Title: Epidemiological significance of *Colletotrichum gloeosporioides* infestation of nursery plants on crown rot of strawberry in the Southeast

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Report Summary: We studied the impact of plant infestation in the nursery on plug production or bare root plant performance in the fruiting field with known amount of infestation. Mother plants in the nursery were inoculated in 0, 5, 10 and 25% with 5 X 10^6 *C. gloeosporioides* spores/ml in RCBD with 4 replicates. Both quiescent infection incidence and severity on leaves showed significant (P=0.0035 & 0.0383) positive correlation with % inoculated mother plants at 40 days after inoculation, which decreased over time with dispersal and multiplication of inocula on new daughter plants. At the end of the nursery growing season, both mother and daughter plants had similar incidence of quiescent infection and dispersal gradients of inoculum from mother to daughter plants showed a good fit in the empirical power law model. Infested tips led to 35% mortality in plug trays, indicating the enormous risk of producing plug plants with infested tips. Plant mortality in the fruiting field due to planting of infested bare root plants from the nursery was significantly affected by inoculum load as quiescent infection. Dipping infested plants from 10% inoculation in selected fungicides (Switch) before fall planting decreased crown rot incidence from 14.06 % to 4.69% with a concurrent increase in plant vigor, biomass production as well as marketable berries. In lower inoculum levels, no plant mortality occurred in the fall and winter but plant vigor and biomass production was significantly (P<0.0001) reduced compared to noninoculated control. Real time PCR protocol could efficiently detect and quantify close to 60 spores or cfu present on leaf. While fungicide Switch and Topsin-M showed significant reduction in plant mortality in field, our invitro fungicide trial identified active ingredient (prochloraz) with superior efficacy against *C. gloeosporioides*.

Objective 1. Determine crown rot severity in fruiting fields from known amounts of infestation of plants by *C. gloeosporioides* in the nursery.

A strawberry nursery was established at the Horticultural Crops Research Station Clinton, NC by using mother plants from the NCSU certification program. Four treatments were inoculation levels of 5%, 10%, 25% of the mother plants using a spore suspension of *C. gloeosporioides* (1 X 10^5 spores/mL) and a noninoculated control in a RCBD in 4 replicates. Leaf samples were collected at 30 and 60 days after inoculation (DAI) from mother and daughter plants at different distances from the point of inoculation and evaluated for the presence of quiescent infections (QI). Plug plants were produced from tips of the 25% inoculation treatment. Bare root plants from each treatment were collected separately from within a 1' radius of the point of inoculation or outside of the 1' area, which constituted 2 different treatments from each inoculation percentage in the fruiting field as shown in Fig 1. Plants from 10% I were subjected to fungicide dip prior to planting in fruiting field.

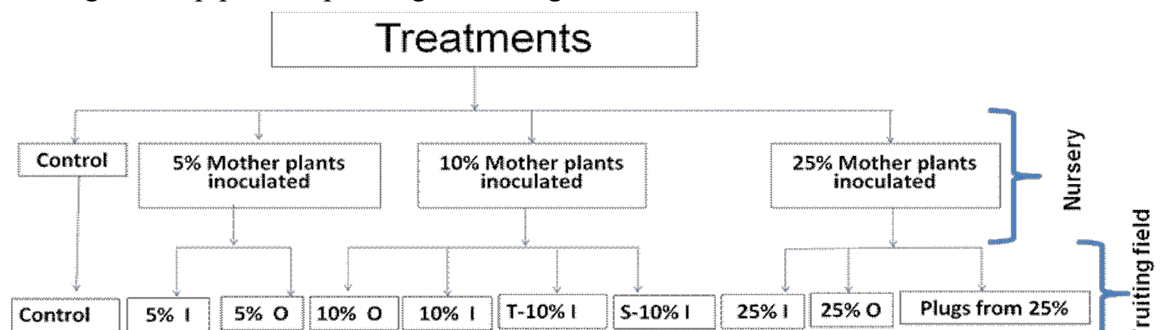


Fig.1. Treatments in the nursery and fruiting field; *I-inside* the radius of 1 ft of the point of inoculation; *O-outside* 1 ft radius, T-Topsin-M, S-Switch

Results: After 30 days of inoculation, QI incidence had a significant correlation with level of inoculation of plants (**Fig. 2**), and this decreased over time. However, at 60 DAI mother and daughter plants had no difference in incidence of QI (**Fig. 3**).

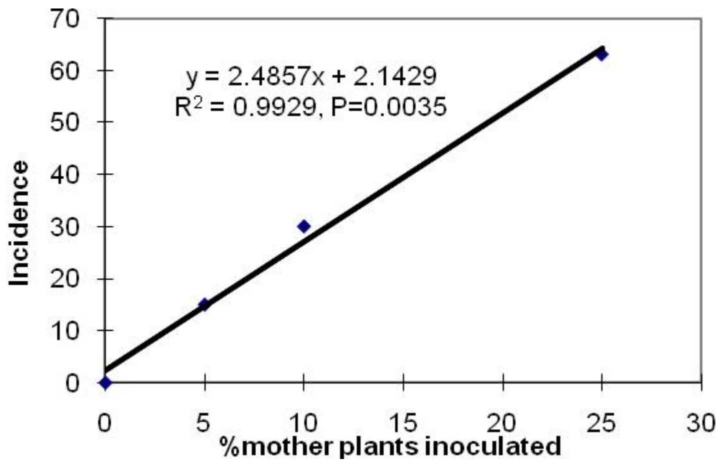
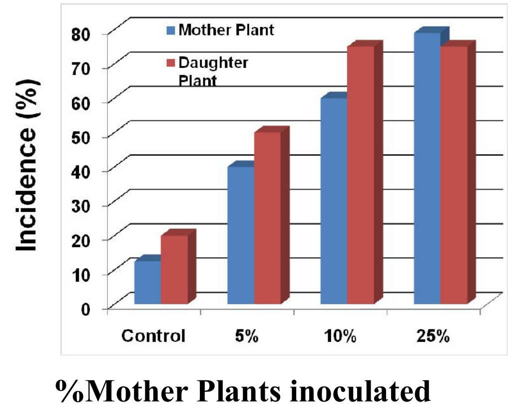


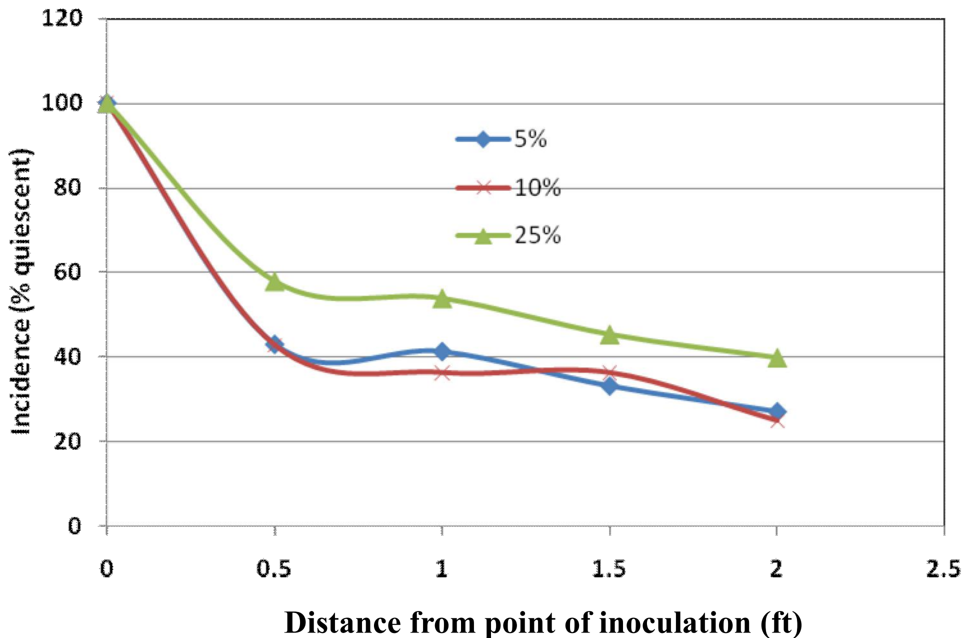
Fig.2. Correlation of *C. gloeosporioides* incidence (QI) in leaves at 30 DAI with % mother plants inoculated in nursery



%Mother Plants inoculated

Fig.3 . Incidence of QI in leaves of mother and daughter plants at 60 DAI before taking bare root plants to the fruiting field

In spite of sprinkler irrigation, leaf samples at 60 DAI indicated that dispersal of inoculum declined sharply from the point of inoculation to a 2' distance (**Fig. 4**).



Distance from point of inoculation (ft)

Fig. 4. Dispersal of *C. gloeosporioides* in strawberry nursery from the source of inoculation at 60 DAI

Spatial dispersal of *C. gloeosporioides* in the nursery showed a good fit in a power law model. Thus roguing could effectively remove the majority of infested plants if infection foci can be determined through proper detection methods.

In the fruiting field, bare root plants from inside the 1' radius had significantly higher plant mortality compared to the outer side of the radius. Although at 60 DAI 10% and 25% had similar incidence, in the fruiting field only 25% I treatment caused plant mortality in an alarming rate throughout the season (**Fig. 5**). Fungicide dipped plants from 10%-I treatment showed significant (P<0.0001) reduction in area under disease progress curve compared to non dipped treatments **(Fig. 6).** Level of infestation in the nursery significantly affected fruit yield in the field. Although at the end of the nursery season all levels of inoculation had incidence of *C. gloeosporioides*, only higher levels with high severity caused plant mortality and reduced yield. Data on plant vigor and biomass production did not show any direct correlation with level of infection as early plant mortality might have compensated by the plant growth due to less competition for space and nutrients (**Table 1**).

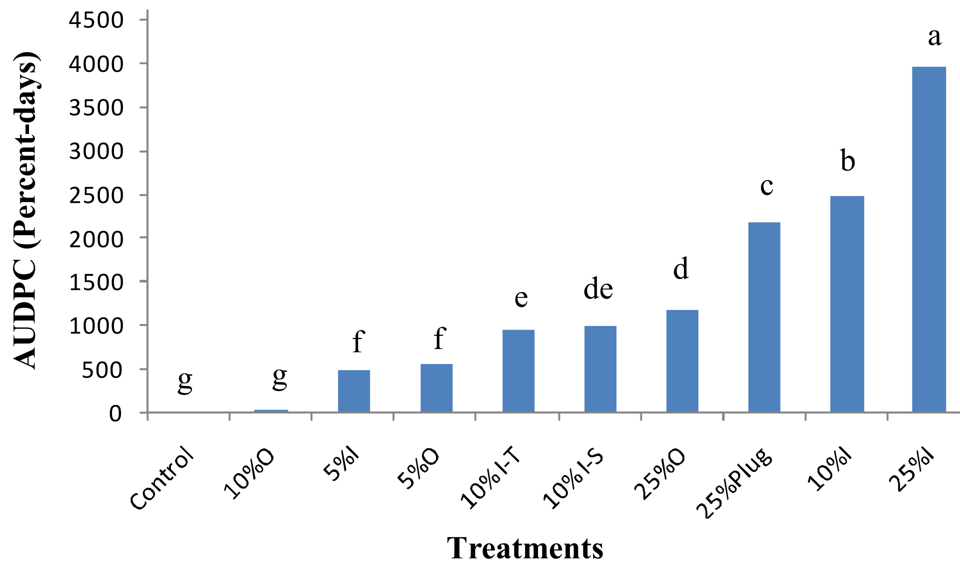


Fig. 6. Area under disease progress curve for the plant mortality in different treatments recorded from planting through the last harvest

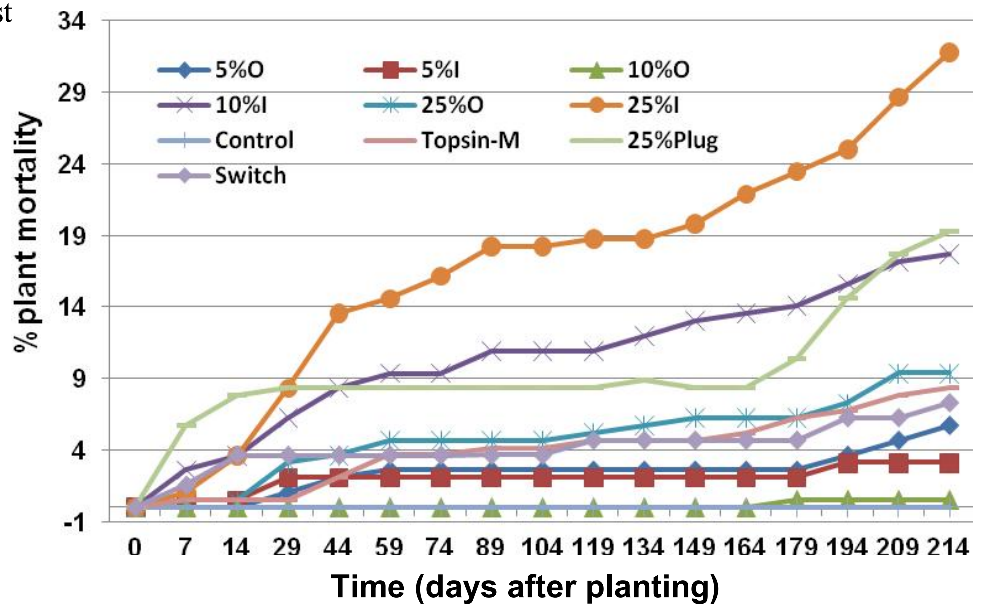


Fig. 5. Plant mortality in the fruiting field as affected by level of infestation in the nursery and fungicide dip before planting.

Pre-plant disinfestation protocols using fungicide treatments can significantly reduce plant mortality in the fruiting field.

Table 1. Fruit yield and plant biomass production in different treatments originating from the nursery inoculation at different levels followed by daughter plants collected from different distances as shown in Fig. 1.

Treatment	Yield (lb/A)	Plant dry wt (g/5 plants)	#crown /plant	Root dry wt (g/5 roots)	Root length (inch)
5% Outer	22848 abc	242.96 a	4.9	21.59 ab	8.80 a
5% Inner	20008 abcd	179.96 b	4.1	19.32 ab	8.16 b
10% Outer	21510 abc	174.87 b	4.2	23.26 ab	8.32 b
10% Inner	14405 d	159.48 b	3.9	17.53 b	8.42 b
25% Outer	18415 cd	179.71 b	3.6	18.47 b	8.04 b
25% Inner	17765 cd	173.74 b	4.6	19.48 ab	8.24 b
Control	25559 a	265.41 a	5.0	28.76 a	8.89 b
10% I-Topsin M	20379 abc	163.65 b	3.9	17.53 b	7.95 b
25% plug	23813 ab	280.37 a	5.9	27.05 ab	9.94 a
10% I-Switch	19943 abcd	273.42 a	4.4	21.29 ab	8.72 b
LSD (α = 0.05)	***5744***	***48.28***	***…***	***9.72***	***0.97***

Lower levels of infestation does not significantly reduce yield in spite of some plant mortality.

Objective 2. Assessing potential risk involved with plug production from infested tips

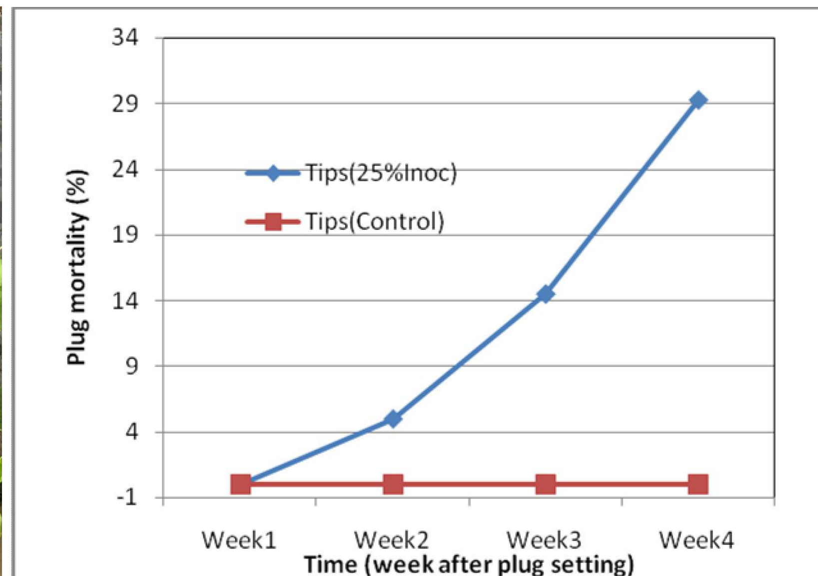


Fig.5. Plant mortality in the plug tray from tips during the 4-week propagation phase; planting was done by using only the apparently healthy plants.

Objective 3: Implementation of PCR-based technology to detect and quantify *C. gloeosporioides* in strawberry production systems especially from asymptomatic infection;

We have developed real time PCR primer and probe sets to detect and quantify anthracnose causing fungi *Colletotrichum acutatum* (fruit anthracnose/rot) and *C. gloeosporioides* (crown anthracnose/rot). This primer set and probe was optimized for Taqman protocol for *Colletotrichum* DNA extracted from pure culture as well as from latently infected strawberry tissue with varied success from different tissues. Strawberry plants from NCSU registered stock were grown in the greenhouse controlled environment to make sure the plants were free from any kind of previous infection. Leaves from each plant were selected to represent 3 different growth stages such as young, middle age and old. Leaves as well as petiole from each growth stage were inoculated with 5 different concentrations (5, 10, 20, 50, and 500 spores/leaf disk) by placing small droplets on pre-selected areas. Petiole inoculation was done by spraying spore suspension of different concentrations. Immediately after inoculation, plants were covered with plastic bags for 72 hr after which time leaves were sampled in 4 replicates. Leaf disks from the inoculation sites of 2 replicate samples of each treatment were cut out and DNA was extracted by MoBio Power plant kit. Another 2 replicate samples were subjected to paraquat (induction of senescence of surface sterilized leaves by dipping in paraquat) protocol followed by a short incubation in a crisper layered with moist paper towel. After a week of incubation leaf disks were cut out for similar DNA extraction method. A real time PCR Taqman cycle was run in ABI 7000 machine following default cycle parameter.

DNA extract from green leaf disks did not amplify the target region of 95 bp at any spore concentrations. But extracts from leaf tissues that was subjected to a bio-amplification phase was detected with a very low cycle threshold and in ascending order with the decrease of # of initial spores placed on the leaf indicating the detection of latent infection of *Colletotrichum* is possible by a Taqman protocol in a quantitative manner. Leaf stage appears to be a significant determinant of successful detection and quantification of latent infection. In spite of inoculation of leaves of different stages, only middle age leaves showed a meaningful change in cycle threshold value with changes in spore numbers and had showed lowest Ct value at each level of inoculum compared to other 2 leaf stages (Table 2).

Table 2. Real time PCR Ct values obtained from a Taqman protocol with 3 different leaf stages and 5 levels of initial spore concentration as latent infection on leaf surface.

	# of spores placed on leaf				
Leaf stage	5	10	20	50	500
Young (just fully open)	22.7	27.5	28.1	28.49	20.6
Middle age (1-2 months old)	20.5	19.2	17.7	15.95	13.15
Old (leaf just prior to senesce)	28.15	28.5	28.13	25.36	19.05

This result will provide the essential directions for sampling strawberry foliage from field for the evaluation of latent infection.

Our goal is to detect and quantify quiescent infection directly from foliage so that time consuming protocol such as the paraquat dip and incubation step can be avoided. After having an indication that middle age leaves are most suitable for molecular detection, we inoculated leaves following the above mentioned protocol with 3 levels of spores, 1,000, 5,000 and 10, 000 to obtain the following Ct values in two different real time protocol (Table 3).

Table 3: Ct value using two different labeling methods and three spore concentrations

# of spores added on leaf	Ct value (Taqman protocol)	Ct value (Syber green protocol)
1,000	26.24	28.32
5,000	24.01	25.25
10,000	22.44	20.85

This result suggests that direct quantitative detection of latent infection is possible when #cfu or # of cells on a sampled leaf area is close to 1000. DNA extract from the leaf sample containing 1000 spores was eluted in 80 µL buffer from which 5 µL was used for real time PCR indicating that it may be possible to detect 16 times lower cfu (~60 spores) with our method. This work is in progress. In the second year, we are attempting similar quantification from field leaves.

Objective 4. Develop tools to manage anthracnose or to reduce disease risk in strawberry fruit production fields.

Current recommendations for anthracnose control did not provide satisfactory result in some of the growers' fields. We determined the efficacy of the product Sportak (a.i. prochloraz) known to be effective against *C. gloeosporioides* causing end rot of mango and is registered in Europe or Australia. Results from this invitro study is very promising. This product can be pursued through IR4 to make it available to strawberry growers. Treatments were 1) Captan; 2) Pyraclostrobin; 3) Captan + Topsin-M which is the common recommendation we use for crown rot control; and 5) Sportak (a.i. Prochloraz). Sportak provided excellent invitro suppression of *C. gloeosporioides* even at 0.01 ppm

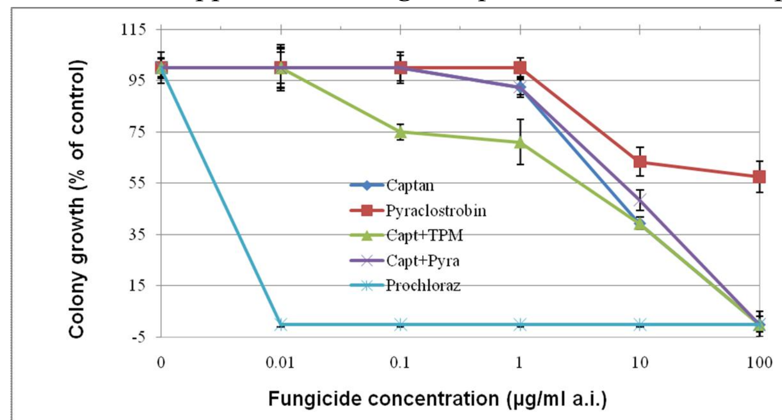


Fig. 6. Invitro efficacy of fungicides against C. gloeosporioides

Summary: The biology, ecology and management of *Colletotrichum gloeosporioides* were studied in the nursery and associated risk in fruiting fields. Our research discovered new information about this serious pathogen in the nursery and fruiting fields suggesting the pathogen is much more manageable than *C. acutatum*. Steep dispersal gradients suggest rouging could play an important role in the nursery, whole plant dips are helpful just prior to transplanting if plants are infested and a low infestation rate is of minor risk in the fruiting field. PCR based approaches to detect this serious pathogen were advanced but technical issues remain. There is a need to repeat this work for a second year and to further advance the practical implementation of PCR based detection systems.