

Determining the Genetic Variation within *Neopestalotiopsis* Populations in Florida and Mexico

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Summary

Comparative whole genome sequencing analysis revealed distinct genetic groupings among *Neopestalotiopsis* isolates associated with strawberry disease outbreaks in the U.S. and Mexico. Isolates from both groups were equally aggressive on 'Florida Brilliance' (highly susceptible) and 'Felicity' (moderately susceptible). Further studies are needed to investigate whether the pathogen's genetic variability would interfere with the breeding of cultivar resistant to *Neopestalotiopsis* sp.

Methods

Objective 1: To study the genetic variation within *Neopestalotiopsis* and determine whether the populations from Florida and Mexico are distinct.

We conducted a comparative whole genome sequencing (WGS) analysis on 49 *Neopestalotiopsis* isolates, comprising isolates recovered from strawberry in Florida and other U.S. states and Mexico. The isolates used for the WGS were cultivated on culture media and subjected to DNA extraction. To enhance genetic comparisons at the species level, we included in the phylogenetic analysis the ex-type species of *Neopestalotiopsis*, which were acquired from a mycology bank along with the strawberry isolates from different geographic regions.

Objective 2: To compare the virulence of *Neopestalotiopsis* isolates from Florida and Mexico

For this study, we chose one isolate representing the new *Neopestalotiopsis* sp. (21-264) and another isolate from Florida (21-266) that clustered with the Mexican isolates in the phylogenetic tree (Figure 1).

Five plants of 'Florida Brilliance' and 'Felicity' were inoculated in a growth room using spore suspensions prepared separately for each isolate. Subsequently, plants were evaluated weekly for three weeks for incidence (%) and severity of leaf spots on a scale ranging from 0 (healthy) to 6 (dead). Non-inoculated plants were included as controls. Five plants per cultivar x isolate combination and four repetitions were used in a completely randomized design, and data were subjected to statistical analysis.

Results

Objective 1

The comparative whole genome sequencing (WGS) analysis revealed distinct genetic groupings among *Neopestalotiopsis* isolates associated with strawberry disease outbreaks in the U.S. and Mexico. Specifically, out of the 11 isolates recovered from severe outbreaks in Florida, ten were grouped as the new *Neopestalotiopsis* sp. On the phylogenetic tree, isolates from Mexico formed a separate clade, while two non-aggressive isolates and one aggressive isolate from Florida clustered with these. The spread-out distribution of the non-aggressive isolates on the tree indicates that they were likely isolated as secondary pathogens rather than the primary causal agent of the disease on strawberry (see Appendix). These results suggest that the most aggressive isolates responsible for disease outbreaks on strawberry in Florida and the U.S. belong to the new *Neopestalotiopsis* sp., as reported in 2017. However, genetically similar isolates to those from Mexico were also identified in Florida.

Objective 2

Disease incidence caused by isolates 21-264 (FL group) and 21-266 (Mexico group) reached 100% two days after inoculation in both 'Florida Brilliance' and 'Felicity', and most plants died after 21 days. No significant difference in disease severity caused by each isolate was observed on either cultivar at any evaluated time point (Figure 2). Both isolates were equally aggressive on 'Florida Brilliance' and 'Felicity'. Further studies are needed to include more resistant cultivars or breeding lines to determine whether the genetic variability of these groups of isolates would interfere with the development of durable cultivar resistance against *Neopestalotiopsis* sp. of strawberry.

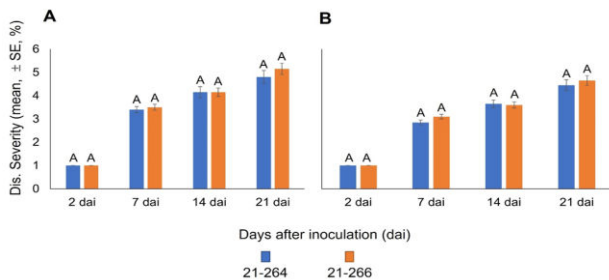


Figure 2. Disease severity of *Neopestalotiopsis* isolates on 'Florida Brilliance' (A) and 'Felicity' (B) was evaluated over several days after inoculation (dai) for isolates 21-264 (blue; FL group) and 21-266 (orange; Mexico group). Severity ratings were assigned based on a leaf spot rating scale (0-6). Columns with the same letter are not significantly different, as determined by Dunn's test.

Takeaways

The main finding of this study is that comparative whole genome sequencing (WGS) revealed distinct genetic groupings of *Neopestalotiopsis* isolates associated with strawberry disease outbreaks in the U.S. and Mexico. Notably, both groups of isolates were found in Florida and both were equally aggressive on susceptible strawberry cultivars. Testing a diverse range of strains on breeding lines with resistance to the new *Neopestalotiopsis* sp. is crucial to develop cultivars with durable resistance across different *Neopestalotiopsis* species groups. Furthermore, ongoing studies monitoring these populations in Florida are still needed to understand how their genetic variability may affect the biology and epidemiology of the pathogen.

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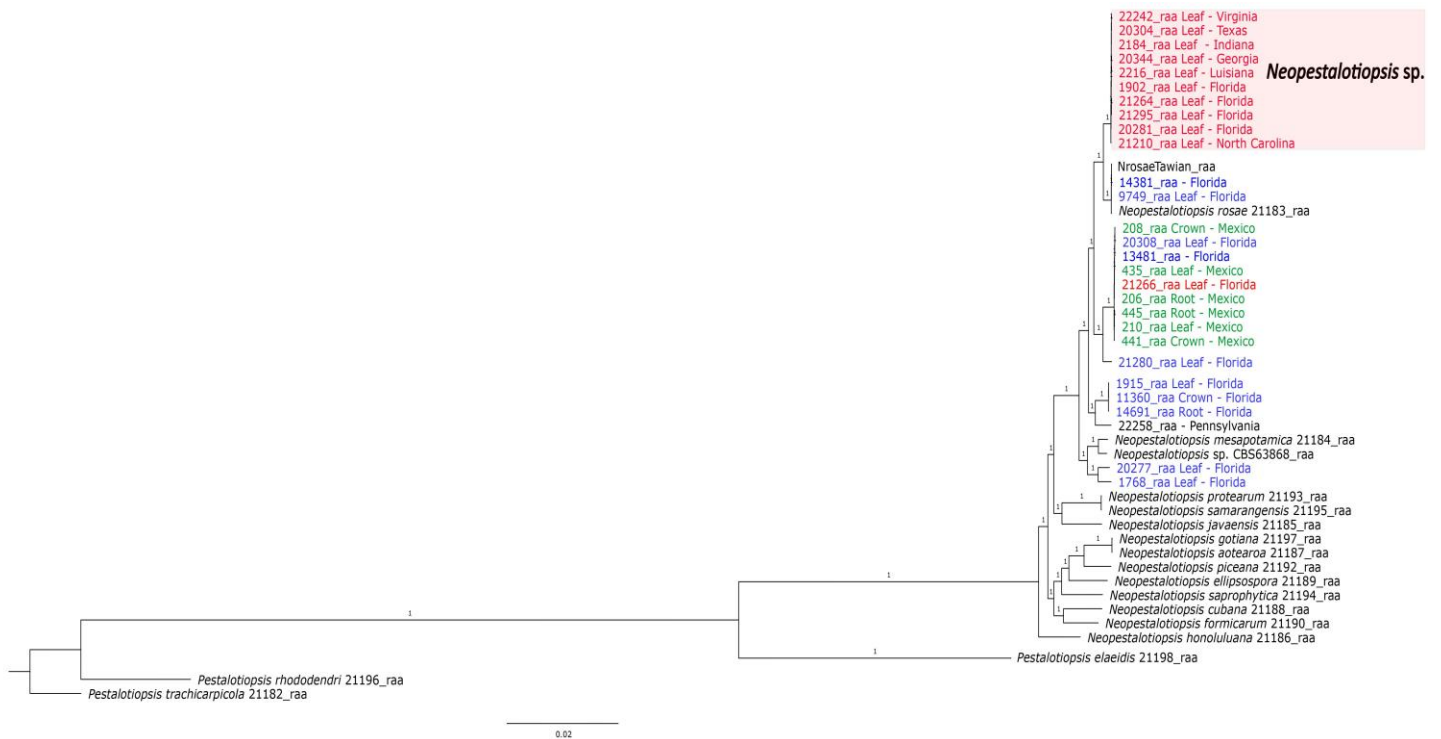


Figure 1. Phylogeny from whole genome sequencing analysis of 49 isolates of *Neopestalotiopsis* species. Isolates marked in red were recovered from disease outbreaks in Florida and other states in the U.S. These isolates were found to be aggressive on strawberry, as evidenced by pathogenicity assays; Isolates marked in blue were collected from Florida but were not associated with disease outbreaks and were not aggressive on strawberry; Isolates marked in green were obtained from outbreaks reported in Mexico and were considered aggressive based on plant inoculations. Bootstrap support values (100%) from 10,000 replicates are given at the nodes. The scale bar indicates 0.02 substitutions per nucleotide position.