Root Necrosis Caused by *Colletotrichum acutatum:* Evaluation of a Field Detection Tool and Management Strategies

Marcus Marin, Carolina Rebello, and Natalia A. Peres

Summary

A field trial at GCREC evaluated fungicide applications via plant dip and planting hole to manage Root Necrosis (RN) caused by *Colletotrichum acutatum*. Eight treatments were evaluated on 'Florida Beauty'. The most effective treatment was Switch applied as a dip, reducing disease to 5% compared to 75% in the nontreated control. Zivion and Omega dips showed moderate control, whereas planting hole applications were ineffective. Moreover, a new molecular LAMP assay was developed to detect *Colletotrichum acutatum*, the RN pathogen. It proved highly sensitive, identifying infections earlier than current methods, even in asymptomatic plants. This tool may offer rapid, field-friendly diagnostics to support early intervention and improve disease management.

Methods

Objective 1. Evaluate the effectiveness of applying fungicide products via plant dip or in the planting hole to control Root Necrosis.

A replicated field trial was conducted at GCREC including the following treatments: 1) Omega 20 fl oz + Orondis 28 fl oz (planting hole), 2) Omega 20 fl oz + Ridomil 1pint (planting hole), 3) Zivion (planting hole), 4) Zivion (dip), 5) Switch 8 fl oz (dip), 6) Omega (dip), 7) inoculated control, and 8) non-inoculated control. Twenty-plant plots of 'Florida Beauty' were arranged in a randomized complete block design with four blocks in neighboring rows, planted in late October, and irrigated and fertilized throughout the season following commercial standards. The application in the planting holes was carried out prior to planting using syringes with specific amounts of product according to the treatment in a 5 ml volume. For dipping, the plants were organized in bundles and dipped in a 3-gallon suspension of fungicide according to their treatment before transplanting. Overhead irrigation was applied during the day for 10 consecutive days to promote plant establishment, and drip tapes located under the plastic were used for further irrigation and fertilization until the end of the experiment. Disease incidence (severely stunted, wilted, and dead plants) and phytotoxicity were evaluated weekly. Marketable yield (lb/A) was obtained by harvesting fruit twice a week from Nov 19 to Jan 16 (17 harvests). Data was analyzed using the statistical software SAS, and means were separated according to Fisher's Protected LSD test ($\alpha = 0.05$).

Objective 2. Develop and deploy a LAMP assay for detecting the strawberry pathogen *C. acutatum* on asymptomatic plants

We evaluated sets of primers that have previously been used to detect *C. acutatum* through other methods using the LAMP detection tests. Tests were carried out on the Genie III (OptiGene) equipment. This device is tailor-made for outdoor usage, equipped with weather-proofing and portability features. First, the LAMP assay was tested on 20 isolates that were recovered from RN cases on 'Florida Medallion' during the 2023-24 season. DNA was extracted to evaluate how these primers performed with the current pathogen population. Isolates from other *Colletotrichum* species were

added as controls to ensure no cross-reaction of the assay. After the tests using pure DNA, field samples were collected from a trial that was previously inoculated with *C. acutatum.* Plants with varying symptoms of development were selected to include the full spectrum of RN symptoms. Tissues were collected from roots, crowns, and petioles, and DNA was extracted for LAMP assays. For the field samples, culturing and isolations were also performed to confirm the presence of *C. acutatum.*

Results

Objective 1

Among the fungicide treatments, Switch applied as a dip provided the best control (5% disease incidence) and the highest yield (2,646 lb/A). Zivion and Omega, both applied as dip, resulted in moderate disease levels (25–27.5%) and intermediate yields, with 1,353 and 1,451 lb/A, respectively. However, when Zivion and Omega were applied in the planting hole, disease incidence was high, reaching 69% with yields ranging from 349 to 627 lb/A, which were not significantly different from the inoculated control, which had 75% disease incidence and yielded only 349 lb/A.

Objective 2

We identified a highly sensitive and specific primer set for the LAMP assay to detect *C. acutatum*. This primer set successfully amplified all 20 tested isolates of *C. acutatum* and showed no cross-reactivity with other *Colletotrichum* species included in the study. Using purified DNA from these isolates, the LAMP method demonstrated a detection sensitivity as low as 10 fg. In field samples, LAMP reliably detected *C. acutatum* in all cases confirmed positive by culture isolation, including those at early stages of symptom development when diseased tissue is minimal. At these initial stages, the current HRM assay often yields negative results due to its detection limit, whereas LAMP consistently outperforms HRM in sensitivity. Despite this limitation, HRM remains a valuable diagnostic tool in our clinic for early disease diagnosis, whereas LAMP shows promise for field deployment, given its straightforward sample preparation and rapid results within 20 minutes.

Summary and recommendations

Management of root necrosis caused by *C. acutatum* remains a challenge, as it is often difficult to determine whether plants are infected before planting. In case of known infected transplants, based on our field trials, Switch applied as a plant dip was the most effective treatment. Unfortunately, applications of Zivion and Omega in the planting hole were not effective. It is crucial to follow the label recommendations for dip applications since phytotoxicity has been reported. The newly developed LAMP assay provides a rapid, sensitive, and field-deployable diagnostic tool that can detect infections even in asymptomatic plants. The adoption of this tool may enable timely interventions and improved decision-making for managing root necrosis. We continue to encourage growers to submit samples to the GCREC Plant Diagnostic Clinic for accurate pathogen identification since accurate diagnosis is key to effective disease management.

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-mail: nperes@ufl.edu

APPENDIX

Table 1. Effectiveness of fungicides applied by dip or in the planting hole in controlling root necrosis caused by *Colletotrichum acutatum*.

Treatment (product and rate/A)	**Application method**	**% DI[x]**	**Yield (lb/A)[y]**
Not treated, non-inoculated control	-	2.5 c[z]	3208 a
Switch 8 fl oz	dip	5.0 c	2646 b
Zivion	dip	25.0 b	1353 c
Omega	dip	27.5 b	1451 c
Zivion	planting hole	66.3 a	390.4 d
Omega 20 fl oz Orondis 28 fl oz	planting hole	68.8 a	349 d
Omega 20 fl oz Ridomil 1pint	planting hole	70.0 a	627 d
Not treated, inoculated control	-	75.0 a	349 d
Probability of a greater F value		<0.0001	<0.0001

[x] %DI = percentage of disease incidence (severely stunted, wilted, and dead plants).

[y] Average of yield in lb/acre for seventeen harvests performed from 19 November 24 to 16 January 2025.

[z] Means in a column followed by the same letter are not significantly different by Fisher's Protected LSD test (α=0.05).