative humidity (RH) and rainfall (Fig 4). Throughout the growing season the crop was monitored for the appearance of spear rot.

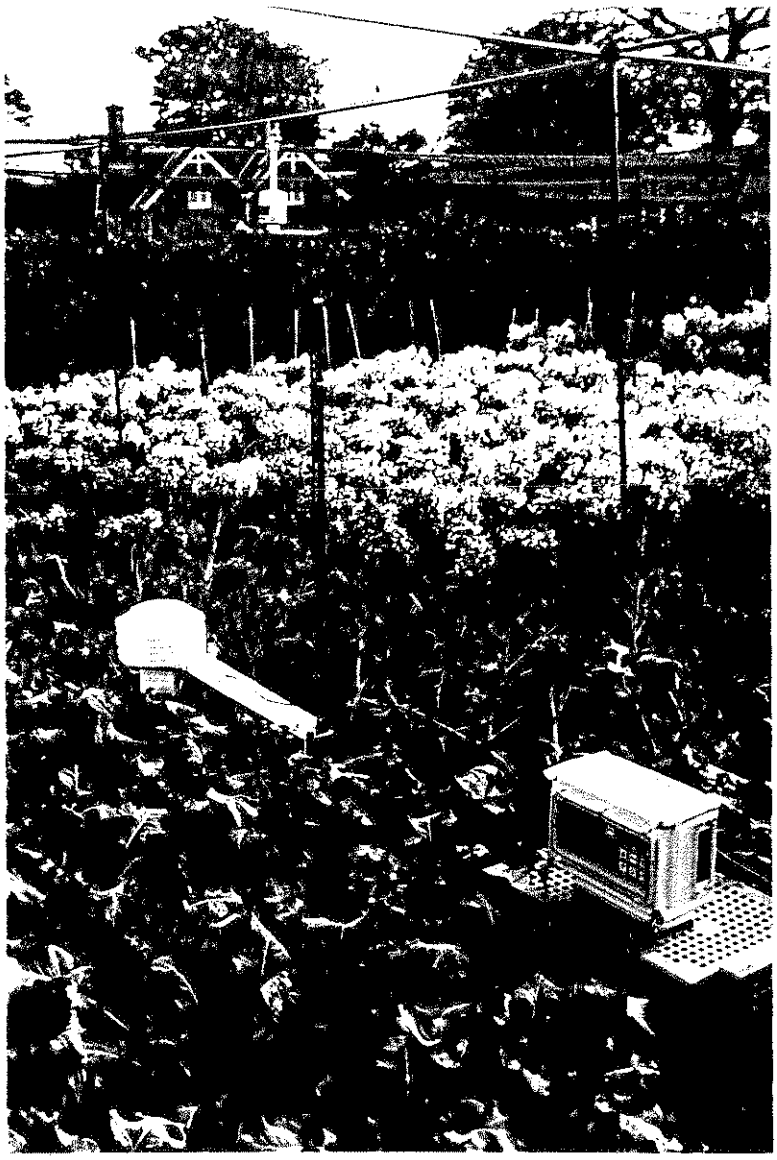


Fig 4: Calabrese trial at Bush Estate, Penicuik, Midlothian with Delta T® weather station

Growers' crops

Thermohygrographs, recording temperature and RH, were placed in commercial crops as follows: Alan and Douglas Neill, Thorngreen Farm, Blairgowrie (Perthshire), John Elgin, Westerton of Rossie, Montrose (Angus), John Millar, Firth Farm, Carmyllie (Angus) and Ron Wilson (Gowrie Growers), Waterybutts, Errol (Perthshire). As well as thermohygrographs, raingauges were sited at Blairgowrie and Montrose. The thermohygrographs were regularly calibrated on-site and charts from these and the raingauges were collected weekly from 26/4/93 to 19/10/93. During the once - weekly visits to the sites, the date (week) of first occurrence of spear rot was recorded; thus a record was obtained of the temperature and RH (and rainfall at two sites) leading up to the disease outbreak.

Controlled environment experiments

Glasshouse-grown spears of Skiff and Shogun were exposed to differing continuous or alternating (16h/8h; day/night) temperature regimes and differing periods of surface wetness in controlled environment cabinets (Fisons). Photoperiod was 16h. The excised spears were supported in sealed 'Magenta' vessels and surface wetness was simulated by the application to the spear surface of sterile squares of lint impregnated with either a bacterial suspension of the pathogenic *Pseudomonas fluorescens* isolate 5038 or distilled water (see 2.1 for further details of this technique). Differing periods of surface wetness were created by removing the lints from 4 replicate spears after 3, 4, 5 or 6 days. The spears then remained in the sealed vessels for a further 48h when spear rot symptoms were assessed according to the four point scale as in 2.1.

Results

Bush Estate

The first incidence of spear rot was on 27 September in the July planting. This followed four days during which there was no rain, but the surfaces of the spears were wet with dew for between 11 and 14 hours in each 24 hour period (Fig 5). Dew formation occurred after day/night temperature inversions (sharp drops in temperature) and when RH rose to 90% or greater (Fig 5). Persistence of surface wetness was governed by duration of the lower temperature, with evaporation occurring when temperature increased to 10 - 12 ^{0}C (Fig 5). During the four days leading up to disease, maximum daytime temperatures were about 14^{0}C, minimum night temperatures were between -1 and 7^{0}C, and the maximum temperature inversion was 15^{0}C (ie difference between daily maximum and minimum temperatures, which occurred on 26 Sep when the temperature dropped from 14^{0}C to -1^{0}C).

Growers' crops

The thermohygrograph data of RH, rainfall and temperature leading up to the onset of disease in the commercial crops were interpreted in the light of the above information from the Delta T environmental records and in the knowledge that periods of surface wetness are used in most plant disease indices (Wilks & Shen, 1991). Dew periods are assumed to correspond to periods of RH of 90% and over (Sutton *et al.* 1984, and as shown by the Delta T records at Bush Estate). The number of hours per day with RH ≥ 90% was calculated for the two to three weeks leading up to the appearance of spear rot and for some time thereafter. In keeping with other predictive schemes (Parvin *et al*, 1974; Bailey *et al*, 1994), the minimum daily temperatures during these periods were also determined. Rainfall data were not used because the relative humidity would in any case be over 90% during periods of precipitation. The data are presented in Figs 6 to 10 and the time when spear rot appeared in the crop is also indicated. It should be noted that the crops were only assessed once per week, and so these times refer to the fact that the disease had appeared some time during the previous 7 days.

Spear rot appeared at the riverside site of Errol in two crops, in cv Skiff in July and in cv Marathon in October, (Figs 6 and 8). In July, spear rot appeared on Skiff in a week in which there were three consecutive days when RH was over 90% for more than 18h of each day, including a 48h period without any evaporation (Fig 6). The temperature at this time did not fall below 12°C. Periods of ≥ 90% RH had also occurred every day in the week before spear rot was evident. In early October, in the days leading up to the appearance of spear rot on cv Marathon, the RH was ≥ 90% for more than 12h of every day (Fig 8). When spear rot was recorded, prolonged daily periods of surface wetness had occurred for at least 5 days including two consecutive days of 24 and 23h. The temperature had not fallen below 10°C during this time (Fig 8).

At the coastal site of Montrose, spear rot appeared on cv Marathon in the first week of September (Fig 7). During the previous two weeks there had been a gradual increase in the daily duration of high RH such that spears could have been wet for at least 12h of every day. In .the week of the appearance of disease, there were two consecutive days where the RH was over 90% for 22h. On these days, minimum temperatures of 7 and 8°C were recorded (Fig 7). At Blairgowrie and Carmyllie, both inland sites, spear rot was apparent on the variety Shogun during the first week of October (Figs 9 and 10). At both sites, there were consecutive 24h periods of high RH: two at Blairgowrie (Fig 9) and five at Carmyllie (Fig 10). The minimum temperatures at these times were between 9 and 10°C and 9 and 11°C respectively.

At all sites, disease appeared following an increase in the persistence of periods with RH ≥ 90%.

Controlled environment experiments

The time taken for disease to appear on excised spears under differing conditions of temperature is shown below in Table 2.

Table 2 Days to expression of spear rot symptoms[1] at different temperatures under conditions of continuous surface wetness.

	Temperature (°C)							
Cultivar	**5**	**10**	**15**	**20**	**25**	**15/5[2]**	**20/10**	**20/5**
Skiff	>6	6	5	5	nd [3]	5	4	3
Shogun	>6	>6	>6	6	nd	>6	5	4

[1] a mean score of at least 3.0 on four replicate heads: (0 = healthy, 4 = black soft rot).
[2] for 16h day/ 8h night.
[3] no data due to fungal growth.

This experiment was designed to show i). the effect of temperature on the rate of symptom appearance and ii). the minimum period of continual surface wetness necessary for symptoms to appear at different temperatures.

Overall, Skiff was the more susceptible cultivar; symptoms were expressed in both cultivars more rapidly at the higher temperatures (Table 2). However, the progression of symptoms was favoured by alternating high and low temperatures, in particular where 20°C was maintained during the 16h photoperiod. The regime with the greatest difference in day/night temperatures (20/5°C) gave the most rapid symptom expression on both cultivars: a spreading rot was evident under the lints after only 3 or 4 days. On the other hand, even after 6 days under wet lints, a continuous temperature of either 5, 10 or 15°C for Shogun, and 5°C for Skiff, did not result in symptoms. Where lints were removed from heads prior to symptom appearance, symptoms did not subsequently appear, despite favourable temperatures; the number of days in the table therefore represent the minimum required at continuous surface wetness for symptoms to appear.

Discussion

Controlled environment experiments

Skiff was more susceptible in these experiments than Shogun, as is seen by growers under field conditions and as we have observed in previous experiments (Robertson *et al.*, 1993). Increased temperatures led to an increased rate of symptom appearance; *P fluorescens* has an optimum temperature for growth in culture of 25-30^{0}C (unpublished results). The rate of symptom appearance was further increased by alternating high and low temperatures. A regime of 20^{0}C day/5^{0}C night with continuous wetness gave the fastest appearance of symptoms (3 days). In the field, alternating temperatures will lead to dew formation which will increase the onset of disease (see below). However, in these controlled environment experiments, surface wetness was maintained under conditions of both alternating and continuous temperature. Alternating temperatures *per se* therefore increased the speed of symptom appearance. Possible explanations for this are: i) plant stress; ii) increased aggressiveness of the pathogen via temperature induced enhanced gene expression (Jones and Inouye 1994); iii) ice nucleation activity at 5^{0}C, damaging the head tissue.

Field data

The data from the Bush Estate trial site showed that spear rot can develop after periods of head surface wetness arising only from dew. At this site, there was no rain during the week before spear rot appeared but there were four consecutive days in which dew periods occurred. These periods arose from nighttime drops in temperature of around 10°C and greater; the more rapid decline leading to quicker and therefore longer dew periods. Prolonged periods of surface wetness (implied from an RH of 90% or greater) were always present on consecutive days before the onset of spear rot in the crops on grower sites. Obviously, the higher the temperature during these periods, the more rapid would be the bacterial multiplication and therefore disease occurrence: this was seen in the controlled environment experiments and from Errol in July, where spear rot appeared in a week with 3 consecutive days of surface wetness for more than 16h each day.

However, generally and as also found at most of our monitored growers' crops, spear rot is more prevalent in the autumn. At this time of the year, there is an overall tendency for the *difference between daily maximum and minimum temperatures* to increase and this would result in extended periods of free water on the spear surface. In the controlled environment experiments above, symptoms appeared faster as the difference between maximum and minimum temperatures was increased, ie symptoms developed faster at 20^{0}C/5^{0}C than at 15^{0}C/5^{0}C or 20^{0}C/10^{0}C. Temperature therefore has two roles in disease development, (i) to encourage dew formation and persistence and (ii) a *direct* effect on growth of bacteria and perhaps plant stress.

Interpretation of the thermohygrograph data in autumn indicates that surface wetness for at least 12h of each consecutive day, for at the most 7 days, is necessary for spear rot to develop. Results from the controlled environment experiments and from the early outbreak at Errol, suggest that this time limit of 7 days can be, and is likely to be, shorter. This may particularly be the case where, each day, spears would have been wet for at least 75% or 100% of the time on consecutive days e.g. Blairgowrie, Carmyllie and Errol in the first week of October and Montrose in September.

In many predictive schemes, the duration of host surface wetness is treated as a critical factor in the development of disease eg. potato late blight (Beaumont, 1947; Krause & Hyre, 1975; Wallin, 1962), apple scab (Mills, 1944; Preece & Smith, 1961), onion (Vincelli & Lorbeer, 1988, 1988a) and carrot (Gillespie & Sutton, 1979), leaf blights, tomato early blight (Madden *et al*, 1978) and peanut leaf spot (Jensen & Boyle,1966; Bailey *et al*; 1994). However, in these schemes, other criteria are also known, such as host (cultivar) susceptibility, stage of maturity, presence of the pathogen and its multiplication rate. Any accurate predictive scheme would need to take account of all these factors. Although there was no time in this study to investigate these determinants, it is still possible to make reasonable assumptions as to when disease is likely to occur using information from spear incubation (see 2.3) and predictions of day/night temperatures and/or rainfall. If the pathogens are present on the growing crop, as indicated by incubation of spears, then depending upon the time of year, the grower should take into account the predicted day/night temperatures for the following 3 - 5 days and not just the predicted rainfall. A more informed judgement may then be made as to the necessity for preventative measures.

This study has not enabled us to provide the kind of predictive scheme, as for example with potato blight, whereby we could predict the *inevitable* occurrence of disease, given the fullfillment of certain criteria. With spear rot, this expectation is unrealistic and probably unattainable for several reasons. Calabrese crops are not uniform: growers make use of different cultivars with different growth habits and, as we have shown, different susceptibilities; successional plantings are made, meaning different stages of crop maturity; plant spacings, irrigation and nitrogen inputs will differ. Many of these factors apply to vegetable Brassica crops in general, and present problems in developing predictive schemes (Humpherson - Jones, 1991).

Regarding the pathogens themselves, spear rot is a bacterial disease and one which is moreover an opportunistic disease caused by more than one pathogen, each of which varies in its aggressiveness and probably multiplication rate. These are crucial considerations in any predictive scheme. Unlike fungal pathogens, bacteria depend more heavily upon optimal conditions of temperature and free water availability for their multiplication almost up to the time of symptom appearance. There will of course be a threshold reached beyond which symptoms will inevitably appear, but the intervening time is likely to be so short (hours) for this disease that no timely action would be possible.

The best long - term prospect for disease control lies in the development of resistant cultivars. To this end with our current state of knowledge of this disease, further research is required to understand and exploit the findings already gained in this project on the basis for host resistance and pathogen aggressiveness.

References

Bailey, J. E., Johnson, G. I. & Toth, S. J. Jr. (1994). Evolution of a weather - based peanut leaf spot spray advisory in North Carolina. *Plant Disease* **78**, 530 - 535.

Beaumont, A. (1947). The dependence on the weather of the dates of potato blight epidemics. *Transactions of the British Mycological Society* **31**, 45 - 53

Gillespie, T.J. & Sutton, J. C. (1979). A predictive scheme for timing fungicide applications to control *Alternaria* leaf blight in carrots. *Canadian Journal of Plant Pathology* **1**, 95 - 99

Humpherson - Jones, F.M. (1991). The development of weather - related disease forecasts for vegetable crops in the UK. Problems and prospects. *Bulletin OEPP/EPPO Bulletin* **21**, 425 - 429.

Jensen, R.E. & Boyle, L.W. (1966). A technique for forecasting leaf spot on peanuts. *Plant Disease Reporter* **50**, 810 - 814.

Jones, P.G. & Inouye, M. (1994). The cold - shock response - a hot topic. *Molecular Microbiology* **11** (5), 811 - 818.

Krause, R.A. & Hyre, R.A. (1975). Blitecast: A computerized forecast of potato late blight. *Plant Disease Reporter* **59**, 95 - 9 8.

Lelliot, R.A. & Stead, D.E. (1987). Methods for the Diagnosis of Bacterial Diseases of Plants. Oxford: Blackwell Scientific Publications.

Madden, L., Pennypacker, S.P. & MacNab, A.A. (1978). FAST, a forecast system for *Alternaria solani* on tomato. *Phytopathology* **68**, 1354 - 1358

Mills, W.D. (1944). Efficient use of sulfur dusts and sprays during rain to control apple scab. *Cornell Extension Bulletin No. 630* Cornell University.

Parvin, D.W. Jr., Smith, D.H. & Crosby, F.L. (1974). Development and evaluation of a computerized forecasting method for Cercospora leaf spot of peanuts *Phytopathology* **64**, 385 - 388.

Preece, T.F. & Smith, L.P. (1961). Apple scab infection weather in England and Wales, 1956 - 60. *Plant Pathology* **10**, 43 - 51

Robertson, S, Brokenshire, T, Kellock, LJ, Sutton, M, Chard, J and Harling, R (1993). Bacterial spear (head) rot of calabrese in Scotland: causal organisms, cultivar susceptibility and disease control. *Proceedings Crop Protection in Northern Britain*, Dundee, Scotland 1993, 265 - 270.

Sutton, J.C., Gillespie, T.J. & Hildebrand, P.D. (1984). Monitoring weather factors in relation to plant disease. *Plant Disease* **68**, 78 - 84.

Vincelli, P.C. & Lorbeer, J.W. (1988). Comparison of predictive systems for timing the initial fungicide application to control *Botrytis* leaf blight on onion. *Plant Disease* **72**, 632 - 635

Vincelli, P.C. & Lorbeer, J.W. (1988a). Relationship of precipitation probability to infection potential of *Botrytis squamosa* on onion. *Phytopathology* **78**, 1078 - 1082.

Wallin, J.R. (1962). Summary of recent progress in predicting blight epidemics in the United States and Canada. *American Potato Journal* **39**, 306 - 312

Wilks, D.S. & Shen, K.W. (1991). Threshold Relative Humidity Duration forecasts for plant disease prediction. *Journal of Applied Meteorology* **30**, *463* - 477

Figure 5 Temperature, relative humidity and surface wetness (dew periods) at hourly intervals in calabrese. (Bush estate, Penicuik)

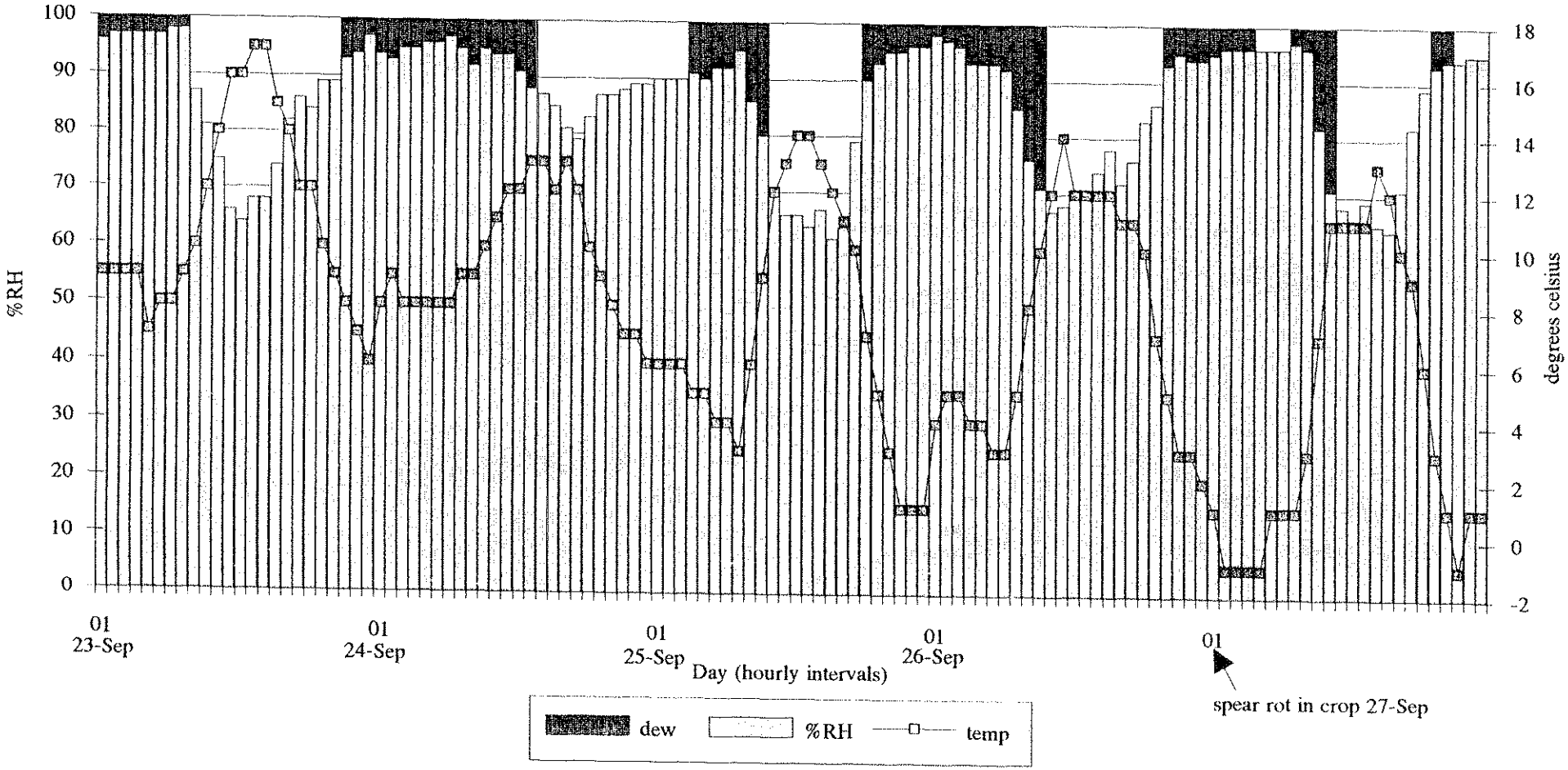


Figure 6 **Errol 2-July to 12 August 1993**

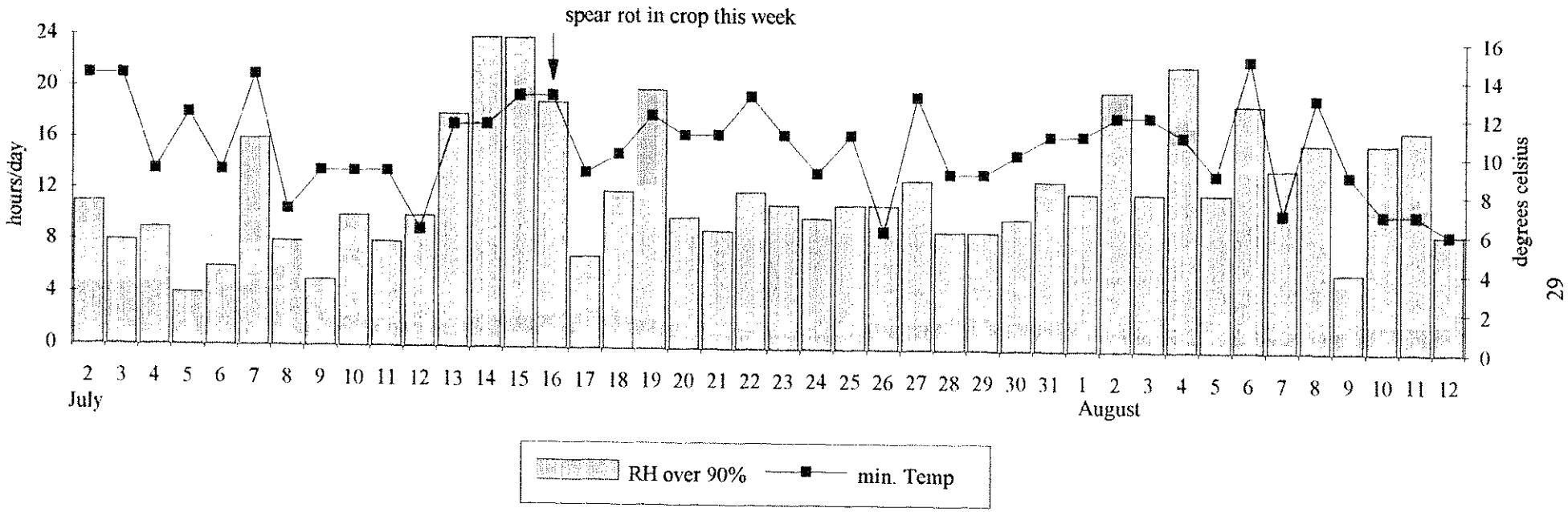


Figure 7 Montrose 17-August to 13-September 1993

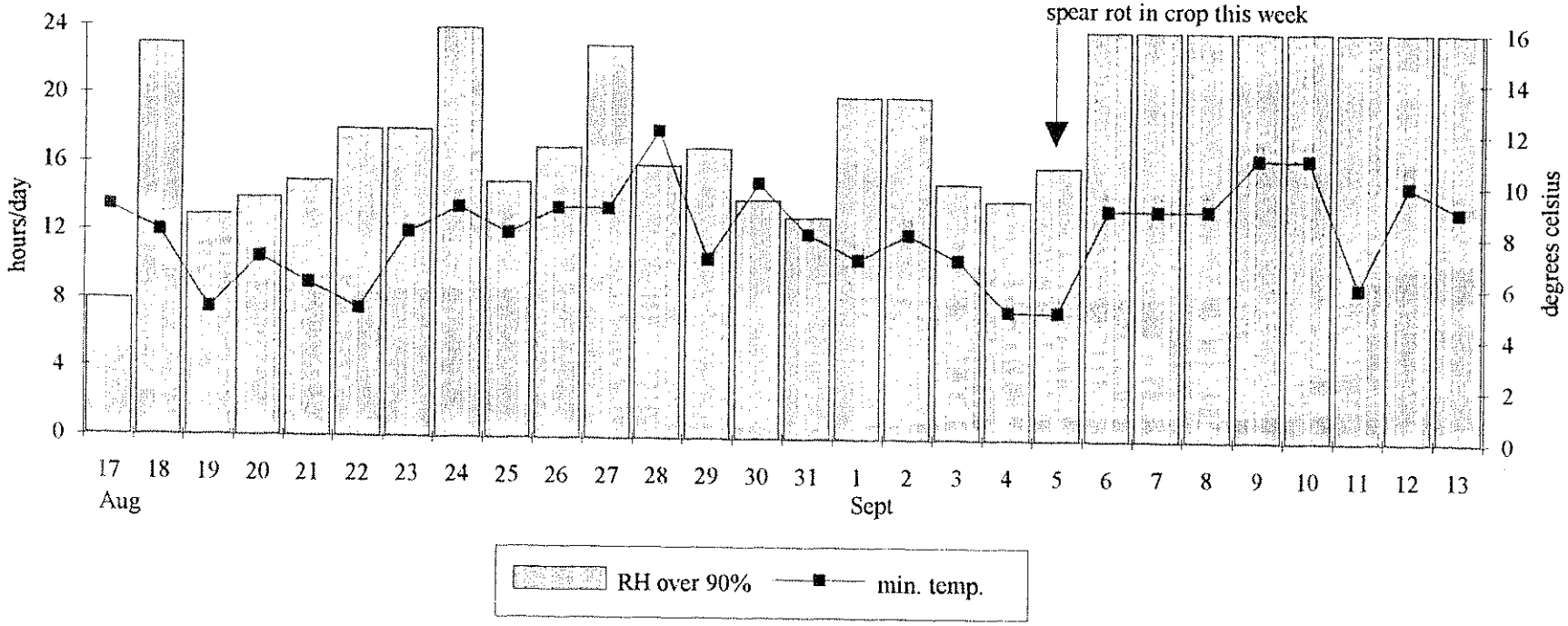


Figure 8 Errol 20-September to 10-October 1993

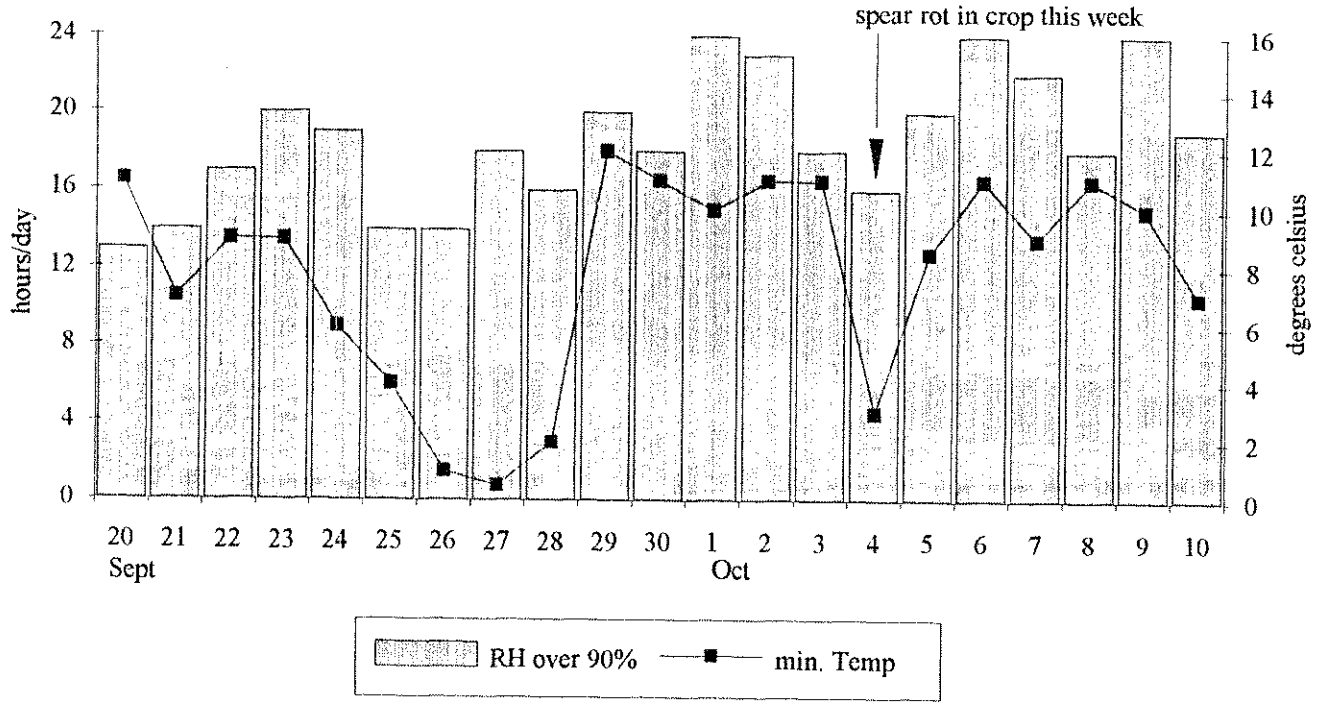


Figure 9 **Blairgowrie 20-September to 11 October 1993**

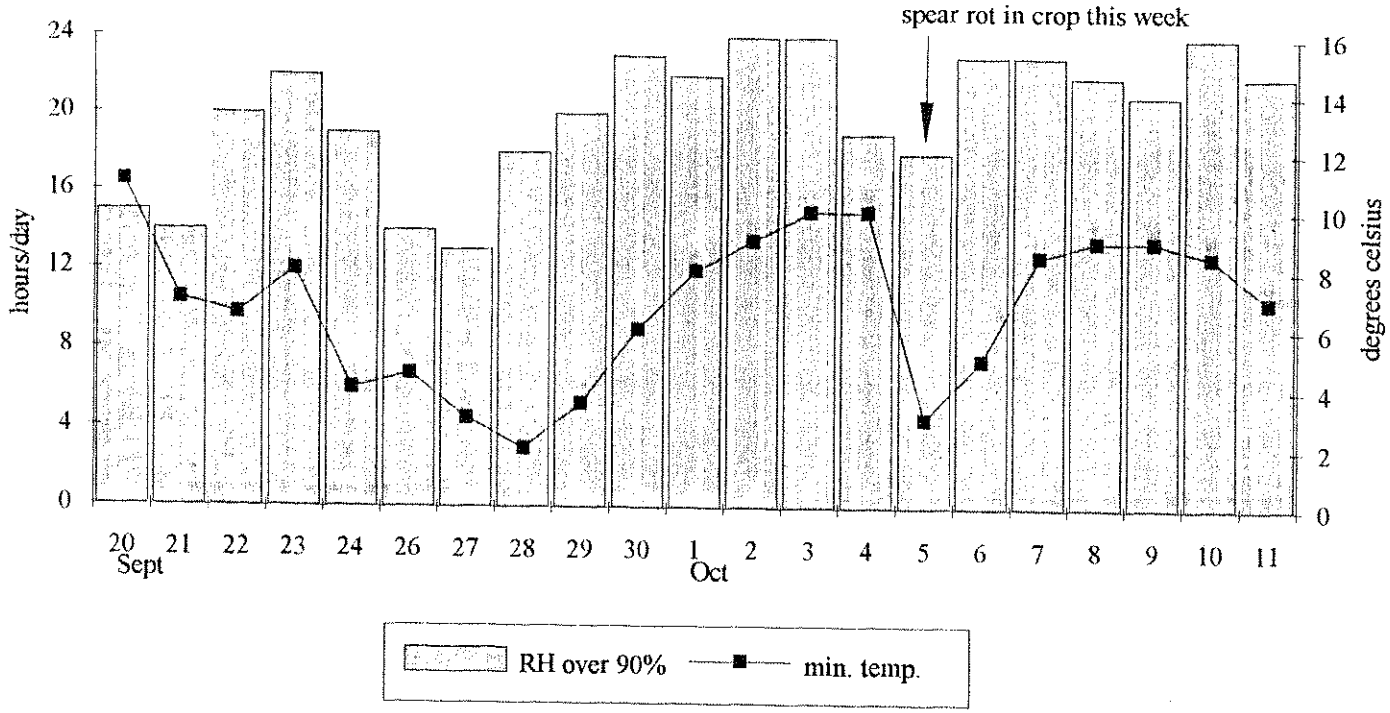
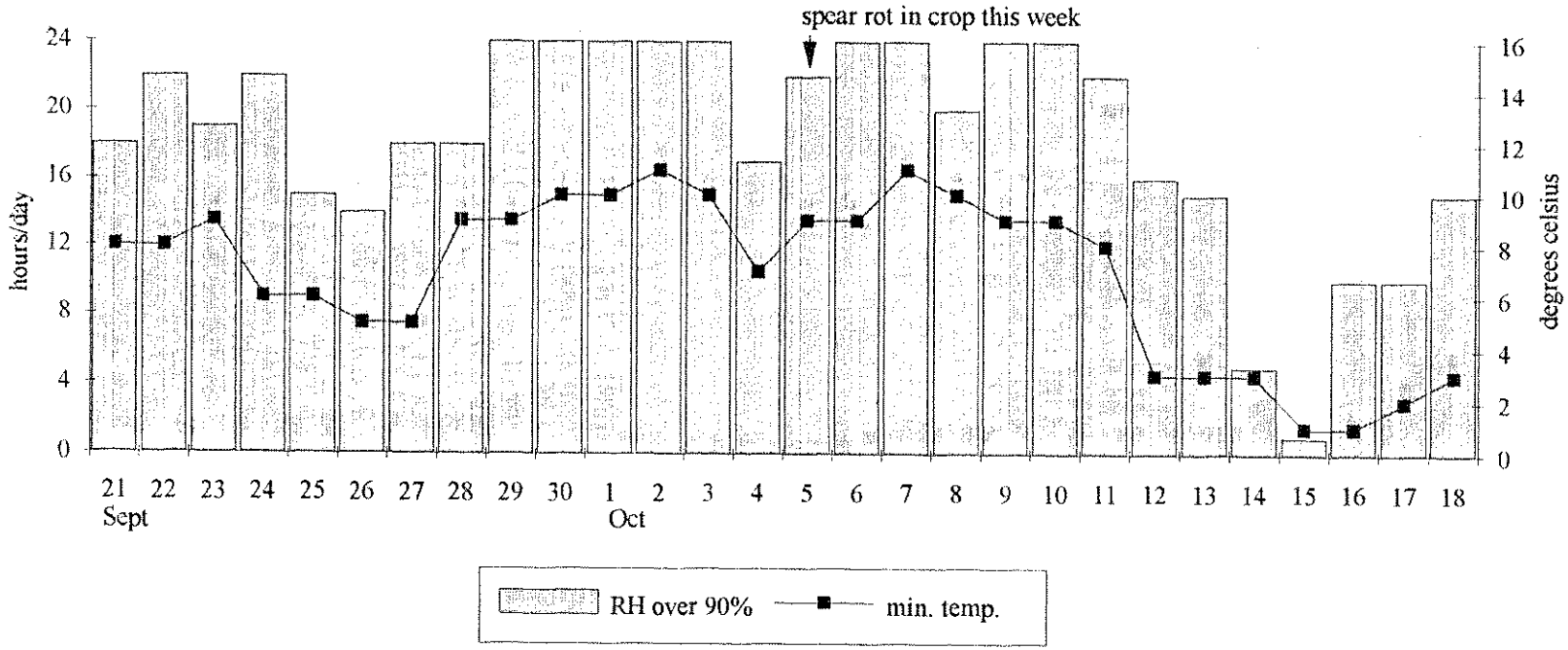


Figure 10 Carmyllie 21-September to 18-October 1993



2.3 PRESENCE OF PATHOGENIC BACTERIA ON CALABRESE HEADS: DEVELOPMENT OF A SIMPLE DIAGNOSTIC TEST FOR USE BY GROWERS

Introduction

In 2.1 we showed that calabrese heads can carry a bacterial population of great diversity; only some of these bacteria are capable of causing spear rot. To aid disease management, it is important to know if pathogenic bacteria are present on the maturing heads; unfortunately the antisera which were raised for this purpose, in the form of a rapid assay by conjugation to *Staphylococcus aureus*, were unsuitable for various reasons as discussed in 2.1.

During the reviewing process of this project an alternative method for the identification of the presence of pathogenic bacteria was proposed. It was felt by the HDC review panel that any such test should be simple, rapid and available for use by growers themselves. Immature spears carry populations of pathogenic bacteria which multiply under conducive environmental conditions; spear rot may be found on immature spears themselves under the right conditions. In this section we test the proposition that by removing immature spears from the growing crop and placing them under conditions which favour spear rot development, we can induce symptoms and thereby identify the presence of pathogenic bacteria in the crop before disease is seen. This is *not* the same as saying disease *will occur* if pathogens are shown to be present; after sampling the immature spears, the bacteria present in the crop *require the right environmental conditions* to actually cause disease.

The incubation of young field grown spears to detect pathogenic organisms was first carried out during the latter half of the 1993 growing season. In this case, immature spears from commercial crops were collected on a weekly basis and kept in contact with water soaked sterile lints in magenta vessels (as described in 2.1). These were incubated at 20°C day/10°C night (16 hour photoperiod) as for the pathogenicity tests. Some of these immature spears developed spear rot. These tests had shown that it was possible to demonstrate the presence of potential rot causing organisms in a crop in which disease then occurred.

During the 1994 growing season a more extensive programme of similar tests was carried out. A more simplistic method was adopted with the idea that this should ultimately be a diagnostic test for use by growers themselves; this test is described below.

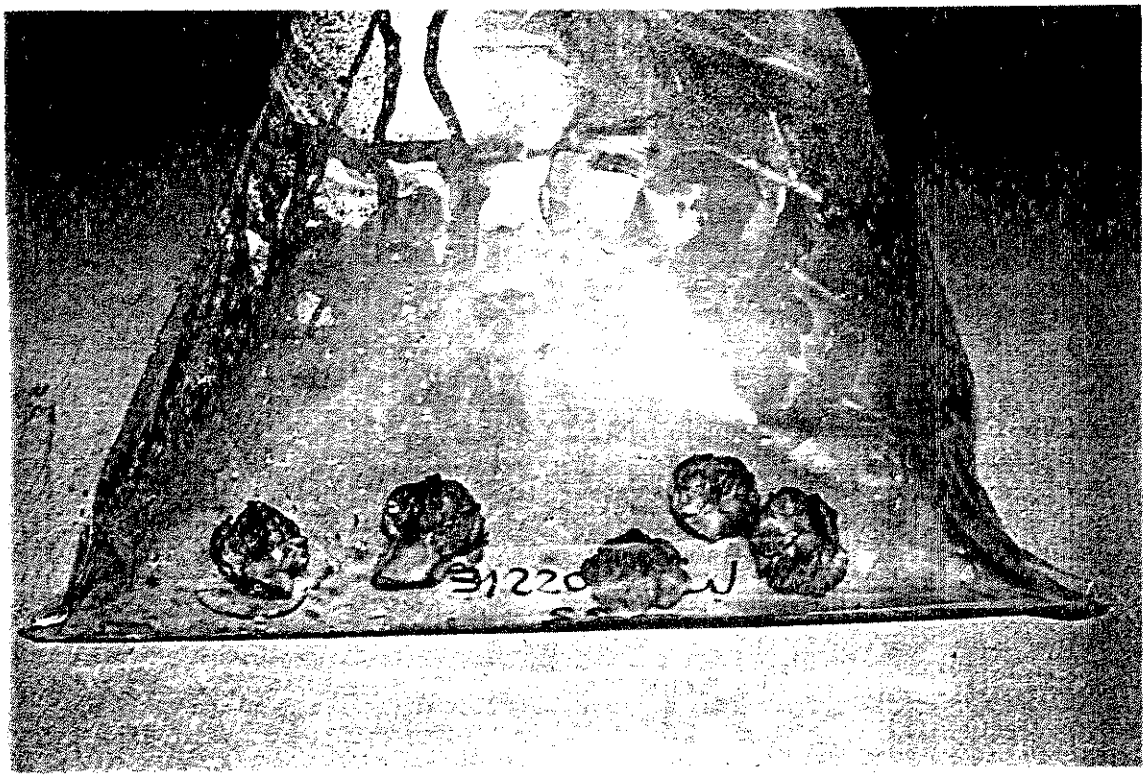
Materials and Methods

Young spears, or portions of spears of more mature heads, were incubated in polythene bags in conditions of high and low temperatures as might be found in a kitchen and in a refrigerator. Half the sample was placed in a Fison's environmental cabinet, set to a temperature regime of 20°C day/4°C night (16 hour photoperiod). The other half of the sample was left outside in a shady position and was therefore subject to natural day/night temperature fluctuations. The polythene bags were sealed by a knot at the neck after trapping air by inflation in the bag. This was to provide a large internal surface area in which water condensed and also to reduce spear to spear contact. Every week, from 15/6/94 to 9/9/94, samples of 12 spears each from commercial crops were sent by post to our laboratory in Edinburgh and arrived the next day. In total 100 samples were received from Angus district and Tayside. Three representative areas were sampled: coastal (Balstout and Wester Rossie sites, near Montrose), riverside (Waterybutts, near Errol) and inland (Firth at Carmillie; Westfield and Slatefield, near Forfar). All the successive plantings at these sites were sampled. The spear development and maturation period available for assessment varied between 3 and 5 weeks for each planting.

Results and Discussion

Following incubation of the spears, many samples developed spear rot symptoms, commencing with soft water soaked regions which went on to give a black spreading rot. The rapidity of this appearance varied but was generally evident after only 4 days incubation (Fig. 11). Significantly, many samples remained healthy, even after 7 days incubation (Fig. 12). Generally there was no difference in the detection of symptoms between the two incubation regimes (i.e. in growth cabinets or outside); sometimes symptoms were more advanced on spears kept in the growth cabinets where environmental conditions were controlled.

At the end of sampling, the results were compared with the appearance of spear rot in the sampled crop. The disease only appeared in seven of the plantings and often at a low level (Table 3); in these cases, the presence of the disease - causing organisms had been demonstrated before the appearance of disease in the field (Table 3). However in many other cases, incubated spears developed spear rot without subsequent appearance of disease in the crop (false positives). This meant that field conditions were not conducive to disease at that time. Of the samples which remained healthy within the bags, spear rot did not subsequently develop in the field. This allayed fears that even uncontaminated spears would rot given these conditions in high humidity and high temperature, and also that the test would fail to indicate the presence of pathogens in crops which subsequently developed disease (false negatives).



Figs 11 and 12: Simple diagnostic test to detect presence of pathogenic bacteria in the crop
Immature calabrese heads are incubated in sealed polythene bags
Fig 11 (top): Rotted heads after 4 days (pathogens present)
Fig 12 (bottom): Healthy heads after 7 days (pathogens not present)

Table 3 Disease on incubated spears before the first appearance of disease in the sampled crop

*total of both bags

Site	Planting	Sample date	*no. (%) of diseased spears in bags	Disease (%) in sampled crop 7 days later
Waterybutts	5	25-Jul	0	0
Waterybutts	5	01-Aug	40	0
Waterybutts	5	08-Aug	20	25
Balstout	5	29-Jul	70	0
Balstout	5	05-Aug	100	0
Balstout	5	12-Aug	10	0
Balstout	5	18-Aug	40	5
Waterybutts	6	01-Aug	50	15
Wester Rossie	3	05-Aug	0	0
Wester Rossie	3	12-Aug	70	0
Wester Rossie	3	18-Aug	100	2
Wester Rossie	4	05-Aug	0	0
Wester Rossie	4	12-Aug	80	0
Wester Rossie	4	18-Aug	60	2
Wester Rossie	5	12-Aug	0	0
Wester Rossie	5	18-Aug	100	0
Wester Rossie	5	26-Aug	90	0
Wester Rossie	5	02-Sep	80	10
Firth	n/a	12-Aug	100	0
Firth	n/a	19-Aug	100	2

This method proved to be a simple, rapid and inexpensive method to demonstrate the presence of spear rot organisms in the growing crop. It does not however follow that the infected crop will go on to develop disease in the field: this depends upon the weather conditions between the time of sampling and spear maturation. For the reasons described in 2.2, it has not been possible within this project to provide the kind of forecasting scheme which predicts whether disease will or will not occur. The test alerts the grower to the presence of pathogenic organisms in the crop; this information can be used to help decide whether treating the crop with bactericide, or preparing to harvest as soon as possible, would be justified.

Proposed use of the diagnostic test. To carry out the tests, 10 to 20 clean, freshly cut spears should be taken at random through the crop. Random sampling is essential because field observations show that spear rot spreads from small discrete foci often spread throughout the crop. Spears can be sampled any time from the stage of 1.5 cm diameter. They are placed in polythene bags, 5 spears to a bag approximately 30 by 45 cm, the bag inflated slightly by mouth and tied at the neck. During the day, the bag should be kept in a warm place such as a kitchen, and then transferred to a refrigerator overnight. Additional bags may also be left out in the shade, preferably off the ground near the crop, thereby subjecting the spears to similar temperature changes as in the field. Symptoms become evident after 3 to 4 days if pathogenic bacteria are present.

If pathogenic bacteria were detected on the young spears, as indicated by rotting symptoms, and the weather following sampling shows/or is predicted to show large and sharp drops in day/night temperatures leading to persistent dew, or there is persistent rainfall, the inexpensive two - spray programme with copper oxychloride (Cuprokylt) should be commenced immediately. This consists of a spray for the developing spears at about 2.0 - 2.5cm diameter, and a second spray 7 days later; both sprays should be of 5 kg product/ha in 600 l water.

As a predictive tool on its own, the test showed too many false positives (i.e. pathogens were detected as present but disease did not develop subsequently in the crop) for growers to rely on it as an indicator of disease. However, most importantly, we did not detect any false negatives, i.e. pathogens were not demonstrated in the test but disease subsequently developed in the crop. Because of the difficulties with forecasting for this disease, and because these are data from one season and a limited number of crops, we cannot be confident about recommending growers take *no* action if *no* pathogens are detected. Growers may wish to sample and test again as the crop matures; as shown in 2.2, disease can occur in only 3 days from the time of arrival of the bacteria under conditions of ideal temperature and humidity, so growers would need to keep a close eye on the weather as the crop matures. We would like to see growers evaluating the method for themselves and accumulating more experience with it before we can make a recommendation for no action.

2.4 DISEASE CONTROL

Introduction

During the course of the project we had the opportunity to test two new methods of disease control:

1) *New chemical products.* Kasugamycin is an antibiotic with a wide spectrum of activity against a number of bacterial (and fungal) diseases, including Crucifer black rot (*Xanthomonas campestris pv. campestris*) and soft rot (*Erwinia carotovora ssp. carotovora*). It is a product of Hokko Chemical Industry Company Ltd., Tokyo, Japan. We requested, and were kindly sent, small samples of two products: Kasumin ® Liquid (a.i. kasugamycin 2%) and Kasumin Bordeaux WP® (also known as Kasuran WP®; kasugamycin 5% + copper oxychloride 75.6%).

2) *Use of a plastic mulch.* As well as being an excellent means of weed control (used in organic production) a mulch will eliminate rain splashing of soil and debris onto the growing crop. This will cause a reduction in a major source of inoculum from pathogens which are soil - borne or have a soil - borne phase in their life cycle, for example the fungal pathogens *Botrytis cinerea* and *Alternaria* species, and bacteria such as *Pseudomonas* and *Erwinia*. In a trial in Canada, a straw mulch around the base of a calabrese crop significantly reduced spear rot (Paul Hildebrand, personal communication).

Materials and Methods

1) *New chemical products.* Plants of cv Skiff were planted out on 25/6/92 at Castle Huntly, a site in the Tay Valley near Dundee. Spacing was 25 cm between plants and 50 cm between rows. Each plot consisted of 16 plants in each of 3 rows and plots were replicated 3 times for each treatment. Plots were treated with preparations of fertiliser and pesticides according to usual commercial practice.

On 14/9/92 (heads 2 - 2.5cm dia.) and 21/9/92 Kasumin Liquid, Kasumin Bordeaux and Cuprokylt were applied with hand held sprayers at rates of 2 litres/ha, 800g/ha and 5kg/ha respectively in 600 litres of water/ha with wetter (Agral). Control plots were left untreated. When heads began to develop, the trial was irrigated daily for one hour from overhead sprinklers to encourage spear rot. The trial was assessed for spear rot symptoms on 5/10/92.

2) *Use of a plastic mulch.* This experiment was carried out at the SAC/University of Edinburgh field station at Bush Estate, Penicuik, during 1993 (see 2.2 for experimental details). Plantings were checked daily for spear rot and assessed according to a disease index which takes account of disease

severity. Heads were assessed when wet from dew: it is more difficult to assess disease when heads are dry. When dry, the intermediate stages of disease from watersoaking to brown rot are less clear; watersoaking is not evident and appears instead as "pepper - spotting"; these are tiny black necrotic lesions over the florets. This represents bacterial infection which has not yet turned into a soft rot.

Mature and immature heads were scored according to the following categories: 0 - healthy; 1 - watersoaking / loss of waxy bloom; 2 - watersoaking becomes brown and covers a considerable area of the spear; 3 - brown soft rot; 4 - black soft rot.

Each head was assigned to one of these categories and a percentage disease index calculated by the following equation:

$$\frac{\Sigma \text{ (disease category x no. of heads within that category)}}{\text{total no. of heads x 4}} \times 100$$

Results and Discussion

New chemical products

Table 4: Chemical control of spear rot, Castle Huntly, Dundee 1992

	percentage disease index				
treatment	**rot[1]**	**watersoaked**	**brown[2]**	**total with symptoms**	**no symptoms**
Kasumin Liquid (40g a.i. kasugamycin/ ha)	58.3	18.5	2.8	79.6	20.4
Kasumin-Bordeaux (40g a.i. kasugamycin/ ha 605g a.i. copper oxychloride/ha)	33.3	21.3	13.9	68.5	31.5
Cuprokylt (2,500g a.i. copper oxychloride/ha)	25.7	23.1	23.4	72.2	27.8
Control (untreated)	55.7	18.9	7.3	81.9	18.1
SED +/-	16.9	5.1	8.4	15.5	15.5

[1] black/brown soft rot

[2] these spears did not show soft rot but appeared to have dried out after previously showing watersoaking

Products containing copper afforded the best control: plants treated with Kasumin - Bordeaux or Cuprokylt had fewer rotted spears than plants from both the untreated control and the kasugamycin (Kasumin Liquid) only (Table 4). Note, however from Table 4 that that the amount of copper oxychloride applied per hectare with Cuprokylt was much greater than with Kasumin - Bordeaux. In control plots, about 50% of plants became affected by spear rot, defined as a black/brown soft rot. The level of variation in the trial was high, reflecting patchy disease occurrence. The trial was irrigated to ensure disease, but this also represented a severe test of the products.

Use of a plastic mulch

No spear rot occurred on the first (May) planting. Spear rot appeared on 27/9/93 on Skiff in the second (June) planting in unmulched plots. Watersoaking appeared in Shogun in the unmulched treatment 2 days later but spear rot was not apparent in this treatment until 7/10. This was also the date when spear rot appeared in the mulched plot of Skiff. The spears of Shogun planted through mulch did not show rotting symptoms until 4 days later, i.e. 11/10. The overall incidence of disease in this second planting was as follows: Skiff (no mulch) 12 rotted heads; Skiff (mulched) 5 rotted heads; Shogun (no mulch) 5 rotted heads; Shogun (mulched) 2 rotted heads.

Spear rot appeared in the third (July) planting on 20/10/93; disease assessed on this date is shown in Table 5.

Table 5: Control of spear rot with a plastic mulch, Bush Estate, Penicuik, 1993 (third planting)

Treatment	Total no. of heads	% mature heads	% spear rot[1] mature heads	% spear rot[1] immature heads	mean % spear rot
Shogun + mulch	178	30.3	20.4	10.3	15.3
Shogun - mulch	158	14.6	53.2	25.4	39.3
Skiff + mulch	171	2.3	31.2[2]	15.7	23.4
Skiff - mulch	192	0	-[3]	35.3	35.3

[1] using disease index

[2] sample size only 4

[3] no mature heads

Spears grown through the plastic mulch matured earlier. In the mulched plots, compared to unmulched, there was approximately 50% less spear rot in immature heads of both cultivars and in mature heads of Shogun. There were too few mature heads of Skiff for comparison. Skiff was more susceptible than Shogun and this became more evident as Skiff matured. The lower incidence of disease in mulched plots is consistent with the explanation that soil splash is a major source of bacterial inoculum. Mulching also, however, reduces weed competition, retains soil moisture and

solarizes the roots - these are factors which may enhance the plants' ability to withstand disease. There was no difference in nitrogen or mineral content (including boron) of tissue of mulched and unmulched plants of Shogun and Skiff (results not shown).

2.5 QUESTIONNAIRE DISTRIBUTED TO GROWERS

In January 1993, 257 questionnaires (Fig 13) were sent to growers in England and Scotland, requesting information about the 1992 growing season. The objective was to collate anecdotal information on spear rot occurrence in relation to aspects of crop husbandry, eg cultivar grown, irrigation, application of copper oxychloride etc. Growers were also asked to supply rainfall data, if available.

Forty five completed questionnaires were received from Scotland (21) and England (24) . The areas represented were Fife (14 replies), Lincolnshire (12), Norfolk (8), Lothians, Borders, Angus, and Perthshire (7) and Lancashire, Derbyshire and Cambridgeshire (4). Overall, this represented around 277 plantings (a planting is a field, or part of a field, with one variety) from the 1992 growing season, 101 from Scotland, 176 from England and covered 18 varieties. Marathon was the most extensively grown variety (144 plantings), then Shogun (46), Greenbelt (30) and Caravelle (15). The other varieties represented were Cruiser (8 plantings), Arcadia (6), Regilio and Lazer (5 plantings each), Citation, Corvet, and Packman (3 plantings each), Skiff and Sumosun (2 plantings each) and Dixie, Emerald City, Legend, Southern Comet and Topgreen with one planting of each.

The periods when spear rot appeared in crops in Scotland and England and the growers' estimated average loss to spear rot each month is represented in Figs 14 and 15 The disease appeared first in July with losses declining in the following month. Thereafter, disease levels increased until they reached a peak in October with the same average loss (approx. 60%) recorded from both Scotland and England. The overall loss to spear rot was estimated at 26.7% in England and 32.1% in Scotland giving an average loss of around 29.4%. This equates to a loss in 1992 of around 13,950 t. marketed output with an average value of £9,577,904 (Basic Horticultural Statistics, 1993, HMSO).

From the figures supplied by the growers, Caravelle was the most susceptible variety (mean 37% loss) followed by Shogun (26.3% loss), Cruiser (19% loss) , Arcadia and Marathon (14% loss). It should be stressed these are estimates, not values derived from controlled experiments.

The average monthly rainfall figures taken from questionnaire returns are given for both Scotland and England in Fig 16. The results show that July and August in England, and August and September in Scotland, were the wettest months. Note how rainfall was much lower in October, yet growers reported greatest losses to spear rot during this month. From our results in 2.2, the most likely explanation of this is persistence of dew on the crop, as a result of increasing range between day/night temperatures, and the direct effects on disease of alternating temperatures themselves.

There was no effect on spear rot incidence which could be related to whether the crop was exposed or sheltered. Most crops were reported as being exposed and disease was not reduced in this situation when compared with sheltered crops.

Covers were only used on 9 plantings out of the 277 and were only used in the early part of the season when there was no spear rot.

In England only 22 plantings were irrigated compared to 62 in Scotland. Where crops were irrigated during heading (5 plantings in England, 34 in Scotland), 3 plantings developed the disease in England and 10 in Scotland.

Ten plantings were sprayed with copper oxychloride in Scotland but spear rot subsequently occurred in all ten; the average loss to spear rot in sprayed plantings was estimated to be was 38%. In England, 32 plantings were treated; no disease occurred in 7 of these and in the remainder losses due to spear rot averaged around 48% .

Field no.	Date of harvest	Variety	Planted or drilled?	Sheltered/ exposed	Covers: yes/no	Total Nitrogen applied: units/acre or kg/ha	Was irrigation applied: 1) after establishment, 2) only at heading, or 3) both	Time of day irrigated (am, midday,pm) and approx daily amount (in or cm)	Were copper sprays applied at heading?	If a wetter was used please state which	Spear rot: yes/no	Estimate % yield loss to spear rot
1												
2												
3												
4												
5												
6												
7												
8												
9												

Rainfall each month:

If you used copper sprays at heading did you consider them worthwhile? YES/NO

Use more sheets if necessary

Fig 13: Questionnaire sent to growers for information on spear rot in the 1992 crop

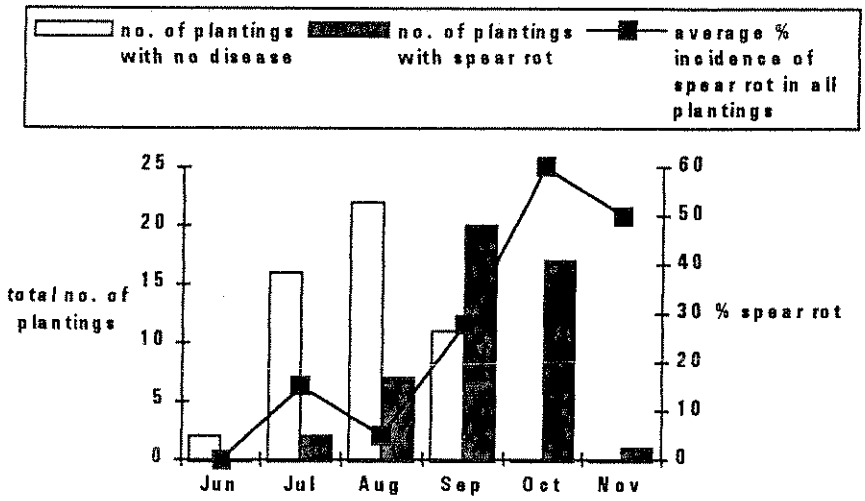


Fig 14: Growers' estimates of losses to spear rot in Scotland, 1992

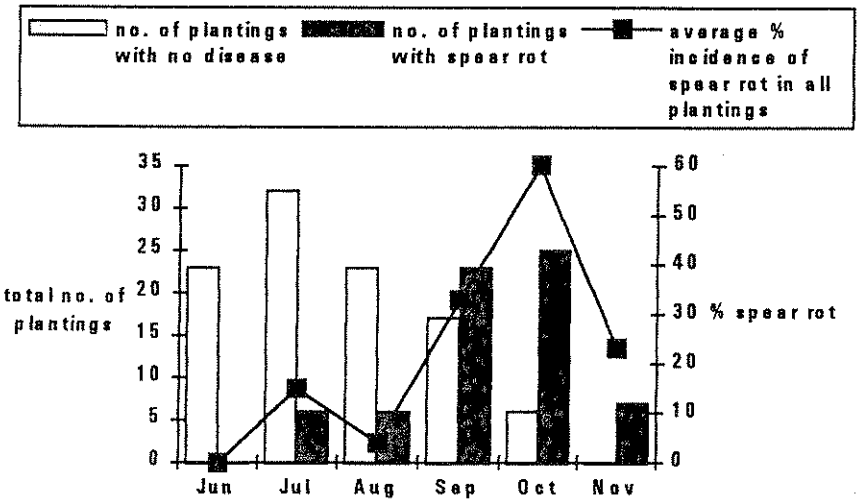


Fig 15: Growers' estimates of losses to spear rot in England, 1992

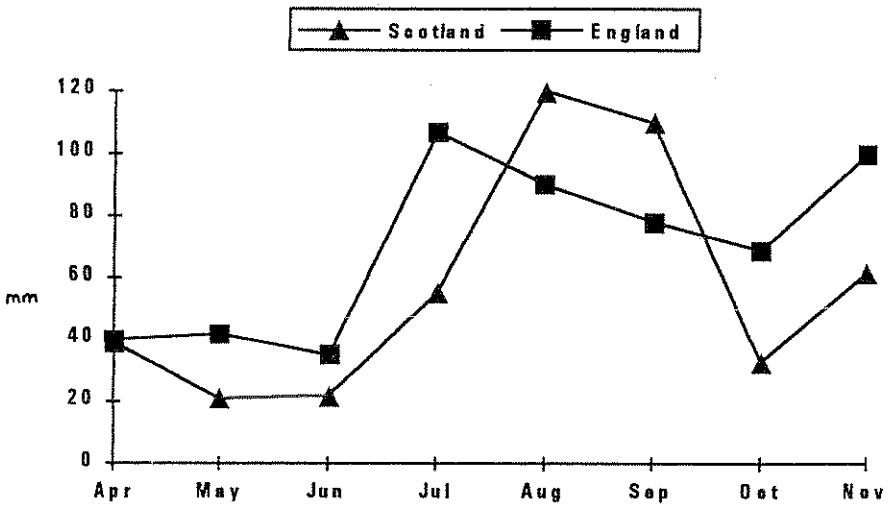


Fig 16: Average rainfall at calabrese sites in Scotland and England 1992, compiled from growers' information

3. ACKNOWLEDGEMENTS

We would like to thank the following growers for their assistance with trials: Alan and Douglas Neill, Thorngreen Farm, Blairgowrie; John Elgin, Westerton of Rossie, Montrose; John Millar, Firth Farm, Carmyllie and Ron Wilson (Gowrie Growers), Waterybutts, Errol.

We would also like to thank:

- Roy Kennedy, Nigel Lyons and John Taylor of Horticulture Research International, Wellesbourne, for their helpful discussions with parts of this work.
- Jeannette Taylor, Brian Pool for technical assistance.
- Denise Darling, Robert Simpson for help with the pathogenicity tests.

4. APPENDIX

4.1 Soil analyses from trial site, Bush Estate, Penicuik
(see following sheets)

REPORT & RECOMMENDATIONS from SOIL ANALYSIS

SAC

PLANT DISORDER

Farm sampled: CC93/498

For: BOB HARLING
CALABRASE PLOTS AT BUSH ESTATE

Field sampled: EXP PLOTS AT START

Last crop:
Next or current crop: . . .

Laboratory ref no: 93070326

Analysis	*Value*		*Recommendations*
pH	6.0		**Lime requirement (as ground limestone) for:** Arable Crops/Temporary Grass: 3 t/ha (1.2 t/ac) Permanent Grassland: no lime required
Extractable	mg/l	Status	
Phosphorus	23	HIGH	
Potassium	121	MOD	
Magnesium	137	MOD	
Sulphur	8.1	MOD	
Nit-N cont	18		
Amm-N cont	6.1		
Manganese	3.7	MOD	
Copper	7.4	MOD	
Boron	0.83	MOD	
Organic Matter (%) - Loss on Ignition	9.7		

General Comments

From: Crop Clinic, Edinburgh,
Reported: 23-Jul-93

ANALYSIS CARRIED OUT BY SAC

REPORT & RECOMMENDATIONS from SOIL ANALYSIS

SAC

PLANT DISORDER

Farm sampled: BUSH EXPERIMENTAL PLOTS

For: ROB HARLING, CST.
BUSH EXPERIMENTAL PLOTS
CALABRESE

Field sampled: BREAK 1 MULCHED

Last crop:

Next or current crop:

Laboratory ref no: 93111303

Analysis	*Value*		*Recommendations*
pH	6.1		**Lime requirement (as ground limestone) for:** Arable Crops/Temporary Grass: 2 t/ha (0.8 t/ac) Permanent Grassland: no lime required
Extractable	mg/l	Status	
Phosphorus	24	HIGH	
Potassium	101	MOD	
Magnesium	158	MOD	
Sulphur	6.7	MOD	
Manganese	2.7	MOD	
Copper	7.8	MOD	
Boron	0.76	MOD	
Molybdenum	0.040	LOW	
Total	mg/kg	Status	
Nitrogen	2301		
Organic Matter (%) - Loss on Ignition	9.6		

General Comments

From: Crop Clinic, Edinburgh,
Reported: 01-Dec-93

ANALYSIS CARRIED OUT BY SAC

REPORT & RECOMMENDATIONS from SOIL ANALYSIS

SAC

PLANT DISORDER

Farm sampled: **BUSH ESTATE**

For: R HARLING
BUSH ESTATE
CALABRESE PLOTS
RESAMPLE

Field sampled: BREAK 1 MULCHED

Last crop: ...
Next or current crop: ...

Laboratory ref no: 93120248

Analysis		*Value*	Status
pH		6.0	
Extractable			Status
Amm-N cont	mg/kg	8.6	
NIT-N cont	mg/kg	1.6	
Total			Status
Nitrogen		2250	

Recommendations

Lime requirement (as ground limestone) for:
Arable Crops/Temporary Grass: 3 t/ha (1.2 t/ac)
Permanent Grassland: no lime required

General Comments

From: Crop Clinic, Edinburgh,
Reported: 25-Feb-94

ANALYSIS CARRIED OUT BY SAC

REPORT & RECOMMENDATIONS from SOIL ANALYSIS

SAC

PLANT DISORDER

Farm sampled: **BUSH EXPERIMENTAL PLOTS**

For: ROB HARLING, CST.
BUSH EXPERIMENTAL PLOTS
CALABRESE

Field sampled: **BREAK 1 UNMULCHED**

Last crop:

Next or current crop:

Laboratory ref no: 93111304

Analysis	*Value*		*Recommendations*
pH	6.2		**Lime requirement (as ground limestone) for:** Arable Crops/Temporary Grass: no lime required Permanent Grassland: no lime required
Extractable	mg/l	Status	
Phosphorus	24	HIGH	
Potassium	132	MOD	
Magnesium	165	MOD	
Sulphur	5.7	LOW	
Manganese	2.8	MOD	
Copper	8.4	MOD	
Boron	0.76	MOD	
Molybdenum	0.050	MOD	
Total	mg/kg	Status	
Nitrogen	2563		
Organic Matter (%) - Loss on Ignition	9.4		

General Comments

From: Crop Clinic, Edinburgh,
Reported: 01-Dec-93

ANALYSIS CARRIED OUT BY SAC

REPORT & RECOMMENDATIONS from SOIL ANALYSIS

SAC

PLANT DISORDER

Farm sampled: BUSH ESTATE

For: R HARLING
BUSH ESTATE
CALABRESE PLOTS
RESAMPLE

Field sampled: BREAK 1 NO MULCH

Last crop: ...
Next or current crop: ...

Laboratory ref no: 93120249

Analysis		*Value*	*Recommendations*
pH		6.2	Lime requirement (as ground limestone) for: Arable Crops/Temporary Grass: no lime required Permanent Grassland: no lime required
Extractable		Status	
Amm-N cont	mg/kg	9.6	
NIT-N cont	mg/kg	1.8	
Total		Status	
Nitrogen		2200	

General Comments

From: Crop Clinic, Edinburgh,
Reported: 25-Feb-94

ANALYSIS CARRIED OUT BY SAC

REPORT & RECOMMENDATIONS from SOIL ANALYSIS

SAC

PLANT DISORDER

Farm sampled: **BUSH EXPERIMENTAL PLOTS**

For: ROB HARLING, CST.
BUSH EXPERIMENTAL PLOTS
CALABRESE

Field sampled: **BREAK 2 MULCHED**

Last crop:

Next or current crop:

Laboratory ref no: 93111305

Analysis	*Value*		*Recommendations*
pH	6.0		**Lime requirement (as ground limestone) for:** Arable Crops/Temporary Grass: 3 t/ha (1.2 t/ac) Permanent Grassland: no lime required
Extractable	mg/l	Status	
Phosphorus	22	HIGH	
Potassium	175	MOD	
Magnesium	149	MOD	
Sulphur	9.1	MOD	
Manganese	2.1	LOW	
Copper	7.5	MOD	
Boron	0.75	MOD	
Molybdenum	0.040	LOW	
Total	mg/kg	Status	
Nitrogen	2416		
Organic Matter (%) - Loss on Ignition	9.5		

General Comments

From: Crop Clinic, Edinburgh,
Reported: 01-Dec-93

ANALYSIS CARRIED OUT BY SAC

REPORT & RECOMMENDATIONS from SOIL ANALYSIS

SAC

PLANT DISORDER

Farm sampled: **BUSH ESTATE**

For: **R HARLING**
BUSH ESTATE
CALABRESE PLOTS
RESAMPLE

Field sampled: **BREAK 2 MULCHED**

Last crop: ...
Next or current crop: ...

Laboratory ref no: 93120250

Analysis		*Value*	*Recommendations*
pH		6.0	Lime requirement (as ground limestone) for: Arable Crops/Temporary Grass: 3 t/ha (1.2 t/ac) Permanent Grassland: no lime required
Extractable			Status
Amm-N cont	mg/kg	9.6	
NIT-N cont	mg/kg	2.6	
Total			Status
Nitrogen		2700	

General Comments

From: Crop Clinic, Edinburgh,
Reported: 25-Feb-94

ANALYSIS CARRIED OUT BY SAC

REPORT & RECOMMENDATIONS from SOIL ANALYSIS

SAC

PLANT DISORDER

Farm sampled: **BUSH EXPERIMENTAL PLOTS**

For: **ROB HARLING, CST.**
BUSH EXPERIMENTAL PLOTS
CALABRESE

Field sampled: **BREAK 2 UNMULCHED**

Last crop:

Next or current crop:

Laboratory ref no: 93111306

Analysis	*Value*		*Recommendations*
pH	6.0		**Lime requirement (as ground limestone) for:** Arable Crops/Temporary Grass: 3 t/ha (1.2 t/ac) Permanent Grassland: no lime required
Extractable	mg/l	Status	
Phosphorus	20	HIGH	
Potassium	106	MOD	
Magnesium	144	MOD	
Sulphur	10	MOD	
Manganese	2.0	LOW	
Copper	7.1	MOD	
Boron	0.73	MOD	
Molybdenum	0.050	MOD	
Total	mg/kg	Status	
Nitrogen	2374		
Organic Matter (%) - Loss on Ignition	9.6		

General Comments

From: Crop Clinic, Edinburgh,
Reported: 01-Dec-93

ANALYSIS CARRIED OUT BY SAC

REPORT & RECOMMENDATIONS from SOIL ANALYSIS

SAC

PLANT DISORDER

Farm sampled: **BUSH ESTATE**

For: R HARLING
BUSH ESTATE
CALABRESE PLOTS
RESAMPLE

Field sampled: **BREAK 2 NO MULCH**

Last crop: ...
Next or current crop: ...

Laboratory ref no: 93120251

Analysis		*Value*	Status
pH		6.1	
Extractable			Status
Amm-N cont	mg/kg	12	
NIT-N cont	mg/kg	3.3	
Total			Status
Nitrogen		2700	

Recommendations

Lime requirement (as ground limestone) for:
Arable Crops/Temporary Grass: 2 t/ha (0.8 t/ac)
Permanent Grassland: no lime required

General Comments

From: Crop Clinic, Edinburgh,
Reported: 25-Feb-94

ANALYSIS CARRIED OUT BY SAC

REPORT & RECOMMENDATIONS from SOIL ANALYSIS

SAC

PLANT DISORDER

Farm sampled: **BUSH EXPERIMENTAL PLOTS**

For: **ROB HARLING, CST.**
BUSH EXPERIMENTAL PLOTS
CALABRESE

Field sampled: **BREAK 3 MULCHED**

Last crop:

Next or current crop:

Laboratory ref no: 93111307

Analysis	*Value*		*Recommendations*
pH	6.1		**Lime requirement (as ground limestone) for:** Arable Crops/Temporary Grass: 2 t/ha (0.8 t/ac) Permanent Grassland: no lime required
Extractable	mg/l	Status	
Phosphorus	20	HIGH	
Potassium	101	MOD	
Magnesium	163	MOD	
Sulphur	5.4	LOW	
Manganese	1.9	LOW	
Copper	7.6	MOD	
Boron	0.74	MOD	
Molybdenum	0.050	MOD	
Total	mg/kg	Status	
Nitrogen	2406		
Organic Matter (%) - Loss on Ignition	9.7		

General Comments

From: Crop Clinic, Edinburgh,
Reported: 01-Dec-93

ANALYSIS CARRIED OUT BY SAC

REPORT & RECOMMENDATIONS from SOIL ANALYSIS

SAC

PLANT DISORDER

Farm sampled: BUSH ESTATE

For: R HARLING
BUSH ESTATE
CALABRESE PLOTS
RESAMPLE

Field sampled: BREAK 3 MULCHED

Last crop: ...
Next or current crop: ...

Laboratory ref no: 93120252

Analysis		*Value*	*Recommendations*
pH		6.0	Lime requirement (as ground limestone) for: Arable Crops/Temporary Grass: 3 t/ha (1.2 t/ac) Permanent Grassland: no lime required
Extractable			Status
Amm-N cont	mg/kg	8.6	
NIT-N cont	mg/kg	3.6	
Total			Status
Nitrogen		2550	

General Comments

From: Crop Clinic, Edinburgh,
Reported: 25-Feb-94

ANALYSIS CARRIED OUT BY SAC

REPORT & RECOMMENDATIONS from SOIL ANALYSIS

SAC

PLANT DISORDER

Farm sampled: **BUSH EXPERIMENTAL PLOTS**

For: ROB HARLING, CST.
BUSH EXPERIMENTAL PLOTS
CALABRESE

Field sampled: BREAK 3 UNMULCHED

Last crop:
Next or current crop:

Laboratory ref no: 93111308

Analysis	*Value*		*Recommendations*
pH	6.1		**Lime requirement (as ground limestone) for:** Arable Crops/Temporary Grass: 2 t/ha (0.8 t/ac) Permanent Grassland: no lime required
Extractable	mg/l	Status	
Phosphorus	20	HIGH	
Potassium	116	MOD	
Magnesium	151	MOD	
Sulphur	7.4	MOD	
Manganese	2.1	LOW	
Copper	7.6	MOD	
Boron	0.71	MOD	
Molybdenum	0.050	MOD	
Total	mg/kg	Status	
Nitrogen	2511		
Organic Matter (%) - Loss on Ignition	9.9		

General Comments

From: Crop Clinic, Edinburgh,
Reported: 01-Dec-93

ANALYSIS CARRIED OUT BY SAC

REPORT & RECOMMENDATIONS from SOIL ANALYSIS

SAC

PLANT DISORDER

Farm sampled: BUSH ESTATE

For: R HARLING
BUSH ESTATE
CALABRESE PLOTS
RESAMPLE

Field sampled: BREAK 3 NO MULCH

Last crop:
Next or current crop:

Laboratory ref no: 93120253

Analysis		*Value*	
pH		6.2	
Extractable			Status
Amm-N cont	mg/kg	8.2	
NIT-N cont	mg/kg	3.9	
Total			Status
Nitrogen		2400	

Recommendations

Lime requirement (as ground limestone) for:
Arable Crops/Temporary Grass: no lime required
Permanent Grassland: no lime required

General Comments

From: Crop Clinic, Edinburgh,
Reported: 25-Feb-94

ANALYSIS CARRIED OUT BY SAC