TITLE: Detection and Management of Anthracnose and Phomopsis blight in Strawberry Production in the Southeast

PROGRESS REPORT

GRANT CODE: SRSFC 2007-05

RESEARCH PROPOSAL

NAME AND ADDRESS of PI: Frank J. Louws, Box 7616 North Carolina State University, Raleigh NC 27695

SUMMARY: We have modified a DNA extraction protocol from infected strawberry tissues to remove potential inhibitors and amplify DNA of *Colletotrichum* spp by using species specific primers in PCR. We have evaluated strawberry genotypes for fruit anthracnose resistance and implemented fungicide evaluation trials to manage phomopsis blight of strawberry.

PART I: Selective amplification of *Colletotrichum acutatum* and *C. gloesporioides* from diseased strawberry tissue by PCR.
Strawberry leaf and crown tissues with or without symptom were collected from disease specimen submitted to NCSU Plant Disease and Insect Diagnostic Clinic. Tissues were subjected to a modified extraction protocol by adding PVP or a special detergent or by running an extra purification step to remove inhibitors from the extracts. PCR amplification of extracted DNA with species-specific primers (CaInt + ITS4 for *acutatum* and CgInt + ITS4 for *gloeosporioides*) amplified a 490 bp fragment.

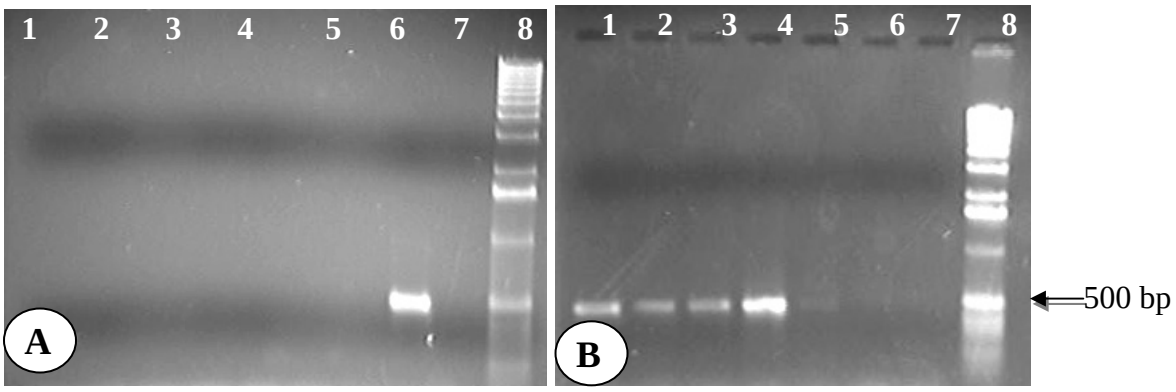

Fig. 1. PCR amplification of *Colletotrichum gloesporioides*; (A) before removal of inhibitors from tissue extract, only +ve control (pure culture) amplified; (B) after removal of inhibitors-lane 1, extract from nonsymptomatic leaf; lane-2, extract from symptomatic (black lesion) leaf; lane-3, extract from symptomatic crown; lane-4, +ve control (pure culture); lane 5, 6-other than *Colletotrichum*; lane-7 –ve control (no template), lane-8, 1 kb ladder.

This protocol is now ready to be used by PDIC which will not only save time but also increase the accuracy of diagnosis, since isolation and culture based identification sometimes are very difficult.

We have also PCR amplified Glutamine synthetase gene of *Colletotrichum* isolates collected from latent infections of leaf samples from growers' fields (including fields with no apparent symptoms) as well as fruit anthracnose samples followed by sequencing of the 1kb fragments. Multiple alignments of the sequences showed that isolates from fruit anthracnose and latent leaf infections are identical. Pathogenicity trials of the isolates from latent leaf infections on detached strawberries invitro showed these isolates are as pathogenic as isolates derived from fruit anthracnose. This study shows the importance of latent infection of strawberry foliage as a source of infection for fruits. Evaluation of latent infection thus will help growers to adopt appropriate preventative measures in case of favorable environment for disease development. In addition we digested this 1 kb fragment with restriction enzyme *pst-I* that showed distinct banding pattern for *Colletotrichum acutatum* (2 fragments of 610 and 390 bp) and *C. gloesporioides* (4 fragments of 400, 250, 200 and 160 bp). This protocol is also useful for detection and discrimination of *acutatum* and *gloesporioides* (Fig. 2).

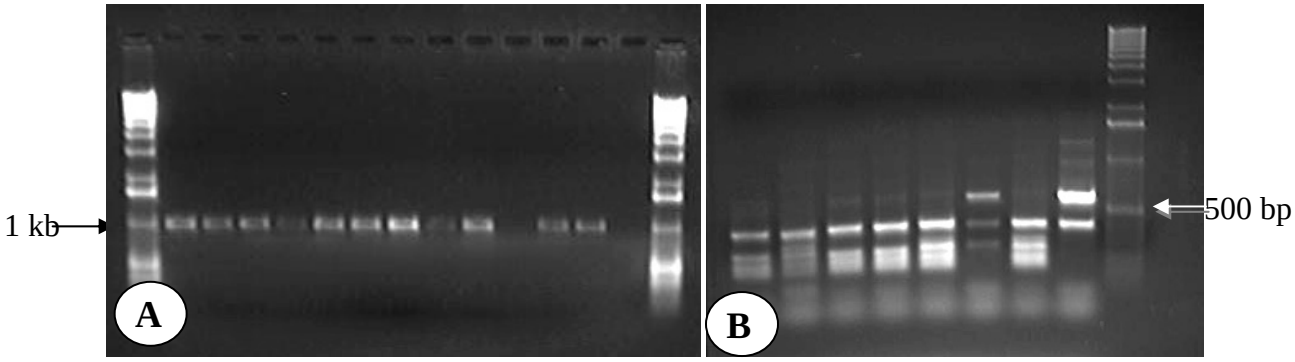

Fig. 2. Amplification of glutamine synthetase gene and pst digest; (a) 1 kb fragment; (B) lanes 1-5 &7, C. gloesoprioides; lane-6 unknown *Colletotrichum* sp, lane-8, *C. acutatum*, lane-9, 1 kb fragment

The ability to detect latent infections, link these infection levels to levels of risk or management decisions and to rapidly detect and identify the two species are critical steps to developing management programs for these serious pathogens.

Part II. Evaluation of breeding lines and cultivars for anthracnose resistance. The test was located at the Horticultural Crops Research Station in Clinton, NC. Plots were established 10 Oct 06 with non-inoculated plants, and consisted of four 6-in. tall, 27-in. wide, plastic mulched beds on 60- in. centers. Plots were 5 ft long and contained 10 plants on a 12-in. spacing staggered in two rows 12 in. apart. Treatments were randomized in four complete blocks. Two inoculated plants (3 *C. acutatum strains*) were inter-planted 10 Oct 06 between plots to allow "natural" spread of disease throughout the growing season. Commercially recommended fertilization and insect management practices were followed. No fungicides were applied throughout the growing season. Fruit were harvested weekly from 5 Apr through 22 May. Total yield and percent of marketable and cull fruit (undersized, misshapen) were calculated based on weight.

The emphasis of this trial was to evaluate strawberry varieties for resistance level to anthracnose fruit rot. Chandler is the commercially standard cultivar; NCC numbered lines are products of the NCSU breeding program. Anthracnose fruit rot incidence was low throughout most of the harvest season among all treatments, which led us to inoculate the plots with *C. acutatum* spore suspension @ 5 $X10^6$ spores/ml at the middle of the harvest season. Spray inoculation of varieties greatly increased disease incidence. Chandler was highly susceptible with an average fruit rot incidence of over 70% and several North Carolina breeding lines were highly resistant and showed superior total yields (Table 1).

Table 1: Yield, anthracnose ripe fruit rot incidence and average berry size for multiple strawberry lines: sorted by incidence level of anthracnose ripe fruit rot.

Strawberry genotype	Breeding program	Yield (g/plant)	Anthracnose incidence (%)	Average berry weight (g)
Chandler	U.C. Davis	158.9 abc	73.6 a	11.1 c
Albion	N.C. State Univ.	63.0 d	67.6 a	15.8 a
Seascape	U.C. Davis	103.9 cd	62.2 ab	10.5 c
Festival	Univ. Florida	119.5 bcd	54.7 abc	11.5 c
NCL-03-05	N.C. State Univ.	157.0 abc	54.3abc	11.4 c
Camino Real	U.C. Davis	206.3 a	52.3 abcd	14.9 ab
NC-93-05	N.C. State Univ.	119.6 bcd	43.5 bcde	13.3 abc
NC-99-13	N.C. State Univ.	175.2 ab	38.0 cdef	10.8 c
Bish	N.C. State Univ.	119.9 bcd	32.7 cdef	10.7 c
NCL-03-06	N.C. State Univ.	157.2 abc	29.9 def	12.4 bc
Winter Dawn	U.C. Davis	126.7 bcd	26.2 efg	12.6 bc
Araza	Unknown Line	164.7 abc	20.2 efg	11.8 c
NC-99-27	N.C. State Univ.	184.8 ab	18.3 fg	11.0 c
Pelican	USDA	199.9 a	16.3 fg	10.9 c
NC-02-63	N.C. State Univ.	171.8 abc	4.9 g	10.2 c

Values followed by the same letter within a column are not significantly different based on Fisher's protected LSD.

Part III. Chemical control of Phomopsis blight of strawberry 2007

No new chemistries were available last spring for determining efficacy against anthracnose. This led us to test a couple of promising new chemistries for Phomopsis blight management which is becoming a bigger problem during the hot summers in nurseries, badly blighting strawberry leaves. The test was located at the Horticultural Crops Research Station in Clinton, NC. Plots were established 10 Oct 06 with healthy plug plants, and consisted of four 6-in. tall, 27-in. wide, plastic mulched beds on 60- in. centers. Plots were 5 ft long and contained 10 plants on a 12-in. spacing staggered in two rows 12 in. apart. Six treatments (5 different fungicides and one untreated control as shown in Table 1) were randomized in four complete blocks. Commercially recommended fertilization and insect management practices were followed. No fungicides were applied throughout the growing season (Oct to May). At the end of the spring harvest season plastic mulches were removed from the bed to allow runners and tips to establish around the plants. Treatment applications were started on 19 Jun followed by bi-weekly applications until 5 Sept for a total of 6 applications. Plots for phomopsis blight were evaluated on 27 Aug and 15 Sept by visual assessment on a 1-6 scale where 1= <10%; 2=11-20%; 3=21-30%; 4=31-40%; 5=41-50%; 6= >60% leaves were symptomatic. Percent leaf area blighted were also considered in determining disease severity.

The summer was abnormally dry and hot. However, phomopsis leaf blight progressed especially toward the end of the summer. Pristine and Indar, not currently labeled for strawberry phomopsis blight control, showed very good efficacy against phomopsis blight, similar to the industry standard fungicide Nova. Topsin-M and Captan did not reduce blight severity compared to the unsprayed control.

Table 2: Treatment effect on Phomopsis blight incidence.

Treatment	Rate/acre	Disease severity	
		27 Aug	15 Sept
Nova 40 WP	4 oz	1.3 b	1.2 b
Indar 75 WSP	2 oz	1.2 b	1.7 b
Captan 50WP	3.5 lb	4.7 a	4.8 a
Topsin M 70WP	1 lb	4.7 a	4.8 a
Pristine	20 oz	1.7 b	2.0 b
Unsprayed control	-	5.3 a	5.5 a

Values followed by the same letter within a column are not significantly different based on Fisher's protected LSD at P=0.05.