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**Research Proposal**

**PROJECT TITLE:** Evaluation of Rate-Reducing Resistance and Defense Responses in Strawberry Genotypes to *Colletotrichum gloeosporioides* and *C. acutatum*.

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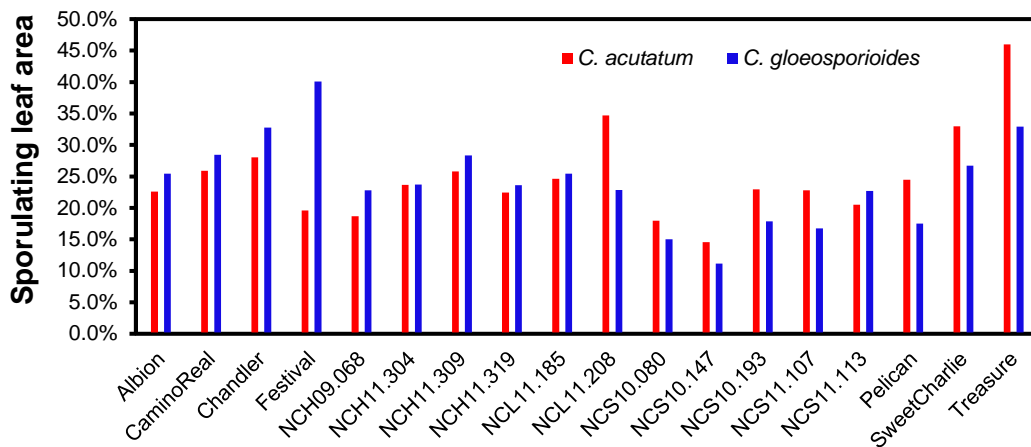
**ABSTRACT:** We evaluated 18 strawberry genotypes (7 commercial varieties and 11 NCSU selections) for resistance to foliar hemibiotrophic infections (HBI), caused by *Colletotrichum acutatum* (*Ca*) and *C. gloeosporioides* (*Cg*). The 'NCS 10-147' line and commercial cultivar 'Chandler' exhibited resistance and susceptibility to both *Ca* and *Cg*, respectively. To gain insight into molecular mechanisms for resistance to *Ca* and *Cg*, we conducted greenhouse experiments to investigate the genotypes × *Ca* and *Cg* HIB interactions and determine if 'NCS 10-147' is consistently resistant to both *Ca* and *Cg* HBI. Leaf samples were collected at seven time-points (0, 12, 24, 48, 96 hours, and 7 days, and 14 days after inoculation) and fungal development in each cultivar was determined by a paraquat assay (induces sporulation of asymptomatic infections) and quantitative real time PCR (qPCR) assay (detects levels of fungal DNA in sample tissues). Disease severity (percentage of total leaf area covered by sporulation) on paraquat-treated was assessed using the Horsfall-Barret rating scale and measured using the ImageJ software. Fungal biomass (DNA) estimated in each sampling time by qPCR was expressed as pg fungal DNA in 100 mg of leaf tissue analyzed. These results showed that 'NCS 10.147' consistently had the lowest disease severity and fungal biomass of both *Ca* and *Cg* in all sampled dates, indicating that this line could be a potential source of resistance to *Ca* and *Cg* HBIs and utilized in breeding programs. In addition, this genetic stock will be useful to investigate mechanisms of resistance to two distinct pathogens of strawberry.

**JUSTIFICATION:** Anthracnose fruit rot and crown rot of strawberry caused by *Colletotrichum acutatum* (*Ca*) and *C. gloeosporioides* (*Cg*), are major diseases in strawberry production regions in the southeastern United States. Chemical control is effective but reduces economic profitability and in the last few years resistance to the most important fungicides has emerged. Breeding strawberry for resistance to anthracnose is considered one of the primary means for managing these diseases. Recently, we estimated the genetic parameters of crown rot resistance to *Cg* using a partial diallel mating design derived from strawberry genotypes from a diverse origin (Pattison et al, *unpublished*). Both *Ca* and *Cg* appear to have a HBI strategy meaning initially these pathogens colonize host tissue with intimate host contact in a biotrophic phase followed by a necrotrophic phase associated with symptoms. This unique aspect of these pathogens has the ability to cause long latent infections (quiescent) during which the pathogens interact with the strawberry as a 'hemibiotroph'. If we can stop or slow down the initial colonization and multiplication of the pathogens, especially on green leaves, then we can substantially reduce risk associated with these pathogens. We have demonstrated this proof of concept from lab to field for *Ca* (Rahman et al., 2013). The high level of inoculum build up during the vegetative stage of production has raised two questions: Can effective management of the HBI phase in leaves and management of early cycles of sporulation limit the epidemic and subsequent anthracnose incidence in plants and on fruits? Can we select resistant strawberry varieties that reduce pathogen

multiplication on vegetative tissues? Our main goal is to advance our understanding of host - pathogen interactions particularly host genetic variation in response to active phases of disease and deploy rate-reducing resistance in strawberry breeding program. The main objective of this project was to determine if strawberry genotypes that confer resistance to HBI by one pathogen confer equal resistance against the other.

**METHODOLOGIES:** The 18 strawberry genotypes (7 commercial varieties and 11 selections developed by NCSU) were evaluated for resistance to foliar hemibiotrophic infections (HBI), caused by *Colletotrichum acutatum* (*Ca*) and *C. gloeosporioides* (*Cg*). Plug plants were transplanted into 4-inch pots and overwintered in a  $19 \pm 5^\circ\text{C}$  greenhouse. Conidia of three isolates each of *Ca* and *Cg* were revived on PDA plates and harvested by flooding distilled water. Inoculum concentration of each isolate was adjusted to  $1 \times 10^6$  using a hemacytometer, then combining equal volumes of the three isolates of each species. Strawberry leaves were spray-inoculated with a handheld sprayer until runoff. Plants inoculated with distilled water served as controls. A total of 45 clonal plants of each genotype were arranged randomly in split split-plot design where fungal pathogen assigned to whole-plots and strawberry genotypes assigned to split-plots. Three leaves were sampled from each plot on each of seven time-points: 0, 12, 24, 48, 96, 168 (7 days), and 336 hours (14 days) after inoculation. Disease assessment consisted of two assays: a paraquat assay (induces sporulation of asymptomatic infections) and quantitative real time PCR (qPCR) assay (detects levels of fungal DNA in sample tissues) that were performed independently. Leaves showing any visible growth of salmon color or acervuli was rated as positive (incidence values; selected colonies were validated as the target pathogen) and severity of colonization was assessed using the Horsfall-Barret rating scale. Genomic DNA from freeze-dried and ground leaf tissues from each time-point were extracted using DNeasy plant mini kit (Qiagen, Inc.; Valencia, CA) and analyzed using real-time PCR based on SYBR Green assay (Garrido et al., 2009). Duplicates of each sample were analyzed and threshold cycle ( $C_T$ ) values were recorded. Quantity of fungal DNA in each sample was expressed as pg fungal DNA in 100 mg of leaf tissue used. HBI incidence and severity values were subjected to two way analysis of variance (ANOVA) using the general linear models procedure (SAS 9.2, SAS Institute Inc., Cary, NC).

**RESULTS:**



**Fig. 1.** Reactions of 18 strawberry genotypes to *Ca* and *Cg* infections.

Among 18 genotypes evaluated, NCSU selection ‘NCS 10.147’ consistently exhibited the lowest severity in percent sporulating leaf area for both *Ca* and *Cg* HBI. The commercial cultivar ‘Chandler’ ranked among the highest in percent sporulating leaf area with double the scores of ‘NCS 10-147’ (Fig. 1). Based on these data, we selected ‘NCS 10-147’ as a resistant genotype and ‘Chandler’ as a susceptible genotype, and a subsequent experiment was initiated to determine the early stages of HBI establishment by both *Ca* and *Cg* in a resistant and susceptible cultivar. Neither ‘Chandler’ nor ‘NCS 10-147’ display any significant capacity to produce secondary conidia at 7 days after inoculation. It is only at 14 days that we see a spike in the production of conidia as visualized by the paraquat assay. This may indicate a lag period of 7 to 14 days after penetration before HBI are capable of producing secondary conidia. The large difference in genotypic effect was apparent. Both ‘Chandler’ and ‘NCS 10-147’ exhibited an increase in overall sporulation at 14 days; however, this response is severely constrained in the resistant genotype. At 14 hours, the susceptible cultivar ‘Chandler’ had more than 4 times the percent sporulating leaf area of the resistant genotype ‘NCS 10-147’. This result confirmed that each genotype responded similarly to both *Ca* and *Cg* HBI. These two genotypes are being used for an experiment that utilizes RNA-seq analysis to identify candidate genes associated with resistance to *Ca* and *Cg*.

In the long run, we believe we can secure high levels of effective resistance to both *Ca* and *Cg*. Currently, most programs in the world pursue one or the other and these are independently governed by different gene sets. By combining HBI resistance to both and QTLs that affect each independently, we envision a robust genetic package for SE growers.

**References:**

- Garrido, C., Carbu, M., Fernandez-Acero, F. J., Boohman, A., Colyer, A., Cantoral., J. M., and Budge, G. 2009. Plant Pathology 58:43-51.
- Rahman, M. Ballington, J., and Louws, F.J. 2013. Annal. Appl. Biol. 163:102-113.

