Understanding the biology and epidemiology of *Neopestalotiopsis* spp. (*Pestalotiopsis* spp.)

Juliana S. Baggio, Nan-Yi Wang, Marcus V. Marin, James C. Mertely, and Natalia A. Peres

Summary

Recently, Florida strawberries were severely affected by a disease caused by a new species of *Neopestalotiopsis*, which seems to be more aggressive than the old population of this fungus. An isolate recovered from rhododendron was able to infect strawberry and was as aggressive on leaves and fruit as the new *Neopestalotiopsis* strawberry isolates. Isolates from other hosts and the old population from strawberry were not as aggressive, nor did they cause any disease. All commercial Florida strawberry cultivars were susceptible to the disease, but 'Florida Beauty' was the most susceptible. The fungicides Switch® and Thiram® showed the best results for supressing disease.

Methods

Molecular characterization of *Neopestalotiopsis* isolates from strawberry

Sixty-seven strawberry isolates collected between 1997 and 2020 and stored in our culture collection at UF-GCREC were used for molecular characterization. DNA was extracted and three small genetic regions (ITS, *β-tub* and *tef1*) were amplified using polymerase chain reaction (PCR) and sequenced. Afterward, these isolates were compared with strawberry isolates from other strawberry-producing areas, isolates from alternative hosts, and other *Pestalotiopsis* and *Neopestalotiopsis* species from the NCBI GenBank and literature.

Characterization of cultures of *Neopestalotiopsis* and *Pestalotiopsis* isolates from alternative hosts

Neopestalotiopsis and *Pestalotiopsis* isolates from alternative hosts (statice, oak, smilax, mountain laurel, rhododendron, blackberry, and pomegranate) were cultured on potato-dextrose-agar (PDA) and colony and spore characteristics were evaluated. The same isolates were incubated at 10, 15, 20, 25, 30, and 35°C for seven days. Colony diameters (isolate growth) were measured and spore production was quantified.

Pathogenicity and aggressivenss of *Neopestalotiopsis* and *Pestalotiopsis* isolates from alternative hosts and strawberry

a) Fruit inoculation: Isolates recovered from statice, oak, smilax, mountain laurel, rhododendron and strawberry (new population, recent outbreaks) were inoculated on green Sensation® 'Florida127' strawberry fruit. Six fruit per isolate were inoculated with 40 µl of a spore suspension (1 x 10^4 spores/ml) and incubated at 23°C (73°F) in sealed humid chambers. Water was used in the control treatment. Three replicates per isolates was used and disease incidence was assessed 7 days after inoculation.

b) Field trials: Isolates recovered from statice, oak, smilax, mountain laurel, rhododendron, blackberry, and pomegranate were inoculated (1 x 10^4 spores/ml) on Sensation® 'Florida127' strawberry plants in field trials. In addition, three strawberry isolates collected prior (old population) and after (new population) the recent outbreaks were used and four spore concentrations (1 x 10^3, 5 x 10^3, 1 x 10^4, and 1 x 10^5 spores/ml) were tested. Water was used as control treatment. Plants were arranged in plots (6 plants/plot) and four plots (replications) per isolate were used. Experiments were performed twice. Foliar and fruit disease incidences were evaluated throughout the season.

Evaluation of cultivars for disease resistance
Six strawberry cultivars commercialy grown in Florida were assessed for disease resistance. Plants were inoculated by spraying a mixed spore suspension (5 x 10^4 spores/ml) of *Neopestalotiopsis* isolates collected during the outbreaks (new population). Plants were arranged in plots (12 plants/plot) and four plots (replications) per cultivar were used. Foliar and fruit disease incidences were evaluated throughout the season.

Efficacy of fungicides in controlling *Neopestalotiopsis*
Strawberry plants arranged in plots (12 plants/plot) were inoculated by spraying a mixed spore suspension (5 x 10^4 spores/ml) of *Neopestalotiopsis* isolates collected during the outbreaks (new population). Fungicides were sprayed weekly over the plants according to label rates and four plots (replications) per treatment were used. The inoculated control was sprayed with the pathogen suspension but did not receive any fungicide treatment. Fungicides were selected based on preliminary screening tests in the laboratory and a previous field trial (2018-19 season). Two experiments were conducted: one started in October 2019 ('Florida Beauty' plants) and the other in February 2020 (Sensation® 'Florida127').

Results

Molecular characterization of *Neopestalotiopsis* isolates from strawberry
Isolates reported in the past as *Pestalotiopsis longisetula*, including the isolates recovered in Florida before the outbreaks, should be identified as *Neopestalotiopsis rosae.* These isolates are most commonly found causing symptoms on roots and crowns during plant establishment. Our studies indicate that isolates from the recent outbreaks (last three seasons) are more aggressive and may belong to a new *Neopestalotiopsis* species. However, we do not know yet the species name and are referring to isolates as *Neopestalotiopsis* sp. (Fig. 1).

Characterization of cultures of *Neopestalotiopsis* and *Pestalotiopsis* isolates from alternative hosts
Fungal colony morphology and color varied among isolates regardless of the host. Spores were five-celled with three median colored cells. However, strawberry isolates and most isolates from alternative hosts had spores with two upper median cells darker than the lowest median cell, and they were classified as *Neopestalotiopsis* (Fig. 2A). The mountain laurel isolate produced spores with equally colored three median cells and it was classified as *Pestalotiopsis* (Fig. 2B). Optimum temperatures for mycelial growth and sporulation were 25 and 30°C, respectively, for isolates from alternative hosts and strawberry.

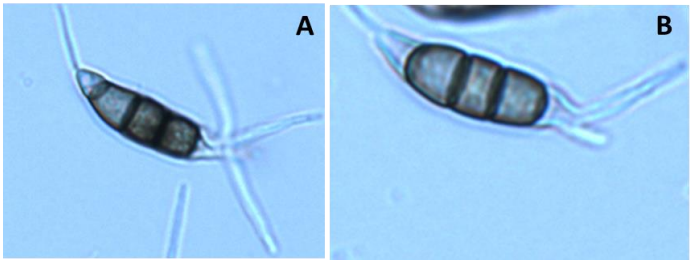

Figure 2. Spores of a *Neopestalotiopsis* isolate recovered from rhododendron (A) and *Pestalotiopsis* isolate recovered from mountain laurel (B).

Pathogenicity and aggressivenss of *Neopestalotiopsis* and *Pestalotiopsis* isolates from alternative hosts and strawberry
a) Fruit inoculation: All tested isolates from alternative hosts were able to produce symptoms on inoculated fruit. However, disease incidence was higher in fruit inoculated with the strawberry isolate from the recent outbreak (new population) than fruit inoculated with isolates from other hosts.

b) Field trials: Only strawberry plants inoculated with an isolate originating from rhododendron sampled around a strawberry nursery field associated with the outbreak showed leaf and fruit incidence levels similar to those caused by strawberry isolates from recent outbreaks (new population). Incidence on plants inoculated with past strawberry isolates (old population) and isolates from all other hosts were low and did not differ from the non-inoculated control (Fig. 3).

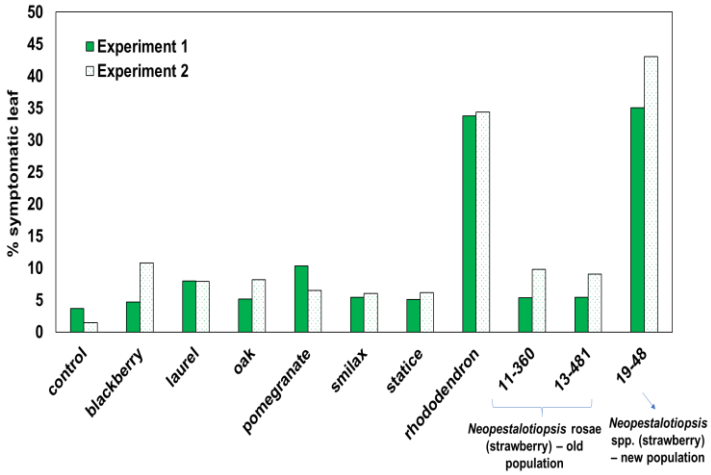

Figure 3. Disease incidence (%) on leaves of strawberry plants inoculated with *Neopestalotiopsis* and *Pestalotiopsis* isolates originating from alternative hosts and strawberry isolates from the old and new *Neopestalotiopsis* species.

Evaluation of cultivars for disease resistance

All the cultivars evaluated were susceptible to the disease. During the early season, 'Florida Beauty' and 'Florida Brilliance' were significantly more affected than Sensation® 'Florida127'. However, over the entire season, 'Florida Beauty' was the most affected. 'Florida Radiance' and Sensation® 'Florida127' showed intermediate susceptibility to the disease. 'Florida Brilliance' and the older cultivars Strawberry Festival and Winterstar™ were less susceptible (Fig. 4).

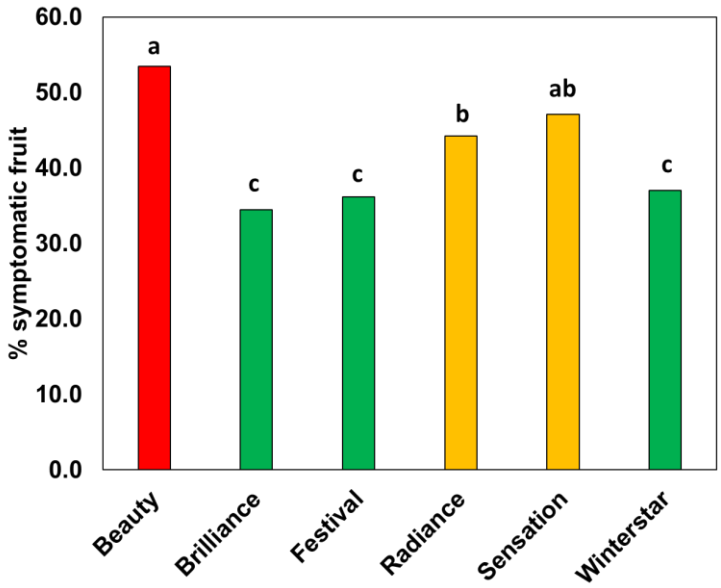

Figure 4. Disease incidence (%) on fruit of different strawberry cultivars inoculated with the new *Neopestalotiopsis* sp.

Efficacy of fungicides in controlling *Neopestalotiopsis*

The fungicides **Switch 62.5 WG** (fludioxonil + cyprodinil) and **Thiram SC** (thiram) significantly reduced disease incidence in both fungicide trials (Tables 1 and 2, Fig. 5). In the first trial, Bravo (chlorothalonil), Omega (fluazinam), and Miravis Prime (fludioxonil + pydiflumetofen) also significantly reduced disease compared to the non-treated inoculated control. Please note that the letters by the numbers for each treatment indicate whether treatments were significantly different according to the statistical analysis. In the case of the first trial, treatments that do not have an 'a' or a 'b' were statistically better than the non-treated control, which had an 'ab'. Bravo and Omega are not registered for strawberry production fields, but these could be good options for nurseries (Omega is in the process of registration for nursery use). In the second trial, in addition to Switch and Thiram, Manzate Pro-Stick (mancozeb) also decreased disease incidence (Table 2). However, mancozeb is not registered for strawberry (neither fruit nor nursery). Unfortunately, our industry already relies greatly on Switch for control of Botrytis fruit rot (BFR). The overuse of this product can lead to increased selection for fungicide resistance; thus, applications need to be limited to the maximum recommended according to the label, and we need to continue seeking alternatives.

Figure 5. Fungicide efficacy trials at GCREC: strawberry plants inoculated with *Neopestalotiopsis*: non-treated strawberry plants (A) and plants treated with Switch (B).

Disclaimer

The use of trade names in this publication is solely for the purpose of providing specific information. UF/IFAS does not guarantee or warranty the products named, and reference to them in this publication does not signify our approval to the exclusion of other products of suitable composition.

Contact

Dr. Natalia A. Peres
UF/IFAS Gulf Coast Research and Education Center
P: 813.419.6602
E: nperes@ufl.edu

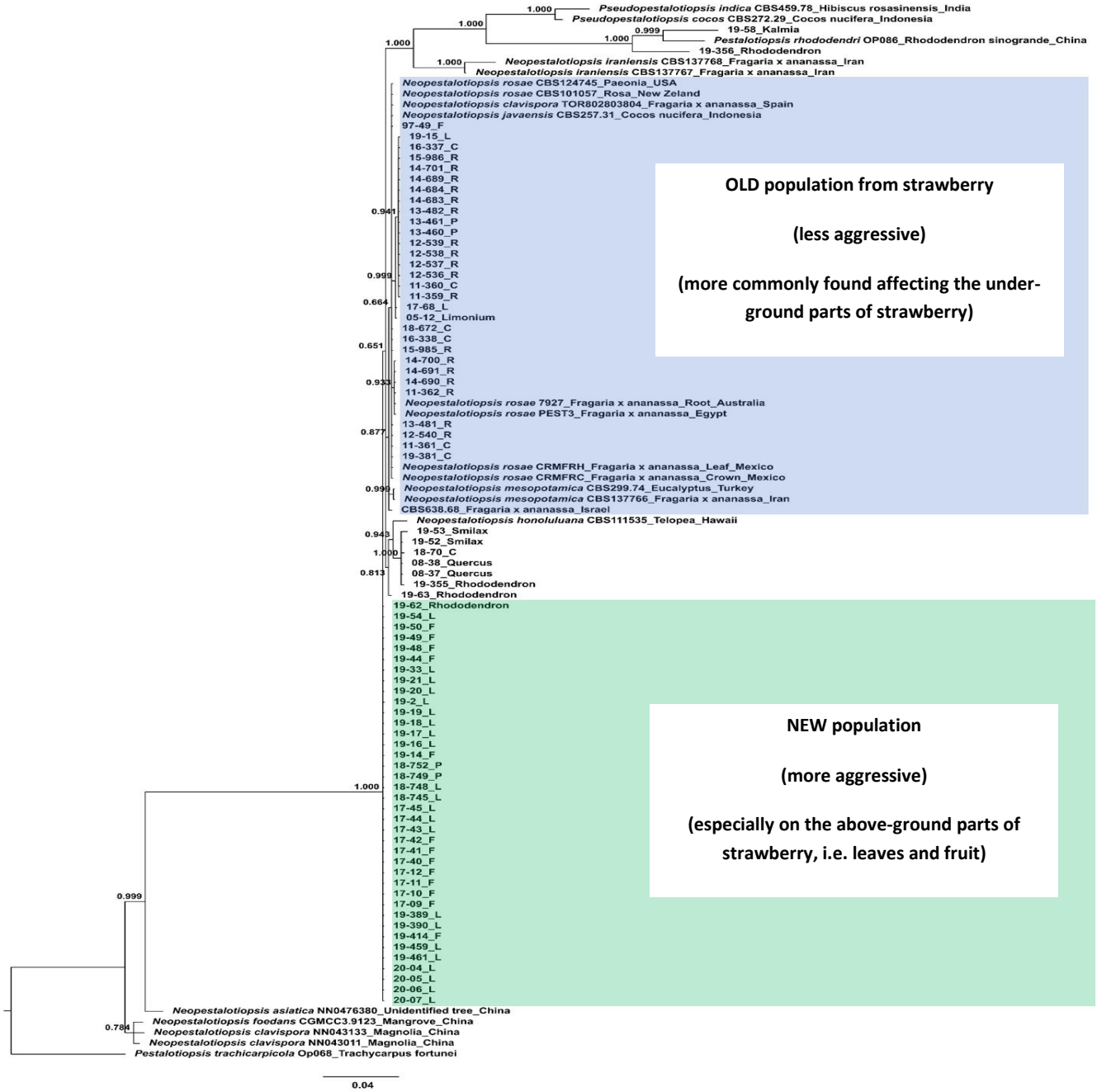

Figure 1. Phylogenetic tree of the old (*Neopestalotiopsis rosae*) and the new *Neopestalotiopsis* sp. isolates of strawberry.

Table 1. Effect of different fungicides on disease incidence (% symptomatic fruit) caused by the new *Neopestalotiopsis* sp. (**Field trial 1**).

Treatment	Fruit rot incidence (%)	
Switch 62.5WG (14 oz)	9.5	de
Thiram SC (2.6 qt)	13.0	cde
Bravo Weather Stik (1.5 pt)	13.8	cd
Omega 500F (20 fl oz)	14.7	cd
Miravis Prime SC (13.4 fl oz)	14.7	cd
Rhyme (7 fl oz)	16.4	bc
Captan Gold 4L (3 qt)	17.9	abc
Mettle 125ME (5 fl oz)	18.4	abc
Oso (=Tavano) (13 fl oz)	20.5	abc
Protexio SC (19 fl oz)	24.2	ab
Abound Flowable (15.5 fl oz)	25.8	ab
Control, inoculated	25.9	ab
Oxidate 2.0 (1% max = 1 gal)	26.0	ab
Topsin 4.5FL (20 fl oz)	26.7	a
Pr > F	<0.0001	

Table 2. Effect of different fungicides on disease incidence (% symptomatic fruit) caused by the new *Neopestalotiopsis* sp. (**Field trial 2**).

Treatment	Fruit rot incidence (%)	
Thiram SC (2.6 qt) + NuFilm P (12 fl oz)	13.8	gf
Switch 62.5WG (14 oz)	16.0	efg
Thiram SC (2.6 qt)	16.7	defg
Manzate Pro-Stick (2 lb)	17.3	cdefg
Inspire (7 fl oz)	18.6	bcdef
Aprovia (10.5 fl oz)	20.1	abcde
Thymox (0.5% = 2 qt)	20.8	abcde
Cueva 1.5 gal)	22.1	abcde
Suffa (1 gal)	22.9	abcd
Tilt (4 fl oz)	23.0	abcd
Rovral 4F (2 pt)	23.0	abcd
Quadris Top (14 fl oz)	23.1	abcd
Uptake (12.5 fl oz)	23.1	abc
Control, inoculated	23.8	ab
Procure 480SC (8 fl oz)	24.1	ab
Actigard 50WG (0.5 oz)	24.4	ab
Rhyme (7 fl oz)	24.5	ab
Inspire Super (20 fl oz)	26.2	a
Pr > F	0.0002	