Detection of *Phytophthora* species in Strawberry in Plantations in S. Italy by a PCR-based Diagnostic

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Introduction

Up to 5 Phytophthora species have been recorded on strawberry in Europe. P. fragariae and P. cactorum, which cause red stele and crown rot respectively, are the commonest and most important species. Crown rot has been recorded in Italy but red stele has not.

Plantations around Lamezia Terme in S. Italy at the end of 2001 were affected by root rot, collapse and typical symptoms of crown rot but several attempts to isolate Phytophthora from them failed. Samples of two main stocks of Camarosa, one from California and the other Belarus, were tested with a polymerase chain reaction diagnostic (PCRD) for Phytophthora. The basis of the test is explained in the poster and the results from S. Italy are given in the poster below

Strawberry red stele disease

Strawberry crown root

Materials and Methods

Samples

The samples came from a single large plantation near Lamezia Terme. The area had been planted with Camarosa with nursery stock from California and Belarus. Patches of poor growth and general plant collapse were found throughout the field shortly after planting. Some affected plants had discoloured crowns typical of crown rot and most plants evidently had failed to make any growth after planting, suggesting that they were affected at time of planting. Samples of crowns and roots were inserted into apples to try and recover live Phytophthora cultures and isolations were attempted onto selective medium.

PCR testing

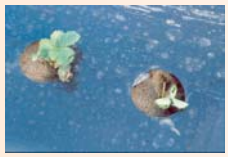
The polymerase chain reaction diagnostic (PCRD) is based on DNA sequence differences in the Internally Transcribed Spacer (ITS) regions of Ribosomal DNA (Bonants et al., 1995). The PCRD uses nested, or two-round, PCR in which DNA of any member of the Peronosporales present in the sample , e.g. a Phytophthora, Pythium or downy mildew, is amplified in the first round but DNA from other microorganism and plants is not amplified. Thereafter, in the second round PCR primers specific for particular Phytophthora species and internal to the amplicon from the first round can be used to test for particular species of interest, in this case P. fragariae and P. cactorum. Roots and crowns were frozen in liquid nitrogen and then ground very finely in a mortar and pestle before DNA extraction and purification.

PCR specificity and sensitivity

Primers for nested PCR detection of Phytophthora

	Sequence	Name	Annealing t temperature
1st round primers for all Peronosporales			
	forward 5'GAGGGACTTTGGGGTAATCA 3'	DC6	Tm – 62oC
	reverse 5' TCCTCCGCTTATTGATATGC 3'	ITS4	
2nd round primers for specific for various Phytophthora species			
P. fragariae	forward 5'ACTTAGTTGGGGGCCTGTCT 3'	DC1	
P. fragariae/cambivora/cinnamomi *			
	reverse 5'CGCCGACTGGCCACACAG 3'	DC5	

* Different forward primers are required to detect P. cambivora and P. cinnamomi
Tm= 62oC



Pictures from Lamezia Terme

1. Plants being planted
2. Wilted plants
3. plants with rotted roots
4. Rotting in the crowns

Results

Apple baits and selective medium

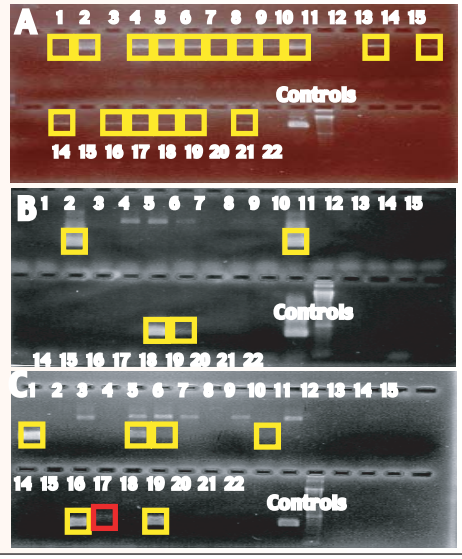
No Phytophthora was recovered from the apple baits or the selective agar, perhaps because the material was very badly rotted by the time it was available for testing.

Nested PCR

Phytophthora was detected in several of the samples by PCR. Both P. fragariae and P. cactorum, were detected in samples from the stocks from Belarus, whereas only P. cactorum was detected in one sample from California. The same PCR techniques have been applied in several surveys in different countries. Typical results obtained on testing strawberry nursery stock with the PCRD are shown in the panel at left. The top panel shows the results after the first round of nested OCR using the Peronosporales primers DC6 and ITS4. There are many positives possibly because of Pythium and relatives being present in roots. the middle panel shows the second round of nested PCR using the DC1 and DC5 primers (see above), are specific for P. fragariae – four of the sample are positive. The bottom panel shows the same samples but this time, the primers specific for P. cactorum have been used (ADF1 and ADR in the panel above). There are six positives and one sample with an abnormal signal (highlighted with a red box), a very unusual occurrence. One sample (number 10) is positive for both pathogens.

Improved version of PCRD The top panel

In recent versions of the PCRD the DC6/ITS4 primers have been replaced with a set of primers that are specific for all Phytophthora species. These primers have increased sensitivity and eliminated many of the positives previously obtained in the first round of



Conclusions

P. cactorum was detected by the PCRD in strawberry plants showing poor growth and typical crown rot symptoms in the plantations at Lamezia Terme in Italy, although the pathogen was not isolated by either bait plants or selective media, mostly probably because of the poor condition of the plants at sampling. P. cactorum was detected in nursery plants from both California and Belarus; P. fragariae was also detected in plants form Belarus although there was no obvious symptoms of red core. These results and those of much

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