

# Exploring the role of phytotoxins in the aggressiveness of *Pestalotia* leaf and fruit rot caused by *Neopestalotiopsis* spp. on strawberry

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## Summary

A compound from strawberry tissue inoculated with *Neopestalotiopsis* spp. was isolated and identified as oxysporone which could be a potential phytotoxin. We are following up with additional research to determine if this is indeed the phytotoxic compound that makes the new species more aggressive and whether there are other compounds involved.

## Methods

Strawberry transplants of 'Florida Brilliance' were inoculated with the new aggressive *Neopestalotiopsis* sp. and *N. rosae*, respectively. Symptomatic tissues were collected for RNA extraction and also sent to Dr. Yu Wang's lab at the Citrus Research and Education Center in Lake Alfred to "fish out" and identify the potential phytotoxins. Using specialized equipment, UHPLC-Q/Orbitrap MS/MS was applied combined with an untargeted metabolomics workflow. Moreover, whole genome sequencing was performed using representative isolates to identify the genes that regulate the toxin production, and roles they play in the infection process.

## Results

Only plants inoculated with the aggressive strain of *Neopestalotiopsis* sp. developed symptoms, whereas those inoculated with *N. rosae* had no visual symptoms (Figure 1). Data analysis, which was performed using mathematical-statistical modeling for differential metabolite screening, followed by the systematic metabolite identification strategy based on the high-resolution mass spectrometry (HRMS),

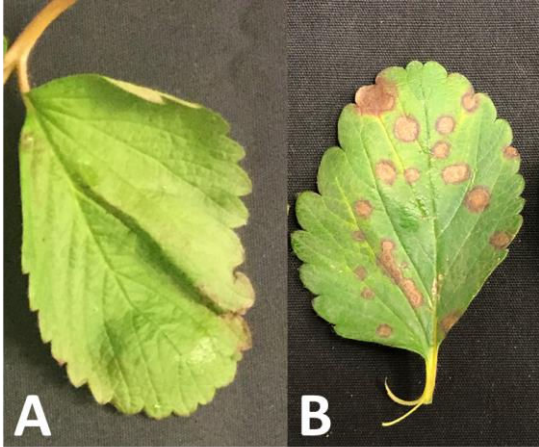
indicated oxysporone as a potential toxin. Currently, 13 different phytotoxins have been identified in some species of *Pestalotiopsis/Neopestalotiopsis*.

Oxysporone is produced by *P. longiseta* (causing tea gray blight), *P. oenotherae* (causing evening primrose leaf spot), and *N. clavispora* (causing *Kadsura coccinea* ring spot). We are currently improving our self-built database search to refine data comparisons and the likelihood of identifying whether only one or more than one phytotoxins is being produced by *Neopestalotiopsis* spp.

Thanks to the financial support from the FSREF through another funded project, we have the draft genomes of the new *Neopestalotiopsis* sp. and *N. rosae* (less aggressive species), which allowed us to identify a unique secondary metabolite cluster that might be responsible for the toxin production. These findings will be validated with the next funded project for the 2022-23 season.

Results from this project indicated that we might be on the right path in exploring the role of phytotoxins in the aggressiveness of *Neopestalotiopsis* spp. on strawberry. For the next funded project, we are going to continue building a database to refine our toxin discovery. We also plan to use a comparative genomic approach to identifying and characterizing phytotoxin-related gene clusters using the RNA sequencing data. If successful, we expect to substantially contribute to the development of rapid screening of strawberry seedlings for resistance to *Neopestalotiopsis* in the breeding program. We could also potentially identify chemicals/enzymes to inactivate phytotoxins and expand the arsenal of

disease management strategies. Moreover, we could potentially manipulate the genes to improve the resistance of current or future cultivars.



**Figure 1.** Strawberry leaves inoculated with *N. rosae* had no symptoms developed after 7 days(A), whereas several leaf spots were observed when the aggressive *Neopestalotiopsis* strain was used.

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