WELLESBOURNE

CONTRACT REPORT

Brassicas: Development of Screening System to detect *Xanthomonas campestris* in seed and evaluation of pathogen resistance in seed parents of winter cauliflower

HDC FV 186
Final Report 1995-1998

S J Roberts, J D Taylor, S Redstone, M Fuller

Project title:	**Brassicas: Development of Screening System to detect *Xanthomonas campestris* in seed and evaluation of pathogen resistance in seed parents of winter cauliflower**
Report:	**Final Report (October 1998)**
Project Number:	**FV 186**
Project Leader:	**Dr S J Roberts** **Horticulture Research International** **Wellesbourne** **Warwick** **CV35 9EF**
Key workers:	**Dr Mick Fuller (University of Plymouth, Seale-Hayne Faculty, Newton Abbot, Devon TQ12 6NQ) – field inspections and assistance with project design and management.** **Ms Sarah Redstone (West Cornwall LEADER project, Rosewarne, Camborne, Cornwall TR14 0AB) - seed testing, field inspections** **Dr S J Roberts (Horticulture Research International, Wellesbourne, Warwick CV35 9EF) – seed testing, training in seed testing technology, race typing, interpretation of seed tests and epidemiological data and project management** **Dr J D Taylor (Horticulture Research International, Wellesbourne, Warwick CV35 9EF) – race typing, resistance screening and project management**
Location:	**HRI Wellesbourne and Rosewarne, Cornwall**
Project Coordinator:	**Mr John Jemmett**
Date Commenced:	**01 October 1995**
Date Completed:	**30 September 1998**
Keywords:	**Cauliflower, Black rot, *Xanthomonas campestris* pv. *campestris*, seed testing, disease resistance**

TABLE OF CONTENTS

PRACTICAL SECTION FOR GROWERS

Objectives and background

Black rot, caused by the bacterium *Xanthomonas campestris* pv *campestris* (*Xcc*), is a very serious problem on cauliflower in Cornwall and increasingly on all brassicas in other parts of the country. The increasing incidence of *Xcc* in the UK may be due in part to a succession of warmer summers but other factors including the uniform susceptibility of F_1 hybrids and increased likelihood of pathogen dissemination (from infected seed) in modern propagating systems have also been implicated.

Field symptoms in module propagated plants usually appear simultaneously throughout the crop with almost all plants infected with uniform severity. This differs from typical seed-borne infection in direct-drilled crops where infection occurs in randomly distributed patches, initiated from individual infected seeds and with evidence of disease gradients as the disease spreads and successive plants becoming infected.

The observation of uniform symptoms in crops grown from module propagated plants suggests that infection, possibly derived from infected seeds, has undergone an initial dissemination within the propagating system.

This report describes a project on *Xanthomonas campestris* pv *campestris* in Cornish cauliflowers with specific objectives to:-

- Evaluate seed test procedures
- Set up a seed-testing facility at Rosewarne
- Train Ms. Sarah Redstone, based at Rosewarne, in seed-testing procedures
- Detect and quantify seed-borne infection in a selection of the most widely grown cultivars
- To follow up a number of field crops which had been grown from both apparently-healthy and infected seed which had been tested during the previous year
- Identify and race type isolates of *Xanthomonas campestris* pv *campestris* from seeds, crops and cruciferous weeds as a possible means of determining the source(s) of infection
- To carry out glasshouse resistance screening of a selection of the most widely-grown cauliflower cultivars

Summary of results

Seed tests

- The methods used were based on an International Seed Testing Association (ISTA) protocol but modified to improve the detection threshold, both in terms of % seed infection and pathogen numbers detected.
- A laboratory at Rosewarne was equipped to carry out seed tests and staff were trained
- Seed samples (12 x 5000 seeds of each lot) were tested at HRI Wellesbourne and the Rosewarne Laboratory, Camborne.
- Infection was found in 12 of the 49 seed lots tested; 6 out of 27 in the first year and 6 out of 22 in the second year
- Infection levels were generally low and in most cases would not have been detected following the International Seed Testing Association procedures.

- The majority of infected seed lots contained only 0.0017% infected seeds (i.e. 1 infected seed in 59,000). The highest level detected was 0.011% (i.e. 1 infected seed in 9,000)
- In the second year infection was found in seed lots which had tested negative in the previous year.

Crop follow-ups

- Follow up of crops in the field which had been grown from seed tested in the previous year was inconclusive: disease was found in all crops examined regardless of seed infection level, cropping history or plant-raising system.
- Infected weeds were found, but it is likely that they were infected from the crop.

Race typing

- In the UK only two races have been detected with certainty.
- Race 1 (1A) was predominant, Race 4 was the earliest detected race (present as an isolate in the HRI culture collection from 1983).

Resistance screening

- All of the twenty-five varieties tested were fully susceptible to Races 1A and 4.
- All cultivars were less susceptible to Race 0 than turnip
- Some variability in susceptibility to Races 1B and 1C was found in both open pollinated and F_1 hybrid varieties, but is of little practical significance.
- Mature plants were more resistant than young plants.

Action points for growers

- Uniform crop infections with no evidence of infection patches or disease gradients are associated with module-raised transplants from propagating units.
- Growers and plant-raisers should only use tested seed
- No seed lot can be guaranteed to be free from infection even if it has been tested
- A negative result from a seed test implies that a seed lot has an infection level below the detection threshold of that test
- Levels of seed-borne infection are generally low suggesting that under propagating house conditions more stringent tolerance levels may be required than provided by the current ISTA protocol
- Seed tests can be carried out by HRI-Wellesbourne or NIAB (NB specify tolerance standard required)
- Only two races (Race 1 (1A) and Race 4) of *Xanthomonas campestris* pv *campestris* have been detected in Cornwall over the past 12 years with Race 1 predominant.
- Race 1 has recently been sub-divided into 1A, 1B, 1C. The frequency of each of these sub-types is unknown.
- The importance of non-seed sources of infection is unknown
- Circumstantial evidence suggests that cruciferous weeds are more likely to be infected by contact with infected *Brassica* crops than vice versa.

SCIENCE SECTION

Introduction

Black rot of Brassicas, caused by *Xanthomonas campestris* pv. *campestris* (*Xcc*), has been causing major problems to growers over the last few years. Originally it was thought to cause significant problems only in the South West of England, but it is becoming increasingly clear that the disease is causing concern in all the Brassica growing regions of England.

Symptoms of black rot are most frequently seen in the field as wedge-shaped yellow necrotic lesions developing from the edges of leaves, but may also appear as necrotic/yellow leaf spots or larger areas. The pathogen colonizes the vascular system giving rise to characteristic blackened veins. Infection often leads to premature defoliation, particularly in cauliflower, and plants may be stunted and crop quality reduced. The disease also results in increased susceptibility to *Alternaria* and to secondary bacterial soft-rots which may result in complete crop loss.

In addition to *Xcc* there are two other pathovars of *X. campestris*, which affect cauliflower and cabbage, however the taxonomic status of these other pathovars is currently in a state of flux and it is possible that they should all be considered as strains of *Xcc.*.

The disease is considered to be primarily seed-borne, and although there may be other sources of infection (e.g. soil, weeds, crop debris, machinery), their relative importance has not been established. Plant to plant spread in the field is by water splash from rain or irrigation and machinery. The bacteria enters the plant through the hydathodes at the leaf margin, the stomata or through wounds to the root system (Krtizman, G. & Ben-Yephet, Y., 1990). Some work has been done on survival in the field in the USA (Schaad & White, 1974) in Israel (Kritzman and Ben-Yephet, 1990) and in Russia but in conditions quite different from the UK. Plant refuse and machinery have also been found to transfer the bacterium (Hayward & Waterston, 1965; Krieg and Holt, 1990; Walker, 1969). In addition, a number of secondary hosts have been identified and these could act as a reservoir for disease (Krieg, N. R. & Holt, J. G., 1990; Brock, T. D. & Madigan, M. T., 1991; Walker, J. C., 1969; Ram-Kishun *et al*., 1988).

There are no approved chemicals available for control of black rot in the UK. Throughout the world, most effort to control the disease has focused on seed testing and/or resistance breeding.

The majority of internationally-traded commercial brassica seed is tested for the presence of *Xcc,* often by the seed companies themselves. Generally the method used follows an International Seed Testing Association (ISTA) protocol or a variation of it. This protocol implies a tolerance standard for infection of 0.01%. One possible reason for the recent increase in the disease may be that this currently applied quality standard of 0.01% infection (1 in 10,000 seeds) is inadequate for the current intensive and centralised transplant production systems, where opportunities for pathogen dissemination are rife.

Some control of the disease can be obtained by using hot water treatment of seeds, but this technique is not totally reliable and F_1 hybrids and cauliflowers are particularly sensitive to damage.

At the LEADER seminar on Brassicas (8 December 1993) which was attended by growers, packers, merchants and seedsmen from Cornwall and Devon, Thanet, Jersey, Wales and Lincolnshire, *Xanthomonas campestris* was identified as the primary concern in the area of plant health. Growers raised the possibility of establishing an independent *Xanthomonas* testing service at Rosewarne to screen new varieties and local seed. It was postulated that new seed material of non-provenance could be even more susceptible to *Xanthomonas* and thus produce an epidemic of the disease from which the industry could take many years to recover. This is particularly important as continuous cropping is widespread and this system provides the opportunity for perpetuation and intensification of the pathogen.

The main objectives of this project were:

1. Evaluate/improve seed test procedures.
2. Set up a seed-testing facility at Rosewarne.
3. Train Ms. Sarah Redstone, based at Rosewarne, in seed-testing procedures.
4. Detect and quantify seed-borne infection in a selection of the most widely grown cultivars
5. To follow up a number of field crops which had been grown from both apparently healthy and infected seed which had been tested during the previous year.
6. Identify and race-type isolates of *Xanthomonas campestris* pv *campestris* from seeds, crops and cruciferous weeds as a possible means of determining the source(s) of infection.
7. To carry out glasshouse resistance screening of a selection of the most widely-grown cauliflower cultivars.

This final report contains results of work carried out throughout the project.

Materials and Methods

Training of Rosewarne staff and setting up laboratory facilities

Ms Sarah Redstone was trained in microbiological and seed testing methods at HRI-Wellesbourne. The equipment and consumables needed to establish a testing facility at Rosewarne were identified and purchased.

Seed stocks

Nine seed companies were approached to supply seed of the 40 most widely grown Autumn/ Winter cauliflower cultivars in Cornwall.

Serology

Several antisera, produced at HRI-Wellesbourne, were examined for their utility in routine *Staphylococcus aureus* (*S.a.*) slide agglutination for identification of suspect *Xcc*.

Seed test methods

A well established ISTA (International Seed Testing Association) method for testing Brassicas for *Xcc* was already in existence and widely accepted. The ISTA working sheet for this test was therefore used as the basis for the protocol used in this project, but with modifications to obtain greater sensitivity both in terms of the proportion of infected seeds which can be detected (i.e. more seed tested) and the mean numbers of the pathogen per seed

(smaller sub-sample sizes). In addition, suspect *Xcc* colonies were screened using *S.a.* conjugated antisera raised at HRI-Wellesbourne prior to pathogenicity testing using methods developed by A. Ignatov at HRI-Wellesbourne.

In the first year of the project the seed testing effort was split equally between HRI-Wellesbourne (Dr S J Roberts) and Rosewarne (Ms S Redstone), in the second year all of the testing was done at Rosewarne (Ms S Redstone). All confirmatory identification of isolates and pathogenicity testing was done at HRI-Wellesbourne (Dr S J Roberts). Anti-serum and a control positive isolate for the seed tests were supplied to Rosewarne by HRI-Wellesbourne. A number of blind control-positive and -negative samples were also prepared at HRI Wellesbourne and sent to Rosewarne for testing to ensure reliable performance of the test procedures.

A detailed protocol for the seed testing was drawn up in the first year and then modified in the second year to clarify some aspects and improve standardisation. In outline, the test protocol used was as follows (the detailed protocol is given as an Appendix). Five-thousand-seed sub-samples were suspended in 50 ml of saline and shaken for 5 min when a sample was removed, centrifuged and plated on NSCAA and FS media. Seed was then allowed to soak for a further 2.5 h before dilution and plating on NSCAA and FS media. Plates were incubated for 3 d at 30°C and the number of suspect *Xcc* and other colonies recorded. Suspect colonies were sub-cultured to sectored plates of YDC medium, incubated for 24-48 h at 30°C and tested for agglutination with *S.a.* conjugated antiserum. Identity of isolates was confirmed by testing pathogenicity on a susceptible host (cabbage cv. Wirosa, turnip cv. Green Globe, cauliflower cv Miracle).

The number of positive and negative sub-samples was used to estimate the percentage of infected seeds using maximum likelihood statistical methods in the form of a stand-alone computer program (STpro) developed at HRI (Ridout & Roberts 1995)

Field Inspections

Seed companies were contacted and asked to provide lists of farmers who were growing crops derived from the particular seed lots which had been tested in the first year of the project. The aim was then to select a number of crops for inspection representing seed lots which had given positive and negative seed test results and different transplant raising methods (i.e. modules or pegs).

A standard field inspection record form was drawn up. A number of cauliflower crops in Cornwall were then inspected for the presence of black rot, the level of infection, disease distribution, presence of cruciferous weeds, black rot on weeds and records were made of cropping history and plant-raising methods. Samples of diseased cauliflower leaves (and weeds if appropriate) were collected from each field inspection site and isolations attempted in the laboratory.

Isolations

Small sections (2-4 mm^2) of leaf tissue/veins from the margins of lesions were comminuted in a drop of sterile water on a sterile microscope slide. Samples were then allowed to stand for a few minutes to allow egress of bacteria and examined under a light microscope before streaking out on plates of YDC agar. Plates were examined after incubation for 2 d at 30°C and suspect *Xcc* colonies were sub-cultured to further plates of YDC. Identity of isolates was

confirmed by slide agglutination with *Staphylococcus aureus* conjugated antiserum and pathogenicity testing on a susceptible host.

Race typing

This was carried out at HRI-Wellesbourne. Whenever possible isolates of *Xcc* from seed tests and field outbreaks were inoculated into a series of differential cultivars of *Brassica oleracea, B. napus, B. rapa* and *B. juncea.* to determine their race type. The inoculation methods were devised and the differential series selected as part of MAFF- and BBSRC-funded studies.

Resistance screening

This was carried out at HRI Wellesbourne. Twenty-five cultivars were selected from amongst the seed lots received in the first year of the project to represent both open pollinated and F1 hybrid types. These cultivars were then tested for their susceptibility to five key races of *Xcc* (Races 0, 1A, 1B, 1C, 4). 'Young' plants of each cultivar were inoculated 3-4 weeks after sowing and 'mature' plants of each cultivar were inoculated at 11 weeks after sowing when plants were approx. 40 cm high with 12 leaves.

Three 'young' and two 'mature' plants of each cultivar were inoculated with each of five isolates (6181, Race 0; 3811, Race 1A; 5212, Race 1B; 3880, Race 1C; 1279A, Race 4). Plants were grown in 7 cm pots of Fisons M2 compost. Isolates were grown for 48 h at 30°C on plates of King's medium B. A small amount of growth was then scraped off and used to make a turbid suspension (approx. 10^8 cells/ml) in sterile saline. The youngest three leaves on each plant were inoculated by clipping the major veins around the leaf margins with a pair of rat's-toothed tweezers wrapped in absorbent cotton wool dipped in the suspension. Sterile saline was used as a negative control. Differential cultivars with known susceptibilities to each of the races were also included as controls in each batch of inoculations.

Following inoculation, plants were maintained in a glasshouse with a heating regime of 15°C night and 20°C day, a venting regime of 17°C night and 22°C day and supplementary lighting (as necessary) to give a minimum 14 h daylength.

Symptoms were recorded on plants at 2 and 3 weeks after inoculation. The number of inoculation sites, number of lesions, and the maximum lesion severity score (0-3 scale) was recorded for each inoculated leaf.

Results & Discussion

Training of Rosewarne staff and setting up laboratory facilities

Ms Sarah Redstone spent two weeks at HRI-Wellesbourne in November 1995 working in the laboratory of Dr S J Roberts. During her stay she received training in general microbiological methods and aseptic technique, preparation and sterilisation of microbiological media. In addition she also received specific training in the protocols for testing of Brassica seed for *Xanthomonas campestris* pv *campestris* and carried out seed tests under the supervision of HRI-Wellesbourne staff. Emphasis was placed on maintaining a clear audit control of procedures including checks and controls wherever appropriate.

The equipment and consumables needed to establish a testing facility at Rosewarne were identified and purchased as necessary. Considerable delays were experienced in getting certain equipment and consumable items delivered to Rosewarne.

Further problems and delays were experienced in the second year of the project as a result of difficulties with and the poor quality of laboratory facilities at Rosewarne. The lack of telephone facilities at the Rosewarne laboratory also presented considerable communication difficulties. Work was also disrupted by building work in the laboratory at Rosewarne. At the outset of the project it seemed feasible to develop a seed-testing facility at Rosewarne, which would be able to provide a rapid service to local growers and seed companies. The reality, however, was that, due to these infrastructure problems, seed testing, diagnosis and identification of symptoms was and could be performed much more rapidly and cost-effectively at HRI-Wellesbourne.

Serology

None of the antisera examined have proved to be entirely satisfactory. Inconsistent results were obtained in agglutination of colonies picked directly from spread plates of selective media, making it necessary to sub-culture colonies to plates of YDC to ensure consistency. Some antisera appear to be too specific, i.e. they do not react with all strains of the pathogen, whereas others appear to be too non-specific, i.e. they react with non-pathogenic isolates. For this reason all serological identifications were confirmed by pathogenicity tests.

Seed testing

In the first year, seven companies responded with offers/promises to supply seed, and finally seed of 27 cvs was obtained from five companies and tested. In the second year, seed of 22 cultivars were obtained from six seed companies.

In the first year, seed testing was split between HRI-Wellesbourne, but in the second year all of the seed testing was done at Rosewarne. The results for both years of testing are summarised in Table 1. The maximum likelihood estimate of the percentage infected seeds was based on the combined results from both laboratories in the first year. Where all of the tests were negative, the infection level given is the upper 95% confidence limit, i.e. the level above which at least one positive result would be obtained in 95% of cases. Infection was found in 12 out of the 49 seedlots tested: 6 out of 27 in the first year, and 6 out of 22 in the second year .

The standard ISTA protocol specifies a test on 3 x 10,000 seeds (total 30,000); we tested 12 x 5,000 seeds (total 60,000), hence our method has a lower detection threshold than the ISTA procedure both in terms of % infected seeds and numbers of the pathogen per seed. In most (10 out of 12) of the positive cases, the estimated infection level was very low and below the detection threshold of the ISTA protocol (0.01%). In half of the positive cases the numbers of the pathogen detected were also very low and would not have been reliably detected by the ISTA protocol. However, two of the seed lots tested (039, 040) had infection levels of around 0.01%, the detection threshold of the ISTA protocol, and the numbers of the pathogen present would certainly have been detected by the ISTA protocol. Some of the positive seed lots are known to have been tested previously by the seed companies for *Xcc* following the ISTA protocol. It is thus clear that infection can still be present, albeit at relatively low levels, in seed which has previously been tested to ISTA protocols, however the

epidemiological significance of such infection levels is at present uncertain and is the subject of current MAFF-funded research.

Two of the six positive seed lots in the second year had been tested as negative in the previous year. Clearly this is cause for some concern, but may be attributable to the fact that the seed of the these particular lots which was tested in the first year had been treated, whereas the seed of the same lots tested this year was untreated. This might suggest that fungicidal seed treatments may affect the results of seed tests to give false negatives. However, positive results were obtained for other treated seed lots and throughout these seed tests we included checks for inhibition of *Xcc* by seed treatments, and did not obtain any indications of a detrimental effect on the test results. Thus, although apparently not necessary, the cautious approach is to recommend that reliable results can only be obtained for un-treated seed, and further investigation should be carried out to clarify the situation with respect to treated seed. Alternatively, this could suggest that infection is not uniformly or randomly distributed throughout the seed lot, in which case sampling procedures prior to testing become paramount. Unfortunately we were not able to control sampling of the seed prior to testing.

The blind testing of positive and negative control samples gave the expected results, indicating that test procedures were being carried out correctly at Rosewarne.

Field inspections

The follow up of field crops derived from infected seed was unsatisfactory. Unfortunately, only very limited information on the location of crops grown from tested seed was obtained from the two main seed co-operatives in Cornwall. In part this was because much of the seed which they had supplied for testing in the first year had not been used for commercial crops. Following these problems and delays in the first year, information on the location of crops derived from infected seed was obtained from one of the major commercial seed companies but by this time many crops had already been harvested. Thus, only four field crops were inspected for black rot in the first year. In the second year more crops were examined, but still only two farms were visited.

The results are shown in Table 2. Infection was found in all of the field crops regardless of the infection level in the seed. There appeared to be no relationship between field infection and seed infection levels, cropping history or the presence of cruciferous weeds. Infected weeds were found occasionally and were infected with the same race as the field crop, but as there was no evidence of disease gradients it appears more likely that they were infected from the crop. Infection appeared uniform throughout the crop in ten out of eleven module-raised crops but was patchy in all three of the peg-raised crops suggesting that significant disease spread had occurred in module-raised plants prior to planting, but not in the peg plants. The race (1 or 1A) found in the field crops derived from infected seed matched the race found in the seed.

It seems likely that as transplants were raised from both infected seedlots and apparently healthy seedlots by the same plant raisers that spread of infection between seedlots occurred during plant raising. Although, in 1997 Race 4 was found in a field crop, but had not been detected in the seed it is possible that this infection may still have originated with infected seed, as the numbers of isolates which we were able to race type was limited and we have previously found mixed race seed infections. This highlights the need for an alternative

molecular fingerprinting approach to the typing isolates from outbreaks whereby many more individual isolates could be typed from each crop/seedlot and allowing more precise tracing of strains and identification of the origin of infection in particular outbreaks.

Race typing

Race typing was carried out on isolates from field crops and from seed tests, results are shown in the appropriate tables and summarised together with data from MAFF and BBSRC-funded projects in Table 4. Only Races 1 and 4 have been found so far in seed or field crops in Cornwall, with Race 1 predominating.

At the start of this project five races of *Xcc* had been identified worldwide (Races 0, 1, 2, 3, and 4), but, during the course of this project, MAFF- and BBSRC-funded studies have tentatively identified additional races. These new races are effectively sub-divisions of the original Race 1 to give Races 1A, 1B and 1C. Thus, during the earlier part of this project isolates were typed as Race 1, but during the latter part as Race 1A. Some earlier isolates were also re-typed according to the new scheme, but due to resource limitations it was not possible to re-type all isolates. To date, all isolates from Cornwall which have been typed according to the new scheme conformed to Race 1A.

Resistance screening

Results of the resistance screening of both 'young' and 'mature' plants are summarised in Table 3 as an overall score derived by multiplying the proportion of inoculation sites with symptoms and the mean of the maximum disease scores for each inoculated leaf. The isolates of *Xcc* used for the resistance screening included representatives of the newly discovered races which sub-divide Race 1 (i.e. 1A, 1B and 1C). In the majority of cultivars mature plants had significantly lower disease scores than young plants, indicating that resistance increases with plant age.

All of the cultivars tested can be considered fully susceptible to Races 1A and 4 and all cultivars showed reduced susceptibility to Race 0 when compared to turnip. On the basis of the young plant inoculations, a number of cultivars (highlighted in Table 3) from amongst both open pollinated and F1 cultivars were identified as having some level of at least partial resistance or reduced susceptibility to Races 1B and 1C. However, this was not always consistently shown in the mature plants. As only a relatively small number of plants were tested with each race, it is not clear whether this is due to variability between individual plants within a cultivar or, contrary to the majority, an increase in susceptibility with age.

It is unlikely that the reduced susceptibility to Races 1B and 1C is any commercial benefit as these races were not found in either seed or field crops and these cultivars are fully susceptible to Races 1A and 4.

Conclusions

There seems to be little benefit in having a local seed-testing facility for Cornwall based at Rosewarne, as testing was and can be done more quickly and efficiently at HRI-Wellesbourne.

Xcc was found in 25% of seed lots of the main cultivars of autumn/winter cauliflowers grown in Cornwall. At least some of these seed lots are thought to have been tested previously according to standard ISTA procedures. Infection levels in positive seed lots were

low and ranged from 0.0017 to 0.011% and most of the positive seedlots would have been unlikely to have been detected following the current ISTA test procedures. In a few cases, however, some seed lots would certainly have tested positive following the ISTA protocol, implying either that the seed had not been tested by the seed company/supplier or had not been tested according to ISTA protocols.

The frequency of positive tests in this study implies that significant numbers of seed stocks which currently test negative in routine seed tests may not be free of disease. Indeed many of the 37 seed stocks which tested negative in the current test programme could still have harboured infection. The significance of such low levels of seed infection is not yet clear and is the subject of current MAFF-funded research at HRI-Wellesbourne.

Race typing demonstrated the presence of two distinct races (Races 1 (1A) and 4) in Cornish Cauliflower crops, with Race 1 predominant and only Race 1 found in seed in the present test series. Given the predominance of a single race, the race typing has retrospectively proved to shed little light on the importance of different sources of infection. There is an urgent need for alternative approach to typing of strains using molecular fingerprinting techniques in order to allow typing of larger numbers of isolates and to clarify sources of infection in indvidual outbreaks.

It is difficult to draw conclusions from the limited inspections of field crops grown from tested seed. However, there was no evidence to contradict the hypothesis that seed is the primary source of infection and that uniform infections of field crops result from rapid spread of the pathogen during plant-raising in module trays. Thus, the critical question of the importance of 'non-seed' sources of infection (e.g. soil, crop debris, module trays) in UK conditions remains unanswered. Experiments aimed at answering this could appropriately be funded by HDC and could be most efficiently carried out alongside the current MAFF programme.

If it is assumed that the majority of field outbreaks of black rot result from rapid spread during plant raising, then control efforts would need to be targeted towards this stage in production. Application of copper sprays to transplants or production under a capillary watering system provide two alternative approaches which could be examined.

The resistance screening indicated that there is some variability in susceptibility to some strains of *Xcc* (Races 1B and 1C) both within and between cultivars. As these races do not appear to be present in field crops and as all cultivars were susceptible to both of the races which have been found (Races 1A and 4) this would seem to be of no practical commercial value. Although there is no useful tissue resistance within cauliflower to *Xcc*, it is feasible that there may be useful differences in field susceptibility to black rot. Especially as the 'mature' plants showed reduced susceptibility compared to 'young' plants. This would require extensive field trials inoculated with isolates/races of known virulence.

Acknowledgements

This project was also supported by CEC funding from the West Cornwall Leader Project. We are grateful to the various seed companies and co-operatives who supplied seed for testing, and who have been cooperative in providing information.

References

Brock, T. D. & Madigan, M. T. (1991). *Biology of Micro-organisms 6th edition*. Prentice-Hall International. pp. 753-756;

Hayward, A.C. & Waterston, J.M. (1965) *CMI Descriptions of plant pathogenic fungi and bacteria no. 47*. Xanthomonas campestris. Commonwealth Mycological Institute, Kew, Surrey, England.

Krtizman, G. & Ben-Yephet, Y. (1990). Control by methan sodium of *Xanthomonas campestris* and the pathogen's survival in soil. *Phytoparasitica* 18: 217-227.

Krieg, N. R. & Holt, J. G. (1990). Bergey's Manual of Systematic Bacteriology, Volume 1;

Mariano, R.I.B. et al., (1985). Detection of *Xanthomonas campestris* in batches of commercial brassica seeds. *Fitopatologia Brasiliera* 10: 423-426

Ram-Kishun et al., (1988). Epiphytic survival of *Xanthomonas campestris* on Centella asiatica. *International Journal of Tropical Plant Diseases* 6: 189-193.

Ridout, M.S. & Roberts, S.J. (1995) STpro: Seed Test analysis program. 1.0. HRI.

Schaad, N.W. and White, N.W. (1974) Survival of *Xanthomonas campestris* in soil. *Phytopath*. **64**, 1518-1520.

Walker, J. C. (1969). *Plant Pathology 3rd edition.* McGraw Hill, New York;

Table 1. Summary of seed test results for *Xanthomonas campestris* pv. *campestris* in Cornish Cauliflower seed, 1996 and 1997. Sample size was 5000 seeds in all cases.

HRI No	Rosewarne No	Coating	No +ve	No samples	% Inf[1]	Race	cfu/ml[2]
1996 Season[3]							
484	001	None	0	12	<0.005		
485	002	None	0	12	<0.005		
486	003	None	0	12	<0.005		
487	004	None	1	12	0.0017	Race 1	1
488	005	None	0	12	<0.005		
489	006	None	0	12	<0.005		
490	007	None	1	12	0.0017	Race 1	1
491	008	None	0	12	<0.005		
492	009	None	0	12	<0.005		
493	010	None	1	12	0.0017	Race 1	300
494	011	None	0	12	<0.005		
495	012	None	0	12	<0.005		
496	020	Treated	0	12	<0.005		
497	022	Treated	0	12	<0.005		
498	024	Treated	0	12	<0.005		
499	021	Treated	0	12	<0.005		
500	023	Treated	0	12	<0.005		
501	019	Treated	1	12	0.0017	Race 1	1
506	013	None	3	12	0.0058	Race 1	3000, 10, 20
507	014	None	0	12	<0.005		
508	015	None	0	12	<0.005		
509	016	Treated	0	12	<0.005		
510	017	None	1	12	0.0017	Race 1	1
511	018	None	0	12	<0.005		
512	025	Treated	0	12	<0.005		
513	026	Treated	0	12	<0.005		
514	027	Treated	0	12	<0.005		

[1] Maximum likelihood estimate of the proportion of seeds infected.
[2] Numbers of *Xcc* per ml of seed extract for positive sub-samples.
[3] 1996 season: six samples tested at HRI-Wellesbourne, six samples tested at Rosewarne.
[4] 1997 season: all samples tested at Rosewarne.

Table 1 continued. Summary of seed test results for *Xanthomonas campestris* pv. *campestris* in Cornish Cauliflower seed, 1996 and 1997. Sample size was 5000 seeds in all cases.

HRI No	Rosewarne No	Coating	No +ve	No samples	% Inf[1]	Race	cfu/ml[2]
1997 season[4]							
523	028	Th/Cz/Ip	1	12	0.0017	Race 1A	2
524	029	Th/Cz/Ip/Met	0	12	<0.005		
525	030	Ip/Ben	0	12	<0.005		
526	031	Ip/Ben	0	12	<0.005		
527	032	Th/Cz/Ip	0	12	<0.005		
528	033	Th/Cz/Ip	1	12	0.0017	Race 1A	4
529	034	Th/Cz/Ip	2	12	0.0036	Race 1A	100, 100
530	035	Th/Cz/Ip	0	12	<0.005		
531	036	Th/Cz/Ip	0	12	<0.005		
532	037	Th/Cz/Ip	0	12	<0.005		
533	038	None	1	12	0.0017	Race 1A	100
534	039	None	4	12	0.0081	Race 1A	1000, 13000, 60, 2000
535	040	None	5	12	0.011	Race 1A	1, 2, 2, 2, 7000
536	041	Blue	0	12	<0.005		
537	042	Green	0	12	<0.005		
538	043	Blue	0	12	<0.005		
539	044	Green	0	12	<0.005		
540	045	Blue	0	12	<0.005		
541	046	Blue	0	12	<0.005		
542	047	Blue	0	12	<0.005		
543	048	Blue	0	12	<0.005		
544	049	Blue	0	12	<0.005		

[1] Maximum likelihood estimate of the proportion of seeds infected.
[2] Numbers of *Xcc* per ml of seed extract for positive sub-samples.
[3] 1996 season: six samples tested at HRI-Wellesbourne, six samples tested at Rosewarne.
[4] 1997 season: all samples tested at Rosewarne.

Table 2. Field inspections of cauliflower crops in Cornwall, grown from seed tested in the previous reporting period .

Inspection Date	Site	HRI Seedlot	Seed Infection		Field Inf.	Distrib.	Confirmed	Prev. crop	Plant raising	Cruciferous weeds	Weeds inf.?
			%	Race							
1996 Season											
26/11/96	1	506	0.0058	Race 1	>75%	patchy	Yes, Race 1	cabbage	pegs	Yes	No
26/11/96	1	507	0 (<0.005)	-	<25%	patchy	Yes, Race 1	cabbage	pegs	No	No
07/02/97	2	?	?	-	?	uniform	No	cauli.	module	Yes	No
07/02/97	2	487	0.0017	Race 1	40%	patchy	No	w. barley	pegs	v. few	No
1997 Season											
05/11/97	3	528	0.0017	Race 1A	100%	uniform	Yes, Race 1A	brassica	module	Yes	?
05/11/97	3	529	0.0036	Race 1A	100%	uniform	Yes, Race 1A	brassica	module	Yes	No
05/11/97	3	530	0 (<0.005)	-	100%	uniform	Yes, Race 1A	brassica	module	Yes	No
05/11/97	3	531	0 (<0.005)	-	100%	uniform	Yes, Race 4	brassica	module	Yes	?
05/11/97	3	532	0 (<0.005)	-	50%	patchy	Yes, Race 1A	brassica	module	No	No
05/11/97	4	528	0.0017	Race 1A	100%	uniform	Yes, Race 1?	grass	module	Yes	Yes, Race 1A
05/11/97	4	529	0.0036	Race 1A	100%	uniform	Yes, Race 1A	grass	module	No	No
05/11/97	4	530	0 (<0.005)	-	50%	uniform	Yes, Race 1	grass	module	No	No
05/11/97	4	531	0 (<0.005)	-	100%	uniform	Yes, Race 1A	grass	module	Yes	Yes, Race 1A
05/11/97	4	532	0 (<0.005)	-	100%	uniform	Yes, Race 1A	grass	module	No	No

Table 3. Summary of results of resistance screening of 25 Cornish cauliflower cultivars, 21 d after inoculation. Values presented are an overall score obtained by multiplying the proportion of wounds with symptoms by the severity score (Max value = 3.0).

HRI No./Cultivar	6181 (Race 0)		3811 (Race 1A)		5212 (Race 1B)		3880 (Race 1C)		1279A (Race 4)	
	Young	Mature	Young	Mature	Young	Mature	Young	Mature	Young	Mature
484	1.8	0.9	3.0	2.9	3.0	2.7	2.9	2.4	3.0	2.9
485	1.8	0.3	3.0	0.3	2.9	0.5	2.2	0.0	3.0	0.9
486	2.2	0.9	3.0	3.0	2.4	2.5	1.8	1.8	3.0	2.9
487	2.8	1.4	3.0	2.5	2.0	not tested	1.3	2.3	3.0	2.8
488	1.7	0.7	3.0	3.0	2.0	2.9	2.1	2.6	3.0	2.2
489	2.0	0.8	3.0	1.2	1.4	1.6	2.3	0.1	3.0	0.9
490	2.7	0.9	3.0	1.8	2.9	2.3	2.1	1.2	3.0	2.3
491	2.0	0.5	3.0	1.6	2.5	0.9	0.9[3]	0.5	3.0	0.9
492	1.3	0.2	3.0	2.5	2.3	2.0	0.9[3]	2.7	3.0	2.0
493	2.2	0.1	3.0	1.1	2.2	1.4	2.2	0.8	3.0	1.0
494	1.7	0.4	2.8	3.0	3.0	2.8	2.4	1.7	2.9	2.9
495	1.9	0.5	2.9	2.6	2.1	0.8	0.9	0.0	3.0	2.6
496	1.3	0.5	3.0	2.9	0.3[1]	0.05	0.1[1]	0.1	3.0	2.5
497	0.7	0.1	3.0	1.8	0.4[1]	0.01	0.5[1]	0.1	3.0	2.0
498	1.5	0.2	2.7	2.9	0.6[2]	1.9	1.0[2]	0.8	3.0	2.8
499	0.8	0.02	3.0	1.2	3.0	1.4	2.9	1.1	3.0	1.9
500	1.1	0.05	3.0	2.8	1.0[2]	0.00[1]	1.1[2]	0.05	3.0	2.0
501	2.1	0.3	3.0	0.5	3.0	1.2	2.5	0.4	2.8	1.1
506	2.0	0.6	3.0	2.5	1.4[2]	2.2	1.7[2]	2.4	3.0	2.8
507	1.1	0.03	3.0	0.3	2.9	0.4	1.4	0.1	3.0	0.7
509	2.5	1.2	3.0	3.0	0.1[1]	1.8	1.1[2]	2.3	2.9	2.6
510	1.4	0.6	3.0	2.8	2.9	1.6	2.7	0.4	2.9	0.5
511	2.3	0.2	3.0	2.5	2.0	1.1	1.7	1.6	3.0	1.9
512	2.2	0.04	3.0	2.8	2.6	1.2	1.8	1.3	2.9	1.8
513	2.1	0.5	3.0	2.8	2.7	2.1	1.4	1.0	2.9	1.9
Differentials:										
Bohmerwaldkohl	1.0	0.8	2.9	2.1	0.0	0.0	0.0	0.0	2.3	1.7
Cobra	2.8	1.2	3.0	0.6	2.2	0.2	2.6	0.9	0.0	0.0
Florida Mustard	1.0	0.6	0.0	0.0	0.0	0.0	0.1	0.4	0.0	0.0
Just Right Turnip	2.9	2.7	3.0	2.9	2.8	2.2	2.8	2.9	0.0	0.0
Wirosa	2.7	0.9	3.0	2.3	2.9	1.2	2.2	2.3	2.9	2.0

[1] All plants showing some partial resistance
[2] Susceptible, but with reduced 'take'
[3] 1/3 plants resistant

Table 4. Distribution of race-typed isolates of *Xanthomonas campestris* pv. *campestris* from the HRI culture collection amongst the currently defined races. Note: data for outside Cornwall were obtained outside the scope of this project.

	Race 0	Race 1[1]	Race 1A	Race 1B	Race 1C	Race 4
Cornwall	0	42	16	0	0	16
UK	0	93	22	2	0	31
World	15	125	26	4	1	68

[1]Race 1 includes isolates which were further typed to the sub-divisions of Race 1.

APPENDIX I: SEED TEST METHOD FOR *XANTHOMONAS CAMPESTRIS* PV. *CAMPESTRIS* IN *BRASSICA* SEED

Based on ISTA working sheet, Mohan and Schaad, Franken

S J Roberts *Horticulture Research International, Wellesbourne, Warwick, CV35 9EF*

Revised: 15 Feb 1996 - no tween added, centrifugation done in microfuge tubes, only one plate of each medium per dilution, inoculated seed as control, spiked sample to check for antagonists/inhibition.

Revised: 8 July 1997 - modifications specific to seed testing for HDC Xanthomonas in Brassicas in Cornwall project.

Before starting. Ensure you are familiar with hazard data and take appropriate precautions, especially during preparation of media, autoclaving, weighing out of antibiotics.

Materials Needed:

Flasks of sterile saline (0.85% NaCl) for soaking (need to fit on shaker) - 50 ml per 5,000 seeds
70% ethanol for wiping down instrument/benches/pipettes, etc.
Universal bottles containing 4.5 ml of sterile 0.85% NaCl for dilutions (2 per sample, only 1 needed for ISTA method)
Plates of NSCAA and FS media (ISTA method uses 4 plates for each of 3 dilns, this is excessive, we use 1 plate of each media for each of 4 dilutions)
Plates of YDC for sub-culture
Slopes of YDC in bijou bottles for sending isolates to Wellesbourne
Sterile 1.5 ml micro-centrifuge tubes, micro-centrifuge
Sterile bent glass rods (hockey sticks)
Pipettes and sterile tips
Known strain of Xcc (3818A) as control and/or inoculated seed
Orbital shaker Incubator at 30°C
Sterile Glycerol and sterile screw cap 2 ml microfuge tubes for storage of extracts

Preparation of samples

1. This can be done in advance of the assay.

2. It is vital to exclude any possibility of cross-contamination between seed samples, it is therefore essential to disinfect all surfaces, containers, hands, etc. both before and after handling each sample. This is perhaps best achieved by swabbing/spraying equipment and gloved hands with 70% ethanol.

3. If seed of a single lot is received in several packets, these should be combined by emptying into a new, clean polythene bag and mixing by hand to give a composite sample.

4. Remove a few grams (5-10) of seed from the composite sample and count the number of seeds. Estimate the Thousand Seed Weight (TSW or TGW) as:

$$TGW = \frac{Wt\ of\ seed}{No\ of\ seed} x1000$$

5. Based on the TSW, weigh out 5,000 seed sub-samples into new, clean polythene bags

Method

1. 5,000 seeds are suspended in 50 ml sterile saline (pre-chilled to 2-4ºC) in a 100 ml conical flask. Adjust volumes of according to number of seeds. If using inoculated seed as control put one or two seeds in 1 ml of saline.

2. Shake on orbital shaker for 5 min at 100-125 rpm at room temperature.

3. Flasks then kept in lab at room temp.

4. *Centrifugation step*

   4.1. After shaking, pipette 2 x 1 ml samples of seed extract into a sterile micro-fuge tubes and keep in fridge until processed.
   4.2. Centrifuge the samples at 13,000 rpm for 10 min [used to do in a coldroom].
   4.3. Remove approx. 0.9 ml from the micro-tubes, vortex to re-suspend the pellet and then spread entire tube contents on plates of FS and NSCAA.

5. After 2.5 h at RT, two tenfold dilutions are prepared from the extract. Extract and each dilution spread onto plates of FS and NSCAA.

6. Pipette 2 x 1.5 ml of the seed extract into sterile 2ml screw-cap microfuge tubes and add 0.23 ml (approx. 15%) of sterile glycerol (due to the viscosity of the glycerol it is helpful to remove the end few mm of the pipette tip with sterile scissors before pipetting). Cap, mix and freeze at -20˚C or below

7. *To check for antagonists/inhibition (by e.g. seed treatment)* (SJR's method) Take a small (1 ml) sample of the seed extract and add a single inoculated seed to the sample. Leave to soak for 2.5 h then dilute and plate as normal.

8. A suspension of a known isolate of Xcc should be diluted and plated onto each of the media as a control (if inoc seed not used). The suspension should be diluted sufficiently to give clearly separated colonies on the spread plate, i.e. a turbid suspension containing 10^8 cfu/ml needs to be diluted at least 6 times.

9. If required, the tested seed can be redried by spreading on several layers of paper towel and leaving to dry in air-flow/fume cupboard. But NB the seed may be infected, therefore take appropriate precautions to avoid cross-contamination.

10. Plates incubated for 3-4 d at 30ºC.

11. Examine plates for presence of yellow (NSCAA) or light green (FS) mucoid colonies surrounded by a zone of starch hydrolysis. Compare with control plates of known strain. Suspect colonies may be tested by *S.a.* slide agglutination, but this has not been very reliable.

12. Sub-culture suspected Xcc colonies and positive control to sectored plates of YDC. To avoid the potential for cross-contamination of isolates, use a new sectored plate for each sub-sample. The precise numbers of colonies sub-cultured will depend on the number of suspect colonies, but reasonable to sub from six to twelve.

13. Incubate sectored plates for 24-48 h at 30˚C.

14. Compare appearance of growth with positive control, and perform *Staph.. aureus* slide agglutination test (NB Some *Xcc* isolates give unreliable results in agglutination, therefore send all isolates which look right to Wellesbourne)

15. Sub-culture all positives from sectored plates to slopes of YDC and send to Wellesbourne for further confirmation of identity and pathogenicity testing.

Additional Notes

1. *Numbering of samples/isolates.* Upon receipt, each seed lot/sample should be allocated a unique sequential sample number and details recorded, i.e. source, lot no, TGW, seed treatments, etc. Each sub-sample of a lot should also be given number, e.g. 123/1 (seed lot no 123 sub-sample 1). Keep the sub-sample numbers within a seedlot unique, i.e. there should only be one 123/1 regardless of the test date. Each colony sub-cultured from a sub sample should also be given a number and should be unique within the sub-sample, regardless of medium and dilution. e.g. 123/1.1 (lot 123, sub sample 1, colony 1) and colonies 1-3 could come from FS and colonies 4-6 could come from NSCAA. Record where they have come from on the dilution plate recording sheet.

2. Assign a unique assay/experiment number to all the tests done on one day, and label all plates/recording sheets with that assay number.

Suggested weekly schedule for seed testing:

Day	Activity
Wed/Thurs	Prepare/sterilise media, flasks, dilution bottles, weigh/count seed
Friday	Soak seed, dilute and plate
Monday	Record dilution plates and sub suspect positives to sectors
Tues/Wed	Record sectored plates (appearance, agglutination), sub positive sectors to slopes and send to wellesbourne

NSCAA medium for *Xanthomonas campestris* pv. *campestris*

Compound	g/l	g/500 ml
Difco Nutrient Agar	23	11.5
Soluble Starch (Aldrich No. 17,933-0)	15	7.5
Cycloheximide[1]	0.200 (200 mg)	0.100
Vancomycin[2]	0.0005 (0.5 mg)	0.00025
Nitrofurantoin[3]	0.010 (10 mg)	0.005
Distilled water	1000 ml	500 ml

Heat to dissolve and autoclave at 121°C, 15 psi for 15 min.

Add antibiotics to cooled molten medium after autoclaving.

[1] Dissolve 2 g Cycloheximide in 10 ml 70% EtOH. Add 1 ml/l (0.5 ml/500 ml)
[2] Dissolve 5 mg of Vancomycin in 10 ml of distilled water. Filter sterilise and add 1 ml/l (0.5 ml/500 ml).

[3] Dissolve 0.1 g Nitrofurantoin in 5 ml of Dimethyl Formamide (DMF) (Care! - use glass bottles). Add 0.5 ml/l (0.25 ml/500 ml).

FS (Fieldhouse & Sasser) agar medium for Xanthomonas campestris pv. campestris

Compound	g/l	g/500 ml
Soluble starch (Aldrich No 17,933-0)	10.0	5.0
Yeast extract	0.1	0.05
K_2HPO_4	0.8	0.4
KH_2PO_4	0.8	0.4
$MgSO_4.7H_2O$	0.1	0.05
1% aq. Methyl Green	1.5 ml	0.75 ml
Bacto Agar	15.0	7.5
Distilled water	1000 ml	500 ml
Cycloheximide[1]	0.2 (200 mg)	0.1 (100 mg)
D-methionine[2]	0.003 (3 mg)	0.0015 (1.5 mg)
Pyridoxine-HCl[3]	0.001 (1 mg)	0.0005 (0.5 mg)
Cephalexin[4]	0.05 (50 mg)	0.025 (25 mg)
Gentamicin[5]	0.0004 (0.4 mg)	0.0002 (0.2 mg)
Trimethoprim[6]	0.03 (30 mg)	0.015 (15 mg)

Heat to dissolve and autoclave at 121°C, 15 psi for 15 min. Add antibiotics to cooled molten medium after autoclaving.

[1] 200 mg/ml Cycloheximide: 2 g in 10 ml 70% EtOH. Add 1 ml/l (0.5 ml/500 ml).
[2] 1 mg/ml D-Methionine: 20 mg in 20 ml distilled water. Filter sterilise and add 3 ml/l (1.5 ml/500 ml).
[3] 1 mg/ml Pyridoxine: 20 mg in 20 ml distilled water. Filter sterilise and add 1 ml/l (0.5 ml/500 ml).
[4] 10 mg/ml Cephalexin: 200 mg in 20 ml distilled water. Filter sterilise and add 5 ml/l (2.5 ml/500 ml).
[5] 1 mg/ml Gentamicin: 10 mg in 10 ml distilled water. Filter sterilise and add 0.4 ml/l (0.2 ml/500 ml).
[6] 10 mg/ml Trimethoprim: suspend 200 mg in 20 ml EtOH (70%). NB does not dissolve (not true, now does seem to), vortex immediately before adding 3 ml/l (1.5 ml/500 ml)

Antibiotic stock solutions for FS medium

Antibiotic	Stock Conc. (mg/ml)	Amount for 1000 ml (ml)	Amount for 500 ml (ml)
Cycloheximide[1] (70% EtOH)	200	1.0	0.5
D-methionine[2] (aq.)	1	3.0	1.5
Pyridoxine-HCl[3] (aq.)	1	1.0	0.5
Cephalexin[4] (aq.)	10	5.0	2.5
Gentamicin[5] (aq.)	1	0.4	0.2
Trimethoprim[6] (70%EtOH)	10	3.0	1.5

YDC Agar for Xanthomonas campestris

Compound	g/l	g/500 ml
Yeast Extract	10	5
$CaCO_3$ (light powder)	20	10
Bacto Agar	15	7.5
Glucose[1]	20	10

[1] Autoclave glucose separately as a 10% solution (we seem to be able to get away without doing this), and reduce volume of water for other components accordingly and add to molten medium before pouring.

Make up in oversize bottle/flask (i.e. 250 ml medium in 500 ml bottle/flask) to allow swirling of $CaCO_3$ just before pouring.

Cool to <50°C before pouring and swirl to ensure even distribution of $CaCO_3$.

Seed test log sheet		Exp:		Date:		Person:	
Code No	Sub-sample	TGW (g)	Wt of seed (g)	No of seeds	Vol. of liquid (ml)	Time on (hh:mm)	Time off (hh:mm)

XANTHOMONAS SEED TEST RESULTS SHEET			EXPERIMENT NUMBER		Suspect Pathogen: *Xanthomonas campestris* pv *campestris*		
DATE		RECORDED BY		ANTISERUM			
REF NO.	Medium		x 10^{-1} (Centrifug)	x 10^{0} (undiluted)	x 10^{1}	x10^{2}	Notes
	FS	Suspect:					
		Others:					
	NSCAA	Suspect:					
		Others:					
	FS	Suspect:					
		Others:					
	NSCAA	Suspect:					
		Others:					
	FS	Suspect:					
		Others:					
	NSCAA	Suspect:					
		Others:					
	FS	Suspect:					
		Others:					
	NSCAA	Suspect:					
		Others:					

Exp No		Date		Person				
Isolate code/ No	Appearance on YDC	Reaction with A/s		Pathogenicity on:			Sent to W	Notes

APPENDIX II: DETAILED RESISTANCE SCREENING RESULTS

Summary of results of resistance screening of 25 Cornish cauliflower cultivars, 21 d after inoculation. Young plants were inoculated 3-4 weeks after sowing; Mature plants were inoculated 11 weeks after sowing. Prop^n , the proportion of inoculation points with visible lesions. Score, the mean of the maximum disease scores for each leaf (0-3 scales).

HRI No/Cv.	6181 (Race 0)				3811 (Race 1A)				5212 (Race 1B)				3880 (Race 1C)				1279A (Race 4)			
	Young		Mature		Young		Mature		Young		Mature		Young		Mature		Young		Mature	
	Prop^n	Score	Prop^n	Score	Prop^n	Score	Prop^n	Score	Prop^n	Score	Prop^n	Score	Prop^n	Score	Prop^n	Score	Prop^n	Score	Prop^n	Score
484	0.7	2.6	0.3	3.0	1.0	3.0	1.0	3.0	1.0	3.0	0.9	3.0	1.0	3.0	0.8	3.0	1.0	3.0	1.0	3.0
485	0.6	3.0	0.2	2.0	1.0	3.0	0.2	1.5	1.0	3.0	0.2	2.2	0.7	3.0	0.1	0.7	1.0	3.0	0.3	3.0
486	0.7	3.0	0.4	2.3	1.0	3.0	1.0	3.0	0.8	3.0	0.8	3.0	0.6	3.0	0.6	3.0	1.0	3.0	1.0	3.0
487	0.9	3.0	0.5	3.0	1.0	3.0	0.8	3.0	0.8	2.7	*	*	0.6	2.3	0.8	3.0	1.0	3.0	0.9	3.0
488	0.6	2.6	0.2	3.0	1.0	3.0	1.0	3.0	0.7	3.0	1.0	3.0	0.7	3.0	0.9	3.0	1.0	3.0	0.7	3.0
489	0.7	3.0	0.3	2.5	1.0	3.0	0.4	3.0	0.5	2.6	0.6	2.8	0.8	3.0	0.1	1.0	1.0	3.0	0.3	3.0
490	0.9	3.0	0.3	2.5	1.0	3.0	0.6	3.0	1.0	2.9	0.8	3.0	0.7	3.0	0.5	2.5	1.0	3.0	0.8	3.0
491	0.7	3.0	0.2	2.2	1.0	3.0	0.5	3.0	0.9	2.9	0.3	2.8	0.4	2.0	0.2	2.5	1.0	3.0	0.3	3.0
492	0.4	3.0	0.1	1.3	1.0	3.0	0.8	3.0	0.8	2.9	0.7	3.0	0.4	2.4	1.0	2.8	1.0	3.0	0.7	3.0
493	0.7	3.0	0.2	0.8	1.0	3.0	0.4	3.0	0.7	3.0	0.5	3.0	0.8	2.8	0.3	3.0	1.0	3.0	0.4	2.5
494	0.6	3.0	0.2	2.3	0.9	3.0	1.0	3.0	1.0	3.0	1.0	2.8	0.8	3.0	0.6	3.0	1.0	3.0	1.0	3.0
495	0.6	3.0	0.2	2.8	1.0	3.0	0.9	3.0	0.7	3.0	0.5	1.5	0.4	2.0	0.1	0.7	1.0	3.0	0.9	3.0
496	0.4	3.0	0.2	2.3	1.0	3.0	1.0	3.0	0.2	1.8	0.1	0.8	0.1	1.0	0.2	0.7	1.0	3.0	0.8	3.0
497	0.3	2.5	0.1	1.2	1.0	3.0	0.6	3.0	0.2	2.0	0.0	0.5	0.2	2.2	0.2	0.5	1.0	3.0	0.7	3.0
498	0.6	2.5	0.2	1.0	1.0	2.7	1.0	3.0	0.3	2.0	0.7	2.8	0.5	2.1	0.4	2.0	1.0	3.0	0.9	3.0
499	0.3	2.7	0.0	0.8	1.0	3.0	0.4	3.0	1.0	3.0	0.5	3.0	1.0	3.0	0.4	3.0	1.0	3.0	0.6	3.0
500	0.4	3.0	0.1	0.8	1.0	3.0	0.9	3.0	0.4	2.4	0.0	0.0	0.4	2.6	0.1	0.7	1.0	3.0	0.7	3.0
501	0.7	3.0	0.2	2.0	1.0	3.0	0.2	2.5	1.0	3.0	0.4	3.0	0.8	3.0	0.2	2.0	0.9	3.0	0.4	3.0
506	0.7	3.0	0.3	2.0	1.0	3.0	0.9	2.8	0.6	2.4	0.7	3.0	0.6	2.9	0.8	3.0	1.0	3.0	0.9	3.0
507	0.4	3.0	0.0	1.0	1.0	3.0	0.1	2.5	1.0	3.0	0.2	1.8	0.6	2.4	0.1	1.2	1.0	3.0	0.2	3.0
509	0.8	3.0	0.5	2.2	1.0	3.0	1.0	3.0	0.1	1.3	0.7	2.5	0.4	3.0	0.8	3.0	1.0	3.0	0.9	3.0
510	0.5	2.9	0.2	2.5	1.0	3.0	0.9	3.0	1.0	3.0	0.5	3.0	0.9	3.0	0.2	1.8	1.0	3.0	0.2	2.3
511	0.8	3.0	0.2	1.5	1.0	3.0	0.8	3.0	0.7	2.9	0.4	3.0	0.6	2.8	0.5	3.0	1.0	3.0	0.7	2.7
512	0.7	3.0	0.1	0.7	1.0	3.0	0.9	3.0	0.9	3.0	0.4	2.8	0.6	2.9	0.5	2.7	1.0	3.0	0.6	2.8
513	0.7	3.0	0.2	2.3	1.0	3.0	0.9	3.0	0.9	3.0	0.7	3.0	0.5	3.0	0.4	2.5	1.0	3.0	0.6	3.0
Differentials:																				
Bohm	0.4	2.6	0.4	2.4	1.0	3.0	0.7	3.0	0.1	0.7	0.1	0.7	0.0	0.3	0.1	0.7	0.8	3.0	0.6	3.0
Cobra	0.9	3.0	0.5	2.2	1.0	3.0	0.3	1.8	0.7	3.0	0.2	1.2	0.9	3.0	0.4	2.3	0.0	0.2	0.0	0.3
FM	0.4	2.7	0.3	2.2	0.0	0.4	0.0	0.0	0.0	0.2	0.0	0.0	0.1	0.9	0.2	1.9	0.0	0.0	0.0	0.0
JRT	1.0	3.0	0.9	3.0	1.0	3.0	1.0	3.0	0.9	3.0	0.7	3.0	0.9	3.0	1.0	3.0	0.0	0.0	0.1	0.2
Wirosa	0.9	3.0	0.4	2.3	1.0	3.0	0.8	3.0	1.0	3.0	0.4	3.0	0.8	2.8	0.8	3.0	1.0	3.0	0.7	3.0