Project title:	Meristem culture for the elimination of the strawberry crown rot pathogen *Phytophthora cactorum*
Project number:	SF 78
Project leader:	Adam Whitehouse
Report:	Final report, April 2007
Previous reports:	None
Key workers:	Ceri Govan Kirsty Hammond Daniel Sargent
Location:	East Malling Research East Malling Kent ME19 6BJ
Project Co-ordinators:	Rupert Hargreaves Hargreaves Plants Ltd. Cowpers Gate Long Sutton Spalding Lincolnshire PE12 9BS Sylvia Peterson GenTech Propagation Ltd. Gemini Crescent Dundee Technology Park Dundee DD2 1SW
Date commenced	1 April 2006
Date of completion:	30 April 2007
Keywords	Meristem, micropropagation, crown rot, strawberry, *Phytophthora cactorum*, *Fragaria* x *ananassa*

The results and conclusions in this report are based on a series of experiments conducted over a one-year period. The conditions under which the experiments were carried out and the results have been reported in detail and with accuracy. However, because of the biological nature of the work it must be borne in mind that different circumstances and conditions could produce different results. Therefore, care must be taken with interpretation of the results, especially if they are used as the basis for commercial product recommendations.

AUTHENTICATION

We declare that this work was done under our supervision according to the procedures described herein and that the report represents a true and accurate record of the results obtained.

Adam Whitehouse
Plant Breeder
EMR

Signature…… Date

David Simpson
Research Team Leader
EMR

Signature…… Date

Report authorised by:

Chris Atkinson
Head of Science
EMR

Signature…… Date

CONTENTS

Grower summary

Headline

Meristem culture was effective in eliminating crown rot (*Phytophthora cactorum*) from latently infected stolons of three strawberry cultivars tested using a PCR method.

Background and expected deliverables

Meristem culture and micropropagation are currently routinely used to produce healthy mother plants for propagators and for initial entry of new cultivars into the Plant Health Propagation Scheme. This technique is widely used throughout the world and is considered to be effective for eliminating fungal pathogens and viruses.

Recent problems with crown rot (*Phytophthora cactorum*) in stocks of Malling Pearl and Malling Opal have provided circumstantial evidence that meristem culture may not be totally effective in eliminating crown rot if the meristems are dissected from runners of latently infected mother plants. Work done at EMR in 1992/3 provided strong evidence that micropropagation did eliminate crown rot (Harris *et al* 1997) but this relied on conventional plating techniques to detect the pathogen.

New molecular techniques, using PCR (Ward *et al* 2006; Causin *et al* 2005), now provide a sensitive test for presence of the pathogen, even in minute quantities. Modern propagation techniques, particularly glasshouse propagation of everbearers, make it even more essential that cryptically infected plants are not distributed to propagators.

This investigation aimed to identify whether meristem culture was effective in eliminating crown rot from latently infected strawberry plants. Detection of the pathogen was performed using PCR tests.

Summary of the project and main conclusions

This investigation demonstrated the suitability and reliability of meristem culture for the production of strawberry plants free from crown rot, confirming the results obtained by Harris *et al* (1997). The results of this investigation are immediately relevant to the industry, particularly propagators and the Nuclear Stock Association.

Main conclusions

- A symptomless, (latent) infection was achieved in 13% of all stolons inoculated with crown rot.
- Spraying a 10^{-4} ml zoospore suspension was the most effective method for inoculating stolons with crown rot.
- 784 meristems were successfully excised from stolons of 3 cultivars which had been inoculated with crown rot.
- DNA was successfully extracted from 505 viable meristem cultures, 46 of which had been excised from latently infected stolons.
- All meristems (784) tested negative for crown rot.
- A subset of samples tested using real-time PCR by CSL gave identical results to those achieved using conventional PCR at EMR.

Financial benefits

Meristem culture proved to be effective in eliminating crown rot so no additional costs will need to be incurred by the industry.

Action points for growers

- The industry, particularly propagators, can have confidence that micropropagation is effective in eliminating crown rot
- There are no recommended changes to current practice for the micropropagation of strawberry shoots via meristem culture

Science section

Introduction

The pathogen *Phytophthora cactorum* causes the disease crown rot of strawberries. There is evidence that the pathogen has the ability to establish a latent, symptomless infection in propagation material, which has been reported as a major factor in the spread of the disease throughout Europe (Harris & Stickels, 1981; Parikka, 1991; de los Santos *et al*., 2002) and other parts of the world (Huang *et al*., 2004). The use of meristem culture to eliminate *P. cactorum* from strawberry plants has previously been reported (Harris *et al*., 1997; Molot *et al*., 1973). However recent problems with crown rot in stocks of newly released cultivars provide circumstantial evidence that meristem culture may not be effective in eliminating crown rot when cultures are initiated from stolons of latently infected plants. Previous work to test the effectiveness of this procedure relied on conventional plating techniques to identify the pathogen (Harris *et al*., 1997). These techniques have now been superseded by more sensitive molecular techniques such as Polymerase Chain Recation (PCR), which have proved to be effective in identifying the presence of *P. cactorum* (Ward *et al*. 2006; Causin *et al*., 2005) and latent infections of other diseases (Parikka & Lemmetty, 2004; Zimmermann *et al*., 2004) in strawberry.

The aim of this investigation was to identify whether meristem culture is effective in eliminating crown rot (*Phytophthora cactorum*) from latently infected strawberry plants. Detection of the pathogen was performed using PCR tests.

Materials and methods

1. Plant material

Elite grade cold-stored runner plants of cultivars 'Elsanta' and 'Rosie', and AH grade cold-stored runner plants of 'Malling Pearl' were used in all experiments.

2. Inoculation

A mixture of two *Phytophthora cactorum* isolates, P412 and P413 were used in all experiments. P412 and P413 are known to be pathogenic to 'Malling Pearl' and 'Malling Opal', respectively having been isolated from infected plants of these cultivars which were sent to the East Malling Research Plant Clinic in 2005.

2.1. Spray method

10mm discs were cut from the margins of actively growing cultures of *P. cactorum* on V8 agar. Discs were immersed in a minimum quantity of a non-sterile compost extract and incubated for 2-3 days at 20˚C in an illuminated incubator. A 10^4 zoospores per ml suspension was produced following the method described by Harris (1986).

Ten cold-stored runners of each cultivar were potted up into 9cm pots using a standard compost mix. These plants were placed into a warm glasshouse (22˚C day, 17˚C night, and 16h photoperiod). The position of each plant was determined using a randomised block design. Stolons that developed from these plants were inoculated by spraying them with a suspension with 10^4 zoospores per ml of *P. cactorum*. Visible effects of inoculation were recorded after 7 days and 50 stolon tips from each cultivar were removed to be used to establish meristem cultures.

2.2. Nutrient Film Technique (NFT) method

10mm discs were cut from the margins of actively growing cultures of *P .cactorum* on V8 agar. Disks were placed onto PDA medium and incubated for 7 days at 22˚C in a dark incubator, and then transferred to a 4˚C dark fridge for 21 days. Twenty-fours before inoculation the disks were placed at room temperature (c. 20˚C) in the dark. A 10^7 sporangia per ml suspension was produced.

Ten cold-stored runners of each cultivar had their roots wrapped in rockwool and were then placed into a nutrient film technique (NFT) system within a warm glasshouse (22˚C day, 17˚C night, and 16h photoperiod). The position of each plant was determined using a randomised block design. The roots of the plants were immersed in a circulating nutrient solution (Solufeed H). A suspension of 500 ml of 10^7 sporangia per ml of *P. cactorum* was added to 80 L of nutrient solution when stolons started to emerge. Visible effects of infection were recorded after 14 days, and 50 stolon tips from each cultivar were removed to be used to establish meristem cultures.

The most effective method was identified by isolating sub-crown tissue from beneath each dissected meristem and testing this for the presence of *P. cactorum* using the PCR method described later in this section. The method that gave the highest incidence of latent infection was adopted for subsequent inoculations. A further inoculation experiment ensured that 200 stolons per cultivar were inoculated.

A minimum of 20 plants of each cultivar were used as controls (untreated).

3. Excision of apical meristem

Inoculated stolons were washed under running tap water prior to dissection. A single apical meristem (0.2-0.5mm in size) was excised from each individual stolon and placed onto a strawberry initiation medium (Appendix A). All procedures were carried out aseptically and cultures were examined weekly for any microbial growth. Meristem cultures were maintained in a growth room (22°C and 16h photoperiod) and DNA was extracted from viable cultures after 8 weeks.

4. Dissection of sub-crown tissue

A section of tissue measuring 5-10mm was removed from an area directly below the meristem immediately after meristems were dissected. The outer surface of the stolon was removed to eliminate the risk of inoculated tissue contaminating the sample. The sub-crown was frozen using liquid nitrogen and stored until DNA extractions could be performed.

5. DNA extraction from strawberry tissue

All DNA was extracted using the DNeasy plant DNA miniprep extraction kits (Qiagen) following the manufacturer's protocols. For a positive control, DNA was extracted from a single plate of *Phytopthora* mycelium that was scraped into a 2 ml microcrentrifuge tube, snap-frozen and ground with 2 ball-bearings then extracted following the plant tissue protocol using the plant miniprep extraction kit for individual samples. *Phytopthora* DNA was diluted 1:50 for use in PCR. DNA was extracted from sub-crown tissue using the DNeasy 96 extraction miniprep kit (for high-throughput extraction of 96 samples simultaneously) and was subsequently diluted 1:20 for use in PCR. Meristems of samples testing positive for *Phytopthora* contamination in sub-crown tissue were extracted using the DNeasy plant miniprep extraction kit (for individual samples) and diluted 1:50 for PCR. Meristems from those samples that tested negative for *Phytopthora* contamination were extracted using the DNeasy 96 miniprep kit and were diluted 1:100 for subsequent use in PCR.

6. PCR analysis

All PCRs were performed in a final reaction volume of 12.5μl comprising 2 μl template DNA, 1x PCR buffer, 1.5mM Mg^{2+}, 200μM dNTPs, 0.2μM each primer and 0.25U *Taq* polymerase (Invitrogen). For the amplification of *Fragaria* genomic DNA, primer pair EMFxaDFR2 (Sargent *et al* 2007) (EMFxaDFR2F: 5'-caccggagtgtttcatgtcg-3' EMFxaDFR2R: 5'- aacctccgaactgtctttgc-3') was used. PCR products were amplified using the touchdown protocol of Sargent *et al* (2003): an initial denaturation step of 94°C (2min), then 10 cycles of 94°C (30s), 55-50°C annealing temperature decreasing by 0.5°C per cycle (45s) and 72°C (1min), followed by 25 cycles of 94°C (30s), 50°C (45s) and 72°C (1min), and a final elongation step of 72°C (5min). For the

amplification of *Phytopthora* DNA from *Fragaria* tissue samples, DNA primers Phy1 (5'-ttccacgtgaaccgtatcaa-3') and Phy2 (5'-tgttcagccgaagccaacca-3') were used. PCR products were amplified using the following protocol: an initial denaturation step of 94°C (2min), then 35 cycles of 94°C (30s), 58°C (45s) and 72°C (1min), and a final elongation step of 72°C (10min).

PCR products amplified using each set of primer pairs were mixed together and co-loaded onto a 1.2%w/v TAE agarose gel. Products were electrophoresed at 110V for 2h and visualised over UV light following staining in ethidium bromide solution (0.5μg per ml).

Results & Discussion

1. Inoculation of stolons

1.1. Spray method

Plants and stolons of 'Malling Pearl' and 'Rosie' were healthy in appearance 7 days after infection; however plants and stolons of 'Elsanta' showed symptoms of powdery mildew (*Podosphaera aphanis*). No necrosis or wilting was observed on either stolons or plants. Sub-crown tissue was successfully dissected from 50, 49 and 51 stolons of 'Elsanta', 'Malling Pearl' and 'Rosie' respectively. The results of PCR tests on sub-crown tissue samples are shown in Table 1.1.

Table 1.1. The numbers of sub-crown tissue samples testing positive for *Fragaria* genomic DNA (DFR) & *P. cactorum* after inoculation using the spray method (values in parenthesis are for untreated (control) samples)

Cultivar	No of samples	Number of samples testing positive		% of samples testing positive for *P. cactorum*
		DFR	*P. cact*orum	
Elsanta	50 (9)	38 (0)	2 (0)	5 (0)
Malling Pearl	49 (9)	40 (9)	7 (0)	18 (0)
Rosie	51 (9)	46 (9)	12 (0)	26 (0)
Totals	150 (27)	124 (18)	21 (0)	Mean = 16 (0)

The percentage of samples testing positive for *P. cactorum* was calculated using the number of successful *Fragaria* genomic DNA (DFR) extractions which are indicative of a successful DNA extraction.

The spray method was successful in inducing a mean latent infection rate of 16%.

1.2. Nutrient Film Technique (NFT) method

Plants and stolons of 'Malling Pearl' and 'Rosie' were healthy in appearance 14 days after infection, but as with the spray test plants and stolons of 'Elsanta' showed symptoms of powdery mildew. No necrosis or wilting was observed on either stolons or plants. The production of runners between cultivars was more erratic in the NFT system than noted with potted plants and only 45 and 48 runners of 'Malling Pearl' and 'Elsanta' respectively were inoculated. One hundred runners in total were taken from 'Rosie' to compensate for the shortfall. The results of PCR analysis on sub-crown tissue samples are shown in Table 1.2.a

Table 1.2.a. The numbers of sub-crown tissue samples testing positive for *Fragaria* genomic DNA (DFR) & *P. cactorum* after inoculation using the NFT method (values in parenthesis are for untreated (control) samples)

Cultivar	No of samples	Number of samples testing positive		% of samples testing positive for *P. cactorum*
		DFR	*P. cact*orum	
Elsanta	48 (6)	36 (0)	0 (0)	0 (0)
Pearl	45 (6)	33 (6)	7 (0)	21 (0)
Rosie	100 (6)	57 (4)	4 (0)	7 (0)
Totals	193 (18)	126 (10)	11 (0)	Mean = 9 (0)

The NFT method was successful in inducing a mean latent infection rate of 9%.

The inoculation method resulting in the highest infection rate was the spray method and so this was therefore adopted for a larger scale inoculation experiment to bring the total number of inoculated stolons to 200 per cultivar.

1.3. Large scale inoculation using spray method

A further 146 'Elsanta', 139 'Malling Pearl and 156 'Rosie' stolons were inoculated using the spray method. All plants and stolons of 'Elsanta', 'Malling Pearl' and 'Rosie' were healthy in appearance 7 days after infection. The results of PCR analysis on sub-crown tissue samples are shown in Table 1.3.

Table 1.3. The numbers of sub-crown tissue samples testing positive for *Fragaria* genomic DNA (DFR) & *P. cactorum* after inoculation using the spray method (values in parenthesis are for untreated (control) samples)

Cultivar	No of samples	Number of samples testing positive		% of samples testing positive for *P. cactorum*
		DFR	*P. cact*orum	
Elsanta	146 (6)	112 (5)	18 (0)	16 (0)
Malling Pearl	139 (5)	41 (3)	2 (0)	5 (0)
Rosie	156 (8)	120 (5)	12 (0)	10 (0)
Totals	441 (19)	273 (13)	32 (0)	Mean = 12 (0)

1.4. Combined results

Data from both inoculation methods is combined in the following section in order to summarise the overall results. The results of PCR tests on sub-crown tissue samples taken from all experiments are shown in Table 1.4. As all untreated (control) treatments tested negative for *P.cactorum* these results have been excluded from the combined tables.

Table 1.4. The combined numbers of sub-crown tissue samples testing positive for *Fragaria* genomic DNA (DFR) & *P. cactorum* after inoculation using both inoculation methods.

Cultivar	No of samples	Number of samples testing positive		% of samples testing positive for *P. cactorum*
		DFR	*P. cact*orum	
Elsanta	244	186	20	11
Malling Pearl	233	114	16	14
Rosie	307	223	28	13
Totals	784	523	64	Mean = 13

784 stolons were inoculated in total, although DNA extraction was only successful from 523 (67%) of the samples. Sixty-four (13%) stolons from which DNA was successfully extracted were found to be latently infected with *P. cactorum*, which represented a higher rate of latent infection than was achieved by Harris *et al.* in 1997 (8.9%).

2. Meristem cultures

2.1. Meristems derived from the spray method

150 apical meristems appeared healthy at the time of excision, but subsequently a proportion failed to establish (dead) on SMI medium or were discarded due to microbial contamination. The numbers of contaminated, dead and viable meristem cultures after 8 weeks are shown in Table 2.1.a.

Table 2.1.a. The numbers of contaminated, dead and viable meristem cultures after 8 weeks. Meristems were derived from stolons inoculated using the spray method (values in parenthesis are for untreated (control) samples)

Cultivar	No of meristems excised	Number of meristems			% meristems that were discarded
		Contaminated	Dead	Viable	
Elsanta	50 (9)	34 (8)	14 (1)	2 (0)	96 (100)
Malling Pearl	49 (9)	0 (0)	5 (0)	44 (9)	10 (0)
Rosie	51(9)	2 (0)	1(0)	48 (9)	6 (0)
Totals	150 (27)	36 (0)	20 (0)	94 (18)	Mean = 37 (33)

A high proportion (96%) of 'Elsanta' meristems were discarded due to microbial contamination or failure to establish on SMI medium. This was also observed with meristems taken from untreated (control) stolons suggesting a cultivar specific factor rather than experimental or operator error. Contaminants comprised of a miscellaneous collection of fungi, bacteria and yeasts but there was no evidence of contamination with *P. cactorum*. It is also unlikely that the presence of powdery mildew on the stolons was a cause, being an obligate fungus that is known not to actively grow on micropropagation media.

Of 93 successful DNA extractions from viable meristems, including 17 taken from stolons testing positive for *P. cactorum*, none were found to be infected with the pathogen (Table 2.1.b.).

Table 2.1.b. The number of meristem samples testing positive for *Fragaria* genomic DNA (DFR) and *P. cactorum* after inoculation using the spray method (values in parenthesis are for untreated (control) samples)

Cultivar	No of samples	Number of samples testing positive		% of samples testing positive for *P. cactorum*
		DFR	*P. cact*orum	
Elsanta	2 (0)	2 (0)	0 (0)	0 (0)
Malling Pearl	44 (9)	43 (9)	0 (0)	0 (0)

Rosie	48 (9)	48 (9)	0 (0)	0 (0)
Totals	94 (18)	93 (18)	0 (0)	Mean = 0 (0)

2.2. Meristems derived from the Nutrient Film Technique (NFT) method

All apical meristems appeared healthy at the time of dissection. The numbers of contaminated, dead and viable meristem cultures after 8 weeks are shown in Table 2.2.a.

Table 2.2.a. The numbers of contaminated, dead and viable meristem cultures after 8 weeks. Meristems were derived from stolons inoculated using the NFT method (values in parenthesis are for untreated (control) samples).

Cultivar	No of meristems excised	Number of meristems			% meristems that were discarded
		Contaminated	Dead	Viable	
Elsanta	48 (6)	29 (3)	18 (3)	1(0)	98 (100)
Malling Pearl	45 (6)	5 (0)	0 (0)	40 (6)	11 (0)
Rosie	100 (6)	10 (2)	2 (0)	88 (4)	12 (33)
Totals	193 (18)	44 (5)	20 (3)	129 (10)	Mean = 40 (44)

Again high proportions (98%) of 'Elsanta' meristems were discarded either due to microbial contamination or failure to establish on SMI medium.

Of the 122 successful extractions taken from viable meristems, including 11 taken from stolons testing positive for *P. cactorum*, none were infected with the pathogen (Table 2.2.b.)

Table 2.2.b. The number of meristem samples testing positive for *Fragaria* genomic DNA (DFR) and *P. cactorum* after inoculation using the NFT method (values in parenthesis are for untreated (control) samples)

Cultivar	No of samples	Number of samples testing positive		% of samples testing positive for *P. cactorum*
		DFR	*P. cactorum*	
Elsanta	1 (0)	1 (0)	0 (0)	0 (0)
Malling Pearl	40 (6)	38 (6)	0 (0)	0 (0)
Rosie	88 (4)	83 (4)	0 (0)	0 (0)
Totals	129 (10)	122 (10)	0 (0)	Mean = 0 (0)

2.3. Large scale inoculation using spray method

Meristems taken from stolons of 'Elsanta' and 'Malling Pearl' appeared healthy at the time of excision; however 12 meristems excised from stolons of 'Rosie' were found to be necrotic. These meristems were not included in the main experiment but were nevertheless placed on SMI medium. All these meristems failed to develop, and a white fungal growth appeared on the surface of the medium after 7 days. A sample of the fungus was plated out onto a selective medium and was confirmed to be *P. cactorum*.

The numbers of contaminated, dead and viable meristem cultures after 8 weeks are shown in Table 2.3.a. and the results of PCR analysis on viable meristems are shown in Table 2.3.b.

Table 2.3.a. The numbers of contaminated, dead and viable meristem cultures after 8 weeks. Meristems were derived from stolons inoculated using the spray method (values in parenthesis are for untreated (control) samples).

Cultivar	No of meristems excised	Number of meristems			% meristems that were discarded
		Contaminated	Dead	Viable	
Elsanta	146 (6)	16 (2)	39 (1)	91 (3)	38 (50)
Malling Pearl	139 (5)	0 (0)	13 (1)	126 (4)	9 (20)
Rosie	156 (8)	32 (3)	22 (0)	102 (5)	35 (38)
Totals	441 (19)	48 (5)	74 (2)	319 (10)	Mean = 27 (54)

Of 290 successful extractions taken from viable meristems, including 18 taken from stolons testing positive for *P. cactorum*, none were infected with the pathogen (Table 2.3.b).

Table 2.3.b. The number of meristem samples testing positive for *Fragaria* genomic DNA (DFR) and *P. cactorum* after inoculation using the spray method (values in parenthesis are for untreated (control) samples).

Cultivar	No of samples	Number of samples testing positive		% of samples testing positive for *P. cactorum*
		DFR	*P. cactorum*	
Elsanta	91 (3)	82 (3)	0 (0)	0 (0)
Malling Pearl	126 (4)	118 (4)	0 (0)	0 (0)
Rosie	102 (5)	90 (5)	0 (0)	0 (0)
Totals	319 (10)	290 (10)	0 (0)	Mean = 0 (0)

2.4. Combined results

Data from both inoculation methods is combined in the following section in order to summarise the overall results. The overall numbers of contaminated, dead and viable meristem cultures excised from all the inoculated stolons are shown in Table 2.4.a.

Table 2.4.a. The combined numbers of contaminated, dead and viable meristem cultures after 8 weeks. Meristems were taken from stolons inoculated using both inoculation methods (values in square parenthesis are for meristems excised form latently infected stolons).

Cultivar	No of meristems excised	Number of meristems			% meristems that were discarded
		Contaminated	Dead	Viable	
Elsanta	244 [20]	79 [3]	71[8]	94 [9]	75 [55]
Malling Pearl	233 [16]	0 [0]	18 [1]	215 [15]	10 [6]
Rosie	307 [28]	44 [0]	25 [2]	238 [22]	20 [7]
Totals	784 [64]	123 [3]	114 [11]	547 [46]	Mean = 35 [23]

547 viable meristems in total were produced from the 784 stolons that were inoculated with *P. cactorum*, representing a mean survival rate of 65%. The results of PCR analysis on these viable meristems are shown in Table 2.4.b.

Table 2.4.b. The combined number of meristem samples testing positive for *Fragaria* genomic DNA (DFR) and *P. cactorum* after inoculation using both methods.

Cultivar	No of samples	Number of samples testing positive		% of samples testing positive for *P. cactorum*
		DFR	*P. cactorum*	
Elsanta	94 [9]	85 [9]	0 [0]	0 [0]
Malling Pearl	215 [15]	199 [15]	0 [0]	0 [0]
Rosie	238 [22]	221[22]	0 [0]	0 [0]
Totals	547 [46]	505 [46]	0 [0]	Mean = 0 [0]

DNA was successfully extracted form 505 (92%) of viable meristems but none were found to be infected with *P. cactorum* including 46 samples taken from stolons that were known to been latently infected with the disease.

A HDC report (Ward *et al*., 2006) published within the duration of this project reported the increased sensitivity of real-time (TaqMan®) PCR assay for the detection of *P. cactorum* compared to conventional PCR (as used in this investigation). In order to validate the results obtained from this investigation, a set of DNA samples taken from both sub-crown and meristem tissue, along with negative and positive controls, were submitted to Central Science Laboratory, York (CSL) for testing using real-time PCR (Appendix B). The sample included 20

samples of DNA taken from meristems derived from latently infected plants (40% of the total). The results obtained using real-time PCR were identical to those achieved using the conventional PCR method at East Malling Research (EMR), indicating the PCR method used was effective in detecting the pathogen.

We can conclude that meristem culture was effective in eliminating *P. cactorum* from latently infected stolons, confirming the findings of Harris *et al.* (1997) and Molot *et al.* (1973). The PCR method detected a higher rate of infection than reported by Harris *et al*. (1997) and was as effective, based on subset of samples, in detecting the pathogen as real-time PCR as tested by CSL. Our data provide no evidence to support the hypothesis that *P. cactorum* can be transmitted as latent infection through meristem culture.

Conclusions

- A symptomless, (latent) infection was achieved in 13% of all stolons inoculated with *P.cactorum.*
- Spraying a 10^{-4} ml zoospore suspension was the most effective method for inoculating stolons with *P.cactorum.*
- 784 meristems were successfully excised from stolons of 3 cultivars that had been inoculated with *P.cactorum.*
- DNA was successfully extracted from 505 viable meristem cultures, 46 (9%) of which had been excised from latently infected stolons.
- All (784) meristems tested negative for *P.cactorum*.
- A subset of samples tested using real-time PCR by CSL gave identical results to those achieved using conventional PCR at EMR.

In summary, this investigation demonstrated the suitability and reliability of meristem culture for the production of strawberry plants free from *P. cactorum*, confirming the results obtained by Harris *et al* (1997). The results of this investigation are immediately relevant to the strawberry industry, particularly propagators and the Nuclear Stock Association.

TECHNOLOGY TRANSFER

A project update was made to the HDC Soft Fruit Panel on 6th March 2007. The findings will also be presented to the industry via article for HDC news (April 2007) and at an EMRA/HDC event or at the British Berry Conference.

REFERENCES

de los Santos, B., Porras, M., Blanco, C., Barrau, C. & Romero, F. (2002). First report of *Phytophthora cactorum* on strawberry plants in Spain. *Plant Disease* **86**, 1051.

Causin, R., Scopel, C., Grendene, A. & Montecchio, L. (2005). An improved method for the detection of *Phytophthora cactorum* (LC) Schroeter in infected plant tissue using SCAR markers. *Journal of Plant Pathology* **87**(1), 25-35.

Harris, D.C. (1986). Methods for preparing, estimating and diluting suspensions of *Phytophthora cactorum* zoospores. *Transactions of the British Mycological Society* **86**, 482-486

Harris, D.C. & Stickels, J.E. (1981). Crown rot (*Phytophthora cactorum*) in glasshouse-grown strawberries at East Malling Research Station. *Plant Pathology* **30**, 205-212.

Harris, D.C. (1993). Project Report Summary For: SF23 Crown rot of strawberry: transmission of the pathogen (*Phtyophthora cactorum*) in micropropagation. HDC Final Report 1992/93.

Harris, D.C., Simpson, D.W. & Bell, J.A. (1997). Studies on the possible role of micropropagation in the dissemination of the strawberry crown rot pathogen *Phytophthora cactorum*. *Journal of Horticultural Science* **72**(1), 125-133.

Huang, H., Jeffers, S.N. & Layne, D.R. (2004). AFLP analysis of *Phytophthora cactorum* isolates from strawberry and other hosts: Implications for identifying the primary source of inoculum. *Plant Disease* **88** (7), 714-720.

Molot, P.-M., Nourrisseau, J.-G., Leroux, J.P., Navatel, J.C. & Germain, P. (1973). Le *Phytophthora cactorum* sur fraisier. Connaissances actuelles. Mise au point des methods de lutte. CTIFL-Documents No. 40, 1-12.

Murashige, T. & Skoog, F. (1962). A revised medium for the rapid growth and bioassays with tobacco tissue cultures. *Physiologia Plantarum* **15**, 473-97.

Parikka, P. (1991). *Phytophthora cactorum* on strawberry in Finland. *Nordisk Jordbruksforskning*, **73,** 121.

Parikka, P. & Lemmetty, A. (2004). Tracing latent infection of *Colletotrichum acutatum* on strawberry by PCR. *European Journal of Plant Pathology* **110**(4), 393-398.

Sargent, D..J, Rys, ., Nier, S., Simpson, D.W., Tobutt, K.R. (2007). The development and mapping of functional markers in *Fragaria* and their transferability and potential for mapping in other genera. *Theoretical and Applied Genetics*. **114**, 373-384.

Sargent, D.J., Hadonou, A.M., Simpson, D.W. (2003). Development and characterization of polymorphic microsatellite markers from *Fragaria viridis,* a wild diploid strawberry. *Molecular Ecology Notes* **3**, 550-552.

Ward, L., Beales, P., Budge, G., Barnes, A. & Downe, G. (2006). SF 82 Development and evaluation of a real-time (TaqMan®) PCR assay of or detection of *Phytopthora cactorum* (Crown rot) in Strawberry Tissue. HDC Final Report 2006.

Zimmermann, C., Hinrichs-Berger, J., Moltmann, E. & Buchenauer, H. (2004). Nested PCR (polymerase chain reaction) for the detection of *Xanthomonas fragariae* in symptomless strawberry plants. *Journal of Plant Diseases and Protection* **111**(1), 39-51.

ACKNOWLEDGEMENTS

The financial contribution made by Meiosis Ltd. and the Nuclear Stock Association is gratefully acknowledged.

Gratitude is also extended to Giles Budge, Central Science Laboratory, York who performed the real-time PCR assays.

APPENDICES

APPENDIX A

Strawberry Initiation Medium (SMI)

Murashige and Skoog (1962) medium including vitamins (Duchefa Biochemie M0222.00050)	4.4.g	
Sucrose	30.0g	
Oxoid agar No.3	7.5g	
Water		make to 1 litre

Sterilised at 121°C for 15 mins. at 103 KPa

APPENDIX B

Samples tested for *P.cactorum* by Central Science Laboratory using real-time (TaqMan®) PCR assay (+ positive, - negative for *P. cactorum*).

EMR code	Sample tested at CSL	Cultivar	Inoculation method	EMR result (+ or -)		CSL result (+ or -)
				sub-crown	meristem	
6b	meristem	Rosie	spray	+	-	-
6g	meristem	Rosie	spray	+	-	-
13c	meristem	M. Pearl	spray	+	-	-
18a	meristem	Rosie	spray	+	-	-
18f	sub-crown	Rosie	spray	+	-	-
20b	meristem	Elsanta	spray	+	-	-
22a	meristem	Rosie	spray	+	-	-
23b	meristem	M. Pearl	spray	+	-	-
24c	meristem	M. Pearl	spray	+	-	-
26d	meristem	Rosie	spray	+	-	-
29b	meristem	M. Pearl	spray	+	-	-
31c	meristem	M. Pearl	spray	+	-	-
56d	meristem	Elsanta	spray	+	-	-
60b	meristem	Rosie	spray	-	-	-
60c	meristem	Rosie	spray	+	-	-
63b	meristem	Elsanta	spray	+	-	-
65a	meristem	Elsanta	spray	+	-	-
88j	meristem	M. Pearl	spray	-	-	-
92b	meristem	Rosie	spray	-	-	-
116b	meristem	Elsanta	spray	-	-	-
158h	meristem	Elsanta	spray	-	-	-
N20a	meristem	M. Pearl	NFT	+	-	-
N30c	meristem	Rosie	NFT	+	-	-
N33b	meristem	M. Pearl	NFT	+	-	-
N38b	meristem	M. Pearl	NFT	+	-	-
NC14a	meristem	M. Pearl	NFT control	-	-	-
NC14a	sub-crown	M. Pearl	NFT control	-	-	-
FRA[1]	n/a	n/a	DFR control	-	-	-
H20[2]	n/a	n/a	Water -ve control	-	-	-
Phyt[3]	n/a	n/a	*P. cactorum* +ve control	+	+	+

[1,2] Two samples of *Fragaria* genomic DNA and water were included as negative controls.

[3] Five samples of *P. cactorum* DNA were included as positive controls.