2004 REPORT

Specific Detection of Phytophthora Crown Rot and Anthracnose in Strawberry

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PRODUCTION RESEARCH

BRIEF INTRODUCTION TO THE PROBLEM:

Strawberry growers face numerous production challenges. High capital costs to establish a strawberry crop make growers keenly aware of the need to adequately manage weeds, diseases, nematodes, insects and mites that prove to be a constant threat. Disease problems are a primary focus of pesticide input and crop losses in strawberry production systems. In particular, anthracnose caused by *Colletotrichum* species, and Phytophthora crown rot caused primarily by *P. cactorum* (also causal agent of leather rot in northern production regions) are serious concerns in the industry. In our region, we have experienced severe losses in the last three years in particular, due to either or both of these pathogens and they are typically introduced on contaminated transplants. There is a real need to develop rapid diagnostic assays to allow rapid disease identification and to develop plant quality assays to ensure reduced risk of planting contaminated plants. We have initiated development of standard and real-time PCR assays and sampling protocols to aid in our goal to ensure the use of disease free plants.

DESCRIPTION OF THE EXPERIMENTAL DESIGN:

Phytophthora Work:
Identifying *Phytophthora cactorum* morphologically requires familiarity with the shapes and sizes of oomycete sexual structures. It can take several days and may require multiple steps to encourage cactorums to reproduce sexually. Even under good conditions some specimens of *cactorum* may not form these structures and could require molecular identification. To make PCR based identification faster and cheaper, we have combined four primers (multiplex-PCR); CACTF1, CACTR1, DC6 and ITS2. Together, they form four bands for cactorums, one band for other oomycetes and no band for all other organisms. Eddie Croom (undergraduate research assistant); L.M. Ferguson; L. Leandro and F.J. Louws. Work in progress.

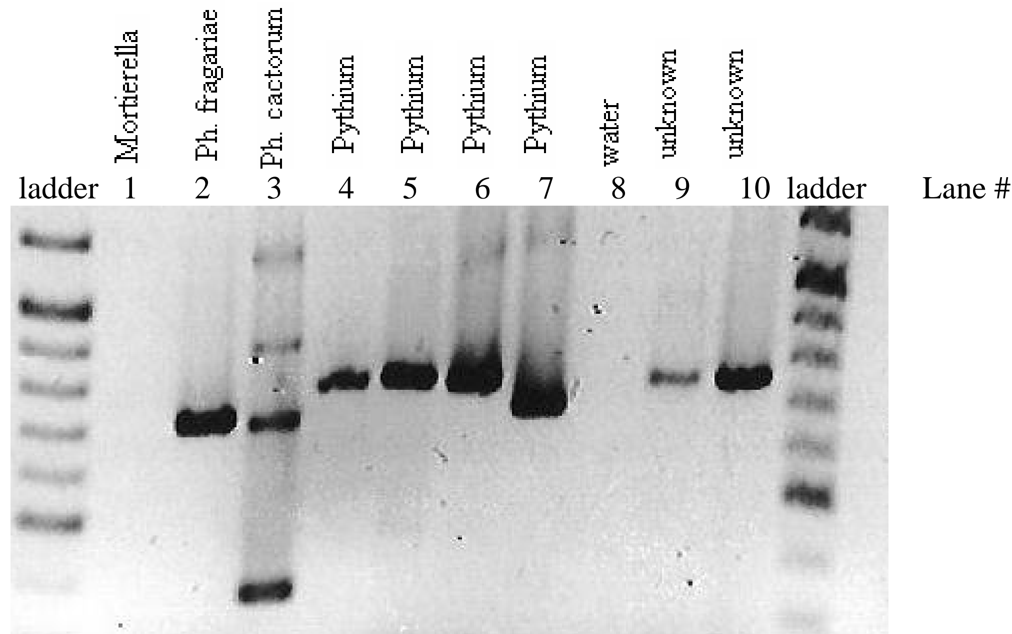
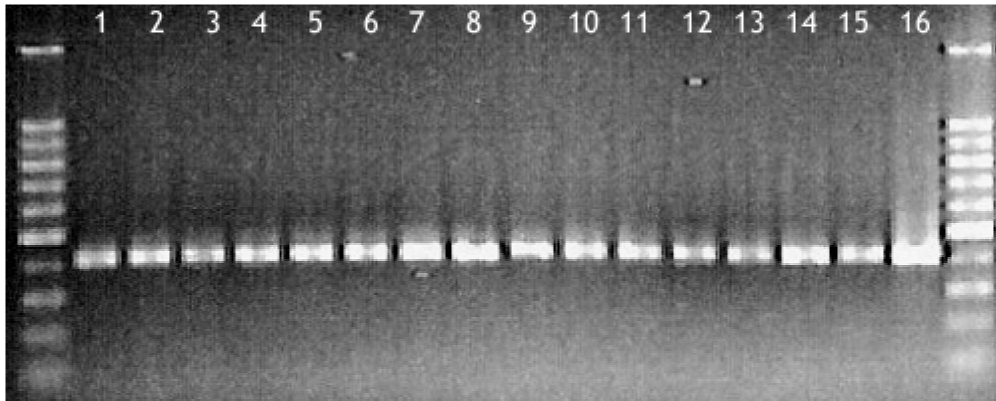


Figure 4:The ladders are the 100bp size standards on either end. For lanes 1 and 8 the samples do not contain any oomycete DNA. They have no bands. In lane 3, the sample is *cactorum* and

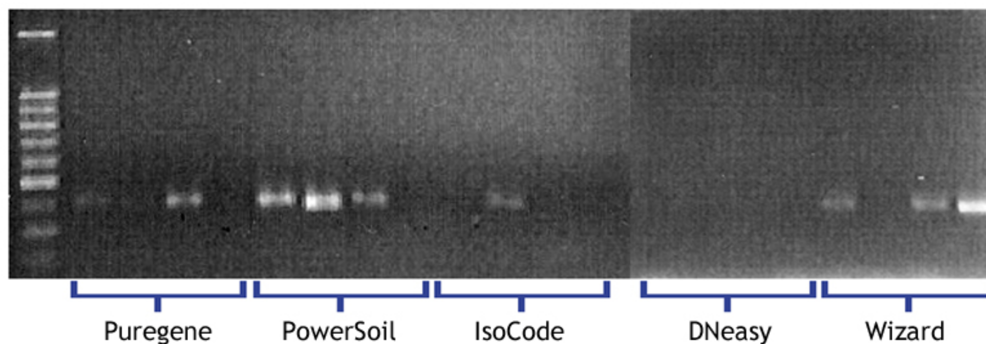
has four bands. Three of these bands are unique for *cactorum*. All other lanes have the one band that is formed from oomycete DNA.

Colletotrichum Work:

Mycelium of *Colletotrichum* strains isolated by the NCSU Plant Disease and Insect Clinic (99-884-A1, 00-4818, and 01-1551) and mycelium of the *Trichoderma* strain MT2-3-H1-2 (courtesy of Dr. Leonor Leandro) were grown in potato dextrose broth. DNA was extracted from the mycelium using the MoBio PowerSoil Kit, the MoBio UltraClean Microbial DNA Kit, the MoBio UltraClean Soil DNA Kit, the Schleicher and Schuell IsoCode Stix system, the Gentra PUREGENE Kit for DNA purification from cells, tissue, body fluids, and Gram-negative bacteria, the Gentra PUREGENE Yeast and Gram-Positive Bacteria Kit, a CTAB-based extraction protocol provided by Dr. Leandro, the Qiagen DNeasy Plant Mini Kit, the Pierce Lyse N Go Kit, and the Promega Wizard Genomic DNA Purification Kit. The PCR products from these isolation protocols were compared to those resulting from positive control DNA obtained from Dr. Stanley Freeman. Of all the kits, only the MoBio PowerSoil kit yielded consistent bands.

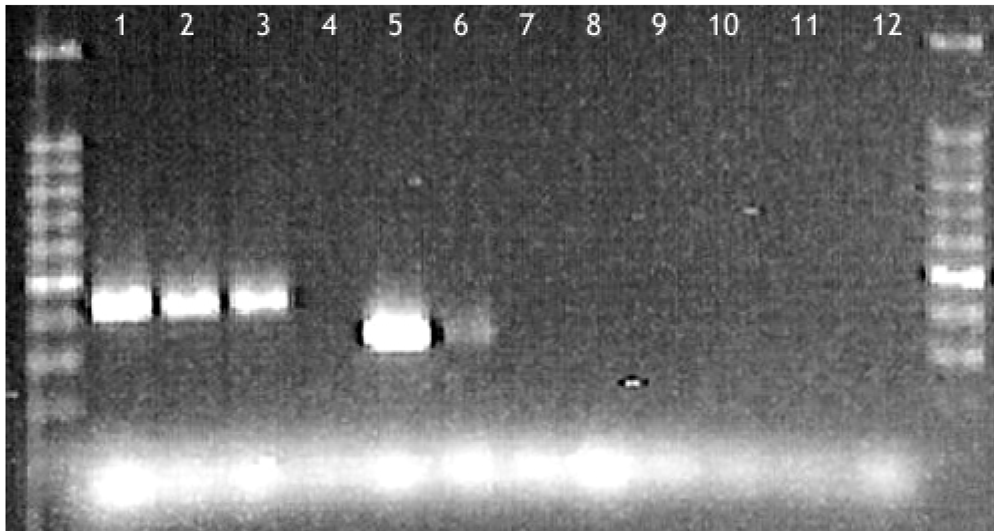


Positive controls amplified by *Col1/Col2*.



Three *Colletotrichum* strains and a *Trichoderma* strain isolated using various kits.

Primer sets from the literature were then tested using the positive control DNA in the last experiment and DNA isolated from Clinic strains using the MoBio kit. All control samples were amplified using the *Colletotrichum* spp. specific primers *Col1/Col2*. Most of the Clinic isolates were confirmed to be *C. acutatum*. The one strain which was not amplified by the *C. acutatum* primers was also not amplified by the *C. fragariae* primers; the strain may have been *C. gloeosporoides*, or may not have been amplified sufficiently during PCR.



C. acutatum, C. fragarie, and *C. gloeosporioides* strains amplified by:
Lanes 1-3: Col1/Col2 (*Colletotrichum*-specific)
Lanes 5-7 Acut1/Col2 (*C. acutatum*-specific)
Lanes 9-11 Fra1/Fra2 (*C. fragariae*-specific)

The same kits used for DNA isolation from mycelium were also used to isolate DNA from *Colletotrichum* conidia. No DNA resulted from any of these kits. In light of this result, various lysis methods were used before beginning the MoBio protocol, including alkaline lysis, microwaving, repeated freezing and thawing, longer mechanical disruption periods, and boiling. None of these procedures produced a significant amount of DNA, suggesting that some factor other than the means of lysis was responsible for the lack of product. Six concentrations of spore suspension ($1x10^2$ to $1x10^8$ spores/ml) were used to determine whether the initial amount of material was insufficient or overloading the protocol, but none of the concentrations produced an appreciable amount of DNA. Two protocols for removing the mucilage (which contains DNases) were also used; washing the spores with TE was not successful, but boiling the spores in SDS prior to washing resulted in a very high DNA yield. However, the resultant DNA did not produce a band, which may indicate that this protocol removed DNases but not all of the inhibitors which were present. Alternative protocols for inhibitor removal should be pursued.

A real-time PCR protocol was initiated. Primers and probes have been designed and are currently being evaluate din PCR experiments.

SUMMARY OF RESULTS AND CONCLUSIONS:

Primers have been adapted or developed for the specific detection of *Phytophthora cactorum* and *Colletotrichum* species. We have used these protocols for rapid diagnosis and identification of these serious pathogens in commercial clinic samples. A real-time PCR protocol has been initiated for *C. acutatum*. We plan to adapt these protocols for rapid detection and identification of plant material. We have met some challenges in extracting pathogen DNA from some of the tissues in attempts to develop protocols that would be useful in “clean plant” programs. Work will continue. Good progress has been achieved and a second year of continued funding will be requested.