Validation and Exploitation of a PCR-based Diagnostic for Detecting *Phytophthora* in Strawberry and Raspberry

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Introduction

Up to 13 *Phytophthora* species have been recorded on soft fruit around the world. *Phytophthora fragariae* varieties *fragariae* and *rubi* are amongst the most damaging to strawberry and raspberry crops respectively. In the absence of fully effective host resistance, control depends on cultural measures and agrochemicals. *Phytophthora* infection is difficult to detect and there is no doubt that contaminated plant material has been the main means of disease dissemination. Provision of disease-free planting material would provide the ultimate control measure as land remains free of contamination. DNA-based diagnostic methods has enabled the development of rapid and sensitive tools capable of detection very low levels of *Phytophthora* contamination. SCRI has developed such a method and validation and exploitation in a Scottish disease survey is reported on this poster.

Strawberry red stele in S. Germany

Raspberry root in Scotland.

Materials and Methods/Results

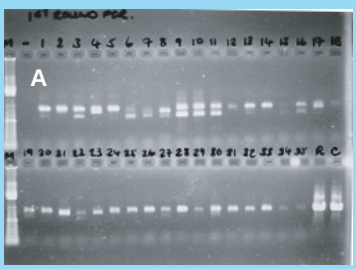
PCR testing

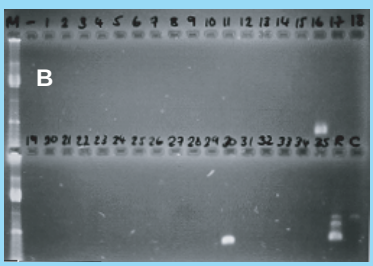
1st Round

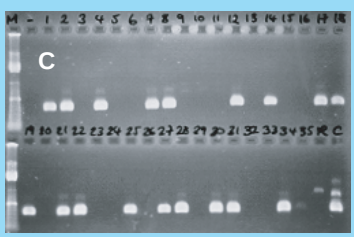
A polymerase chain reaction (PCR) detection system was developed on the basis of DNA sequence differences in the Internal Transcribed Spacer regions of Ribosomal DNA (rDNA) (Bonants et al., 1995). Root samples are finely chopped in a domestic grinder and homogenised in liquid nitrogen before DNA extraction and purification. The PCR primers used in the first round of the nested PCR (see box on right side) are designed to amplify specifically DNA of all Peronosporales including *Phytophthora e.g.....* *Pythium* and downy mildews.

2nd Round

In the 2nd round of nested PCR, a series of primer pairs, each of which amplifies only one species or a small range of species, are used to test the 1st round PCR product for the presence of species of interest: for strawberry and raspberry, normally *P. fragariae* and *P. cactorum* or *P. idaei.* A range of these specific primers has been developed (some of which are detailed in the panel at right). All of them can be used in nested PCR.







The three gels in this panel show the results of PCR tests on raspberry root samples from the field using:

A 1st round with general Oomycete primers - DC6/ITS4

B 2nd round with primers for *P. fragariae*

C 2nd round with primers for *P. cactorum/ P. idaei*

Primers for nested PCR detection of Phytophthora

	Sequence	Name	Annealing temperature
1st round primers for all Peronosporales			
	forward 5'GAGGGACTTTGGGGTAATCA 3'	DC6	Tm - 62oC
	reverse 5' TCCTCCGCTTATTGATATGC 3'	ITS4	
2nd round primers for various *Phytophthora* species			
P. fragariae	forward 5'ACTTAGTTGGGGGCCTGTCT 3'	DC1	
P. fragariae/cambivora/cinnamomi			Tm= 62oC
	reverse 5'CGCCGACTGGCCACACAG 3'	DC5	
P. cambivora	forward 5' TTAGTTGGGGGCTAGTCCC 3'	DC4	Tm= 60oC
	reverse (see above)	DC5	
P. cinnamomi	forward 5'AACTGAGCTAGTAGCCTCTC 3'	DC9	Tm= 60oC
	reverse (see above)	DC5	
P. cryptogea/P. drechsleri/P. erythroseptica			
	forward 5'CGGTTTTCGGCTGGCTGGG 3'	CRYFwd	Tm= 60oC
	reverse 5'CAGCTTrCGCCAGAACAGAC 3'	CRYrev	
P. nicotianae	forward 5'CCAATAGTTGGGGGTCTTATT 3'	DC3	Tm= 58oC
	reverse 5'AATTCAAAAGCCAAGCCACC 3'	DC9	
P. cactorum/P. idaei			
	forward 5'TACTGTGGGGACGAAAGTCCT 3'	ADF1	Tm= 64oC
P. cactorum/P. idaei			
	reverse 5'CCGATTCAAAAGCCAAGCAACT 3'	ADR	

During this work, new primers were developed that amplify the DNA of all *Phytophthora* species but no *Pythium.* These primers have been substituted for the DC6 /ITS4 set of primers in the first round of nested PCR. The result is far fewer bands in the first round, probably a reflection of *Pythium* not being amplified. The primers also gave slightly more and stronger positives than DC6/ITS4. In addition, a few bands were produced in the first round that subsequently did not amplify with either the specific primers for *P. fragariae* or *P. cactorum/P. idaei* in the 2nd round of PCR . Restriction digests of these 1st Round bands indicated the presence of other species such as *P. cryptogea* and *P. megasperma sensu stricto* in the samples, although these species were not always isolated. These *'Phytophthora'* primers are being tested further as a method of detecting and distinguishing all and any species in any plant sample.

Practical testing and conclusions

Soft fruit survey

A survey was carried out to assess the extent of *Phytophthora* contamination in Scottish raspberry and strawberry crops. Over 450 samples, collected from more than two hundred commercial plantings, were tested by conventional baiting assays and the PCR methods described above. Preliminary results indicate that:

1. Around a third of strawberry and raspberry plantings are infected with *P. fragariae* varieties *fragariae* or *rubi* respectively.
2. There was a close agreement between the baiting assay and the PCR testing with discrepancies in only 12 of 450 samples. PCR testing gave a few more positives than baiting. Overall, the results were broadly similar to a survey of English and Welsh strawberry stocks using similar procedures (Hughes, Inman & Cooke 2000)
3. Results using the *Phytophthora* primers indicated that less than 0.5% of the samples were infected with a *Phytophthora* other than *P. fragariae, P. cactorum* and *P. idaei.* Subsequent examination revealed for example *P. megasperma* and *P. cryptogea*.

Bonants, P.J.M., Hagenaar De Veerdt, M., Van Gent-Pelzer, M. P. , Lacourt, I. and Cooke D. E. L., & Duncan J.M. (1997) Detection and Identification of *Phytophthora fragariae* Hickman By the Polymerase Chain Reaction. *European Journal of Plant Pathology.* **103**, 345-355.

Hughes K.J.D., Inman A.J., and Cooke D.E.L. 2000 Comparative testing of nested PCR-based methods with bait plant tests for detecting *Phytophthora fragariae* var. *fragariae* (strawberry red core) in infected strawberry roots from fruit crops in the UK. EPPO conference, Wageningen, Feb 2000 *OEPP/EPPO Bulletin* **30,** 533-538.

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