Title: Monitoring of *Neopestalotiopsis* spp. on strawberry cultivars in the Southeastern United States

Final Report: Funded Research Proposal (SRSFC Funding 2025 R-01)

Name, Mailing and Email Address of Principal Investigator(s):

Principal Investigator
Dr. Alejandra Jimenez Madrid
Manager, Plant Molecular Diagnostic Laboratory
Mailing: Dept. of Plant Pathology, University of Georgia – Tifton Campus
115 Coastal Way, Tifton, GA 31794
Phone: 229-386-3372
Email: a.jimenez@uga.edu

Co-Principal Investigator
Dr. Phillip M. Brannen
Professor and Fruit Extension Plant Pathologist
Mailing: Department of Plant Pathology,
2105 Miller Plant Sciences Bldg., University of
Georgia, Athens, GA 30602
Phone: 706-542-1250
Email: pbrannen@uga.edu

Collaborators
Dr. Ed Sikora, Extension Specialist Professor. Auburn University
Dr. Guido Schnabel, Professor of Plant Pathology. Clemson University
Dr. Mark Hoffmann, Small Fruits Specialist & Associate Professor. North Carolina State University
Dr. Bill Cline, Researcher & Extension Specialist. North Carolina State University
Dr. Mary Helen Ferguson, Extension Agent – Horticulture. LSU AgCenter
Dr. Rebecca Melanson, Mississippi State University Extension Service
Dr. Aaron Cato, Extension Specialist - Horticulture IPM, University of Arkansas

Public Abstract:

Neopestalotiopsis fruit rot and leaf spot, caused by aggressive strains of N*eopestalotiopsis rosae*, have led to significant losses for strawberry growers across the southeastern US in recent years. These strains are highly aggressive on leaves and fruit, although they can also infect all plant tissues, eventually causing the plant to collapse. These novel strains have infected nursery transplants, which are believed to be the primary source of infection and spread to other regions. In 2024, a survey supported by the SRSFC was launched to determine the prevalence of an aggressive *Neopestalotiopsis* strain in the Southeast (US). Strawberry leaf and fruit samples (n=40) were received and tested at the Plant Molecular Diagnostic Lab at the UGA-Tifton campus; 55% of these tested positive for the aggressive Neopestalotiopsis strain. The survey continued into 2025 to monitor regional prevalence and to identify any emergence of new aggressive strains. During 2025, 30 samples were

received from Alabama, Arkansas, Georgia, Louisiana, and Tennessee. Overall, 70% of the samples tested positive for the aggressive strain. The strain was detected in all samples from each participating state. Additionally, 13% of samples were confirmed as a newly reported strain, referred to as the "second aggressive lineage," indicating the emergence and potential spread of new strains across the Southeast. Monitoring the species causing Neopestalotiopsis fruit rot and leaf spot has helped strawberry producers stay informed about critical considerations for the upcoming season.

Objectives:

(1) Monitor the virulent *Neopestalotiopsis rosae* on strawberry in the southeastern United States.
(2) Conduct molecular characterization of *Neopestalotiopsis rosae* strains to facilitate and enhance accurate disease diagnosis.

Introduction:

Neopestalotiopsis fruit rot and leaf spot, caused by *Neopestalotiopsis rosae* strains, was first reported to cause damage in strawberry fields in Florida in 2019 and 2020 (Baggio et al. 2021). Since then, this disease has been observed in most southeastern states (Jiménez Madrid et al. 2024; Conner 2022; Hoffmann 2020; Holland 2021; Morgan 2022) and is considered a major concern due to the severe outbreaks reported and limited management options. These strains are highly aggressive on leaves and fruit (**Fig 1**), although they can also infect all plant tissues. Monitoring the strains responsible for Neopestalotiopsis leaf spot and fruit rot is essential for understanding the disease's epidemiology and improving management strategies.

As a species, *N. rosae* has been known to cause symptoms on strawberry (Rebollar-Alviter et al. 2020), but symptoms can vary greatly, and it is highly recommended that producers send samples to a diagnostic lab or specialist for confirmation. Accurate and reliable identification of the strains associated with the disease can significantly aid in managing it effectively. Morphological features among strains cannot be used for definitive identification, since symptoms and spore types are very similar (**Fig 2**). Therefore, a few molecular tools have been developed based on DNA detection. One such molecular tool is a polymerase chain reaction/restriction fragment length polymorphism (PCR/RFLP) assay that can accurately identify the aggressive *N. rosae* strain from strawberry (Kaur et al. 2023) and determine whether nonaggressive secondary strains are present instead (**Fig 3**). A second aggressive strain has also played a role in recent outbreaks (Peres*, personal communication*); however, to date, a molecular assay for this strain has not been released, and further testing or confirmation is necessary to ensure accurate diagnosis of species/strains. Molecular detection assays can be costly, but they are the only reliable way to confirm the aggressive form of Neopestalotiopsis.

These novel strains infect nursery transplants, which are considered the main source of infection and dissemination to other regions. Based on results from 2024, including initial surveys and sample testing, monitoring continued in 2025 to further track this disease in the Southeast. Strawberry samples (leaf and fruit samples; **Fig 1**) were received and tested at the Plant Molecular Diagnostic Lab at the UGA-Tifton campus. Samples were shipped from Alabama (n=3), Arkansas (n=5), Georgia (n=12), Louisiana (n=6), and Tennessee (n=4). Of all the samples tested in 2025, 70% tested positive for the first aggressive strain using molecular assays. Additionally, 13% (strains from GA and AL) were confirmed as the second aggressive strain.

Figure 1 Symptoms and signs of strawberry samples sent to the molecular diagnostic lab at the UGA-Tifton campus in 2025

Materials and Methods:

Collection and isolation of *Neopestalotiopsis* spp. A field survey was coordinated with Extension Specialists from Alabama, Arkansas, Georgia, Louisiana, and Tennessee in 2025. Symptomatic strawberry plants from fields with significant suspect damage were collected and sent to the Plant Molecular Diagnostic Laboratory at the University of Georgia-Tifton Campus. Clients were required to submit a sample submission form along with the sample to obtain information such as disease incidence, cultivar, nursery providing the plugs or plants, and sample location. Symptomatic plants were carefully examined to document symptoms and/or signs (e.g., dark fruiting bodies, mycelium). Fungal isolations were conducted using standard isolation methods on potato dextrose agar amended with chloramphenicol (75 ppm cPDA). Isolates were incubated at 25°C for 5-6 days. Morphological differences between isolates were noted.

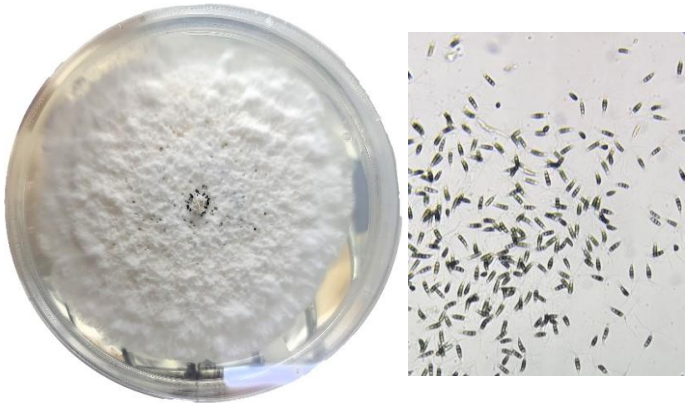

Figure 2. A. Colony and conidial morphology of *Neopestalotiopsis* sp.

Molecular characterization of *Neopestalotiopsis* species. Colony morphology was characterized on cPDA. Features such as culture color, growth pattern, texture, as well as conidia (spore) morphology, including shape, color, and size, were documented for each fungal isolate. Genomic DNA extraction from pure fungal cultures was performed with fungal DNA extraction kits according to the manufacturer's instructions. The molecular identification of *Neopestalotiopsis* spp. was conducted following a polymerase chain reaction/restriction fragment length polymorphism (PCR/RFLP) method as described by Kaur et al. 2023 (**Fig 3**). A previously confirmed agressive *Neopestalotiopsis* sp. isolate (AJ07-2023) and a non-aggressive *Neopestalotiopsis rosae* isolate from Georgia were used as positive controls for the molecular assay (Jimenez Madrid et al. 2024).

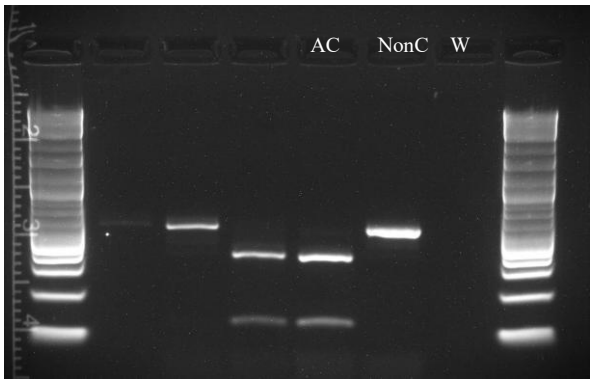

Figure 3. Gel electrophoresis of the β-tubulin gene amplified with primers Bt2a and Bt2b and digested with restriction enzyme BsaWI. Lines 1-2 are positive for the non-aggressive strain. Line 3- is positive for the aggressive strain. AC: aggressive control.

Results

Collection and isolation of Neopestalotiopsis isolates. In total, 30 strawberry samples from Alabama (n=3), Arkansas (n=5), Georgia (n=12), Louisiana (n=6), and Tennessee (n=4) were processed. Most of the samples exhibited clear characteristic symptoms and signs on leaves and/or fruit (**Fig 1**). Crown and root discoloration were also observed on severely infected samples, and reports of plant death in the field were generally provided by the clients who submitted the samples. Disease incidence ranged from 1% to 50%. Foliar symptoms differed according to cultivar, but generally included: irregularly distributed, different-sized spots (dark brown with light brown centers), and dark brown V-shaped necrotic areas starting at the leaf edge. Red discoloration was observed in the crown, and necrotic roots were also noted. Signs of the pathogen (mycelium, acervuli) were observed in severely impacted leaf and fruit samples. Samples were received from January to October 2025, mostly from GA. Fungal isolation was conducted from either leaves, fruit, crowns, and/or roots. For most samples, at least two fungal isolates were recovered and tested using a molecular assay. A total of 94 pure cultures were recovered from all samples (**Table 1**).

Morphological characterization of *Neopestalotiopsis* species. No major differences were observed in colony morphology between the aggressive forms of *Neopestalotiopsis* and the non-aggressive strains. Most isolates exhibited a white/cottony growth with brownish coloration in the center of the plate and black acervuli forming after 8-10 days of incubation at 25C (**Fig 4**). This observation confirms that morphological characteristics cannot be used for definitive identification, since spore types and growth patterns are very similar among *Neopestalotiopsis* species.

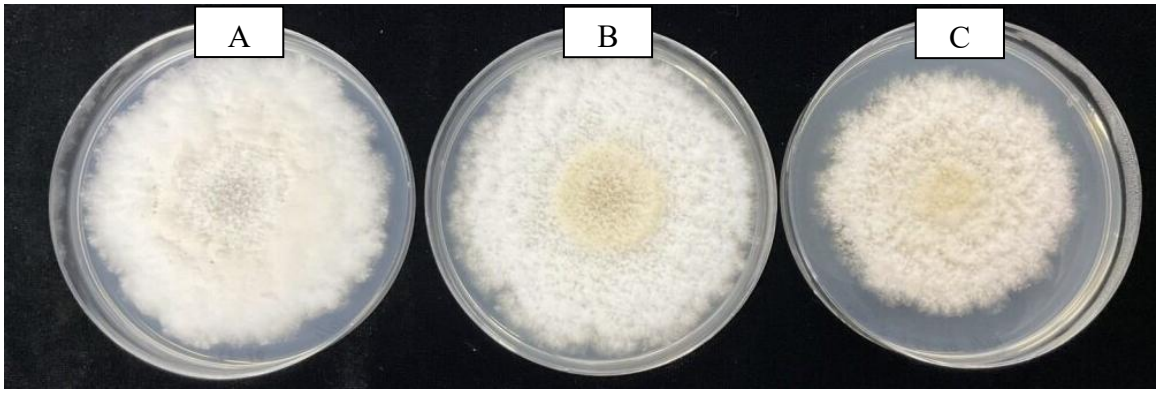

Figure 4. Colony morphology on cPDA of the aggressive strain (A) and non-aggressive strain(B) and second aggressive strain (C) of Neopestalotiopsis after 8 days of incubation at 25C.

Molecular characterization of *Neopestalotiopsis* strains. From all samples, a total of 94 pure cultures were recovered and tested using the polymerase chain reaction/restriction fragment length polymorphism (PCR/RFLP) method. Amplified DNA was digested with the restriction enzyme BsaWI. After gel electrophoresis (2%), if two bands were clearly visualized (~130 bp and ~290 bp), that indicated the samples were positive for *Neopestalotiopsis* aggressive strain 1 (**Fig 3**). From the 94 isolates recovered, 55 were confirmed as the first aggressive strain, and 22 were confirmed as the non-aggressive strain. In addition, 17 were isolated from four samples with severe foliar symptoms. From those, 4 isolates (one from each sample) were confirmed as positive for the second aggressive strain. This confirmation was conducted at the UF, Peres Lab.

Discussion:

The results from these ongoing survey and characterization efforts provided immediate evidence of the prevalence and distribution of *N. rosae* strains causing infections on strawberry plants in the southeastern United States. The aggressive form of Neopestalotiopsis was detected in 70% of the samples tested in 2025 and from every state participating in this effort. The aggressive strain was identified in plants from the field, with incidence rates ranging from 1% to 50% by the time symptoms appeared. Molecular detection tools have facilitated accurate diagnosis for this particular pathogen. However, a new observation of a second lineage (second aggressive strain) in FL (also confirmed in GA and AL) may be contributing to the recent outbreaks reported throughout the Southeast. This second aggressive strain was detected in 13% of samples processed in 2025. Current diagnostic capabilities cannot detect the second lineage of the aggressive strain, so caution is advised when a test result is negative. Testing for the second aggressive strain is being supported on a limited basis by UF, Gulf Coast Research and Education Center, Plant Diagnostic Clinic.

Performing an accurate diagnosis is essential for implementing suitable management strategies and preventing further spread or establishment of the aggressive *N.*

rosae strains within a region. Molecular detection tools are reliable methods for identifying plant pathogens quickly and can help strawberry growers avoid production losses.

Table 1. Sample origin, nursery reported, cultivar and species found for each strawberry sample submitted to the MDL in 2025

Sample Origin (State)	Nursery Reported	Cultivar	Species Found	Total # isolates tested	Total # Aggressive strains
AL	--	Camarosa	Second aggressive	4	1
AL	A	Fronteras	Aggressive strain	4	4
AL	--	--	Aggressive strain	5	5
AR	--	Fronteras	Aggressive strain	2	2
AR	--	SB	Aggressive strain	3	1
AR	--	Sensation	Non-aggressive	3	0
AR	--	X08	Aggressive strain	3	1
AR	--	Ruby June	Aggressive strain	3	3
GA	B	--	Second aggressive	4	1
GA	C	Camerosa	Second aggressive	6	1
GA	--	--	Non-aggressive	1	0
GA	D	--	Aggressive strain	3	3
GA	B	Camerosa	Non-aggressive	4	0
GA	--	Camerosa	Non-aggressive	3	0
GA	--	Fronteras	Aggressive strain	4	4
GA	E	Fronteras	Aggressive strain	6	6
GA	--	Camino Real	Aggressive strain	4	4
GA	--	unknown	Second aggressive	3	1
GA	--	Fronteras	Aggressive strain	1	1
GA	--	Albion	Aggressive strain	1	1
LA	F	Camino Real	Aggressive strain	4	4
LA	G	Brilliance	Aggressive strain	5	5
LA	F	Camino Real	Aggressive strain	3	3
LA	--	Fronteras	Aggressive strain	5	2
LA	H	San Andres	Aggressive strain	3	2
LA	I	Camino Real	Non-aggressive	3	
TN	--	Ruby June or Chandler	Aggressive strain	1	1
TN	--	Ruby June or Chandler	Aggressive strain	1	1
TN	--	Ruby June or Chandler	Aggressive strain	1	1

TN	--	Ruby June or Chandler	Aggressive strain	1	1

Note: nurseries names are not disclosed due to confidentiality. --not reported

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