EVALUATION OF RESISTANCE TO *ALLIUM* WHITE ROT

Project FV4

FINAL REPORT

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1 SUMMARY OF ACHIEVEMENTS

1) A novel standardised glasshouse test was developed to screen *Allium* germplasm against the white rot fungus, *Sclerotium cepivorum* (Objective 1); the test comprised five stages: (a) production of test plants in open-base modular trays, (b) production of *S. cepivorum* inoculum in a similar set of modular trays, (c) inoculation of the test plants with *S. cepivorum*, (d) growth of inoculated plants in a semi-controlled glasshouse and (e) assessment of white rot symptoms. The method had the advantages of (1) separate production of test plants and inocula, (2) minimal opportunities for roots to escape the effects of inoculum, (3) production of typical white rot symptoms, and (4) standardised recording protocols.

2) The method was successfully up-graded for large-scale screening (Objective 2).

3) One hundred and eighty accessions of UK or European *A. cepa* from the Genetic Resources Unit were screened for their reaction to white rot (Objective 3).

4) Plants which survived the primary screen were inoculated a second time (secondary screen) to test for escapes (Additional Objective).

5) Survivors from the secondary screen were transferred to the Plant Breeding Department for incorporation in a Breeding Programme (Additional Objective).

6) The 'best' and 'worst' performers from each batch of sixty accessions were collated and tested together (Additional Objective).

2 Introduction

Allium white rot (AWR) is caused by the fungus *Sclerotium cepivorum* Berk. (SC) and is the most serious soil-borne disease of onions in the UK. The pathogen attacks the root system causing losses at any stage of crop growth, and during storage; the pathogen can be extremely persistent.

Research at HRI demonstrated the high degree of control achieved by combined seed and stem base treatment with dicarboximide fungicides (Entwistle & Munasinghe, 1981) and the treatment continues to form the basis of control measures worldwide. A few years after development, the treatments became unreliable in the UK due, it was subsequently discovered, to the accelerated degradation of fungicidal active ingredient in soil (Entwistle, 1986); loss of control has since been reported from other parts of the world. Other control measures are also unreliable e.g. eradication of sclerotia by germination stimulants or soil fumigants (Adams & Johnston, 1983; Merriman *et al.*, 1980; Entwistle *et al.*, 1991; Entwistle *et al.*, 1990). 'Newer' integrated control measures using a combination of microbial control and the eradication of sclerotia by solar heating ('solarisation'), or aerobic composting offer promise but require testing in commercial conditions (Entwistle *et al.*, 1991). The absence of effective control measures highlighted the need to review the potential of host resistance for control.

There is the possibility that host resistance may provide a component of an integrated control strategy. However, laboratory and field tests give variable results due to a lack of standardisation of such factors as inoculum and environmental conditions (Semb *et al.*, 1978; Utkhede & Rahe, 1978a, b). None of the *A. cepa* cultivars in commercial use are totally resistant to white rot (Entwistle, 1990) but the literature was sufficiently promising to justify re-evaluation of the occurrence of resistance.

The Genetic Resources Unit (GRU) (formerly the Vegetable Gene Bank) at Horticultural Research International, Wellesbourne (HRIW) contained about 900 accessions of *A. cepa* and hence provided the opportunity to screen a substantial and diverse germplasm collection for resistance to white rot. Expertise at HRI provided the basis for developing a standard screening test.

Project HDC FV487 had the following objectives:

(1) Develop a standard method for screening *Allium* plants for response to *S. cepivorum*.

(2) Evaluate the standard method using selected accessions of *A. cepa*.

(3) Evaluate *A. cepa* accessions from the GRU, HRIW by means of the standard method.

3 Objectives and Results

3.1 Objective 1: Development of a standard method for screening *Allium* germplasm against *S. cepivorum*

Summary of Objective 1. A prototype method was successfully developed to screen Allium seedlings against white rot and comprised (a) the production of test plants in modular trays, (b) the production of white rot inocula, (c) inoculation of the test plants with the pathogen, (d) growth of inoculated plants in semi-controlled glasshouses, and (e) standard protocols for evaluating white rot symptoms.

a) Production of plants. Open-base modular trays (QP104; PG Horticulture Ltd., Suffolk, UK) proved to be economical and convenient for the production of standard onion seedlings. Closed base modular trays (Hassy 308) proved to be less satisfactory because of the need to transplant seedlings and the configuration of the root system being unsuitable for inoculation.

b) Production of inocula of the white rot pathogen. *S. cepivorum* hyphae produced on 1-cm discs of onion leaf scale tissue were effective and convenient to prepare; leaf scales from various parts of bulbs of each of up to 8 cultivars/hybrids were tested during the development of the test. The method was used for the remainder of the project.

Other potential forms of inocula were also tested: a) sclerotia added to the peat-based growth medium, (b) mycelial colonies produced on various agar media (2% malt extract, 5% or 10% sucrose, 5% fructose, 5% or 10% glucose, 5% or 10% pectin). Sclerotium germination (hence the production of infection hyphae) was no higher than 12% due to endogenous dormancy and was considered to be unsatisfactorily low for a standard test; success at breaking dormancy was considered unlikely within the resources of the project.

All inocula originated from a single stock of sclerotia of isolate SC9181; sclerotia were produced on onion and stored at 4°C.

c) Inoculation of plants with the white rot pathogen. Trays of 'standard' plants superimposed on trays of inocula suspended on nylon mesh proved to be highly effective for ensuring contact between inocula and roots.

d) Growth of inoculated plants in semi-controlled conditions in a glasshouse. Plants were watered according to the appearance and texture of the compost, and the needs of the plant. It was necessary to adjust for differences in water uptake following infection, by providing additional irrigation to individual modules containing healthy plants. Humidity was maintained in the root-inoculum zone by the use of irrigated bench matting beneath and around the trays; growth of roots into the matting was minimised by raising the trays above the bench. Glasshouse temperatures were controlled by computer at 10-20°C (night/day) and monitored with a Squirrel data logger.

e) Evaluation of white rot symptoms. Standardised protocols were developed for recording seedling growth (numbers, length and appearance of leaves, root growth), *S. cepivorum* inoculum (hyphal growth from onion disc), and white rot symptoms. Following inoculation, susceptible plants were progressively affected by typical white rot symptoms: leaf wilt and tip necrosis, retarded growth, the presence of mycelium and sclerotia on the stem base, and root rot. Inoculum development followed a sequence of active hyphal growth, typically filling the module in 3-5 days, followed by lysis (10-17 days) and the production of sclerotia on the onion disk. Roots were rapidly enveloped in hyphae, became translucent, and collapsed. The development of actively growing *S. cepivorum* hyphae on the roots, following removal from the growth medium and incubation in damp conditions, was confirmation of infection.

3.2 Objective 2: Evaluation of standard method using selected accessions of *A. cepa*.

Summary of Objective 2. The practical aspects of the screening method - production of plants, production of inoculum, inoculation - proved successful when tested on a bigger scale and hence was suitable for large-scale screening.

The prototype screening method was tested with full size modular trays and several accessions and cultivars.

a) Production of plants. Seed of 12 accessions from the GRU, and two commercial cultivars were germinated on damp filter paper for 6d and vigorous seedlings transferred one per module to modular trays pre-filled with Fisons Levington F2 compost. The modular trays were transferred to capillary matting in a glasshouse, irrigated 180 sec.day^{-1}, and the modules watered from above daily, and with nutrient solution twice weekly. Glasshouse temperatures were controlled by computer in the range of 20°C (day) - 10°C (night). Supplementary lighting was provided from Mid-October to March. Additional seedlings were produced in similar trays and

used as replacements for any which failed to establish.

b) Production of inoculum. Inocula were produced on discs of onion cv. Hyton fleshy leaf scales at 22°C for 3 days. Twelve modular trays were prepared on each of two consecutive days. Additional inocula were produced as replacements for any which failed to grow.

c) Inoculation and 'subsequent growth' of plants. Trays of seedlings were transferred to trays of inocula.

d) Experimental design and statistical analysis. Treatments were arranged in four experimental blocks each comprising six trays of 104 plants. Each tray contained one plot each of two test accessions and one comparison accession. Plots were surrounded by a single row of cv. White Lisbon as guards. Test accessions were allocated in pairs to the trays and the position of comparison cvs. allocated at random within each test accession/half trays; the allocation of pairs was randomised within in each block. Two blocks were prepared on each of three consecutive days.

e) Results. The scaled-up method proved to be very satisfactory for screening. The need to prepare the scaled-up experiment in three stages, because of the amount of work involved, was confirmed. The scaled-up method was used for the remainder of the project.

3.3 Objective 3: Screening accessions of A. cepa ('Primary Screen')

Summary of Objective 3. One hundred and eighty accessions of A. cepa were screened against white rot. Accessions were tested in three experiments of 60 accessions, in comparison with four commercial cultivars. Nine accessions showed a reduced susceptibility to white rot.

a) Selection of accessions. One hundred and eighty accessions of European origin with a long day length response were chosen as being most relevant to UK conditions (Appendix 1). Cvs. White Lisbon, Hyper, Hyton and Hysam were included for comparison and were chosen as being representative of those grown in commerce; all except cv. White Lisbon were F1 hybrids.

b) Production of seedlings. Plants were grown for five weeks in Fisons F2 compost in open-base modular trays the trays were placed on similar empty trays to minimise root growth into the

matting; the matting was flooded for 180 sec.day^{-1}. The seedlings were watered as necessary and fertilizer applied once per week (Vitafeed 111, Vitax). Glasshouse temperatures were controlled by computer (10°/20°C heating/venting) and recorded hourly (Squirrel logger; Grant Instruments, Cambridge, UK). Supplementary lighting (14 h photoperiod when the incident light <300 watts.m^{-2}; Osram HD71022s 400W SON/T) was provided from mid-October to mid-March).

c) Production of inoculum, and inoculation. Trays of *S. cepivorum* inocula were produced in the usual manner (6 days). The trays of 5-week old seedlings were then placed on top of the trays of inocula ensuring roots were in contact with mycelium.

d) Experimental design. Accessions were screened in three Experiments of 60 accessions each in comparison with the four standard commercial cultivars. Each experiment comprised three stages of two experimental blocks of 20 trays of seedlings, with each tray containing three test accessions and one standard cultivar. Trays were subdivided into 3 plots of 8 x 3 modules containing one test accession, and one plot of 8 x 4 modules containing one of the standard cultivars. The positions of the plots in a tray and the allocation of accessions were randomized according to an alpha design and test accessions occurred once and standard cultivars five times in each block. Thus accessions were replicated a total of six times.

e) Assessment of inocula and plant symptoms. Growth of the inocula and development of leaf symptoms were assessed as described in Table 1. Root symptoms, together with a final assessment of leaf symptoms, were assessed after about 45 days.

Table 1. Assessment of inoculum, and leaf and root symptoms.

Assessment (days after inoculation)	Score	Description
Inoculum	0	No growth of mycelium on the onion disc
(3, 10, 17 d)	1	Mycelium (< 1 cm diam.) growing on disc
	2	Mycelium (1-2 cm diam.) growing on disc
	3	Mycelium filling the module (>2 cm diam.)
Leaves	0	No symptoms (no wilting and no chlorosis)
(3, 10, 17, 45 d)	1	One or more leaves wilting
	2	One or more leaves wilting and chlorotic
	3	All leaves dead or dying (completely collapsed and chlorotic)
Roots	0	All roots healthy (no collapse, no translucence and no mycelium)
(45 d)	1	Some roots collapsed and translucent but mycelium absent
	2	Some roots collapsed and translucent with mycelium present
	3	All roots collapsed and translucent with or without mycelium

f) Statistical analysis. When the screening of accessions 1-60 (Experiment 1) was complete, a detailed statistical analysis was made of (a) positional effects within and between trays of seedlings, (b) rates of development and severity of foliar symptoms, (c) differences in severity of root symptoms and uniformity in the ranking of accessions between pairs of blocks in each set of three stages. The information was used to make slight improvements to the layout of the plots, chiefly to minimise edge effects in the modular trays.

When screening of the whole 180 accessions was complete, a detailed analysis was made of each of the records. The information was used to develop an Index for ranking the response of accessions to white rot. The Index was based on normalised values of the percentage white rot: the scores were generated from combinations of the foliar and root symptoms (Entwistle *et al.*,

1994). The index is weighted in favour of healthy plants and plants with few symptoms, and against dead plants. Large positive values indicate 'resistance' in comparison with four standard cultivars, and large negative index values indicate 'susceptibility'.

g) Results

At the time of inoculation most plants had two true leaves and the roots extended beyond the base of the module. Three days after host inoculation, most of the inocula filled the base of the cells (inoculum score 3) and lysed after 10-17 days. Two to three days after inoculation the first leaves of a majority of plants were wilting (foliar score=1). After 10 days, wilting was limited to the first leaf of some plants, or the leaves regained turgor. Wilted plants were chlorotic 10 days after inoculation, and necrotic after 10-17 days. By 17 days plants were dying (foliar score=3) and many plants were dead by 45 days. At the end of the experiment, most plants had dead roots (root score=3) which were collapsed and translucent. Plants with dead foliage always had dead roots, but some plants with healthy leaves also had dead roots. A few plants had roots which were all healthy and cream-white (root score=0).

In the test of the first 60 accessions (Experiment 1) the mean incidence of healthy plants per accession remaining at the end of the primary screen varied from 0-28%. Five accessions had higher percentages of healthy plants in comparison with the overall mean of the four standard cultivars (P=0.05; 1-tailed test) (See para. 6, Publications Arising From Research, Entwistle *et al.*, (1990)). The amount of infection was generally higher in Experiments 2 & 3 (Accessions 61-180), probably due to better control of humidity in the infection zone. The experimental design and calculation of the Index took into account differences in white rot severity in different experiments.

The Index for the whole 180 accessions ranged from -10.79 to +14.73. Accessions 1006137, 1000130, 1000115, 1004167, 1004181, 1000201, 1002907, 1005944, 1004186 had an index of $> +6.58$ ($t=1.645$; 1-tailed test;$P=0.05$) indicating a reduced susceptibility to the white rot pathogen.

3.4 Secondary screen (Additional Objective)

Following the final assessment of the primary screen of Accessions 61-120 (Experiment 2) and 121-180 (Experiment 3) plants with healthy leaves ***and*** a mainly healthy root system were transferred to a second series of modular trays and maintained in the same glasshouse. Plants which developed white rot in the next 8-9 weeks were discarded. The remaining healthy plants were re-inoculated using methods similar to those of the primary screen; inoculum was produced on cv. Hyton (Experiment 2) or cv. Senshyu Yellow (Experiment 3). The numbers of available

plants were small and varied between accessions therefore randomized block designs were not used. Growth of inocula was assessed after 3 and 17 days in a similar manner to the primary screen. Leaf, stem base and root symptoms were assessed 45 d after inoculation using a modified system of scoring (Table 2). Plants which were healthy or showed little evidence of damage were retained for further study.

Table 2 Assessments of foliar and stem base symptoms made during the secondary screen (Screening Experiments 2 and 3).

Assessment (time after inoculation)	Score	Description
1. Foliar symptoms (45 d)	0	No healthy leaves present
	1	One healthy leaf present
	2	Two healthy leaves present
	3	Three or more healthy leaves present
'Healthy' indicates no stunting, no wilting and no chlorosis		
2. Stem base symptoms (45 d)	0	Stem base free from mycelium and sclerotia
	1	Mycelium present, sclerotia absent on stem base
	2	Mycelium absent, sclerotia present on stem base
	3	Mycelium and sclerotia present on stem base

3.5 Inclusion of material in Breeding Programme (Additional Objective)

Plants which survived the secondary screen were transferred to the Plant Breeding Department for further investigation and production of seed (MAFF Project HH0906SFV). The proportion of surviving plants which gave seed was low because many plants were small. To achieve better results the tests would have to be timed to produce surviving plants at a time of the year when they had more time to grow before flowering. Inbred lines have been produced from the better performing cultivars but white rot resistance is no longer an objective of the MAFF onion

breeding commission

3.6 Comparison of 'best' and 'worst' accessions

Thirty-nine *A. cepa* accessions and one *A. fistulosum* accession comprising groups of the 'best' and 'worst' accessions ('best': 20/22 accessions with the least white rot, two accessions insufficient available seed; 'worst': 20/23 accessions with the most white rot, three accessions with insufficient seed) were tested in comparison with the standard cultivars cvs. White Lisbon, Hyper, Hyton and Hysam. In addition, twelve of the 'best' and twelve of the 'worst' accessions were tested in a quarantine field. In both tests, the majority of accessions remained in the same group thus substantiating the data from the original experiments.

4 Discussion and Conclusions

Previous research has indicated the need for a uniform screening method (van der Meer, 1987; Rahe, 1987). Variations in the type and amount of inoculum, the environmental conditions of the test e.g. glasshouse versus field are thought to account for variability in data on host response to the white rot pathogen. The methods developed in this project overcame these difficulties and were used successfully to screen 180 *A. cepa* accessions. Future improvements should aim to produce inocula with differing amounts of mycelia as a means of testing for the presence of small differences in host response. Sources of variability in the method still exist e.g. lack of close control of glasshouse temperatures.

The method successfully overcame the difficulties described earlier. However, the method was time-consuming and necessitated splitting each experiment into three stages. Now that a method is available which is capable of detecting differences in white rot response in standardised conditions, it may be possible to streamline future work by targeting the surviving plants, rather than the diseased plants, with consequent saving of resources. Results were promising in that varying proportions of seedlings from different accessions survived inoculation with the white rot pathogen. Moreover, a small number of plants survived a second inoculation, thus eliminating the possibility of escapes, always a possibility with field experiments. Healthy plants which survived the screening procedures either represented isolated individuals from accessions where the overall infection rates were very high, or came from the five accessions with a greater than average survival. Both sources may be of value when evaluating the evidence for the existence of genetically controlled susceptibility to the white rot pathogen. At this stage, no claim is made whether the differences in response represent the involvement of

resistance mechanisms and further research is needed to clarify this aspect by investigating transfer of the response following crossings of test accessions and standard cultivars.

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7 Meetings attended

Entwistle A.R. (1994) Managing onion white rot in Britain. *Invited presentation at the 43rd Annual Conference, Muck Vegetable Growers, Bradford, Canada, 24-25 March 1994.*

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Spence N.J. (1988) Runner up in British Society for Plant Pathology Competition for the P.H. Gregory Prize, awarded annually to the best public presentation by a young scientist.

8 Collaborative Research

Luis Perez Moreno, Escuela de Agronomia y Zootecnia, Guanajuato, Mexico visited HRI Wellesbourne for 6 months in 1992 to train with the Author in methods of screening for resistance to white rot in garlic. The method was successfully adapted for screening radio-induced mutants of garlic.

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Appendices

Appendix 1: Diary

1 July 1987: Appointment of Miss N.J. Spence

July 1987-June 1989. Small scale glasshouse screening test was developed during the first year of the project (Dr A.R. Entwistle and Miss N.J. Spence). Scale-up of screening test. Large scale screening of 180 *A. cepa* accessions from GRU started.

June 1989 Transfer of N.J. Spence to new post within HRI.

June- October 1989: continuation of experiments by A.R. Entwistle.

October 1989: Appointment of Miss K.R. Green

November 1989-January 1990. Large scale screening continued. Ongoing experiments to improve screening method.

August 1990: Initial screening (primary screen) of 180 accessions completed. Method developed for the re-inoculation (secondary screen) of plants surviving the primary screen.

1991: Collation of 'best' and 'worst' accessions for re-testing in quarantine field, HRIW (MAFF FO6A).

8.1 Accessions of Allium cepa used in the screening experiments

GRU accession number	Name	Origin
Experiment 1 (Accesssions 1-60)		
1	1000026	Fortuna	GBR
2	1000077	Elsoms Dominator	GBR
3	1000112	Big Ben	GBR
4	1000113	R. Robusta	GBR
5	1000114	Kaizuka	GBR
6	1000115	Ciboule C8379	GBR
7	1000124	Blood Red	GBR
8	1000126	Exhibition	GBR
9	1000128	Asmer Advance	GBR
10	1000129	Asmer Lancastrian	GBR
11	1000130	Bedfordshire Champion	GBR
12	1000132	R. Enormous	GBR
13	1000135	Hygro (F1)	GBR
14	1000136	Hypee (F1)	GBR
15	1000138	Kaizuka Extra Early	GBR
16	1000140	Reliance	GBR
17	1000141	R. Rivato	GBR
18	1000149	R. Envee	GBR
19	1000156	Giant Zittau	GBR
20	1000157	Royal Oak (F1)	GBR
21	1000159	Autumn Queen	GBR
22	1000162	Oakey	GBR

177	1007983	Happy (F1)	NLD
178	1007984	Quicksilver	NLD
179	1008424	Hysol (F1)	NLD
180	1008426	R. Sublima	NLD

Accessions preceded by 'R.' are of Rijnsburger type
Country of origin: accessions 1-120, from UK seed houses or NIAB and destined for the UK/european market (GBR); accessions 121-180 - UK seed houses/NIAB (GBR), Netherlands (NLD), Denmark (DNK). Accession 1000115, cv. Ciboule C8379, is **not** *A. cepa.*