

Project title: Development of Watercress Lines with Improved Resistance to Crook Root (*Spongospora subterranea* f. sp. *nasturtii*) and Watercress Yellow Spot Virus (WYSV)

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PRACTICAL SECTION FOR GROWERS

Background

Crook root disease of watercress (*Rorippa nasturtium-aquaticum*) is caused by *Spongospora subterranea* f. sp. *nasturtii*. The disease is the most important one currently affecting UK watercress production and occurs in other parts of the world (Walsh & Phelps 1991). The causal organism infects watercress roots, where it proliferates and causes the roots to become swollen and brittle and frequently curved in the shape of a crook (Tomlinson 1958). Roots are often stunted and tend to rot causing, in extreme cases, the plants to become dislodged. This is accompanied by a loss of vigour and a consequent reduction in yield. The disease is present in watercress roots all year round, but is particularly debilitating in winter when plants are growing slowly.

The crook root organism has the added importance of acting as the vector of watercress yellow spot virus (WYSV) which is another important disease affecting watercress production in the UK (Walsh, Clay & Miller 1989). The virus is also known to occur in France (Spire 1962). Like crook root, the virus is present in watercress roots all year round. At certain times of year (sometimes in winter, but mostly in spring) an unknown factor(s) induces the virus to move up into the leaves where it causes the characteristic yellow spot symptoms. Plants with such symptoms are not marketable and therefore commercially unviable.

During the winter months when the effects of crook root on watercress are most severe, growers treat the water flowing through the watercress beds with zinc, mostly in the form of zinc solutions. This practice, which has been adopted since the 1950's has been shown to give good control of crook root. Recent research at Wellesbourne has shown that due to the vector relationship of crook root to WYSV, such treatments also give some control of WYSV. Formerly crook root was controlled by the application of zinc to the spring water supplying watercress beds to give a concentration of 0.1 ppm in water flowing through the crop (Tomlinson 1960). Zinc has been shown to be toxic to freshwater shrimps (*Gammarus pulex*) (Martin & Holdich 1986) and zinc emissions from watercress beds have been implicated in the reduction of numbers of these shrimps in rivers and streams downstream of watercress beds (Roddie, Kedwards & Crane 1992). Currently UK watercress producers are allowed to discharge zinc at only 0.075 ppm.

This project aimed to investigate the potential of using natural plant resistance as an alternative to zinc for the control of crook root and to evaluate the commercial/agronomic acceptability of watercress accessions with improved levels of resistance to crook root and WYSV compared with current commercial lines. It was jointly funded by the Horticultural Development Council (HDC) and the Environment Agency. The project provided the chance to work with the watercress industry to explore possibilities of reducing the amount of zinc discharged from watercress sites, thereby providing the opportunity to affect a direct improvement in water quality of receiving water courses. Experiments were carried out on a grower's site, provided free of charge by Vitacress Salads Ltd.

Preceding Research

Germplasm of watercress and related plant species was collected from around the world (132 seed lots) in 1986-87 (Walsh 1993) and stored in the Genetic Resources Unit at HRI. A technique was developed to evaluate this material for resistance to crook root and WYSV in watercress beds (Walsh & Phelps 1991). Using this technique the different watercress accessions that had been collected, and related species were tested for susceptibility to crook root and WYSV. These experiments identified a number of watercress accessions that were significantly less susceptible to both diseases than current commercial lines. The accessions that showed the lowest susceptibility to both diseases originated from Japan, New Zealand, Spain and the UK (Walsh 1993). The most susceptible accession was the watercress line that is most widely grown in the UK (Walsh 1993). Non-watercress *Rorippa* species were less susceptible to crook root and WYSV than most watercress accessions. *Cardamine* (Walsh 1993) and a *Barbarea* (Walsh & Phelps 1991) species were found to be least susceptible to both diseases; one accession of *Cardamine hirsuta* and one accession of *Cardamine impatiens* appeared not to be hosts of crook root and only poor hosts of WYSV.

Objectives

The specific objectives of this research were as follows:-

- (a) To test selections of watercress made from within accessions (on the basis of their susceptibility to crook root and WYSV) in watercress beds to determine whether selection for enhanced levels of resistance to both diseases had been achieved.
- (b) To multiply accessions of watercress previously identified as less susceptible to crook root and WYSV. Evaluate the accessions of watercress (from Japan, UK, New Zealand and Spain) that have been found to be less susceptible to crook root and WYSV to determine their relative merits with respect to agronomic characteristics including leaf shape, cold tolerance, colour, growth rate and late flowering.
- (c) Attempt to select improved agronomic types from within watercress accessions that had been found to be less susceptible to crook root and WYSV but which were agronomically unacceptable.
- (d) Attempt to hybridise watercress with related plant species that have been shown to be non-hosts of crook root in order to incorporate this resistance into watercress.
- (e) Evaluate new untested watercress accessions for susceptibility to crook root and WYSV.
- (f) Cross less susceptible accessions of watercress with current commercial watercress lines, and look at morphological variation and susceptibility to crook root and WYSV in subsequent generations with a view to producing commercially acceptable lines with improved levels of resistance.

The ultimate aim of the project was to produce watercress lines with improved levels of resistance to crook root and WYSV.

Summary of Results

1. The project was designed to ensure that growers' dependence on zinc treatments for the control of crook root could be reduced. A number of experimental approaches were taken during this project. These included:
 - I taking existing watercress lines, testing them for susceptibility to crook root and watercress yellow spot virus in watercress beds, producing seed from the most resistant and most susceptible plants for each line and then testing these for resistance to both diseases to determine whether it was possible to produce selections with improved levels of resistance
 - II evaluating accessions previously identified as less susceptible to the diseases than current commercial lines for commercial acceptability, including characters such as leaf shape, leaf colour, leaf size, growth rate, uniformity etc.
 - III evaluating accessions previously identified as less susceptible to the diseases than current commercial lines for commercial acceptability
 - IV attempting to hybridise watercress with related plant species that have been shown to be non-hosts of crook root
 - V evaluating new watercress accessions for resistance and making crosses between less susceptible accessions and a commercially acceptable line to produce improved lines.
2. In earlier experiments individual plants had been selected from five different watercress accessions on the basis of having the highest or lowest susceptibilities to crook root and watercress yellow spot virus infection. Following subsequent multiplication, none of the offspring of the plants that had least disease were significantly better in terms of crook root susceptibility than offspring of the plants that had the highest susceptibility. This suggested that it would not be possible to select watercress lines with improved resistance to crook root from within accessions. Selections made from two of the five original accessions were significantly different from each other in terms of watercress yellow spot virus susceptibility, suggesting that it may be possible to select watercress lines with improved resistance to this pathogen from within accessions.
3. Accessions of watercress previously identified as less susceptible to crook root and watercress yellow spot virus (i-vii described on page 11) were multiplied at HRI and subsequently evaluated in watercress beds by growers for agronomic/commercial acceptability. In parallel, experiments on the relative susceptibilities of these lines to the two diseases were carried out. The Japanese accession was found to be the least susceptible followed by the Spanish and New Zealand accessions. The Japanese and Spanish accessions were totally unacceptable commercially. The New Zealand line had potential, but suffered from too pale leaf colour and too large leaves. The New Zealand line performed better in the winter months (when crook root and watercress yellow spot virus are most damaging) than it did in the summer. It was not possible to select improved agronomic types from the Japanese, New Zealand and Spanish accessions that still had resistance to the two diseases.

4. In earlier experiments it was found that a number of plant species supposedly related to watercress, including *Cardamine* spp., *Rorippa* spp., and *Barbarea verna* were very resistant or non-hosts of crook root and/or watercress yellow spot virus. Consequently attempts were made to produce hybrids between these species and watercress. No hybrids were obtained suggesting that watercress is incompatible with these species.
5. Five new accessions of watercress including wild and commercial types were received during the course of this study, consequently they were tested for susceptibility to the two diseases alongside the most widely grown commercial line (Hampshire) and the most resistant (Japanese) accession for comparison. Only one of the new accessions (Wild Dorset) had significantly less crook root than the commercial line, and none of them had significantly less watercress yellow spot virus.
6. Crosses between less susceptible and current commercial watercress lines have produced some hybrids that are intermediate in terms of susceptibility to the two diseases. The morphologies of hybrids suggested that some of the agronomic characters were segregating in some of the populations and that in others the morphologies were intermediate to those of the two parents.
7. A concluding chapter summarises the results of the experiments and suggests that the hybrid watercress lines are unlikely to give sufficient control of crook root and watercress yellow spot virus to be deployed without other forms of control. Recommendations are made to ensure that the results of this work are further developed in order to incorporate them into watercress growing practices.

Action points for growers

- Japanese and Spanish accessions of watercress had the highest levels of resistance to crook root, but were totally unusable commercially due to their morphologies.
- The New Zealand accession has potential, but its leaves were too pale and large; it performed better in the winter months than it did in summer.
- It is unlikely that resistance to crook root and watercress yellow spot virus (WYSV) can be incorporated in to watercress from other plant species in the near future.
- It is unlikely that plant resistance alone will provide sufficient control of crook root and WYSV to completely replace the use of zinc for this purpose.
- Partial resistance to crook root and WYSV in hybrid watercress lines produced during the project could be deployed as one component of an integrated strategy for the control of these diseases.
- Research is needed to identify and evaluate other non-chemical measures (such as cultural practices, physical barriers, biological control etc.) that could be deployed to control crook root and WYSV as part of an integrated control strategy

Practical and financial benefits from study

The work has clearly shown that natural plant resistance alone is unlikely to provide adequate control of crook root and watercress yellow spot virus (WYSV) and consequently growers' reliance on zinc remains.

If used in combination with zinc treatments, partially resistant watercress lines produced as part of the project might allow the amount of zinc used by growers to be reduced.

The work highlights the need to identify and develop further non-chemical control measures for these diseases that could be combined in an integrated programme to give effective control and hence obviate the growers' dependence on zinc for disease control.

The partial plant resistance to the two diseases produced by this project could be used as part of such an integrated programme.

The subsequent part of the report is divided into sections describing the experimental procedures used, the six key activities and the final section provides an overview of the project and makes recommendations for future actions.

Key words: Crook root disease, watercress yellow spot virus, natural plant resistance, hybrid watercress lines.

EXPERIMENTAL SECTION

Experimental site

Small purpose-built experimental watercress beds measuring 2.3 x 20.1 m on a watercress farm in Dorset (National Grid reference SY 852936) (Doddings) where crook root and WYSV were known to occur (Walsh, Clay & Miller 1989) were selected for the evaluation, testing and screening of watercress lines, selections and hybrids. Some of these beds have been free of zinc treatments for many years and contain high levels of crook root and WYSV.

Determination of susceptibility of plants to crook root and WYSV

For determination of the susceptibility of different watercress lines and hybrids to crook root and WYSV, the module method (Walsh & Phelps 1991) developed at HRI was used. This involved raising watercress plants in small plastic modules (Plug tray P576, Plantpak Ltd., UK) cut into arrays of 3 x 5 cells and containing autoclaved coarse sand irrigated with nutrient solution in the glasshouse for 4-6 weeks (depending on the time of year). These modules were placed in larger arrays of the same-sized modules to secure and space them. Following this propagation in the glasshouse, they were transported to the experimental site and placed in plastic crates (45 x 75 cm) with open sides and perforated bases, in the lower half of experimental watercress beds 4 or 5. The crates served to keep the modules clear of the bed base, allowing spring water to circulate freely under them, and prevented watercress growing in the bed from over-growing the modules and engulfing the test plants. The test plants remained in the bed for 6 weeks for infection to take place and were then returned to the laboratory for evaluation and testing.

Plants were removed from the modules, sand washed from their roots and then visually assessed for the presence and severity of crook root. This was scored on a scale of 0-3 where 0 represents none; 1, few; 2, many and 3, abundant crooked roots visible.

Enzyme-linked immunosorbent assay (ELISA) tests were then carried out on the plants to quantify WYSV infection levels (Walsh, Clay & Miller 1989; Walsh & Phelps 1991). This is a serological test that detects the presence of the virus and gives a quantitative readout of the amount of virus present in the sample.

ELISA was performed according to Clark & Adams (1977). Plates were coated with immunoglobulin G from a polyclonal antiserum (produced against WYSV at HRI [Walsh *et al.* 1981]) at 1 $\mu\text{g ml}^{-1}$ for 4 h at 35°C. Sap extracts of plants to be tested were prepared by macerating plants between a pair of electrically powered steel rollers. The coating solution of immunoglobulin was then washed out of the ELISA plates with phosphate buffered saline containing Tween 20 (PBS-T); three washes with a 3 minute soak between each wash. Plant samples were then mixed thoroughly and pipetted into the ELISA plate wells using a clean pipette tip for each sample. Extracts of virus-free glasshouse grown and known virus-infected plants from watercress beds were also pipetted into each ELISA plate to confirm the

efficiency of virus detection. ELISA plates were then left overnight (16 h) at 4°C. The next morning, the plates were washed as described above and the same immunoglobulin used earlier, but this time conjugated to the enzyme alkaline phosphatase and at 1 µg ml⁻¹ was added to each ELISA plate well. The plates were then incubated for 5 h at 35°C. They were then washed as earlier and the substrate for alkaline phosphatase pipetted into each ELISA plate well and left to incubate at room temperature. The absorbences of the substrate solutions in the wells were measured at 405 nm (A_{405}) at various intervals on an Anthos Labtec HT2 microplate reader (Tech Gen International, London, UK) and recorded.

Statistical analyses

Analysis of variance was done on mean crook scores and ELISA values (A_{405}), the latter being transformed to logarithms before calculation of means from the individual plants. The transformation used was $\log ([A_{405} + 0.01] \times 100)$.

Plant crossing

Watercress plants and plants of other *Rorippa* species and *Cardamine* species were grown on to flower. Pollen was taken from some plants using a small fine sable hair paint brush and was brushed on to flowers of other plants that had been emasculated. Insect proof bags were then put onto the flower heads. In many cases, particularly the inter species crosses, the styles dehydrated and no pods were produced. Where pods were produced, these were left to dry on the plant and were then harvested and seed collected. Crosses were also attempted without emasculating flowers in order to prevent the carpels drying out. Seed was grown for visual comparison with the parental lines to determine whether hybridisation had been achieved.

A. GENERAL INTRODUCTION

Background

Crook root disease of watercress (*Rorippa nasturtium-aquaticum*) is caused by *Spongospora subterranea* f. sp. *nasturtii*. This organism was formerly classified as a fungus and a forma specialis of the causal agent of powdery scab disease of potato (Tomlinson 1958) but is now known to be more closely related to the protists (Clay & Walsh 1997). The disease is the most important one currently affecting UK watercress production and occurs in other parts of the world (Walsh & Phelps 1991). The causal organism infects watercress roots, where it proliferates and causes the roots to become swollen and brittle and frequently curved in the shape of a crook (Tomlinson 1958). Roots are often stunted and tend to rot causing, in extreme cases, the plants to become dislodged. This is accompanied by a loss of vigour and a consequent reduction in yield. The disease is present in watercress roots all year round, but is particularly debilitating in winter when plants are growing slowly.

The crook root organism has the added importance of acting as the vector of watercress yellow spot virus (WYSV) which is another important disease affecting watercress production in the UK (Walsh, Clay & Miller 1989). The virus is also known to occur in France (Spire 1962). Like crook root, the virus is present in watercress roots all year round. At certain times of year (sometimes in winter, but mostly in spring) an unknown factor(s) induces the virus to move up

into the leaves where it causes the characteristic yellow spot symptoms. Plants with such symptoms are not marketable and therefore commercially unviable.

A recent survey carried out by HRI in 1992 of the incidence of crook root and WYSV showed that the former was present at all watercress farm sites sampled and the latter at all but two sites. Of 203 individual watercress beds sampled at these different sites in Dorset, Hampshire, Hertfordshire, Wiltshire, Yorkshire and Surrey, 183 (90%) contained plants infected by crook root and 152 (75%) contained plants infected by WYSV. Random sampling of the watercress beds showed that 58% of the plants were infected by crook root and 47% by WYSV.

During the winter months when the effects of crook root on watercress are most severe, growers treat the water flowing through the watercress beds with zinc, mostly in the form of zinc solutions. This practice, which has been adopted since the 1950's has been shown to give good control of crook root. Recent research at Wellesbourne has shown that due to the vector relationship of crook root to WYSV, such treatments also give some control of WYSV. Formerly crook root was controlled by the application of zinc to the spring water supplying watercress beds to give a concentration of 0.1 ppm in water flowing through the crop (Tomlinson 1960). Zinc has been shown to be toxic to freshwater shrimps (*Gammarus pulex*) (Martin & Holdich 1986) and zinc emissions from watercress beds have been implicated in the reduction of numbers of these shrimps in rivers and streams downstream of watercress beds (Roddie, Kedwards & Crane 1992). Currently UK watercress producers are allowed to discharge zinc at only 0.075 ppm.

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both diseases; one accession of *Cardamine hirsuta* and one accession of *Cardamine impatiens* appeared not to be hosts of crook root and only poor hosts of WYSV.

Objectives

The specific objectives of this research were as follows:-

- (a) To test selections of watercress made from within accessions (on the basis of their susceptibility to crook root and WYSV) in watercress beds to determine whether selection for enhanced levels of resistance to both diseases had been achieved.
- (b) To multiply accessions of watercress previously identified as less susceptible to crook root and WYSV. Evaluate the accessions of watercress (from Japan, UK, New Zealand and Spain) that have been found to be less susceptible to crook root and WYSV to determine their relative merits with respect to agronomic characteristics including leaf shape, cold tolerance, colour, growth rate and late flowering.
- (c) Attempt to select improved agronomic types from within watercress accessions that had been found to be less susceptible to crook root and WYSV but which were agronomically unacceptable.
- (d) Attempt to hybridise watercress with related plant species that have been shown to be non-hosts of crook root in order to incorporate this resistance into watercress.
- (e) Evaluate new untested watercress accessions for susceptibility to crook root and WYSV.
- (f) Cross less susceptible accessions of watercress with current commercial watercress lines, and look at morphological variation and susceptibility to crook root and WYSV in subsequent generations with a view to producing commercially acceptable lines with improved levels of resistance.

The ultimate aim of the project was to produce watercress lines with improved levels of resistance to crook root and WYSV.

The Gantt chart in Figure 1 shows the sequence of laboratory, glasshouse and field activities carried out during the course of this project.

The subsequent part of the report is divided into sections describing the six key activities and the final section provides an overview of the project and makes recommendations for future actions.

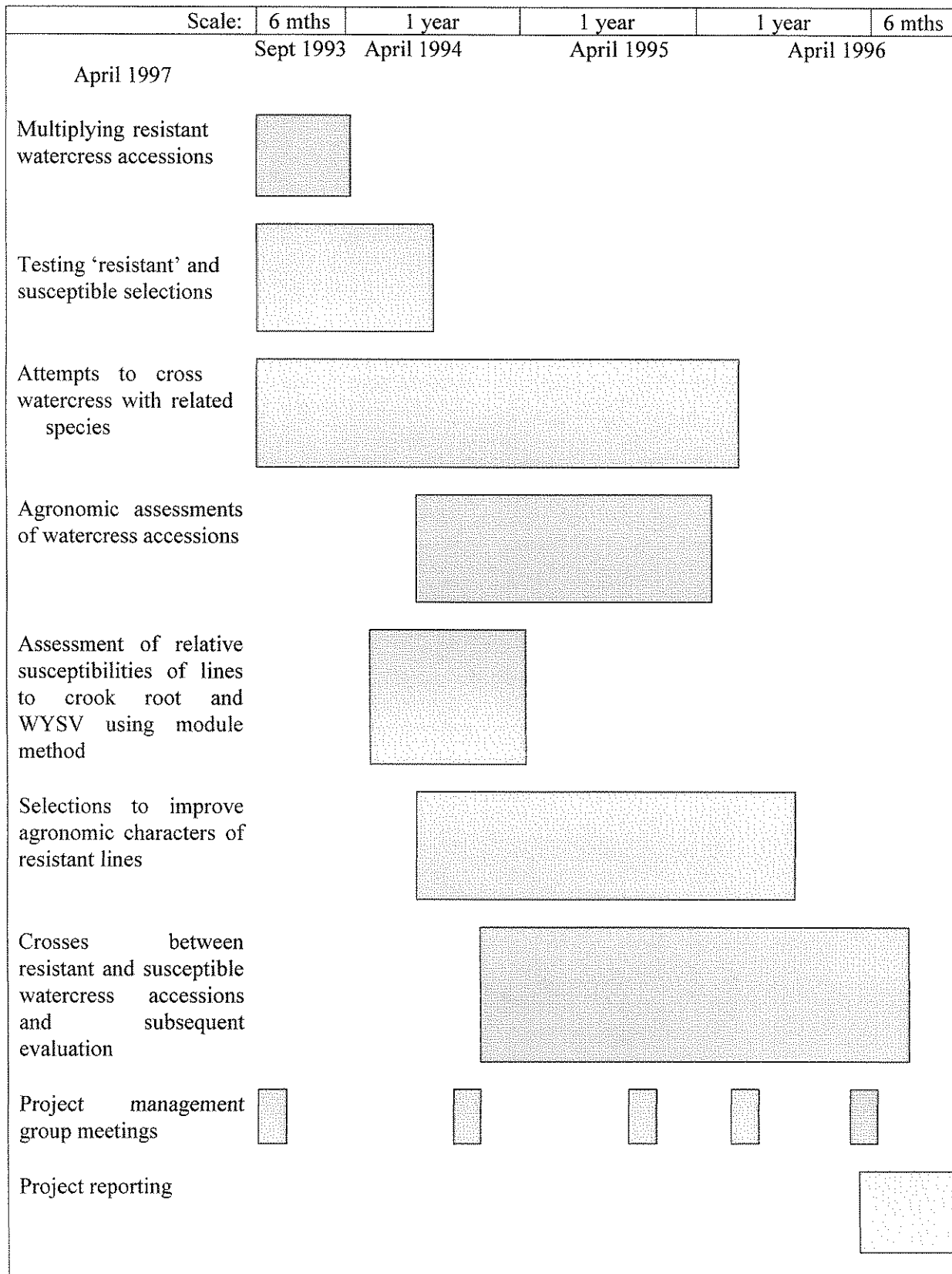


Figure 1. Gantt chart showing the duration of the different activities described in this report.

B. PART I - SELECTION OF LINES WITH IMPROVED RESISTANCE FROM WITHIN WATERCRESS ACCESSIONS

Five watercress accessions showing different levels of susceptibility to crook root and WYSV were selected for this experiment. The accessions comprised the most susceptible and widely grown line in the UK (Hampshire control), the least susceptible (Japanese), two further UK accessions (WC3 and WC5) and a French accession (WC123). The accessions were chosen based on a number of criteria including the statistical distribution of their susceptibilities to both diseases. A conscious decision was made not just to concentrate on the most resistant lines so that a spread of lines with different responses to the two diseases could be examined.

Plants from the lines involved had been tested prior to this project in watercress beds at the Doddings site for susceptibility to crook root and WYSV using the module method described earlier, and the two plants from each accession appearing most susceptible and the two plants appearing most resistant to crook root and WYSV were selected from each of the five accessions and grown on in the glasshouse and seed produced prior to the start of this project. These seed lines were then retested in watercress beds as part of this project using the module method developed at HRI (Walsh & Phelps 1991). Seed was planted into modules on 28.9.93 and 19.10.93 in a glasshouse at HRI. Those planted on 28.9.93 were delivered to Dorset on 9.11.93 and those planted on 19.10.93 were delivered on 30.11.93 and placed in an experimental watercress bed at the Doddings site for natural infection by crook root and WYSV to take place. Modules were recovered on 13 December 1993 and 11 January 1994 and assessed and tested for crook root and WYSV infection as described in the Materials and Methods section.

None of the accessions showed significant differences between 'resistant' and 'susceptible' selections in terms of crook root susceptibility (Table 1). For two of the accessions (WC3 and WC5), one of the 'resistant' selections appeared to be significantly less susceptible to WYSV than the susceptible selections (see Table 1).

Table 1. Analysis of variance on WYSV and crook root infection levels of susceptible and resistant selections of watercress from five watercress accessions.

Accession No. from which resistant and susceptible selections were made (country of origin)	Probability that difference between selections was due to chance (significance)	
	WYSV	Crook root
WC3 (English)	0.023 (*)	0.590 (NS)
WC5 (English)	0.005 (**)	0.334 (NS)
WC11 (Japanese)	0.847 (NS)	0.239 (NS)
WC123 (French)	0.408 (NS)	0.089 (NS)
Control (Hampshire)	0.178 (NS)	0.451 (NS)

(NS resistant and susceptible selections were not significantly different;

* resistant and susceptible selections were different at $p \leq 0.05$;

** resistant and susceptible selection were different at $p \leq 0.01$).

PART II - AGRONOMIC EVALUATION OF LESS SUSCEPTIBLE ACCESSIONS

The accessions of watercress previously identified as less susceptible to crook root and WYSV were multiplied in order to produce enough seed for trials in watercress beds to assess their agronomic acceptability. During multiplication the different accessions were monitored for morphological variation. The Japanese, Spanish and *R. islandica* accessions had pointed leaves, the Spanish accession had small leaves and the New Zealand had large leaves. Based on current agronomic perceptions, most accessions had acceptable (dark green) leaf colour except the Spanish and New Zealand which were lighter. The Japanese, New Zealand, Spanish and *Rorippa islandica* accessions flowered very rapidly whereas the English and Bullington accessions flowered later. Growers require watercress lines that do not flower rapidly so that they can produce leafy watercress in the summer months that is acceptable to supermarkets and consumers.

The accessions were as follows:-	Reason for being included in evaluations:
i) English small grower's own line	Found to be less susceptible to crook root and WYSV by HRI
ii) Bullington, English 'shy flowering' line	Thought by growers to be resistant to crook root
iii) Hampshire Watercress' commercial line	Line most widely grown in UK and most susceptible to crook root and WYSV (susceptible control)
iv) New Zealand, cultivated 'wild strain'	Found to be less susceptible to crook root and WYSV by HRI
v) Japanese	Found to be least susceptible to crook root and WYSV by HRI
vi) Non-watercress <i>Rorippa</i> species (<i>Rorippa islandica</i>)	Only 'non-watercress' accession to resemble watercress morphologically
vii) Spanish, wild small leaf	Found to be less susceptible to crook root and WYSV by HRI

Their morphologies whilst being grown in the glasshouse are summarised in Table 2.

Table 2. Morphological differences between lines whilst being multiplied in the glasshouse.

LINE:	BULLINGTON	ENGLISH	SPANISH	JAPANESE	NEW ZEALAND	'RORIPPA ISLANDICA'
LEAF SHAPE	Slightly pointed	Slightly pointed	Small pointed	Pointed	Rounded	Pointed
LEAF COLOUR	Dark green	Dark green	Mid-green	Dark green	Mid-green	Dark green
GROWTH HABIT	Very thick stems	Very thick stems	Bushy with fragile stems	Very bushy	Bushy with thick stems	Very thick stems
FLOWERING HABIT	Flowered last	Slow to flower	Very small flowers	Flowered readily	Flowered readily	Flowered readily
FINAL SEED WEIGHT (g)	18.9	32.4	181.4	92.5	36.3	20.6

Seed was planted on four occasions (two spring [11.3.94 and 5.4.94] and one autumn sowing, 1994 [4.10.94] and one summer sowing, 1995 [20.6.95]) and seedlings were transplanted into two watercress beds at the Doddings site in Dorset following current commercial practice on 30.3.94, 26.4.94, 27.10.94 and 3.7.95. The plants transplanted on 30.3.94 were washed away following flooding and consequently were replanted by those of 26.4.94. The design of this experiment was discussed and agreed jointly with statisticians and growers. Plants were then assessed on a number of occasions for what were considered by growers to be the most important agronomic/commercial traits, i.e.

- i) General appearance
- ii) Leaf size
- iii) Leaf colour
- iv) Leaf shape
- v) Growth rate
- vi) Growth habit
- vii) Uniformity
- viii) Crook root susceptibility
- ix) Taste

In all, assessments were made on five occasions (28.6.94; 15.3.95; 5.5.95; 25.7.95; 31.8.95). They were carried out by growers and were made 'blind' (the plots were labelled A-G and the origin of the accessions was not revealed). During the winter of 1994, the plots of the spring sowing received zinc treatments whereas those of the autumn sowing did not. Growers scored accessions on a scale of 1-3 for the characters mentioned above where: 1 = not commercially acceptable; 2 = approaching commercial acceptability; 3 = as good as current commercial lines. The plots were trimmed at regular intervals in line with commercial practice.

On most occasions the growers picked out the 'Hampshire Watercress' control commercial line as the best for most agronomic characters except crook root susceptibility and the other commercial lines (English and Bullington) as mostly acceptable. The other accessions varied depending upon time of assessment and date of planting. The accession with most resistance to

crook root and WYSV (Japanese) was commercially unacceptable for most of the traits assessed, except of course crook root susceptibility. Of the other two most resistant accessions, the Spanish was commercially unacceptable for most traits (except crook root susceptibility), whereas the New Zealand was commercially acceptable or approaching commercial acceptability for most traits, the exceptions being leaf colour (too pale) and leaf size (too big). This New Zealand accession appeared to be more commercially acceptable in winter than in summer. Full details of assessments are presented in the tables in the Appendix.

Before disposal of the plants in the experimental plots used for these assessments, they were viewed on 3 November 1995 by Environment Agency personnel along with HRI staff and the site manager.

In addition to these visual assessments in the beds, plants from the different plots were collected at different times. Those in Bed 6 (autumn sowing, no zinc) and Bed 7 (spring sowing, zinc treated) were sampled on 21.2.95. Sixteen plants per line were taken at random and visually assessed for crook root severity and tested by ELISA for WYSV susceptibility at HRI. The Japanese, Spanish and to a lesser extent, the New Zealand accessions had significantly less crook root than most of the other lines in the bed that did not receive zinc treatment, following the winter of 1994. In the bed that did receive zinc treatment, positional effects were detected. Crook root severity increases in watercress beds towards the bottom of beds. In Bed 7, the English small grower's own line was grown at the top of the bed and had lower levels of crook root than more resistant lines such as the Japanese that was grown lower down the bed. In Bed 6, where the English small grower's own line was grown lower down the bed, it had one of the highest crook root scores. Although the Japanese and Spanish accessions had some of the lowest crook root scores, no significant differences were detected, presumably partially due to the effect of zinc on crook root levels. In the watercress bed that did not receive zinc treatment (autumn sowing), the Japanese line contained significantly less WYSV than most of the other lines, otherwise there were no other significant differences in this bed. In the bed that did receive zinc treatment (spring sowing), positional effects on WYSV infection were observed, very high levels of WYSV infection were present and no significant differences between most lines were found. The results are summarised in Tables 3 and 4. Plants with higher scores have more crook root infection than plants with lower scores as explained in the Materials and Methods, section 2.2, Determination of susceptibility of plants to crook root and WYSV.

Table 3. Mean crook root scores for the lines growing in Bed 6 (no zinc) and Bed 7 (zinc).

Line	Bed 6 (no zinc)	Bed 7 (zinc)
English small grower's own line	2.406	1.031
Bullington	2.594	1.312
Hampshire	2.219	1.344
New Zealand	1.906	1.500
Japanese	1.250	1.437
' <i>Rorippa islandica</i> '	1.875	1.625
Spanish	1.169	1.375
Least significant difference (LSD) = 0.263		

Table 4. Mean ELISA values (after transformation) for WYSV infection levels in watercress lines growing in Bed 6 (no zinc) and Bed 7 (zinc).

Line	Bed 6 (no zinc)	Bed 7 (zinc)
English small grower's own line	1.487	3.449
Bullington	1.738	4.410
Hampshire	1.363	4.159
New Zealand	1.287	3.940
Japanese	0.961	4.158
' <i>Rorippa islandica</i> '	1.742	4.430
Spanish	1.688	4.396
Least significant difference (LSD) = 0.507		

In parallel to these experiments on the plants growing in beds, the original seven watercress accessions were tested for their relative susceptibilities to crook root and WYSV in watercress beds using the module method developed at HRI which avoids positional effects (Walsh & Phelps 1991). Seed was sown into modules on 6 April 1994, raised in the glasshouse and transported to the Doddings site on 10 May 1994, where they remained until 21 June 1994 when they were recovered. Plants were then assessed and tested for crook root and WYSV as normal. In this experiment, plants of the Japanese accession had significantly less crook root than all the other accessions, except the Spanish which, along with the New Zealand accession, were the next best in terms of resistance to crook root. These results were also reflected in the WYSV infection levels where the Japanese accession contained significantly less WYSV than all the other accessions except the New Zealand which along with the Spanish accession had the next lowest WYSV content. Full details of crook root and WYSV infection levels are given in Table 5.

Table 5. Mean crook root score and ELISA values for the seven watercress accessions from experiments designed to avoid positional effects

Line	Mean crook root score	Mean ELISA value
English small grower's own line	1.778	4.056
Bullington English 'shy flowering line'	2.250	3.969
Hampshire commercial line	2.000	3.669
New Zealand, cultivated 'wild strain'	1.694	2.350
Japanese	1.083	2.084
'Non-watercress' <i>Rorippa islandica</i>	2.139	3.194
Spain, wild small leaf	1.444	2.989
Least significant difference (LSD)	0.530	0.735

PART III - SELECTION OF IMPROVED AGRONOMIC TYPES FROM WITHIN ACCESSIONS FOUND TO BE LESS SUSCEPTIBLE TO CROOK ROOT AND WYSV

It became apparent that the watercress accessions that were most resistant to crook root and WYSV were commercially unacceptable for most (Japanese and Spanish), or some (New Zealand) agronomic traits. So attempts were made to identify and select individual plants that were more commercially suitable from the plots of these accessions growing in watercress beds.

In addition, plants that were considered typical of the lines were also taken for future comparisons. Details of the selections made were as follows:

Line	Selection made
i) English small grower's own line	Plants with improved growth rate
ii) Bullington English 'shy flowering' line	Typical plants taken as control
iii) Hampshire watercress commercial line	Typical plants taken as control
iv) New Zealand, cultivated 'wild strain'	Darker leaved plants and typical plants also taken
v) Japanese	Plants with improved general appearance and typical plants also taken
vi) 'Non-watercress' <i>Rorippa islandica</i>	No selections made as this line was too susceptible to crook root and WYSV
vii) Spanish, wild small leaf	Plants with improved general appearance and typical plants also taken

The selections were made in August 1994 by growers and plants were used to establish replicated mini-plots in Bed 5 in gravel on top of a plastic membrane (to exclude volunteer watercress plants). These mini-plots were subsequently sampled (two occasions in May 1995) and individual plants were scored for crook root and tested for WYSV infection at HRI.

Only the New Zealand typical selection and the Spanish improved morphology selection had significantly less crook root than the Hampshire commercial/reference line, and then, only on one of the sampling occasions. The Spanish typical selection had the lowest WYSV content on both sampling occasions but it was only significantly better than the Hampshire reference line on the second occasion. The results of these experiments are presented in Tables 6-7. The plots

were left to flower and seed was harvested from the Spanish typical selection in July 1996 for further evaluation. Subsequent experiments, using the module method developed at HRI, to test this Spanish line in watercress beds showed that it appeared to be as susceptible to crook root and WYSV as the Hampshire reference line, and that the original Japanese accession was significantly less susceptible to both diseases than this Spanish selection (Tables 11 and 12). Full details of these experiments and the comprehensive results are given in Part VI.

Table 6. Crook root severity in watercress accessions and selections growing in mini-plots in experimental Bed 5.

Line, and selection made	Mean crook root score	
	Sampling occasion 1 24 May 1995	Sampling occasion 2 31 May 1995
<i>R. islandica</i>	1.500	0.583
Spanish, improved morphology	1.083	0.917
Spanish, typical	1.250	0.417
New Zealand, darker leaf 1	2.500	1.472
New Zealand, darker leaf 2	1.750	1.000
New Zealand, typical	1.083	1.167
Japanese, improved morphology	1.333	0.778
Japanese, typical	1.500	0.833
English, small grower's own line, improved growth rate	1.917	0.833
English small grower's own line, typical	1.750	0.917
Bullington, typical	1.417	0.750
Hampshire watercress, typical	2.000	0.750
Least significant difference (LSD)	0.8151	0.6554

Table 7. Watercress yellow spot virus content (ELISA value) of watercress accessions and selections growing in mini-plots in experimental Bed 5.

Line, and selection made	Mean ELISA value	
	Sampling occasion 1 24 May 1995	Sampling occasion 2 31 May 1995
<i>R. islandica</i>	3.901	2.662
Spanish, improved morphology	4.032	3.859
Spanish, typical	3.375	1.103
New Zealand, darker leaf 1	3.774	3.879
New Zealand, darker leaf 2	3.928	3.798
New Zealand, typical	3.863	3.635
Japanese, improved morphology	4.137	3.778
Japanese, typical	4.162	3.684
English, small grower's own line, improved growth rate	4.239	3.765
English small grower's own line, typical	3.993	3.686
Bullington, typical	4.201	3.991
Hampshire watercress, typical	3.848	3.547
Least significant difference (LSD)	0.483	0.8068

PART IV - HYBRIDISATION OF WATERCRESS WITH RELATED PLANT SPECIES THAT HAVE BEEN SHOWN TO BE NON-HOSTS OF CROOK ROOT

Attempts were made to cross a number of *Cardamine* species (*C. hirsuta*, *C. impatiens*, *C. flexuosa* and *C. amara*) with different watercress accessions. The *Cardamine* species did not produce enough pollen to attempt to pollinate watercress, consequently in all cases, watercress pollen was used to pollinate the *Cardamine* plants. In most cases the styles of flowers dehydrated and no seed pods were produced. Where seed was produced from these attempted crosses, it was grown alongside parental lines to determine whether hybridisation had been achieved. No hybrids were identified.

Reciprocal crosses were also attempted between *Rorippa palustris* and two different watercress accessions, again no hybrids were identified.

Following information obtained at a Steering Group meeting that *Barbarea verna* may be closely related to watercress, reciprocal crosses between it and the Hampshire reference watercress line were attempted, again no hybridisation was achieved.

PART V - EVALUATION OF NEW WATERCRESS ACCESSIONS

During the course of this project, a number of new watercress accessions were received by HRI from growers. These included: a wild Cornish accession; a wild Dorset accession; a French commercial line; a putative 'Sylvasprings' accession and a Danish commercial line. Consequently, they were tested for susceptibility to crook root and WYSV alongside the Hampshire commercially grown reference line and the most resistant Japanese accession, in watercress beds using HRI's module method. Seed was sown into modules on 7 and 21 May 1996, plants were raised in the glasshouse, the modules were transported to the Doddings site on 18 June and 3 July 1996 and recovered on 29 July and 12 August, 1996. Plants were then assessed for crook root and tested for WYSV infection as normal. One of these accessions (wild Dorset) had significantly less crook root than the Hampshire commercial/reference line, but more than the Japanese accession. None of the accessions contained significantly less WYSV than the Hampshire commercial/reference line. Full details of crook root scores and WYSV content of the different accessions are given in Tables 8-9.

Table 8. Crook root infection levels of watercress accessions received during the project.

Watercress accession No.	Origin	Mean crook root score	Significance of difference from Hampshire control
WC135	Danish commercial line	1.692	N.S.
WC136	French commercial line	1.254	N.S.
WC137	Wild Dorset accession	1.123	*
WC138	Wild Cornish accession	1.371	N.S.
WC140	Putative 'Sylvasprings' line	1.320	N.S.
Hampshire	Original control susceptible line	1.679	
Japanese	Most resistant line control	0.708	***

N.S. = Not significantly different; * = $p \leq 0.05$ (significantly different); *** = $p \leq 0.001$ (significantly different) from Hampshire control line

Table 9. WYSV infection levels of watercress accessions received during the project.

Watercress accession No.	Origin	Mean ELISA value	Significance of difference from Hampshire control
WC135	Danish commercial line	3.808	N.S.
WC136	French commercial line	4.140	N.S.
WC137	Wild Dorset accession	4.226	N.S.
WC138	Wild Cornish accession	4.090	N.S.
WC140	Putative 'Sylvasprings' line	3.967	N.S.
Hampshire	Original control susceptible line	4.352	
Japanese	Most resistant line control	3.204	***

Low ELISA values mean not much virus; High ELISA values mean a lot of virus.

N.S. = Not significantly different; *** = $p \leq 0.001$ (significantly different) from Hampshire control line

PART VI - CROSSES BETWEEN LESS SUSCEPTIBLE ACCESSIONS AND CURRENT COMMERCIAL WATERCRESS LINES TO PRODUCE IMPROVED LINES

The Japanese and New Zealand accessions which showed the highest level of resistance to crook root and WYSV were selected as resistant parents for these crosses. The New Zealand accession was commercially more acceptable than the Japanese accession. The Hampshire commercial/reference line, being the most widely grown line in the UK was selected as the commercially acceptable parent.

Reciprocal crosses between individual plants from these accessions were made and seed (F_1 generation) collected from plants. Some of this seed was planted in the glasshouse along with seed that had been produced by selfing (self pollinating) the parents and seed of the original accessions.

The morphologies of the F_1 hybrid plants was compared to those of the selfed parents and the original accessions in the glasshouse. There was some suggestion of the hybrid vigour in F_1 hybrids from the New Zealand x Hampshire cross. The F_1 hybrids from the Japanese x Hampshire reciprocal crosses showed some intermediate morphologies to those of the parental plants (eg. leaf shape) and also some morphologies that were closer to one, or other of the parents (eg. internode length and time to flowering).

This suggested that some traits are under the control of dominant genes and others are not. F_1 plants from all crosses were grown on and seed of the next (F_2) generation collected for further evaluation.

The susceptibility of plants of the F_1 populations, plants resulting from selfed parents and the original Japanese accession and the Hampshire reference line, to crook root and WYSV infection were compared in a watercress bed at the Doddings, Dorset site using the module method developed at HRI (Walsh & Phelps 1991).

Four F_1 populations from Japanese x Hampshire reciprocal crosses, one F_1 population from a New Zealand x Hampshire cross, all their selfed parents and the original Japanese accessions and Hampshire line were included in this experiment. Seed was planted in modules on 7 and 21 May, 1996, grown on in the glasshouse and transported to the Doddings site on 18 June and 3 July, 1996 where they remained in a watercress bed for infection to take place until they were recovered on 29 July and 12 August, 1996. Plants were then assessed and tested for the presence of crook root and WYSV as normal. Two of the Japanese hybrids had significantly less crook root than the Hampshire line and three had significantly less WYSV. Two of the Japanese hybrids had significantly more crook root than the original Japanese accession and one had significantly more WYSV than the original Japanese accession, suggesting that Japanese hybrids were intermediate to their parents in terms of susceptibility to crook root and WYSV. The New Zealand hybrid and its selfed parent did not have significantly different levels of crook root and WYSV to the Hampshire line (see Table 10).

Table 10. Mean crook root scores and WYSV content (ELISA value) for resistant and susceptible watercress lines and hybrids.

Resistant lines		Susceptible lines	
Japanese	0.708***	Hampshire	1.679
Control	3.204***	Control	4.352
<u>Self of resistant parent</u>		<u>Self of susceptible Parent</u>	
<u>Hybrid</u>			
RN95053	0.831*** 2.950***	RN95056 (F ₁)	1.068** 3.489*
		RN95047	1.335 ^{NS} 3.985 ^{NS}
		RN95058(F ₁)	1.097* 4.061 ^{NS}
		RN95048	1.709 ^{NS} 4.363 ^{NS}
RN95050	0.695*** 2.819***	RN95059(F ₁)	1.305 ^{NS} 3.567*
RN95052	0.953** 2.326***	RN95061(F ₁)	1.267 ^{NS} 3.426*
		RN95046	1.423 ^{NS} 2.551***
RN95017 (New Zealand)	1.381 ^{NS} 3.680 ^{NS}	RN95018(F ₁)	1.225 ^{NS} 3.652 ^{NS}
		RN95016	1.211* 4.160 ^{NS}
		RN95042(F ₂)	1.162* 3.954 ^{NS}

Upper values given for each line are mean crook root scores and lower values are mean ELISA values. Significance levels for differences from Hampshire control: ^{NS} not significant; * p ≤ 0.05; ** p ≤ 0.01; *** p ≤ 0.001.

The morphologies of the individual plants within F₂ populations of Japanese x Hampshire reciprocal crosses were compared. Leaf shape seemed to be segregating in one population in that some plants had pointed leaves like the Japanese parent and some had rounded leaves like the Hampshire parent. In other populations some plants were segregating for leaf shape and others had intermediate leaf shape. Similar mixtures of segregation and intermediate types were observed for internode length and time to flowering.

F₂ populations have also been tested for susceptibility to crook root and WYSV in a

watercress bed at the Doddings site, Dorset, using the module method. In all, six F₂ populations from Hampshire x Japanese reciprocal crosses and one F₂ population from the New Zealand x Hampshire cross were tested. Seed was planted into modules on 14 October and 28 October, 1996 and plants were grown on in the glasshouse until 25 November and 9 December, 1996 when they were delivered to the Doddings site and placed in one of the watercress beds. They were recovered on 6 January and 10 February 1997 and assessed and tested for crook root and WYSV as normal. The F₂ population (RN95042) from the New Zealand x Hampshire cross appeared to be intermediate to its parents in terms of susceptibility to WYSV and may be slightly more resistant to crook root than the Hampshire reference line (Table 8.1). Mean crook root scores in F₂ populations from Hampshire x Japanese reciprocal crosses showed that one (RN96034) had a very low score and was similar to the Japanese parent, two others (RN96032 and RN96035) were intermediate but significantly better (lower) than the Hampshire parental line, whereas all others were not significantly different to the Hampshire parental line (Table 11). Due to differences between occasions for ELISA results, it was not possible to put the data from both occasions together and carry out an analysis of variance.

Table 11. Mean crook root scores and WYSV content (ELISA value) for F₂ populations from crosses between the commercial Hampshire line and the resistant Japanese line.

F ₂ population/Plant line	Mean crook root score	Mean ELISA [†] value	
		Occasion 1	Occasion 2
RN96032	0.917**	2.164	
RN96033	1.083 ^{NS}	2.695	
RN96034	0.583***	2.604	
RN96035	1.056*		4.403
RN96036	1.208 ^{NS}		3.787
RN96043	1.125 ^{NS}		-
Hampshire control	1.403	2.744	4.379
Spanish line	1.072 ^{NS}	2.409	4.387
Japanese control	0.392***	2.619	2.429

^{NS} = not significantly different from Hampshire control line; * = p≤0.05; ** = p≤0.01; *** = p≤0.001.

[†] Due to differences between occasions for ELISA results, it was not possible to put the data from both occasions together and carry out an analysis of variance.

This experiment was repeated as a lot of plants died due to frost damage during the experiment, and infection levels on the first of the two sampling occasions were very low. Seeds were planted into modules on 24 February and 10 March 1997 and plants were grown on in the glasshouse for 5 weeks until 1 April and 14 April 1997, when they were delivered to the Doddings site and placed in one of the watercress beds for infection to take place. The crates were recovered 6 weeks later on 12 May and 27 May 1997, and assessed and tested for crook root and WYSV as normal. In the repeated experiment, the same F₂ populations (RN96032 and RN96035) had intermediate crook root scores that were significantly better than the Hampshire parental line (Table 12). One of these F₂ populations (RN96035) along with another (RN96036) had significantly lower WYSV levels than the Hampshire parental line (Table 12).

Table 12. Mean crook root scores and WYSV content (ELISA value) for F₂ populations from crosses between the commercial Hampshire line and resistant Japanese line from the repeat of the previous experiment.

F ₂ population/Plant Line	Mean crook root score	Mean ELISA value
RN96032	1.083***	5.055 ^{NS}
RN96033	1.556 ^{NS}	5.095 ^{NS}
RN96034	1.389 ^{NS}	5.109 ^{NS}
RN96035	1.222**	4.956*
RN96036	1.417 ^{NS}	4.936*
RN96043	1.375 ^{NS}	4.992 ^{NS}
Hampshire control	1.597	5.174
Spanish line	1.444 ^{NS}	5.007 ^{NS}
Japanese control	0.764***	4.809***

^{NS} = not significantly different from Hampshire control line; * = p≤0.05; ** = p≤0.01; *** = p≤0.001

Plants of these F₂ populations were also grown in the glasshouse and their morphologies were observed and recorded to see how they compared with the commercially acceptable Hampshire and morphologically unacceptable Japanese lines. These results along with crook root and WYSV susceptibilities are summarised in Table 13.

Table 13. Morphologies of F₂ populations from crosses between the Hampshire and Japanese lines and their susceptibilities to crook root and WYSV.

F ₂ population	Whether better than Hampshire line for:-		Leaf shape	Internode length	Time to flower
	Crook root susceptibility	WYSV susceptibility			
RN96032	Yes	No	Segregating mostly round	Segregating mostly short	Segregating Mostly long
RN96033	No	No	Segregating mostly round	Segregating equal proportions of long and short	Intermediate
RN96034	Yes/No	No	Segregating mostly round	Segregating mostly short	Segregating equal proportions long and short
RN96035	Yes	Yes	NR	NR	NR
RN96036	No	Yes	NR	NR	NR
RN96043	No	No	NR	NR	NR
Hampshire	-	-	Round	Short	Long
Japanese	Yes	Yes	Pointed	Long	Short

NR = Not recorded due to lack of seed

C. OVERALL CONCLUSIONS AND RECOMMENDATIONS

The experiments carried out during the project to select lines with improved levels of resistance to crook root and WYSV from within accessions demonstrated that there was little variability within the accessions for resistance/susceptibility to the two pathogens. This suggested that accessions may not be as genetically diverse as had previously been thought. There was an indication that a selection with improved resistance to WYSV had been selected from each of two English accessions, but these selections did not have improved resistance to crook root.

Evaluation of the agronomic acceptability of the most resistant accessions showed that the

Japanese and Spanish accessions were totally unusable commercially. The New Zealand accession had potential, but suffered from too pale leaf colour and too large leaves. It should be noted that in the winter months (when crook root and WYSV are most damaging), the New Zealand accession performed better than it did in summer.

Two approaches to producing commercially acceptable watercress lines with improved resistance to crook root and WYSV were then taken: attempting to select improved agronomic types from within resistant accessions and crossing resistant accessions with a commercially acceptable line. The former approach was not successful, selections made for improved appearance were as susceptible to crook root and WYSV as the most widely grown commercial line. The latter approach appears to have some potential, hybrids (F₁ generation) between resistant (Japanese) and commercially acceptable, but very susceptible (Hampshire) accessions, had intermediate susceptibilities to both diseases. F₂ populations appeared to be mostly intermediate also, although there was some evidence of segregation, suggesting that the resistance is a quantitative character possibly controlled by several genes. Some morphological traits appeared to segregate in some lines, whereas others were intermediate to the parents. Detailed morphological studies need to be linked to assessments of crook root and WYSV susceptibility in order to determine whether improved resistance to crook root and WYSV can be combined with commercially acceptable morphologies in segregating populations from Hampshire x Japanese crosses.

Attempts to hybridise watercress with related plant species that are extremely resistant to crook root and WYSV were unsuccessful. New watercress accessions were on the whole no better than the most widely grown watercress line in terms of susceptibility to both diseases.

The levels of resistance to crook root and WYSV provided by the offspring of hybrids from the watercress crosses that have been made are thought unlikely to give sufficient control of crook root and WYSV to be deployed without other forms of control. It is recommended that the following further research and development is required:-

1. The lines RN96032, RN96036 and especially RN96035 are worthy of further evaluation.
2. There is an urgent need to study and identify other non-chemical measures (such as cultural practices, physical barriers, biological agents etc.) that could be deployed to control crook root and WYSV so that integrated control strategies can be developed for the control of these diseases.
3. The resistant lines identified in this study could be a component of such integrated strategies, as could reduced zinc regimes or more benign chemicals. Combining a number of treatments which alone might only have minor beneficial effects often results in synergy, where the effect of the treatments combined is much greater than the sum of the individual component parts.

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E.**BIBLIOGRAPHY**

1. Clark, M. F., & Adams, A. N. (1977). Characteristics of the microplate method of enzyme-linked immunosorbent assay for the detection of plant viruses. *Journal of General Virology* **34**: 475-483.
2. Clay, C.M., & Walsh, J.A. (1997). *Spongospora subterranea* f. sp. *nasturtii*, ultrastructure of the plasmodial-host interface, food vacuoles, flagellar apparatus and exit pores. *Mycological Research* **101**: 737-744.
3. Martin, T. R., & Holdich, D.M. (1986). The acute lethal toxicity of heavy metals to peracarid crustaceans (with particular reference to fresh-water Asellids and Gammarids). *Water Research* **20**: 1137-1147.
4. Roddie, B., Kedwards, T. & Crane, M. (1992). Potential impact of watercress farm discharges on the freshwater amphipod, *Gammarus pulex* L. *Bulletin of Environmental Contamination and Toxicology* **48**: 63-69.
5. Spire, D. (1962). Etude de la maladie des taches jaunes du cresson (*Nasturtium officinale* R. Br.). *Annales des Epiphyties* **13**: 39-45.
6. Tomlinson, J.A. (1958). Crook root of watercress. III The causal organism *Spongospora subterranea* (Wallr.) Lagerh. f. sp. *nasturtii* f. sp. nov. *Transactions of the British Mycological Society* **41**: 491-498.
7. Tomlinson, J.A. (1960). Crook root disease of watercress. A review of research. *N.A.A.S. Quarterly Review* **49**: 13-19.
8. Walsh, J.A. (1993). Final report on HDC Project FV/55: Screening watercress and related germplasm for resistance to crook root and watercress yellow spot virus (WYSV). 10pp.
9. Walsh, J.A., Clay, C.M. and Miller, A. (1989). A new virus disease of watercress in England. *EPPO Bulletin* **19**: 463-470.
10. Walsh, J.A. and Phelps, K. (1991). Development and evaluation of a technique for screening watercress (*Rorippa nasturtium-aquaticum*) for resistance to watercress yellow spot virus and crook root fungus (*Spongospora subterranea* f. sp. *nasturtii*). *Plant Pathology* **40**: 212-220.
11. Walsh, J., Jenner, C. and Pink, D. (1997). Fighting crook root disease with genetics. *HDC Project News* **45** (August 1997): 10-11.

F. APPENDIX

The order of the lines in each table indicates the order of the lines in the beds, the first line listed being uppermost in the bed and the last line listed being lowest.

Table 14. Assessment sheet for grower 1 for the seven watercress lines growing in Bed 7 from assessment of 28 June 1994.

NAME: Grower 1

SEED SOWN INTO MODULES: 5.4.94

SEEDLINGS PLANTED OUT INTO BED: 26.4.94

PLEASE SCORE EACH LINE ON A 1-3 SCALE WHERE:

- 1 = NOT COMMERCIALY ACCEPTABLE
- 2 = APPROACHING COMMERCIAL ACCEPTABILITY
- 3 = AS GOOD AS CURRENT COMMERCIAL LINES

LINE	GENERAL APPEARANCE	LEAF SIZE	LEAF COLOUR	LEAF SHAPE	GROWTH RATE	GROWTH HABIT	UNIFORMITY	CROOK ROOT	TASTE (OPTIONAL)	COMMENTS
English grower's own line	3	3	3	3	2	-	3	3	3	
Bullington	3	3	3	3	2	-	3	3	3	
Hampshire	3	3	2	3	3	-	3	3	3	
New Zealand	3	3	1	3	3	-	3	3	3	
Japanese	1	1	1	1	1	-	2	3	1	
' <i>Rorippa islandica</i> '	2	1	3	1	2	-	2	3	2	
Spanish	1	1	1	1	1	1	1	3	1	

Table 15. Assessment sheet for grower 2 for the seven watercress lines growing in Bed 7 from assessment of 28 June 1994

NAME: Grower 2

SEED SOWN INTO MODULES: 5.4.94

SEEDLINGS PLANTED OUT INTO BED: 26.4.94

PLEASE SCORE EACH LINE ON A 1-3 SCALE WHERE:

- 1 = NOT COMMERCIALY ACCEPTABLE
- 2 = APPROACHING COMMERCIAL ACCEPTABILITY
- 3 = AS GOOD AS CURRENT COMMERCIAL LINES

LINE	GENERAL APPEARANCE	LEAF SIZE	LEAF COLOUR	LEAF SHAPE	GROWTH RATE	GROWTH HABIT	UNIFORMITY	CROOK ROOT	TASTE (OPTIONAL)	COMMENTS
English grower's own line	3	2	3	3	2	2	2	1		Bit slow growing but nice leaf
Bullington	3	2	3	3	3	2	2	1	2	Bit slow growing
Hampshire	3	3	3	3	3	3	2	2		Probably good a few weeks before
New Zealand	2	3	2	3	3	3	2	2	2	A bit pale now
Japanese	1	3	1	1	1	1	1	3	0	Turns to flower quickly
'Rorippa islandica'	1	1	1	1	2	2	2		0	Awful, slow, bad taste
Spanish	1	1	1	1	1	0	0		-	No comment needed

Table 16. Assessment sheet for grower 3 for the seven watercress lines growing in Bed 7 from assessment of 28 June 1994

NAME: Grower 3

SEED SOWN INTO MODULES: 5.4.94

SEEDLINGS PLANTED OUT INTO BED: 26.4.94

PLEASE SCORE EACH LINE ON A 1-3 SCALE WHERE:

- 1 = NOT COMMERCIALY ACCEPTABLE
- 2 = APPROACHING COMMERCIAL ACCEPTABILITY
- 3 = AS GOOD AS CURRENT COMMERCIAL LINES

LINE	GENERAL APPEARANCE	LEAF SIZE	LEAF COLOUR	LEAF SHAPE	GROWTH RATE	GROWTH HABIT	UNIFORMITY	CROOK ROOT	TASTE (OPTIONAL)	COMMENTS
English grower's own line	2	2	3	2	2	2	2	2	3	Small leaf, slow flowering, crook root
Bullington	3	3	3	3	2	2	3	2	3	Best from commercial point of view
Hampshire	3	3	3	3	3	3	3	3	1	Poor taste
New Zealand	1	3	1	3	3	3	3	3	1	Poor colour a lot of flowers
Japanese	1	1	1	1	1	1	1	1	1	Very poor
'Rorippa islandica'	2	1	2	2	1	2	2	1	2	Small leaf
Spanish	0	0	0	0	0	0	0	0	0	

Table 17. Assessment sheet for grower 4 for the seven watercress lines growing in Bed 7 from assessment of 28 June 1994.

NAME: Grower 4

SEED SOWN INTO MODULES: 5.4.94

SEEDLINGS PLANTED OUT INTO BED: 26.4.94

PLEASE SCORE EACH LINE ON A 1-3 SCALE WHERE:

- 1 = NOT COMMERCIALY ACCEPTABLE
- 2 = APPROACHING COMMERCIAL ACCEPTABILITY
- 3 = AS GOOD AS CURRENT COMMERCIAL LINES

LINE	GENERAL APPEARANCE	LEAF SIZE	LEAF COLOUR	LEAF SHAPE	GROWTH RATE	GROWTH HABIT	UNIFORMITY	CROOK ROOT	TASTE (OPTIONAL)	COMMENTS
English grower's own line	3	3	3	3	2	3	2			Very little flower
Bullington	3	3	3	3	2	3	2			Very little flower
Hampshire	3	2*	3	3	3	3	3			In flower
New Zealand	3	2*	2	3	3	3	3			In flower
Japanese	1		Yellow							Very badly in flower
'Rorippa islandica'	-		3		2	3				
Spanish	-1	-1	-1	-1	-1	-1	-1			

ANY FURTHER COMMENTS: * almost too large

Table 18. Assessment sheet for grower 5 for the seven watercress lines growing in Bed 7 from assessment of 28 June 1994

NAME: Grower 5

SEED SOWN INTO MODULES: 5.4.94

SEEDLINGS PLANTED OUT INTO BED: 26.4.94

PLEASE SCORE EACH LINE ON A 1-3 SCALE WHERE:

- 1 = NOT COMMERCIALY ACCEPTABLE
- 2 = APPROACHING COMMERCIAL ACCEPTABILITY
- 3 = AS GOOD AS CURRENT COMMERCIAL LINES

LINE	GENERAL APPEARANCE	LEAF SIZE	LEAF COLOUR	LEAF SHAPE	GROWTH RATE	GROWTH HABIT	UNIFORMITY	CROOK ROOT	TASTE (OPTIONAL)	COMMENTS
English grower's own line	3	2	3	3		2	3			
Bullington	3	2	3	3		2	3			
Hampshire	3	2	3	3		2	3			
New Zealand	2	2	2	2		2	3			
Japanese	1	1	1	1		2	2			
' <i>Rorippa islandica</i> '	1	2	2	2		2	3			
Spanish	-1	-1	-1	-1		-1	-1			

ANY FURTHER COMMENTS:

Table 19. Assessment sheet for grower 6 for the seven watercress lines growing in Bed 7 from assessment of 28 June 1994

NAME: Grower 6

SEED SOWN INTO MODULES: 5.4.94

SEEDLINGS PLANTED OUT INTO BED: 26.4.94

PLEASE SCORE EACH LINE ON A 1-3 SCALE WHERE:

- 1 = NOT COMMERCIALY ACCEPTABLE
- 2 = APPROACHING COMMERCIAL ACCEPTABILITY
- 3 = AS GOOD AS CURRENT COMMERCIAL LINES

LINE	GENERAL APPEARANCE	LEAF SIZE	LEAF COLOUR	LEAF SHAPE	GROWTH RATE	GROWTH HABIT	UNIFORMITY	CROOK ROOT	TASTE (OPTIONAL)	COMMENTS
English grower's own line	3	2	3	3	2	2	3	3		
Bullington	3	2	3	3	2	2	3	3		
Hampshire	2	3	2	3	3	3	3	1		
New Zealand	2	2	2	2	3	3	3	3		
Japanese	1	1	1	1	1	1	2	3		
'Rorippa islandica'	2	2	3	3	2	3	3	2		
Spanish	3	3	3	2	3	3	3	2		

ANY FURTHER COMMENTS:

Table 20. Assessment sheet for grower 7 for the seven watercress lines growing in Bed 7 from assessment of 28 June 1994

NAME: Grower 7

SEED SOWN INTO MODULES: 5.4.94

SEEDLINGS PLANTED OUT INTO BED: 26.4.94

PLEASE SCORE EACH LINE ON A 1-3 SCALE WHERE:

- 1 = NOT COMMERCIALY ACCEPTABLE
- 2 = APPROACHING COMMERCIAL ACCEPTABILITY
- 3 = AS GOOD AS CURRENT COMMERCIAL LINES

LINE	GENERAL APPEARANCE	LEAF SIZE	LEAF COLOUR	LEAF SHAPE	GROWTH RATE	GROWTH HABIT	UNIFORMITY	CROOK ROOT	TASTE (OPTIONAL)	COMMENTS
English grower's own line	Fair	2	3	3	2	3	2	3	Good	A bit small in leaf
Bullington	Good	2	3	3	2	3	3	3	Good	Very good a bit slow growth
Hampshire	Good	3	3	3	3	3	3	3	Fair	Normal strain
New Zealand	Fair	2	1	3	3	3	3	2	Fair	A bit yellow
Japanese	1	1	1	1	1	1	1	2	Fair	Too flowery
'Rorippa islandica'	Fair	2	3	2	1	3	3	2	No Good	Too small
Spanish	1	1	1	1	1	1	1	1	-	No growth

ANY FURTHER COMMENTS:

Table 21. Assessment sheet for grower 8 for the seven waterress lines growing in Bed 7 from assessment of 28 June 1994

NAME: Grower 8

SEED SOWN INTO MODULES: 5.4.94

SEEDLINGS PLANTED OUT INTO BED: 26.4.94

PLEASE SCORE EACH LINE ON A 1-3 SCALE WHERE:

- 1 = NOT COMMERCIALY ACCEPTABLE
- 2 = APPROACHING COMMERCIAL ACCEPTABILITY
- 3 = AS GOOD AS CURRENT COMMERCIAL LINES

LINE	GENERAL APPEARANCE	LEAF SIZE	LEAF COLOUR	LEAF SHAPE	GROWTH RATE	GROWTH HABIT	UNIFORMITY	CROOK ROOT	TASTE (OPTIONAL)	COMMENTS
English grower's own line	2	1	3	2	1	2	1		2	
Bullington	2	1	3	2	1	2	1			
Hampshire	3	3	3	3	3	3	3		3	
New Zealand	2	2	1/2	2	2	3	3		3	
Japanese	1	1	1	1	1	1	1			
'Rorippa islandica'	1	1	1	1	1	1	1			
Spanish	1	1	1	1	1	1	1			

ANY FURTHER COMMENTS:

Table 22. Summary sheet showing mean scores for the seven watercress lines growing in Bed 7 from assessments of 28 June 1994.

SEED SOWN INTO MODULES: 5.4.94

SEEDLINGS PLANTED OUT INTO BED: 26.4.94

PLEASE SCORE EACH LINE ON A 1-3 SCALE WHERE:

- 1 = NOT COMMERCIALY ACCEPTABLE
- 2 = APPROACHING COMMERCIAL ACCEPTABILITY
- 3 = AS GOOD AS CURRENT COMMERCIAL LINES

LINE	GENERAL APPEARANCE	LEAF SIZE	LEAF COLOUR	LEAF SHAPE	GROWTH RATE	GROWTH HABIT	UNIFORMITY	CROOK ROOT	TASTE (OPTIONAL)	NUMBER OF ASSESSORS	OVERALL MEAN
English grower's own line	2.625	2.125	3	2.75	1.86	2.29	2.25	2.4	2.75	8	2.46
Bullington	2.875	2.25	3	2.875	2	2.29	2.5	2.4	2.75	8	2.55
Hampshire	2.875	2.75	2.75	3	3	2.86	2.875	2.4	2.25	8	2.79
New Zealand	2.125	2.375	1.563	2.625	2.86	2.86	2.875	2.6	2.2	8	2.44
Japanese	1	1.29	1	1	1	1.17	1.43	2.4	1.25	8	1.22
'Rorippa islandica'	1.57	1.43	2.25	1.71	1.57	2.29	2.29	2	1.5	8	1.92
Spanish	1.25	1.25	1.25	1.25	1.29	1.25	1.25	1.6	1	8	1.25

ANY FURTHER COMMENTS:

Table 23. Score for the seven watercress lines growing in Bed 6 for grower 1 from assessment of 15 March 1995.

NAME: Grower 1 Bed Number: **6** No Zinc

SEED SOWN INTO MODULES: 5.4.94

SEEDLINGS PLANTED OUT INTO BED: 26.4.94

PLEASE SCORE EACH LINE ON A 1-3 SCALE WHERE:

- 1 = NOT COMMERCIALY ACCEPTABLE
- 2 = APPROACHING COMMERCIAL ACCEPTABILITY
- 3 = AS GOOD AS CURRENT COMMERCIAL LINES

LINE	GENERAL APPEARANCE	LEAF SIZE	LEAF COLOUR	LEAF SHAPE	GROWTH RATE	GROWTH HABIT	UNIFORMITY	CROOK ROOT	TASTE (OPTIONAL)	COMMENTS
Spanish	1	1	2	1	1	2	1	2		Poor growth
' <i>Rorippa islandica</i> '	1	1	1	1	1	1	1	2		Leaf spot
Japanese	1	1	2	1	1	2	1	3		Poor growth – best for lack of crook root
New Zealand	1	1	1	2	2	3	2	1-2		Pale
Hampshire	3	3	2	2	3	3	2	½		
Bullington	2	3	3	2	3	2	2	1		Good colour dark
English grower's own line	1	1								

ANY FURTHER COMMENTS:

Table 24. Score for the seven watercress lines growing in Bed 6 for grower 2 from assessment of 15 March, 1995.

NAME: Grower 2 Bed Number: **6** No zinc.

SEED SOWN INTO MODULES: 4.10.94

SEEDLINGS PLANTED OUT INTO BED: 27.10.94

PLEASE SCORE EACH LINE ON A 1-3 SCALE WHERE:

- 1 = NOT COMMERCIALY ACCEPTABLE
- 2 = APPROACHING COMMERCIAL ACCEPTABILITY
- 3 = AS GOOD AS CURRENT COMMERCIAL LINES

LINE	GENERAL APPEARANCE	LEAF SIZE	LEAF COLOUR	LEAF SHAPE	GROWTH RATE	GROWTH HABIT	UNIFORMITY	CROOK ROOT	TASTE (OPTIONAL)	COMMENTS
Spanish	1	1	2	1	1	1	1	1		
' <i>Rorippa islandica</i> '	3	2	3	2	3	2	2	2		
Japanese	3	3	3	2	3	2	2	2		
New Zealand	3	3	2	3	3	3	3	3		
Hampshire	3	3	3	2	3	3	3	3		
Bullington										
English grower's own line	1	1	1	1	1	1	3	3		

ANY FURTHER COMMENTS:

Table 25. Summary sheet showing mean scores (15 March '95) for the seven watercress lines growing in Bed 6 (autumn sown, no zinc)

SEED SOWN INTO MODULES: 4.10.94

SEEDLINGS PLANTED OUT INTO BED: 27.10.94

PLEASE SCORE EACH LINE ON A 1-3 SCALE WHERE:

- 1 = NOT COMMERCIALY ACCEPTABLE
- 2 = APPROACHING COMMERCIAL ACCEPTABILITY
- 3 = AS GOOD AS CURRENT COMMERCIAL LINES

LINE	GENERAL APPEARANCE	LEAF SIZE	LEAF COLOUR	LEAF SHAPE	GROWTH RATE	GROWTH HABIT	UNIFORMITY	CROOK ROOT	TASTE (OPTIONAL)	COMMENTS	Mean Overall Score
Spanish	1	1	2	1	1	1.5	1	1.5	-	Poor growth	1.25
'Rorippa islandica'	2	1.5	2	1.5	1.5	2	1.5	2	-	WYSV symptoms	1.75
Japanese	2	2	2.5	1.5	1.5	2.5	1.5	2.5		Poor growth least crook root	2.0
New Zealand	2	2	1.5	2.5	2.5	3	2.5	2.3	-	Pale	2.3
Hampshire	3	3	2.5	2	3	3	2.5	2	-		2.6
Bullington	2	3	3	2	3	2	2	1	-	Good colour dark	2.3
English grower's line	1	1	1	1	1	1	1	3	-		1.3

Table 26. Scores (15 March '95) for the seven watercress lines growing in Bed 7 (spring sown, zinc treated)

NB: Assessment from only one grower

SEEDLINGS PLANTED OUT INTO BED: 26.4.94

PLEASE SCORE EACH LINE ON A 1-3 SCALE WHERE:

- 1 = NOT COMMERCIALY ACCEPTABLE
- 2 = APPROACHING COMMERCIAL ACCEPTABILITY
- 3 = AS GOOD AS CURRENT COMMERCIAL LINES

LINE	GENERAL APPEARANCE	LEAF SIZE	LEAF COLOUR	LEAF SHAPE	GROWTH RATE	GROWTH HABIT	UNIFORMITY	CROOK ROOT	TASTE (OPTIONAL)	COMMENTS	Mean Overall Score
English grower's own line	3	3	3	3	3	3	3	2	3		2.9
Bullington	2	2	2	2	2	2	2	2	2	WYSV symptoms	2.0
Hampshire	1	3	2	2	2	2	2	2	3		2.1
New Zealand	2	1	1	1	1	1	2	2	2	Pale	1.4
Japanese	1	1	1	1	2	2	2	3	-	Some flowers	1.6
' <i>Rorippa islandica</i> '	2	2	1	1	1.5	2	2	2	-		1.7
Spanish	2	1	2	1	1	2	2	2	3		1.8

Table 27. Scores (5 May '95) for the seven watercress lines growing in Bed 6 (autumn sown, no zinc)

NB: Assessment from only one grower

SEED SOWN INTO MODULES: 4.10.94

SEEDLINGS PLANTED OUT INTO BED: 27.10.94

PLEASE SCORE EACH LINE ON A 1-3 SCALE WHERE:

- 1 = NOT COMMERCIALY ACCEPTABLE
- 2 = APPROACHING COMMERCIAL ACCEPTABILITY
- 3 = AS GOOD AS CURRENT COMMERCIAL LINES

LINE	GENERAL APPEARANCE	LEAF SIZE	LEAF COLOUR	LEAF SHAPE	GROWTH RATE	GROWTH HABIT	UNIFORMITY	CROOK ROOT	TASTE (OPTIONAL)	COMMENTS	Mean Overall Score
Spanish	1	1	1	1	1	1	1	2	-		1.1
'Rorippa islandica'	1	1	1	1	1	1	1	3	-		1.3
Japanese	1	1	1	1	1	1	1	3	-		1.3
New Zealand	1	1	1	3	1	1	1	2	-		1.4
Hampshire	2	1	2	3	1	2	1	3	-		1.9
Bullington	3	3	3	3	3	3	3	3	-		3
English grower's own line	2	2	2	3	2	2	2	3	-		2.3

Table 28. Scores (5 May '95) for the seven watercress lines growing in Bed 7 (spring sown, zinc treated)

NB: Assessment from only one grower

SEED SOWN INTO MODULES: 5.4.94

SEEDLINGS PLANTED OUT INTO BED: 26.4.94

PLEASE SCORE EACH LINE ON A 1-3 SCALE WHERE:

- 1 = NOT COMMERCIALY ACCEPTABLE
- 2 = APPROACHING COMMERCIAL ACCEPTABILITY
- 3 = AS GOOD AS CURRENT COMMERCIAL LINES

LINE	GENERAL APPEARANCE	LEAF SIZE	LEAF COLOUR	LEAF SHAPE	GROWTH RATE	GROWTH HABIT	UNIFORMITY	CROOK ROOT	TASTE (OPTIONAL)	COMMENTS	Mean Overall Score
English grower's own line	3	3	2	3	3	3	3	3	-	-	2.9
Bullington	3	2	3	3	2	3	3	3	-	-	2.8
Hampshire	3	3	2	3	3	3	3	3	-	-	2.9
New Zealand	3	3	2	3	2	3	3	2	-	-	2.6
Japanese	3	3	3	3	3	3	3	2	-	-	2.9
'Rorippa islandica'	1	1	2	2	1	2	2	2	-	-	1.6
Spanish	3	3	2	3	2	3	3	3	-	-	2.8

Table 29. Scores (25 July '95) for the seven watercress lines growing in Bed 6 (summer sown, no zinc)

NB: Assessment from only one grower

SEED SOWN INTO MODULES: 20.6.95
SEEDLINGS PLANTED OUT INTO BED: 3.7.95

PLEASE SCORE EACH LINE ON A 1-3 SCALE WHERE:

- 1 = NOT COMMERCIALY ACCEPTABLE
- 2 = APPROACHING COMMERCIAL ACCEPTABILITY
- 3 = AS GOOD AS CURRENT COMMERCIAL LINES

LINE	GENERAL APPEARANCE	LEAF SIZE	LEAF COLOUR	LEAF SHAPE	GROWTH RATE	GROWTH HABIT	UNIFORMITY	CROOK ROOT	TASTE (OPTIONAL)	COMMENTS	Mean Overall Score
Spanish	1	1	1	3	1	1	2	-			1.4
' <i>Rorippa islandica</i> '	1	1	2	2	1	3	3	-	-		1.9
Japanese	1	1	1	1	1	1	1	-	-		1.0
New Zealand	1	2	1	3	3	2	2	-	-		2.0
Hampshire	3	3	2	3	3	2	3	-	-		2.7
Bullington	3	3	3	3	3	3	2	-	-	Best line	2.9
English grower's line	3	2	2	3	3	2	2	-	-		2.4

Table 30. Scores (31 August 1995) for the seven watercress lines growing in Bed 6 for grower 1 (summer sown, no zinc)
 NAME: Grower 1

SEED SOWN INTO MODULES: 20.6.95
 SEEDLINGS PLANTED OUT INTO BED: 3.7.95

PLEASE SCORE EACH LINE ON A 1-3 SCALE WHERE:

- 1 = NOT COMMERCIALY ACCEPTABLE
- 2 = APPROACHING COMMERCIAL ACCEPTABILITY
- 3 = AS GOOD AS CURRENT COMMERCIAL LINES

LINE	GENERAL APPEARANCE	LEAF SIZE	LEAF COLOUR	LEAF SHAPE	GROWTH RATE	GROWTH HABIT	UNIFORMITY	CROOK ROOT	TASTE (OPTIONAL)	COMMENTS
Spanish	1	1	1	1	1	1	1			Awful
' <i>Rorippa islandica</i> '	3	2	3	3	2	2	2			Small leaf
Japanese	1	3	1	3	1	2	1			Very pale green – many flowers
New Zealand	3	2	1	3	2	2	2			Light colour
Hampshire	3	3	3	3	3	3	3			
Bullington	2	2	3	3	2	2	2			Slightly smaller than E & G
English grower's line	3	2	3	3	3	3	3			

All have crook root

Table 32. Summary sheet showing mean scores (31 August '95) for the seven watercress lines growing in Bed 6 (summer sown, no zinc)
 NAME: _____ Means of two growers' assessments

SEED SOWN INTO MODULES: 20.6.95
 SEEDLINGS PLANTED OUT INTO BED: 3.7.95

PLEASE SCORE EACH LINE ON A 1-3 SCALE WHERE:

- 1 = NOT COMMERCIALY ACCEPTABLE
- 2 = APPROACHING COMMERCIAL ACCEPTABILITY
- 3 = AS GOOD AS CURRENT COMMERCIAL LINES

LINE	GENERAL APPEARANCE	LEAF SIZE	LEAF COLOUR	LEAF SHAPE	GROWTH RATE	GROWTH HABIT	UNIFORMITY	CROOK ROOT	TASTE (OPTIONAL)	COMMENTS	Mean Overall Score
Spanish	1	1	1	1.5	1	1.5	1	-	-		1.1
'Rorippa islandica'	2.5	2.5	3	2.5	2	2	2	-	-		2.4
Japanese	1	2	1	2	1.5	1.5	1	-	-		1.4
New Zealand	3	2.5	1	2.5	2.0	2.5	2	-	-		2.2
Hampshire	3	2.5	3	3	3	3	2.5	-	-		2.9
Bullington	2.5	2.5	3	3	2	2	2	-	-		2.4
English grower's own line	3	2.5	3	3	3	3	3	-	-		2.9