HDC PROJECT FV/55 FINAL REPORT

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FV/55 Screening watercress and related germplasm for resistance to crook root and watercress yellow spot virus (WYSV).

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INTRODUCTION AND BACKGROUND

The first phase of this project (FV/23) involved collecting germplasm of watercress and related taxa from all over the world and was completed in 1988. A total of 136 different seed lots (accessions) were assembled.

The second phase of the project (FV/23a) lasted six months (1988-1989) and confirmed that the virus infecting watercress in Kent, Dorset and Hampshire was watercress yellow spot virus which had only previously been reported in France. Laboratory and "field" techniques for screening watercress for resistance to the crook root fungus (*Spongospora subterranea* f.sp. *nasturtii*) and WYSV were developed. In consultation with statisticians and plant breeders a schedule was drawn up to evaluate the technique by screening ten watercress accessions. This showed that very high levels of crook root infection (98.8%) and WYSV (88.9%) could be obtained in susceptible lines and differences between lines could be discriminated. Having successfully evaluated the technique it was applied to the screening of further accessions of watercress and related species (FV/55).

OBJECTIVES

1. To test true watercress accessions (*Rorippa nasturtium-aquaticum*) for variation in susceptibility to crook root and WYSV.

2. To select for extremes of susceptibility and resistance within accessions and determine whether there is genetic variation within accessions.

3. To test the remaining watercress accessions and some accessions of species related to watercress for susceptibility to crook root and WYSV.

4. Determine the incidence of crook root and WYSV on watercress farms.

RESULTS

1. <u>Testing true watercress accessions for susceptibility to crook root and WYSV</u>

Forty-eight plants of each of twenty-eight different true watercress accessions together with 192 plants of a known susceptible line (1536 plants in all) were tested over a period of 18 weeks to determine their susceptibility to crook root and WYSV. All plants were scored for crook root susceptibility on a scale of 0-3, where 0 represents no visible crook roots and 3 represents very severe crook root infection in many roots. The virus content of plants was determined by enzyme-linked immunosorbent assay. The 3072 units of data were collated and analysed to compare accessions. Accessions were ranked for their susceptibility to both pathogens (Table 1).

Table 1. The susceptibility of different watercress accessions to crook root fungus and watercress yellow spot virus (WYSV).

	Accession Number	Crook root scores ranked	Accession Number	Virus content ranked
Lowest =	6	0.86	107	-.59
Most Resistant	11	0.91	11	-.53
	106	0.91	101	-.50
	9	0.98	123	-.38
	119	1.01	119	-.33
	15	1.01	8	-.33
	104	1.03	116	-.29
	114	1.06	105	-.27
	18	1.09	104	-.25
	8	1.10	91	-.24
	107	1.10	108	-.21
	3	1.11	106	-.19
	16	1.12	5	-.18
	108	1.13	6	-.16
	5	1.16	9	-.15
	105	1.16	16	-.14
	91	1.17	114	-.12
	101	1.21	128	-.10
	128	1.24	3	-.08
	123	1.27	15	-.04
	116	1.28	18	-.01
Highest =	CONTROL	1.33	103	+0.01
Most Susceptible	103	1.35	CONTROL	+0.08

The data for crook root and WYSV susceptibility have been combined to give combined rankings for both diseases (Table 2).

Table 2. Data for virus content and crook root infection combined and ranked.

	Accession Number	Combined ranking for virus content and crook root score	Origin
Lowest rank =	11	1	Japan
Most Resistant	119	2	Denmark
	107	3	USA/Denmark
	6	4 =	Hawaii
	106	4 =	USA
	8	6 =	Australia
	104	6 =	Italy
	9	8	Hawaii
	101	9	Switzerland
	105	10 =	Italy
	123	10 =	France
	108	12 =	UK
	114	12 =	Brazil
	15	14	UK
	91	15	USSR
	5	16 =	UK
	116	16 =	Holland
	16	18	UK
	18	19	Portugal
	3	20	UK
	128	21	Germany
Highest rank =	103	22 =	UK
Most Susceptible	CONTROL	22 =	UK

From the combined rankings, the two most susceptible accessions were those that are most widely grown in the UK; all UK accessions are in the lower (most susceptible) part of the table. The most resistant accessions were of Japanese and Danish origin, and were significantly less susceptible to both crook root and WYSV than the most susceptible accessions.

2. Selections within accessions

Forty-eight plants of each of five accessions from different countries that had been used in the evaluation of the screening technique were chosen and grown in modules in watercress beds as previously described. However, the plants were not destructively tested for WYSV as in previous experiments. In this case their root systems were destructively tested for WYSV after visual inspection for crook root, and the shoots were propagated. The two plants with the highest crook root score and highest virus content and the two plants with the lowest crook root score and lowest virus content were selected as representing "resistant" or "susceptible" individuals from within the original population of plants. The selected plants were grown on to produce seed. The seed from these plants along with seed from the susceptible control and the least susceptible line so far identified (Japanese) were used to plant up modules in four crates in a fully replicated and randomised experiment to assess the susceptibility of the selections to WYSV and crook root. The mean crook root scores of these selections and the controls are shown in Table 3.

Table 3. Mean crook root scores for "resistant" and "susceptible" selections from five watercress accessions used in the evaluation of the screening technique.

Country of origin of accession	Mean crook root score of "resistant" selections	Mean crook root score of "susceptible" selections
Germany	1.042 0.917	1.083 1.167
Denmark	1.292 0.958	1.167 0.875
New Zealand	1.000 1.000	0.958 0.917
Brazil	0.833	1.000 0.875
France	1.042	1.125 0.917
U.K. (susceptible control)	1.000	
Japan (least susceptible accession)	0.569	

There were no significant differences between or within accessions for the mean crook root scores of plants originating from parents selected as being "resistant" or "susceptible". With one exception, all selections were more susceptible to crook root than the Japanese watercress accession. There were no significant differences within or between accessions in their mean ELISA value (measure of virus content) (Table 4). Six selections were more susceptible to WYSV than the Japanese accession and none were less susceptible.

Table 4. Mean ELISA values (measure of virus content) for "resistant" and "susceptible" selections from five watercress accessions.

Country of origin of accession	Mean ELISA value of "resistant" selections	Mean ELISA value of "susceptible" selections
Germany	4.04 3.27	4.39 3.52
Denmark	3.80 3.89	4.22 3.58
New Zealand	4.64 4.72	4.54 4.54
Brazil	4.16	3.48 3.80
France	3.67	3.77 3.87
U.K. (susceptible control)	3.95	
Japan (least susceptible accession)	3.12	

A range of the watercress accessions (7) that were screened more recently (Table 2) including the Japanese accession, have now also been grown in modules in a watercress bed, non-destructively sampled and selections with high and low crook root scores and ELISA values have been made. Seed has been produced, and these selections now await further evaluation.

3. Screening of further watercress accessions and accessions of related species

Eighty four plants of each of the remaining sixteen true watercress accessions (not previously tested), one accession of each of two related *Rorippa* species (*R. palustris* and *R. sylvestris*) and one accession of each of four *Cardamine* species (*C. amara*, *C. flexuosa*, *C. hirsuta* and *C. impatiens*) were tested over a period of 36 weeks alongside 420 plants of the known susceptible line (2268 plants in all) to determine their susceptibility to crook root and WYSV. The 4536 units of data were collated and analysed to compare accessions. The different accessions have been ranked for their susceptibility to both pathogens (Table 5).

Table 5. The susceptibility of different watercress, non-watercress *Rorippa* species and *Cardamine* species to crook root fungus and watercress yellow spot virus (WYSV).

	Country of origin of watercress accession or species name	Crook root scores ranked	Country of origin of watercress accession or species name	Virus content ranked
Lowest = Most Resistant	*Cardamine hirsuta*	0	*Cardamine impatiens*	1.508
	Cardamine impatiens	0	*Cardamine hirsuta*	1.580
	"Curly cress"	0.056	*Cardamine flexuosa*	2.194
	Cardamine flexuosa	0.083	New Zealand	2.513
	Rorippa sylvestris	0.099	*Rorippa palustris*	2.542
	Rorippa palustris	0.139	*Cardamine amara*	2.619
	Cardamine amara	0.417	U.K.	2.777
	U.K.	0.583	Spain	2.899
	Spain	0.639	*Rorippa sylvestris*	2.937
	New Zealand	0.639	Brazil	3.001
	U.K.	0.708	France	3.016
	New Zealand	0.772	"Curly cress"	3.088
	Belgium	0.778	Belgium	3.102
	France	0.806	U.S.A.	3.146
	Brazil	0.806	U.K.	3.212
	U.S.A.	0.861	New Zealand	3.263
	Brazil	0.875	Belgium	3.346
	U.S.A.	0.972	Belgium	3.365
	Belgium	0.972	Belgium	3.565
	Belgium	1.083	Brazil	3.740
Highest = Most Susceptible	Belgium	1.104	U.S.A.	3.775
	Belgium	1.111	Control (U.K.)	3.932
	Control (U.K.)	1.200	Belgium	4.003

The data for crook root and WYSV have been put together to give combined rankings for both diseases (Table 6).

Table 6. Data for virus content and crook root infection combined and ranked.

	Country of origin of watercress accession or species name	Combined ranking for virus content and crook root score
Lowest rank = Most Resistant	*Cardamine impatiens*	1
	Cardamine hirsuta	2
	Cardamine flexuosa	3
	Rorippa palustris	4
	New Zealand	5
	Cardamine amara	6
	Rorippa sylvestris	7
	"Curly cress"	8 =
	U.K.	8 =
	Spain	10
	France	11
	U.K.	12
	Brazil	13
	New Zealand	14
	U.S.A.	15
	Belgium	16
	Brazil	17
	Belgium	18
	Belgium	19 =
	Belgium	19 =
	U.S.A.	21
Highest rank = Most Susceptible	Belgium	22
	CONTROL (U.K.)	23

Again the most susceptible accession was the one that is most widely grown in the UK The watercress accession that was least susceptible to crook root was a UK watercress producer's own line which had a significantly lower crook root score than the most susceptible accessions. The accession that was found to have the lowest virus content was a wild strain originating in New Zealand. This accession had a significantly lower virus content than the seven most susceptible watercress accessions. The New Zealand accession also appeared to be the least susceptible to a combination of the two diseases based on combined rankings (Table 6). The non-watercress *Rorippa* species seemed to be less susceptible to crook root and WYSV than most watercress accessions. On the whole, the *Cardamine* species seemed to be least susceptible to both diseases; one accession of *Cardamine hirsuta* and one accession of *Cardamine impatiens* appeared not to be hosts of the crook root fungus and only poor hosts of WYSV.

4. <u>The incidence of crook root and WYSV on watercress farms</u>

Sixteen watercress farms were visited between September 1991 and February, 1992 and watercress plants were sampled at random from different beds at each site. Of the farms visited all but two had virus infections. Crook root was found at all sites. Of 203 beds sampled 152 (75%) contained plants infected by the virus and 183 (90%) contained plants infected by crook root. Of all the plants sampled at random from the beds at all sites 47% were infected by virus and 58% by crook root. The results of the survey are summarised in Table 7.

Table 7. Summary of survey of watercress farms for incidence of crook root and watercress yellow spot virus.

Site	Date Sampled	Crook root			Watercress yellow spot virus		
		Range of incidences in beds	No. of beds containing infected plants/no. of beds sampled	% of all plants sampled that were infected	Range of incidences in beds	No. of beds containing infected plants/no. of beds sampled	% of all plants sampled that were infected
1	24.09.91	0-90%	10/15	24.7%	0-100%	4/15	14%
2	03.10.91	10-100%	14/14	52.1%	0- 80%	9/14	24.3%
3	14.10.91	10-100%	15/15	69.3%	10-100%	15/15	57.3%
4	14.10.91	0-100%	13/15	44.7%	0-100%	13/15	58.7%
5	06.11.91	30-100%	15/15	88.7%	0-100%	14/15	68.7%
6	06.11.91	10-100%	15/15	70%	0-100%	14/15	64.7%
7	26.11.91	30-100%	15/15	80%	0-100%	14/15	74.7%
8	26.11.91	0-30%	7/10	11%	0%	0/10	0%
9	10.12.91	0-100%	8/15	19.3%	0- 30%	8/15	12%
10	08.01.92	70-100%	8/8	87.5%	30- 90%	8/8	65%
11	08.01.92	30-100%	15/15	92.7%	20-100%	15/15	77.3%
12	03.02.92	70-100%	9/9	85.5%	0-100%	8/9	67.8%
13	03.02.92	40-100%	8/8	72.5%	0%	0/8	0%
14	10.02.92	10- 80%	15/15	37.3%	10-100%	15/15	42%
15	25.02.92	20-100%	9/9	60%	40-100%	9/9	86.7%
16	25.02.92	20-100%	10/10	42%	0- 90%	6/10	31%

It was interesting that no virus-infected plants were found at two of the sites. At one of these sites (number 8) the absence of WYSV was probably partly due to the low incidence of crook root at this site; with an overall infection level of 11% it was much lower than any of the other sites. This site was the only one visited where zinc treatments are applied all year round. At other sites where zinc was used, applications were not made from March/April to September/November. At the other site where no virus-infected plants were found (number 13), very high levels of crook root were detected. This suggested that the crook root at this site was not transmitting WYSV or that the watercress being grown at this site was susceptible to crook root but totally resistant to WYSV. Consequently seed was obtained from the owner of this site and included in tests for susceptibility (see 3). These tests indicated that watercress from site 13 was both highly susceptible to crook root and WYSV. The suggestion that the crook root fungus at site 13 is not carrying WYSV remains to be investigated further.

FINAL CONCLUSIONS AND RECOMMENDATIONS

All the available watercress accessions have now been tested for their susceptibility to the crook root fungus and watercress yellow spot virus; a number of these have been found to be less susceptible to both diseases than lines of watercress currently under commercial cultivation in the UK. The most promising watercress accessions with respect to their susceptibility to disease are from Japan (Accession 11, Table 2), UK and New Zealand (see Tables 5 & 6). Although none of these three accessions is totally resistant to either disease they may prove useful in permitting a reduction in the amount of zinc needed to provide control of crook root. The deployment of partially resistant material in combination with other control measures may enable the use of zinc to be eliminated. These three accessions will need to be evaluated further to determine their relative merits with respect to agronomic characteristics including: frost hardiness, yield potential, taste, leaf shape, flowering etc. Should they prove unsuitable for commercial production due to any of these characteristics being inappropriate, it should be possible through conventional breeding to incorporate this resistance into lines of watercress commercially acceptable for growing in the UK. The *Cardamine* species tested (Tables 5 & 6) appear to be completely resistant to crook root and very resistant to WYSV. As a *Cardamine* species is thought to have been one of the parents of *Rorippa microphylla* from which cultivated brown cresses were derived it may be possible to use a *Cardamine* accession as a source of resistance in a breeding programme to produce watercress with resistance to crook root and WYSV.

It was striking and of practical significance that in every test, the watercress line most widely grown in the UK was one of the most susceptible to crook root and WYSV.

Selections that have been made from within accessions on the basis of their susceptibility to crook root and WYSV still need to be evaluated in watercress beds to determine whether selection for enhanced levels of resistance to WYSV and crook root has been successful. This is especially important in the case of the Japanese line.

The survey of watercress farms confirmed how widespread crook root is and showed that WYSV is much more widespread than was previously thought. The absence of the virus from two sites suggested that eradication of the virus from a particular location may be feasible. It may then prove possible to keep the site virus-free through good hygiene. Although it may be environmentally undesirable, it seems that crook root can be reduced to very low levels and WYSV may be excluded by applying zinc treatments all year round to watercress beds .

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The following publications have emanated from this work:

Walsh, J.A. & 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.

Walsh, J.A. (1992). Resistant watercress. Grower **118** (6), 18-21.

Walsh, J.A. (1992). Resistance to crook root and watercress yellow spot virus found in a foreign line of watercress. HDC Project News **16**, 4-5.

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