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Understanding the biology, survival, and dissemination of *Neopestalotiopsis* spp. to improve disease control

Adrian I. Zuniga, Andre B. Gama, Juliana S. Baggio, Galvin Alonzo Ortiz, and Natalia A. Peres

Summary

The new *Neopestalotiopsis* species has caused significant yield losses in Florida fields since 2017. The primary source of inoculum has been identified as infected strawberry transplants, but the survivability of the pathogen in Florida was unknown. We found that *Neopestalotiopsis* sp. survived in strawberry leaf debris for 6 months, up until total leaf decomposition. Survival in strawberry crowns was observed for up to 16 months, with inoculum being reduced from 5873.3 to 3.3 colony forming units (CFU) per gram. In the soil, *Neopestalotiopsis* was recovered from non-treated row middles after pre-plant soil fumigation. Our results show that *Neopestalotiopsis* sp. can survive in soil and strawberry crowns and may represent a source of inoculum for the subsequent season. Furthermore, we found that spores from infected fruit and leaves as well as soil can spread over 3 ft with simulated rain + wind. However, spread with wind only was not significant. These results confirm observations from disease spread within commercial fields, in particular after severe storms such as the ETA tropical storm in the 2020-21 season.

Methods

Objective 1. To evaluate the survival of *Neopestalotiopsis* spp. populations in the soil and crop debris over summer in strawberry production fields using different over-summer strategies.

Survival in plant debris. A mixture of three *Neopestalotiopsis* sp. isolates at 1 x 10^5 spores/ml was used to inoculate established strawberry plants in the field. Approximately three months later, the severely affected plants were collected and placed inside hand-made meshed bags and exposed to the environment. Each bag contained 4 plants and 4 bags were collected monthly for up to 16 months. After collection, leaves, and crowns were processed separately.

Survival in the soil. Soil was collected from one or two locations in six commercial fields in Wimauma and Plant City, FL after the 2020-21 season. The sampling sites were surveyed at the end of the season (May), before (July), and after (September) pre-plant soil fumigation for the following season. Soil samples were collected arbitrarily 10 cm deep from the soil or bed surface. Four soil samples composed of three subsamples were collected for each sampling site.

One gram of each sample type (leaves, crowns, or soil) was processed using a semi-selective medium developed for this study to recover *Neopestalotiopsis* spp.. Five days after incubation, colonies were quantified and *Neopestalotiopsis* sp. populations were calculated as colony-forming units (CFU) per gram of sample.

Objective 2. To assess the impact of different inoculum loads of Neopestalotiopsis spp. in the soil on pathogen survival and disease development.

In this lab/greenhouse trial, plastic trays were filled with about 7 lb of pasteurized soil (180 F, two cycles of 8 hours each), and soil was inoculated with a spore suspension of *Neopestalotiopsis* isolates at 1 x 10^2, 1 x 10^3, 1 x 10^4 spores/ml or with infected crowns. The soil was homogenized and each tray received 200 ml of DI water, the spore suspension, or 6 crowns. Afterward, the soil was sampled once a month and processed with the semi-selective medium described for the previous experiment.

Objective 3. To determine the influence of wind, water, and manual harvest on the spread of *Neopestalotiopsis* spp.

Controlled trial. Short-distance dispersal by wind and water was assessed by using a wind/rain tunnel built by our group. Strawberry leaves (green and dried) and fruit showing typical leaf spots and fruit rot caused by *Neopestalotiopsis* sp. and sandy soil inoculated with a spore suspension calibrated to 5 x 10^4 conidia per ml were placed in the wind tunnel, with semi-selective medium plates arranged at about 2 ft, 3.3, 10 ft, 16 ft, and 23 ft from the inoculum source. Winds of 11 and 15 mph were applied for fifteen minutes over the inoculum sources (strawberry fruit, strawberry leaves, dried strawberry leaves, and infested soil). The two wind speeds were applied with or without rainfall. Briefly, the following treatments were applied: 11 mph, 11 mph + rain, 15 mph, and 15 mph + rain. Each inoculum source was exposed to all treatments and experiments were conducted twice. After treatments were applied, plates were sealed and incubated for five days under constant light and at 25 °C. The number of *Neopestalotiopsis* sp. colony-forming units was counted after that period and data were adjusted to negative exponential and power law models to describe the dispersal gradient.

Field trials. Short- and long-distance dispersal by water, wind, and human hands were evaluated under wet and dry conditions, simulating harvest operations performed when plants are wet (in the morning from dew, after rain, or after overhead irrigation) or dry, to determine whether the pathogen spread is influenced by wetness and manual harvest. Strawberry plants (Sensation FL127) were transplanted into marked plots (20 plants per plot). After establishment, the plot located at one end of the bed was inoculated with a mixed spore suspension of *Neopestalotiopsis* spp. (5×10^4 spores/ml). Fruit were harvested unidirectionally from the inoculated plots to the remaining plots in the beds. Fruit were counted and evaluated for disease symptoms twice per week in each plot, and disease incidence was expressed as the percentage of symptomatic fruit based on the total fruit. Disease symptoms on leaves were evaluated throughout the season.

In the same field trial, an automated pollen and spore counting device provided by Pollen Sense was installed to validate a machine learning model that could possibly identify *Neopestalotiopsis* sp. conidia in the air. We worked with the company in analyzing the images collected by the device for the identification of *Neopestalotiopsis* spores.

Results

Objective 1

On leaf debris, *Neopestalotiopsis* sp. survived from February until August 2021 with CFU decreasing from >6000 to 2285. After August, the leaves were totally decomposed and thus *Neopestalotiopsis* sp. could not be recovered. On crowns, CFU declined from 5873.3 to 3.3 from February 2021 to June 2022 (Table 1). Unfortunately, we used up all the samples after 16 months so we could not determine how long it would have taken to reach zero. On soil from the commercial farms, CFU ranged from 48.3 to 2410.8 at the end of the season, 1.7 to 630.8 before soil fumigation, and was mostly zero after soil fumigation. However, 1.7 to 25 CFU were recovered from the non-treated areas in the row middles of all farms sampled (Table 2). From the colonies recovered, the majority was confirmed as the new *Neopestalotiopsis* sp. by our HRM protocol (Figure 1).

Table 1. *Neopestalotiopsis* sp. survival on strawberry plant debris after exposure to the environment.

Year	Month	*Neopestalotiopsis* sp. CFU/g	
		Leaves*	Crowns
2021	February	> 6000.0	5873.3
	March	> 6000.0	5685.0
	April	> 6000.0	5703.3
	May	> 6000.0	5885.0
	June	> 6000.0	3120.0
	July	3746.7	640.0
	August	2285.0	585.0
	September	-	295.0
	October	-	100.0
	November	-	41.7
	December	-	15.0
2022	January	-	8.3
	February	-	5.0
	March	-	3.3
	April	-	3.3
	May	-	1.7
	June	-	3.3

* Leaves totally decomposed in September 2021.

Table 2. *Neopestalotiopsis* spp. over-summering survival in commercial strawberry farms.

Farm[y]	SS[z]	*Neopestalotiopsis* spp. CFU/g soil			
		End of season	Before soil fumigation	After soil fumigation	
				Row middle	Bed
F- 1	A	75.0	15.0	2.5	0.0
F- 2	A	555.0	52.5	25.0	0.0
F- 3	A	547.5	3.3	5.0	0.0
F- 4	A	459.2	125.0	21.7	0.0
	B	48.3	4.2	2.5	0.0
F- 5	A	90.0	2.5	1.7	0.0
	B	1962.5	1.7	2.5	0.0
F- 6	A	603.3	4.2	3.3	0.0
	B	2410.8	630.8	18.3	0.0

[y] Commercial farms.
[z] SS= sampling site

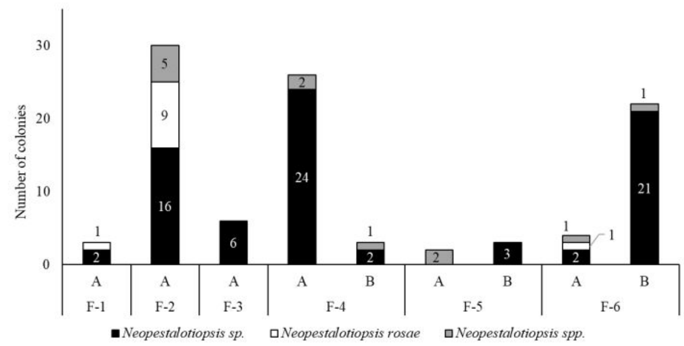

Figure 1. Number of colonies of *Neopestalotiopsis* species recovered from non-treated areas after preplant soil fumigation on 6 commercial farms (F1-F6). ***Neopestalotiopsis sp.***: new strawberry species; ***Neopestalotiopsis rosae***: former *Pestalotia longisetula*, not as aggressive; ***Neopestalotiopsis spp***: other non-identified *Neopestalotiopsis* species.

Objective 2

Unfortunately, this experiment was lost due to an irrigation problem in the greenhouse that flooded the trays. We are currently repeating the experiment and plan to report in the next cycle.

Objective 3

In the experiment conducted under controlled conditions in the wind tunnel, more CFU were observed within 3.3 ft of the source for all inoculum sources, although 3.3 ft was statistically comparable to 10 and 16 ft for strawberry fruit and infested soil (Table 3). Although some CFU were randomly found in the treatments that did not receive rainfall, the numbers were so small that it was not possible to fit any statistical models. For wind combined with rain, models adequately described dispersal gradients. In general, there was more inoculum being moved from strawberry fruit than from strawberry leaves, and conidia for the strawberry fruit exposed to 15 mph + rain moved farther than with winds of 11 mph + rain. The same was observed for strawberry leaves, although CFU numbers were lower. Based on our results, *Neopestalotiopsis* sp. conidia are mostly spread within 3 ft of the inoculum source at wind speeds up to 15 mph, which was the highest wind speed tested. In the field, this could mean that wind speeds higher than 15 mph could spread spores even farther. During the ETA tropical storm in 2020, winds gusts of 55-60 mph were reported, which could have spread spores much farther within strawberry fields.

In fact, during the 2020-21 strawberry season, the tropical storm ETA affected our experimental area in Wimauma. In our dissemination trial, *Neopestalotiopsis* symptoms were observed in almost every plant in the trial where only a few 20-plant plots had been inoculated. Therefore, the experiment was repeated during the 2021-22 season. In plots harvested when plants were wet, disease incidence on fruit was higher in the inoculated plots (22.6 to 30.4%) than in the non-inoculated plots (6.0 to 10.32%). Similar results were observed in plots harvested when plants were dry for inoculated plots (19.8 to 22.9%) and non-inoculated plots (3.1 to 10.3%). Dissemination of the pathogen unidirectionally from the inoculated first plot was not observed regardless of the wetness conditions of the plants at harvest. This might be due to inoculum spread through wind or rain from the inoculated plots within the trial or adjacent areas in the field. Thus, further investigation is needed under controlled conditions.

Table 3. Average number of CFU at each distance considering data from all treatments applied in the wind tunnel experiments for each inoculum source (11 mph, 11 mph + rain, 15 mph, and 15 mph + rain. Means followed by the same letter within the same inoculum source (strawberry fruit, strawberry leaves, dry strawberry leaves, and inoculated sandy soil) do not differ statistically according to results from the Tukey-Kramer post hoc test at alpha=0.05.

Distance from source (ft)	Strawberry Fruit		Strawberry Leaves		Strawberry Dry Leaves		Inoculated Sandy S	
	Avg CFU	*P*<0.01	Avg CFU	*P*<0.01	Avg CFU	*P*<0.01	Avg CFU	*P*<0.01
2	218.9	a	73.6	a	113.7	a	10.8	a
3.3	74.1	ab	17.9	b	61.2	ab	3.9	ab
10	3.8	bc	0.4	b	1.5	b	0.4	bc
16	1.6	bc	0.2	b	0.9	b	0.2	bc
23	1.0	c	0.2	b	0.6	b	0.4	c

[Y]Average number of *Neopestalotiopsis* sp. colony-forming units found at each distance.
[Z]Standard error of the mean.

Contact

Dr. Natalia A. Peres
UF/IFAS Gulf Coast Research and Education Center
P: 813. 419-6602
E-mail: nperes@ufl.edu