FINAL REPORT

To on Report file, please.

HDC Watercress Project: Evaluation of techniques for screening watercress and related germplasm for resistance to crook root fungus and watercress virus.

Project Co-ordinator:	Mr Charles Barter
Project Leader:	Dr John Walsh
Location:	IHR Wellesbourne
Start Date:	05.09.89
Research Worker funded by HDC:	Miss Janet Rowe

Introduction

The first phase of this project which involved collecting germplasm of watercress and related taxa from all over the world was completed in 1988 and a total of 130 different seed lots assembled.

The second phase of the project began very successfully due to the competent and dedicated efforts of Janet Rowe. It was confirmed that the virus infecting watercress in Kent, Dorset and Hampshire was identical to the virus watercress yellow spot (WYSV) occurring in France and consequently should be known by this name rather than watercress chlorotic leafspot virus. Progress was so good that by December 1988 laboratory and "field" techniques for screening watercress had been developed and evaluated. At the beginning of 1989 a screening programme was devised in consultation with statisticians and plant breeders and a schedule drawn up to screen ten watercress lines over a period of six months, sadly during the same week these plans were formulated Miss Rowe sustained a fractured skull and a brain haemorrhage in a road accident and was unable to carry the project through. The work already set in motion was carried out by IHR staff.

Objectives

1. To test different methods of infecting watercress plants artificially with crook root and virus in the laboratory.

2. To investigate methods of raising watercress plants in the glasshouse in winter, transferring them to watercress beds and subsequently screening them for fungal and viral infections.

3. Having selected a suitable method to screen, ten different watercress lines will be tested for resistance to crook root and the virus.

Results

(1) Laboratory infection experiments

Growing healthy rooted watercress cuttings in containers with crook root and virus-infected plants showed that it was possible to infect plants with the fungus and virus in the laboratory. Tests were carried out at different temperatures and plants were sampled at different time intervals (Table 1). Crook root determinations were carried out by visual assessment and virus diagnoses by two methods employing the antiserum produced to the virus at Wellesbourne (immunosorbent electron microscopy [ISEM] and enzyme linked immunosorbent assay [ELISA]). The levels of crook root and virus transmission from infected to healthy plants was very high (Table 1).

Table 1. Transmission of crook root and virus from infected (donor) to healthy (recipient) watercress plants grown in test tubes.

		Number of recipient plants infected by virus or crook root / Number of plants tested					
		VIRUS			CROOK ROOT		
Number of weeks recipient plants left with donor plants		2	3	4	2	3	4
Temperature (°C)	4	4/4	4/4	4/4	4/4	3/4	3/4
	10	3/4	4/4	4/4	4/4	4/4	4/4
	18	4/4	4/4	4/4	4/4	2/4	1/4

Virus was detected in some plants where no crook root was seen by visual assessment (4°C at 3 and 4 weeks; 18°C at 3 and 4 weeks; Table 1).

This serves to underline the fact that subjective methods like visual assessment may not be fully reliable in determining crook root infection. Serological techniques like those used in these tests for the watercress virus (ELISA + ISEM) are much more sensitive and objective than visual assessments. ISEM tests which are much more time consuming than ELISA tests detected the presence of virus in some plants where ELISA failed, demonstrating that this test was more sensitive in the detection of the virus.

It was also possible to infect rooted watercress cuttings with crook root and virus by growing them in mud that had been collected from a watercress bed known to have contained plants infected by both crook root and virus. Again tests were carried out at different temperatures and were sampled at different time intervals (Table 2). Crook root determinations were carried out as before and virus diagnoses by ISEM and ELISA. Again levels of virus transmission were high, however very few crook roots were observed in the roots of these plants (Table 2).

Table 2. Virus and crook root infection of healthy watercress cuttings growing in mud.

		Number of cuttings infected by virus and crook root / Number of plants tested					
		VIRUS			CROOK ROOT		
Number of weeks plants grown in mud		2	3	4	2	3	4
Temperature (°C)	4	3/4	3/4	4/4	0/4	0/4	1/4
	10	3/4	3/4	3/4	0/4	1/4	0/4
	18	1/4	4/4	2/4	0/4	0/4	1/4

Again virus was detected in plants where it was not possible to detect the presence of the crook root fungus by visual assessment. Also as in the previous experiment ISEM tests were more successful in detecting virus infection than ELISA.

As it had been shown that it was possible to infect watercress plants artificially in the laboratory with crook root and virus, attempts were made to do this on a large scale with a view to developing a laboratory based method for screening watercress for crook root and virus resistance. Growing seedlings in spring water and using crooked roots as inoculum it was possible to obtain infection of most seedlings with the fungus and the virus. As in previous tests the amount of virus present in the watercress plants was too low to be detected by ELISA and could only be detected by ISEM. As ISEM testing of plants on a large scale for virus resistance screening was not considered practicable it was decided to concentrate on developing a "field"-based screen.

Tests were also made to determine whether it was possible for the virus to infect healthy watercress cuttings and plants in the absence of the crook root fungus. In initial tests two watercress cuttings were rooted in test tubes of spring water, a suspension of purified virus in distilled water was then added to the spring water and the plants tested for the presence of virus 18 days later. Both cuttings were found to be infected by the virus. A similar test was carried out on two more cuttings only this time, at the end of the experiment both plants were cut into two pieces: roots and shoots, to determine whether virus had moved into the aerial parts of the plant. Again both plants were found to be infected by the virus, with the highest levels of virus occurring in the roots and movement into the upper parts of the plant only occurring in one of the two cuttings (Table 3).

Table 3. The infection of watercress cuttings by virus in the absence of the crook root fungus.

Plant No		Virus present (+) or absent (-)
1	Shoot	-
	Root	+
2	Shoot	+
	Root	+

This was followed by attempts to infect whole watercress plants (as opposed to cuttings) that had grown naturally from seed. These plants were grown in sand in modules and either distilled water (control) or virus suspension in distilled water was watered onto the sand, 28 days later the plants were removed from the modules, those that had virus suspensions added to their growing media were cut into two pieces: roots and shoots before virus testing, whereas the controls were left whole. Yet again virus was recovered from both treated plants with detectable movement into the upper part of only one of the plants (Table 4).

Table 4. The infection of watercress plants by virus in the absence of the crook root fungus.

Treatment	Plant No		Virus present (+) or absent (-)
Virus suspension	1	Shoot	+
		Root	+
	2	Shoot	-
		Root	+
Distilled water	3	Whole Plant	-
	4	Whole Plant	-

A number of conclusions were drawn from these results. Although it was possible to obtain infection of healthy watercress plants in the laboratory, the levels of virus obtained in such plants were low and in some cases could only be determined by ISEM which is too labour intensive, time consuming and costly for routine screening. Visual assessment of crook root infection may not be reliable, and as it has been shown that it is possible for virus infection to take place in the absence of the crook root fungus, it is clear that presence or absence of crook root in watercress plants is not a reliable indicator of the susceptibility or resistance of such plants to the virus. This underlines the importance of screening plants for both crook root and virus resistance. Due to the unsuitability of laboratory screening methods it was decided to investigate "field" methods. "Field" methods were thought to be more promising because they should be less labour intensive and more efficient allowing more plants to be screened in a short period of time. Also, as pointed out earlier, any material identified in a lab screen would subsequently have to be evaluated in the field, so why not just by-pass the lab screen.

(2) Monitoring crook root and virus incidence in watercress beds throughout the year.

As it was known that if resistance to crook root or virus was found in laboratory tests it would eventually have to be evaluated in the field and as it was subsequently decided to concentrate on "field" rather than laboratory screening it was considered important to determine levels of crook root and virus infection throughout the year. This should reveal whether it will be possible to carry out field screening all year round or whether this would be limited to the winter months. During this monitoring it was also possible to study beds with and without zinc treatments to get some indication of the effect of these treatments on levels of virus and

fungus in the crop. These studies were carried out in experimental beds at Bere Regis in Dorset. One bed was studied in detail, it was sampled in three regions, top, middle and bottom. Temperature readings and water samples for pH determination were taken at monthly intervals from each of these regions as were plant samples for determination of the incidence of crook root and virus. As can be seen from Table 5 there were fluctuations in the levels of virus and fungus but the incidences were very high in the middle and bottom regions of the bed throughout the year. This indicated that it should be possible to screen for resistance to both fungus and virus all year round in watercress beds. Temperature was fairly stable at 10-11 ^{o}C in the bed during the winter months but fluctuated in summer reaching 18^{o}C on one occasion (Table 5). pH always increased down the bed and ranged from 6.7 - 7.5 at the top of the bed to 7.4 - 7.95 at the bottom (Table 5).

The effect of zinc treatment on crook root and virus incidence was monitored in two beds and these were compared with two identical beds that did not receive zinc treatment. During the winter months when zinc treatment was continuous there were slightly reduced levels of crook root in treated beds (Table 6) and no detectable virus (Table 7) in zinc-treated beds. However in spring and summer when problems were experienced with the zinc treatment and it was intermittent or totally absent, levels of crook root in these supposedly zinc-treated beds increased (Table 6) and by May, 1989 virus infection was detected and subsequently virus levels increased (Table 7). Again levels of virus and fungus in untreated beds during the summer months indicated that screening for crook root and virus resistance could be carried out all year round.

(3) Development of a screening technique for use in the field

Before screening in the field could start a system had to be devised and tested for growing watercress plants from seed in large numbers in the glasshouse at Wellesbourne whereby they could be easily transported to Dorset, transplanted into or placed in experimental beds in such a manner that high levels of crook root infection and virus transmission occurred and subsequently brought back to the laboratory for examination and testing.

A procedure was devised in which watercress was grown from seed in an inert medium in rigid plastic modules. The modules had holes in the bottom through which the watercress roots could protrude. Plants would be grown in the glasshouse at Wellesbourne, when large enough they would be put into watercress beds for infection to take place and then monitored. Before tests could be carried out it had to be established where the best place in the experimental beds for the modules to be placed was for maximum infection by crook root and virus. Details of the actual procedure that needed to be determined were: would infection by crook root and fungus occur in such a system, and if so what was the best medium for growing the watercress plants in, what was the optimum size and type of module giving maximum infection levels, how old or big should plants be before they are introduced into the watercress beds and how long would plants have to remain in beds for maximum infection levels to be reached.

The two experimental watercress beds allocated for screening were sampled to determine where maximum levels of crook root and virus infection occurred and from preliminary tests the best growing medium was determined. Three different types of modules were chosen for tests to determine which gave the highest levels of crook root and virus infection. Watercress seeds were sown in the growing medium in each cell of each tray. When the plants were large enough they were taken to Bere Regis and placed in the

TABLE 6.

INCIDENCE OF CROOK ROOT IN WATERCRESS BEDS, WITH AND WITHOUT ZINC TREATMENTS FROM JANUARY TO SEPTEMBER 1989

Incidence of crook root (% of plants sampled with crooked roots as determined by visual assessment)

		DATE: 17.1.89	07.2.89	08.3.89	03.4.89	02.5.89	30.5.89	11.7.89	08.8.89	12.9.89
No Zinc	Top	0	20	0	30	20	0	40	70	0
	Middle	100	100	100	100	80	100	70	40	100
	Bottom	100	100	100	100	90	100	30	40	70
	Top	20	0	10	60	20	10	20	50	10
	Middle	100	100	100	100	60	70	70	80	90
	Bottom	100	100	100	100	30	100	30	20	90
Zinc Applied	Top	0	20	10	70	10	20	60	30	0
	Middle	20	100	100	80	70	100	70	30	100
	Bottom	60	100	90	80	30	90	10	0	30
	Top	0	0	0	10	0	0	20	0	0
	Middle	80	100	100	100	50	100	50	0	50
	Bottom	40	80	100	100	20	100	40	0	10

TABLE 7.

INCIDENCE OF VIRUS IN WATERCRESS BEDS WITH, AND WITHOUT ZINC TREATMENTS FROM OCTOBER 1988 TO SEPTEMBER 1989

	DATE:	1988			1989								
		20.10	08.11	29.11	20.12	17.01	07.02	08.03	03.04	02.05	30.05	11.07	08.08	12.09
No Zinc	Top	0	0	0	0	0	0	0	0	0	0	10%	20%	0
	Middle	0	0	0	20%	20%	20%	20%	90%	70%	80%	80%	50%	60%
	Bottom	20%	0	0	20%	60%	80%	80%	80%	80%	60%	90%	30%	40%
	Top	0	0	-	0	0	0	0	0	10%	0	10%	0	0
	Middle	0	0	-	0	0	0	0	10%	50%	20%	50%	50%	40%
	Bottom	10%	0	-	0	0	0	20%	40%	50%	80%	50%	20%	60%
Zinc Applied	Top	0	0	0	0	0	0	0	0	0	0	0	0	0
	Middle	0	0	0	0	0	0	0	0	10%	0	10%	0	30%
	Bottom	0	0	0	0	0	0	0	0	10%	20%	10%	10%	10%
	Top	0	0	-	0	0	0	0	0	0	0	0	0	0
	Middle	0	0	-	0	0	0	0	0	10%	40%	60%	0	30%
	Bottom	0	0	-	0	0	0	0	0	30%	50%	70%	40%	10%

- = not tested.

first experimental bed to be tested. In consultation with a statistician a fully randomised design and sampling regime had been formulated to determine any positional effects that might arise, which of the modules gave highest crook root and virus infection, and how long plants should be left in the watercress bed. To this end modules were placed in nine different positions within the watercress bed to determine infection levels in different regions. The plants in the modules were monitored at regular intervals and when high levels of crook root and virus were detected they were returned to Wellesbourne and fully assessed for crook root and virus infection. Positional effects within the bed were detected in that higher levels of virus and fungus were detected in modules from certain parts of the bed whereas in other areas lower levels were detected and there was a significant effect of module size on both diseases (Table 8) in that infection levels were highest in small modules.

Table 8. Levels of virus and crook root infection in the three different module sizes located in nine different positions within the first experimental watercress bed tested.

VIRUS

POSITION IN BED	Module size LARGE	MEDIUM	SMALL	Percentage infected plants in each position
1	2/9	6/9	8/9	59
2	6/9	7/9	9/9	81
3	8/9	7/9	9/9	89
4	3/9	2/9	8/9	48
5	7/9	6/9	8/9	78
6	7/9	7/9	9/9	85
7	2/9	0/9	4/9	22
8	8/9	7/9	9/9	89
9	5/9	5/9	9/9	70
% infected for module type	59	58	90	

CROOK ROOT

POSITION IN BED	Module size LARGE	MEDIUM	SMALL	Percentage infected plants in each position
1	5/9	8/9	9/9	81
2	8/9	5/9	9/9	81
3	7/9	5/9	9/9	78
4	9/9	8/9	9/9	96
5	8/9	8/9	8/9	89
6	9/9	9/9	9/9	100
7	8/9	7/9	9/9	89
8	8/9	9/9	9/9	96
9	7/9	8/9	9/9	89
% infected for module type	85	83	99	

The positional effects were more severe for virus infection than crook root infection and plants grown in small modules had higher levels of both virus and crook root than those grown in large or medium-sized modules.

This experiment was repeated in the other experimental watercress bed at Bere Regis allocated for screening to determine whether there were differences between beds, which was the best bed for screening and whether positional and module size effects were consistent. Again positional effects were detected and module size had a significant effect on the incidences of both diseases (Table 9).

Table 9. Levels of virus and crook root infection in the three different module sizes located in nine different positions within the second experimental watercress bed tested.

VIRUS

POSITION IN BED	Module size LARGE	MEDIUM	SMALL	Percentage infected plants in each position
1	0/9	1/9	7/9	30
2	4/9	3/9	6/9	48
3	1/9	1/9	5/9	26
4	3/9	0/9	8/9	41
5	1/9	0/9	1/9	7
6	0/9	2/9	4/9	22
7	1/9	3/9	5/9	33
8	4/9	3/9	5/9	44
9	2/9	0/9	0/9	7
% infected for module type	20	16	51	

CROOK ROOT

POSITION IN BED	Module size LARGE	MEDIUM	SMALL	Percentage infected plants in each position
1	7/9	8/9	9/9	89
2	4/9	3/9	9/9	59
3	2/9	3/9	8/9	48
4	6/9	5/9	9/9	74
5	6/9	5/9	9/9	74
6	2/9	6/9	9/9	63
7	6/9	6/9	8/9	74
8	3/9	6/9	8/9	63
9	9/9	8/9	9/9	96
% infected for module type	56	62	96	

In this bed crook root incidences were slightly lower than in the other bed and virus levels were lower (Tables 8 and 9). Again positional effects were more severe for virus infection than crook root and plants in small modules had higher levels of both diseases than those in large and medium-sized modules.

The technique appeared to work very well; high levels of both virus and crook root were obtained, and any differences observed were consistent in both tests. The first bed tested gave higher levels of both crook root and virus than the second, consequently it was this bed that was selected for screening. Positional effects within this bed were detected and these would have to be taken account of in the experimental design.

(4) "Field" screening for resistance to crook root and watercress yellow spot virus.

Having developed a reliable and reproducible "field" test for screening, discussions were held with plant breeders and statisticians at Wellesbourne to determine the best way of employing this test in the search for resistance. Dr David Pink the plant breeder at Wellesbourne responsible for disease resistance breeding in vegetables considered two hundred plants per line a reasonable number to screen for a crop with the characteristics of watercress. After consideration of the logistics of how many plants per week one person could process and discussions with Kathleen Phelps the statistician attached to our department it was decided to attempt to screen 180 plants of each of ten lines. One of these ten lines would be a known susceptible (control), in this case 'Hampshire Watercress' standard seed lot, two UK lines were chosen and the other seven lines were chosen on the basis of their geographical origin in an attempt to screen material from as many different regions of the world as possible. The seven lines chosen were from Belgium, Brazil, Denmark, New Zealand, Switzerland, Tenerife and West Germany. The screening regime was designed so that any positional effects within the bed could be quantified and not allowed to bias the final statistical analyses. Using the "field" screening technique that had been developed and is outlined in the previous section, the first lot of plants for screening were delivered to Bere Regis and placed in the selected watercress bed at the beginning of February, 1989 and visits were made subsequently at fortnightly intervals to deliver more plants and/or recover plants for testing until the middle of June 1989.

In all 1,800 plants were individually assessed by visual examination for crook root infection and they were scored according to the severity of the infection. All the plants were subsequently processed for ELISA testing to determine whether they were infected by virus as well. The data was then entered into the computer and statistical analyses carried out. These analyses revealed that the UK lines of watercress (A, B and control) were the most susceptible to both crook root and virus and line E was most resistant to both diseases (Tables 10 and 11). Line E was quite different morphologically from UK watercress despite the seed packet it was sent in being labelled "watercress". When plants were grown on, it was obvious that it was a different species of plant from UK watercress. Plants were sent to Kew Gardens for identification where it was considered to be a member of the Cruciferae. Kew were unable to identify the plant further until flowering or fruiting specimens could be sent to them. We are currently trying to induce flowering in the glasshouse so that further specimens can be sent to Kew.

The field screen of ten different lines of "watercress" worked well and high levels of virus and crook root infection were obtained in susceptible lines, a whole range of susceptibilities from very susceptible to very resistant were detected. Normal watercress types (Rorippa nasturtium-aquaticum) were mostly susceptible although some of the lines originating from foreign countries were more resistant to both virus and crook root than UK lines. The most resistant line was not the same species as

TABLE 10.

SCREENING "WATERCRESS" LINES FOR RESISTANCE

TO CROOK ROOT

LINE	A	B	C	D	E	F	G	H	J	CONTROL	
CROOKROOT SCORE	1.94	1.73	1.52	1.62	0.1	1.81	1.57	1.63	1.68	1.85	LSD=0.24

High score = very susceptible, Low score = resistant
LSD = least significant difference

TABLE 11.

SCREENING "WATERCRESS" LINES FOR RESISTANCE

TO VIRUS

LINE	A	B	C	D	E	F	G	H	J	CONTROL	
VIRUS CONTENT	0.62	0.70	0.44	0.35	0.11	0.50	0.36	0.37	0.34	0.75	LSR=1.33

High values = susceptible, Low values = resistant
LSR = least significant ratio

Final conclusions and recommendations.

Despite disappointing results from laboratory screening tests a reliable and reproducible "field" screening method was developed ahead of schedule and ten watercress lines were thoroughly screened for both virus and crook root resistance.

Field screening proved to be more reliable, reproducible and less costly and labour intensive than laboratory screening. Visual assessment for crook root may not be totally reliable and it is virtually impossible to confirm total absence of crook root infection by this means. The presence or absence of crook root in the roots of plants may not be a reliable indicator of the susceptibility or resistance of such plants to the virus. It has been shown that virus infection can occur in the absence of crook root. These findings stress the importance of screening plants for both crook root and virus resistance. Monitoring of crook root and virus incidence throughout the year indicated that resistance screening could be carried out all year round in watercress beds. Monitoring also indicated that effective zinc treatment can give good virus control.

These studies suggest a number of options that may lead to the development of lines of watercress with resistance to crook root and virus.

1) Only ten lines have been screened so far and as this is only a small proportion of the 130 lines collected, it would be worth screening more lines in case some of these are more resistant than those already tested.

2) Now the initial screen has been completed it would be useful to go back and look more closely at those cultivars that showed some resistance to the two diseases. Plants within these lines that showed highest levels of resistance could be selected, allowed to flower and seed collected from them. This seed could then be used to determine whether the resistance trait is inherited (i.e. whether it is under genetic control and is passed to successive generations).

3) If the line E with the highest level of resistance to crook root and virus turns out to be closely related to watercress and has the same number of chromosomes it may be possible in conjunction with plant breeders at Wellesbourne to undertake a crossing programme to move the resistance into watercress.

Acknowledgements

I would like to thank Miss Janet Rowe and Mrs Ann Miller who carried out a great deal of this work, Mr Colin Clay who carried out all ISEM testing, Mrs Kathleen Phelps for statistical advice and analyses, Hampshire Watercress for providing and servicing experimental beds, Dr David Pink for advice on screening and breeding, John Cullington for technical assistance and the watercress growers for supporting this work through their contributions to the HDC.

Dr John A Walsh

10th October, 1989