CONTRACT REPORT

Crown Rot of Strawberry
Transmission of the Pathogen in Micropropagation

Undertaken for the
Horticulture Development Council
Project SF 23

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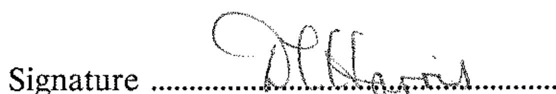
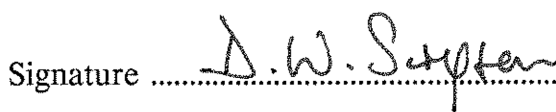
AUTHENTICATION

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

Signature 

Signature 

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Final Report February 1994

HDC SF 23

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Transmission of the Pathogen in Micropropagation

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Coordinator: Mr. H. Darby

Commenced: April 1992

Completed: September 1993

Key Words: strawberry: crown rot: *Phytophthora:*

micropropagation

CONTENTS

	Page
Relevance to growers and practical application	5
Application	5
Summary	5
Experimental Section	8
Introduction	8
Materials and Methods	9
Results	14
Discussion	22
References	23
Glossary	24
Appendix	25

RELEVANCE TO GROWERS AND PRACTICAL APPLICATION

Application

The objective of the project was to examine the possibility that the crown rot pathogen, *Phytophthora cactorum,* can be transmitted during micropropagation of strawberry. No evidence was obtained for the transmission of *P. cactorum* in meristem culture or stolon tip micropropagation of strawberry. Evidently, micropropagation has not contributed to spread of the disease and, on the contrary, may be used as a means of freeing stocks from *P. cactorum.*

Two collateral benefits derived from the project were: the identification of a selective medium which is outstandingly good for the detection of *Phytophthora cactorum* in plant material; and the finding that the inoculation of stolon tips could form the basis of an *in vitro* method for measuring the resistance of strawberry genotypes to crown rot.

Summary

Description and scope of the project

Crown rot of strawberries is a soil-borne disease caused by a specialised strain of the fungus *Phytophthora cactorum.* Until recently it had occurred only locally and sporadically in Britain since its first introduction from continental Europe in the late 1960s. However, since 1989 crown rot has become progressively more conspicuous and damaging in field-grown strawberries in Britain. The great expansion in the cultivation of susceptible

cultivars, particularly Elsanta, has undoubtedly been an important factor in this development.

Another important factor is diseased planting material. There is evidence that the crown rot pathogen has been spread with both imported and British-grown runners. Unless steps are taken to curb the dissemination of disease, crown rot could become as widespread and serious a threat to strawberry production in Britain as it is in continental Europe. A feature of the disease which facilitates spread in planting material is the ability of the pathogen to establish latent infection in the tissues. Observational evidence indicates that the pathogen can remain latent for more than a year, only to cause disease when the infected plant undergoes cropping or other stress.

The presence of crown rot in planting stocks may lead to disqualification from certification with the direct losses that it brings and, when the disease occurs in the higher grade of stocks, there is a severely disruptive effect on the whole system of production.

One possible method for producing and maintaining pathogen-free stocks, particularly at the highest levels of certification is through micropropagation. However, there is a suspicion, based mostly on circumstantial evidence from several sources, that the crown rot pathogen is capable of being spread through micropropagation systems. If true, this would completely undermine the micropropagation approach to control. The objective of this project was to examine the possibility that *P. cactorum* could be transmitted undetected with strawberry tissue during micropropagation.

Experimental Approach

The work was carried out in two phases. In the first phase, possible techniques were investigated for producing latently infected young daughter plants and stolon tips of three cultivars (Elsanta, Cambridge Favourite and Redgauntlet) of high, medium and low field susceptibility to crown rot respectively. In the second phase, the methods developed in phase 1 were used to provide infected plants from which micropropagated cultures of five cultivars (Cambridge Favourite, Elsanta, Pegasus, Redgauntlet and Tamella), representing a wider range of crown rot susceptibility, could be established. After a period of development these cultures were examined for the presence of *P. cactorum*.

RESULTS

P. cactorum was found to be present in meristems (0.8%) and stolon tips (6%) excised from inoculated but apparently healthy plants, but the pathogen invariably grew conspicuously into the culture medium and killed the plant tissue. Of 380 micropropagation cultures successfully established from meristem explants or stolon tips after inoculation with *P. cactorum* not one yielded the pathogen on destructive sampling although other micro-organisms were detected.

Implications

It is impossible to prove a negative beyond all doubt, but the evidence obtained in this investigation indicates that transmission of crown rot through

micropropagation does not happen. Two main conclusions follow from this.

Firstly, where crown rot occurs in association with plants that have been derived from micropropagation, the disease can be assumed to have entered the plants at some stage after they have emerged from culture. The facilities used for weaning micropropagated plants may be contaminated, for instance, or the plants may have been exposed to infection at some later stage.

Secondly, the techniques of micropropagation, whether meristem excision for eliminating viruses, or tip culture for commercial rapid multiplication, can be used to ensure a source of *Phytophthora cactorum*-free plant material.

EXPERIMENTAL SECTION

INTRODUCTION

Micropropagation of strawberries involves establishing a culture from a tissue explant and culturing through successive specially formulated media to produce small plantlets. If it is necessary to free material of virus infection, an apical meristem taken from a stolon tip or a young plant is used as the starting point. For rapid commercial propagation from virus free stocks, stolon tips treated to remove microbial contamination form the starting point (see Glossary).

For this study it was necessary first to develop methods for producing starting material for micropropagation in which there was a sufficient incidence of latent infection with the crown rot strain of *P. cactorum,* and

then to use this material for setting up micropropagation cultures to test for the presence of the pathogen at various stages. The first phase of the project was carried out from April to September in 1992 and the second phase during a similar period in 1993.

MATERIALS AND METHODS

Plant Material

All plants used for experiments were from cold-stored super elite grade certified runners and were grown in pots in a glasshouse under quarantine conditions. Plants were deblossomed and allowed to runner freely to provide material for inoculation and for micropropagation experiments. Plants were from 8 - 11 weeks old at inoculation. Care was taken when watering to minimise the chances of secondary spread of disease.

Inoculation

In all cases plants were exposed to a suspension of zoospores of one or more isolates of *P. cactorum* of proven pathogenicity. The numbers of zoospores in suspension varied with experiments in Phase 1 (see later), but in Phase 2 suspensions were standardised to about 10^4 zoospores/ml. A mixed inoculum of four isolates was used in Phase 1 but a single isolate was used in Phase 2. Stolon tips were dipped in suspension, and plants were sprayed with suspension using a small garden spray on a coarse setting. Zoospores survived application as a coarse spray, but were damaged when applied as a fine spray. Tips received about 0.1 ml of inoculum and plants about 6 ml. In

both cases the inoculated material was enclosed in a polythene bag or sleeve for 24 hours after inoculation in order to maintain moist conditions. Inoculated stolons were tied to a cane so as to minimise zoospore suspensions dribbling down towards the mother plant. Infection by zoospores in wet conditions is probably how most disease arises naturally. The methods of producing and handling zoospores were similar to those described by Harris (1986).

Recording

After from one to four weeks the visible effects of inoculation were recorded and plants or stolon tips were removed and either screened for the presence of *P. cactorum* (Phase 1) or used to establish micropropagated cultures which were subsequently screened for the presence of *P. cactorum* (Phase 2).

The presence of *P. cactorum* in plant tissue was determined by culturing the tissue onto a suitable agar medium, incubating the cultures at 20°C in the dark, and examining cultures for the characteristic growth of the pathogen after one to two weeks.

For experiments where other micro-organisms were likely to be present, such as in the inoculation experiments in Phase 1, a medium highly selective for the growth of *P. cactorum* was used for culturing plant material. This medium was selected from several published recipes on the basis of preliminary experiments. For experiments in Phase 2, where micropropagated material was being screened, a standard *Phytophthora*

culture medium was used. The references for the selective media and a brief account of the experiments in which they were compared are given in the Appendix.

Phase 1

Inoculation Experiment 1

Twenty stolon tips of three cultivars (Cambridge Favourite, Elsanta and Redgauntlet) were inoculated with one of each of four inoculum concentrations: 0, 10^1, 10^2 or 10^3 zoospores/ml. The zoospores were obtained from four crown rot isolates of *P. cactorum* (P154, P372, P373 and P374). One stolon per plant was used and plants were arranged in ten randomised blocks on the glasshouse bench. Stolons were examined weekly for four weeks, after which they were screened for the presence of *P. cactorum*. During the four weeks following inoculation most stolons had continued to develop, producing small plants, and one or more further stolons. Approximately half the stolons inoculated with zoospores which had not died as a result of inoculations were sampled by one method and the remainder by another method. In the first method, representative portions of the stolon and growth from it were excised and transferred to selective medium. These portions were: the original point of inoculation: a transverse crown section of any young plant which had developed: and any stolon tip(s) present. In the second method, the entire stolon and any growth from it were chopped into pieces *c.* 5 mm in length and a representative sample (about one third) of the pieces, was spread on the base of a sterile Petri dish and covered with molten

selective medium at *c.* 50°C. The medium was allowed to set and the Petri dishes were incubated as described.

Inoculation Experiment 2

Twenty young plants of the same three cultivars were inoculated by spraying compost extract or extract containing zoospores at each of three inoculum concentrations: 10^3, 10^4 or 10^5 zoospores/ml. In this case zoospores were obtained only from isolate P372. One daughter plant from each mother plant was used and mother plants were arranged in a replicated block design. One week after inoculation daughter plants were examined for signs of necrosis and removed from the parent plant. The leaves were stripped from each young plant and the crown was cut into 3 - 8 transverse sections which were placed in order on selective medium and incubated.

Growth of *P. cactorum* on micropropagation media

The ability of *P. cactorum* to produce visible growth on the three media (initiation, proliferation and rooting) which are used in the various stages of micropropagation was examined. Plugs of *P. cactorum* culture (isolate P372) were placed in the centre of plates of the three media and of V8 agar, a standard *Phytophthora* culture medium. The mean linear growth (on four radii) of the fungus during a 7-day period was measured. There were six replicate plates for each medium.

Phase 2

Testing for Latent Infection in Micropropagation Cultures

The following procedure was repeated with ten batches of plant material over a period of four months. A number of young plants and stolon tips were inoculated with zoospore suspension (the concentrations of inoculum varied from 0.7 to 1.8 x 10^4 zoospores/ml) as described above. All inocula were produced from isolate P372. Fifty plants and tips were inoculated in the first batch but this number was reduced to 25 in subsequent batches (275 in total). After one week any necrosis was recorded and all inoculated plants and tips which were still viable and any plants or tips which had developed from them were used for micropropagation. Plants were excised by the meristem technique (see Glossary) while stolon tips were treated according to standard commercial micropropagation practices (see Glossary). An additional batch of inoculated plants and stolon tips was excised as above but the explants were transferred to the *Phytophthora* selective medium.

Meristem and tip cultures were incubated for six weeks at 22°C and a 16 hour photoperiod. During this time, cultures were examined weekly for growth and for contamination by micro-organisms. For the first five batches any white fungal growths were transferred to the *Phytophthora* selective medium to check for *P. cactorum;* in subsequent batches all fungus contaminated cultures were transferred to the selective medium. After six weeks incubation, when plant tissue would normally be transferred to proliferation medium, the tissue was plated onto V8 agar to check for any

latent infection by *P. cactorum*. Plant material was cut aseptically into from six to 30 pieces before plating to encourage any *P. cactorum* present to grow into the medium. For the first batch of tissue cultures, half were plated out after six weeks and half after a further two weeks on initiation medium, for subsequent batches all cultures were plated out after six weeks.

RESULTS

Phase 1

Inoculation Experiment 1

The results are summarised in Table 1. Of 180 stolon tips inoculated with zoospores, three Elsanta, five Cambridge Favourite and two Redgauntlet had died back after four weeks; 11 stolon tips exhibited severe and conspicuous necrosis and several stolon/plant combinations, showed various degrees of localised necrosis on stipules, leaflets or the stolon. Similar localised necrosis was evident on several control plants suggesting that factors other than *P. cactorum* might have been responsible for these symptoms. All ten deaths resulted from inoculation with the highest concentration of inoculum. Four Redgauntlet stolons were lost through mechanical damage. Of the 167 stolons screened by the two sampling methods one from the first and seven from the second yielded *P. cactorum*. The one isolation with the first method was from a symptomless plant; none of the five plants with severe necrosis screened in this way yielded the pathogen. Four out of six plants exhibiting severe necrosis screened by the second method yielded *P. cactorum;* the three other isolations were from

TABLE 1. Results of inoculating stolon tips with *P. cactorum* zoospores

Experiment 1 1992

Cultivar	Inoculum*	Number of stolons (+derivative growths)					
		Total	Dead	With severe necrosis		With little or no necrosis	
				Yielding *P.c.*	Not yielding *P.c.*	Yielding *P.c.*	Not yielding *P.c.*
Elsanta	0	20	0	--	--	--	--
	1	20	0	0	1	0	19
	2	20	0	0	0	0	20
	3	20	3	0	0	1	16
Cambridge Favourite	0	20	0	--	--	--	--
	1	20	0	0	1	0	19
	2	20	0	0	1	0	19
	3	20	5	2	1	1	11
Redgauntlet	0	19	0	--	--	--	--
	1	19	0	0	2	1	16
	2	19	0	0	0	0	19
	3	19	2	2	1	1	13

* Zoospores/ml: 0 = 0; 1 = 10^1; 2 = 10^2; 3 = 10^3

TABLE 2. Results of inoculating young plants with *P. cactorum* zoospores

Experiment 2 1992

Cultivar	Inoculum*	Number of plants						
		Total	Dead		Necrotic		Healthy	
			Yielding *P.c.*	Not yielding *P.c.*	Yielding *P.c.*	Not yielding *P.c.*	Yielding *P.c.*	Not yielding *P.c.*
Elsanta	3	20	2	0	1	4	2	11
	4	20	2	0	4	5	1	8
	5	20	4	0	7	3	2	4
Cambridge Favourite	3	20	0	0	3	3	3	11
	4	20	3	0	6	3	3	5
	5	19	3	0	12	3	1	0
Redgauntlet	3	20	0	0	6	2	3	9
	4	20	0	1	9	5	0	5
	5	20	1	0	13	2	1	3
Totals		179	15	1	61	30	16	56

*Zoospores/ml: 3 = 10^3; 4 = 10^4; 5 = 10^5

plants which had shown no signs of necrosis. Three of the four infected symptomless plants had been inoculated with the highest of the three inoculum concentrations.

Inoculation Experiment 2

The results are summarised in Table 2. Of 180 young plants inoculated with zoospores one was lost through mechanical damage and 92 yielded *P. cactorum* one week after inoculation. Of the latter, 76 were dead or had shown signs of necrosis. This necrosis was evident on leaves, on the stolon bearing the plant, on the tips of root initials at the base of the plant, or combinations of these. Sixteen of the plants which yielded the pathogen were apparently healthy. This is equivalent to a 'latent' infection incidence of 8.9% ([number of plants infected showing no symptoms/total plants inoculated] x 100). The incidence of death reflected the relative field susceptibility of the three cultivars to crown rot; the incidences of necrotic plants and of 'latent' infection did not. The incidences of plant death and necrosis were positively correlated with inoculum dose while the incidences of 'latent' infection were negatively correlated with dose.

The frequencies of isolation from the basal section, the apical section and intermediate sections of the 92 infected plants are shown in Table 3. The distribution of the fungus in the plant crowns indicates that most infections originated in the base of the crown in the rooting region. The necrotic tissues of some root initials were examined and found to contain oospores resembling those of *P. cactorum*.

TABLE 3. Distribution of *P. cactorum* in the crowns of 92 infected plants

Numbers of crowns yielding *P. cactorum* from:	
Basal section only	22
Intermediate sections only	8
Apical section only	3
Basal + intermediate sections	30
Basal + apical sections	0
Intermediate + apical sections	3
Basal + intermediate + apical sections	26

Growth of *P. cactorum* on micropropagation media

P. cactorum grew on all three micropropagation media although not as rapidly or as luxuriantly as on V8 agar. The results are shown in Table 4.

TABLE 4. The growth of *P. cactorum* on micropropagation media and on V8 agar

Medium	Mean radial growth in 7 days (mm)
Initiation	6.7
Proliferation	6.5
Rooting	7.5
V8	36.9

Phase 2

Incidence of *P. cactorum* in micropropagation cultures

A number of plants and stolon tips had died or were showing severe necrosis one week after inoculation and the overall incidences of this for the five cultivars for all batches are shown in Table 5.

TABLE 5. Percentages of stolon tips or plants dead or severely necrotic one week after inoculation with *P. cactorum*

Tamella		Elsanta		Cambridge Favourite		Redgauntlet		Pegasus	
Stolon	Plant	Stolon	Plant	Stolon	Plant	Stolon	Plant	Stolon	Plant
66	34	39	4	2	10	13	11	7	0

The comparative amounts of damage to the five cultivars, particularly to stolon tips, broadly reflects their known field susceptibility to crown rot, except that Cambridge Favourite appears more resistant than expected.

Plant material which was obviously necrotic was not used as a source for micropropagation. The results obtained through attempting to establish micropropagation cultures of the remaining material are summarised in Table 6.

A total of 357 meristems was excised from young daughter plants that had survived/developed up to seven days after inoculating the parent material with zoospores. Of these, 235 meristems grew and were apparently healthy

TABLE 6. Summary of micropropagation experiments 1993

Type of explant	Total excised	State of cultures after 6 weeks incubation			Results of microbial examination of cultures		
		Apparently healthy	Failing to grow	Visibly contaminated	No micro-organisms	Bacteria +/or fungus	*P. cactorum*
Meristems	357	235*			218	13	
			122				
				20		17	3
Tips	298	162**			119	30	
			136				
				79		61	18

* 4 cultures lost subsequently

** 13 cultures lost subsequently

after six (or eight) weeks incubation; 122 meristems did not grow and 20 of these developed visible contamination. Of the 20 contaminated meristems, three (0.8% of the total meristems excised) produced growth of *P. cactorum* and the rest produced miscellaneous fungi and bacteria. *P. cactorum* occurred in meristem explants from cvs. Tamella (one) and Elsanta (two). The *P. cactorum* growth in micropropagation culture was distinctive: it was white, sparse, growing on the surface and in the upper 5 mm of agar. Out of 235 apparently healthy meristem cultures, four were lost in error and, of the rest, 11 produced bacterial and two produced fungal growth when cultured to V8 agar. No *P. cactorum* growth arose from these cultures.

A total of 298 stolon tips that survived or were produced following inoculation were excised to set up micropropagation cultures. After six (or eight) weeks incubation, 162 tips were apparently healthy, although 13 of these were lost in error subsequently, 136 had not grown of which 79 had developed fungal or bacterial contamination. In a few instances the tip appeared to be growing in spite of the presence of microbial contamination. The identity of 18 fungal contaminants thought to be *P. cactorum* from their characteristic growth in micropropagation culture was confirmed. The *P. cactorum* growths occurred in tips excised from all five cultivars: Tamella (one), Elsanta (five), Cambridge Favourite (two), Redgauntlet (six) and Pegasus (four). When transferred to V8 agar, the tissue from 30 of the healthy growing tips gave rise to bacterial (16 tips) or fungal growth (14 tips), but in no case was *P. cactorum* detected. Material from the other 119 tips gave no microbial growth.

There was no growth of plant material from any of the meristems or tips

transferred directly to selective medium although three cultures produced fungal growths, none of which was *P. cactorum*.

DISCUSSION

Latent infection by *P. cactorum* in young plants and stolons of strawberry was not reproduced experimentally at a high frequency by the methods used here. Some infection (about 2%) was produced in stolons which showed little or no signs of disease four weeks after inoculation with low doses of zoospores and could therefore be described as latent, but the incidence was too low for experimental requirements. More infection occurred when young plants were inoculated with higher concentrations of zoospores, although much of the infection not apparent after one week may have become visible as necrosis had it been allowed to develop, and could not therefore be truly described as latent. The higher dose and shorter period of incubation were used experimentally to simulate a situation where apparently healthy plants infected with *P. cactorum* might be used inadvertantly for establishing micropropagation cultures. For young plants much of this 'latent' infection had its origins in the emerging root initials.

Using this experimental approach, *P. cactorum* was excised with plant tissue from a small proportion of plants which were not obviously unhealthy one week after inoculation. However, once in micropropagation culture, the pathogen grew in the tissues, killing them, and into the initiation medium producing a visible and characteristic growth. Experiments in pure culture confirm that *P. cactorum* is able to grow on all three of the media used for micropropagation. All the evidence obtained indicates that *P. cactorum* does not establish a benign and latent co-

existence with strawberry tissues in culture to allow it to be propagated with them. In fact, the behaviour of the pathogen means that micropropagation can be used as a method for ensuring freedom from the pathogen in strawberry stocks.

The effects of inoculating stolon tips with moderate doses of zoospores suggest that this could be the basis of an *in vitro* method for measuring the resistance/susceptibility of strawberry selections/cultivars to the crown rot strain of *P. cactorum*.

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GLOSSARY

Meristem Culture. This technique depends on excising a small portion of the stem apex containing the apical meristem and transferring it aseptically to a specially formulated medium (initiation medium). Young strawberry plants or stolon tips may be used. The excision is carried out with the aid of a dissecting microscope. The young leaves and leaf primordia are dissected away until the apical meristem is exposed and approximately 0.5 mm of the stem apex is cut out. The culture is incubated at 22°C in light and if viable and uncontaminated will produce a small nodule of proliferating tissue which after six weeks is ready to be transferred to the proliferation medium.

Commercial Micropropagation. This technique is used for the rapid *in vitro* multiplication of strawberry plants. An approximately 10 mm length of the stolon tip is removed, surfaced sterilised in 10% Domestos for 10 minutes, rinsed three times in sterile distilled water and pressed base down into a plug of initiation medium in a growing tube. The culture is incubated as above. This information was obtained by courtesy of Munton Microplants Ltd., Cedars Factory, Stowmarket, Suffolk IP14 2AG.

APPENDIX

The Choice of a Selective Medium for *P. cactorum*

The selective medium for *P. cactorum* used in this investigation was chosen on the basis of two criteria: the ability of *P. cactorum* to grow in pure culture from mycelial inoculum; and the incidence of recovery of the pathogen from diseased plants from the glasshouse (following inoculation) and from the field (naturally diseased).

Five recipes for selective media for *Phytophthora* spp. were compared. The media were those described by: Kannwischer & Mitchell (1978); Harris & Bielenin (1986) (two recipes); Jeffers & Martin (1986); Pettit & Pegg, (1991).

Small plugs of actively growing cultures of *P. cactorum* (three isolates) on V8 agar were transferred to the centres of plates of the various selective media plus cornmeal agar (CMA), a medium used commonly for *Phytophthora* and as a base for selective media. The linear growth was measured on four radii after six and nine days incubation at 20°C. The growth of *P. cactorum* (average for the three isolates) on the selective media compared with CMA is shown in Table 7.

The roots and leaves were stripped from 30 inoculated and naturally diseased plants and the residual crowns were washed thoroughly. Each crown was sliced longitudinally into ten sections and two sections were transferred to plates of each of the five selective media. After five and eight days incubation the presence of mycelial growth resembling that of *P. cactorum* and of confirming structures (sporangia and/or oospores) was noted. The results are also summarised in Table 7.

TABLE 7. A comparison of five media selective for *Phytophthora*

Medium	Growth in pure culture	Recovery from diseased tissue	
	% of radial growth on cornmeal agar*	% pieces with *P. cactorum* -like mycelial growth	% growths with sporangia +/or oospores
Kannwischer & Mitchell	88	95	95
Harris & Bielenin 1	43	78	35
Harris & Bielenin 2	44	76	53
Jeffers & Martin	59	92	85
Pettit & Pegg	64	67	63

* After 9 days incubation

The Kannwischer & Mitchell (1978) medium gave the highest recovery of *P. cactorum* growth from diseased plant tissue and all growths developed confirmatory structures. This was correlated with the comparative growth of the fungus in pure culture. This medium is marginally superior to the Jeffers & Mitchell (1986) recipe which has a very similar composition, but is significantly better than the medium of Pettit & Pegg (1991) which has been used in ADAS diagnostic laboratories for diagnosis of crown rot and for screening for the presence of *P. cactorum* in plant tissue.

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DCH/AP:3.3.94

Contract between HRI (hereinafter called the "Contractor") and the Horticultural Development Council (hereinafter called the "Council") for a research/development project.

PROPOSAL

1. TITLE OF PROJECT Contract No: SF/23

CROWN ROT OF STRAWBERRY: TRANSMISSION OF THE PATHOGEN (PHYTOPHTHORA CACTORUM) IN MICROPROPAGATION

2. BACKGROUND AND COMMERCIAL OBJECTIVE

Crown rot of strawberries is a soil-borne disease caused by a specialised strain of Phytophthora cactorum. Until recently it has occurred only locally and sporadically in Britain since its first introduction from continental Europe in the late 1960s. However, in 1989 and 1990 crown rot was a much more conspicuous and damaging problem of field-grown strawberries, especially in the south-east. The great expansion in cultivation of susceptible cultivars, particularly Elsanta, has undoubtedly been an important factor in this development.

Another important factor is diseased planting material. There is good evidence that the pathogen has been spread with both imported and with British-grown runners. Unless steps are taken to curb this dissemination of disease, crown rot could become as widespread and serious a threat to strawberry production in Britain as it is in continental Europe.

One possible method for producing and maintaining pathogen-free plant stocks particularly at the highest levels is through micropropagation. However, there is a suspicion, based mostly on circumstantial evidence from several sources, that the crown rot pathogen can co-exist in a latent form with plant material in vitro. If true, this would completely undermine the micropropagation approach to control. This proposal is to evaluate this possibility. If latent infestation of plants is demonstrated and time permits, methods of controlling infestation will also be investigated.

3. POTENTIAL FINANCIAL BENEFIT TO THE INDUSTRY

The presence of crown rot in stocks may lead to disqualification with the direct losses than it brings and, when the disease occurs in the higher grades of stocks, there is an enormous disruptive effect on the whole system of production.

A reliable method of eliminating P.cactorum from stocks through micropropagation would be extremely valuable in the strategy of controlling crown rot through clean planting material.

4. SCIENTIFIC/TECHNICAL TARGET OF THE WORK

To test the hypothesis that the strawberry crown rot pathogen can exist in latent form in micropropagated strawberry plants and be propagated with them _in vitro_ and during weaning-on.

5. CLOSELY RELATED WORK - COMPLETED OR IN PROGRESS

Annual screening for resistance to the raspberry root rot pathogen (_Phytophthora fragariae_ var. _rubi_) is being carried out. A watching brief on possible crown rot infestation of the NSA nuclear stocks at East Malling is constantly maintained. There is experimental work on micropropagation in strawberries, specifically for its application to the maintenance of NSA nuclear stocks, and in other contexts; micropropagation is used routinely in the strawberry breeding programme.

6. DESCRIPTION OF THE WORK

The ability of _P.cactorum_ to grow on the media used in strawberry micropropagation will be examined. Runner tips of strawberry cultivars of different resistances to _P.cactorum_ will be inoculated with zoospores at various stages of development and micropropagated cultures will be obtained from them. Shoot tips of various sizes will be taken, from the smallest meristem explants that are used in virus elimination to the comparatively large explants that are commonly used in commercial micropropagation. Once established, the cultures will be examined for the presence of _P.cactorum_, directly by microscopic methods and indirectly by culturing the tissues on to suitable media. If latent infestation is demonstrated, the strawberry cultures will be taken through the proliferation and rooting stages and then weaned back into conventional culture to see whether the fungus is maintained in the tissues through these stages. If latency in micropropagation is confirmed, various possible methods of control such as hot water and fungicides will be evaluated as time allows.

7. COMMENCEMENT DATE AND DURATION

01.04.92; duration 2 years (a six month sandwich student will be appointed from the 1st April in each year).

8. STAFF RESPONSIBILITIES

A sandwich student working under the joint direction of D C Harris and D W Simpson.

9. LOCATION

HRI-East Malling.

Contract No: SF/23

TERMS AND CONDITIONS

The Council's standard terms and conditions of contract shall apply.

Signed for the Contractor(s)

Signature..

Position..Commercial & Marketing Manager HEI

Date..........21/8/92..........

Signed for the Contractor(s)

Signature..

Position..

Date..

Signed for the Council

Signature..

Position.......CHIEF EXECUTIVE.......

Date..........24.7.92..........