

Enhancing the Management of Important and Emerging Diseases of Strawberry through Rapid and Accurate Diagnostic and Monitoring of Fungicide Resistance

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Summary

During the 2024-25 season, we processed 307 strawberry samples, with the majority showing crown and root rot, followed by leaf spot symptoms. Regrettably, populations resistant to at least one fungicide group were detected for *Botrytis cinerea*, *Phytophthora* spp., and *Colletotrichum acutatum*. Altogether, these results are crucial to aid practical management recommendations.

Methods

Objective 1. To continue providing rapid and accurate diagnosis of strawberry diseases and to develop new HRM assays for *Verticillium dahlia*.

Our HRM assay, designed to detect common crown rot pathogens in strawberries and *Neopestalotiopsis* spp., offers growers benefits such as simplicity, accuracy, reproducibility, and low cost. Thanks to a generous donation of a LightCycler 480 system by the FSREF, this assay became part of the standard diagnostic procedures in the plant diagnostic clinic for strawberry samples, especially those with crown rot and leaf spot symptoms. Samples submitted by growers or consultants undergo DNA extraction and HRM assay, with preliminary results sent within 24-48h. Molecular results are followed by validation through standard culture procedures.

Objective 2. To continue monitoring the resistance of strawberry pathogens to commonly used fungicides.

During the 2024-25 season, 242 isolates of *Botrytis cinerea* were collected from symptomatic fruit tissue

across twenty-one commercial strawberry fields. All isolates were tested using a conidial germination method to assess their sensitivity to fludioxonil (Switch®), and the SDHI fungicides isofetamid (Kenja) and pydiflumetofen (Miravis® Prime). Sensitivity levels were determined based on the number of germinated conidia and the elongation of their germ tubes, resulting in categorizations of sensitive (S), moderately resistant (MR), or highly resistant (HR). Isolates of *Colletotrichum acutatum* and *Phytophthora cactorum* were obtained from samples received at the GCREC Plant Diagnostic Clinic and tested against azoxystrobin (Abound) and mefenoxam (Ridomil), respectively, using mycelial growth assays.

Objective 3. To investigate the resistance mechanisms and develop molecular tools to quickly screen fungicide-resistant populations of *Botrytis cinerea* and *Phytophthora cactorum*.

Botrytis. Fifteen isolates collected from 2018 to 2021 from different commercial fields in Florida during previous monitoring of fungicide resistance were characterized as sensitive or moderately resistant to fludioxonil. To determine the resistance mechanism, molecular primers were designed to amplify the *Mrr1* gene in *B. cinerea*. Additionally, primers from the literature were used to amplify regions of the *Bos1* gene. Mutations in these genes have previously been reported to confer resistance to fludioxonil. Using a FastDNA Kit, DNA was extracted from mycelia and used for PCR reaction. Samples of sensitive and moderately resistant isolates were sent for DNA

sequencing and Geneious Prime was used for assembling, translation, and alignment of sequences.

Phytophthora. In a proposal funded in 2019, we investigated the mechanisms of resistance to mefenoxam in *Phytophthora* isolates, all of which originated from specific nurseries in North Carolina and were characterized as highly resistant. During the 2022–23, 2023–24, and 2024–25 seasons, we began identifying a small number of resistant isolates from nurseries in California and Canada. Unlike the earlier findings, these newer isolates came from diverse locations, and some displayed only moderate resistance. Using the six mutations previously associated with mefenoxam resistance and the primers developed in the earlier study, we sequenced both highly and moderately resistant isolates collected during recent seasons to verify whether the previously identified DNA markers would still work for the newer resistant isolates.

Results

Objective 1

During the 2024-2025 season, we processed 307 strawberry samples, and many samples had more than one pathogen diagnosed. The summary of the diagnostic results is as follows: 117 cases of leaf spots, 204 instances of crown and root rot, and 4 occurrences of fruit rot symptoms (Appendix, Figure 1). This rapid diagnostic approach significantly enhances decision-making, enabling the timely implementation of effective disease management practices. Furthermore, owing to the expertise of our diagnosticians and the specialized molecular tools, particularly the new assays to detect *Neopestalotiopsis* sp., our clinic has become a reference lab for strawberry samples. In 2024, we received 139 strawberry samples from commercial growers and nurseries across different U.S. states, including Massachusetts, North Carolina, New York, Alabama, Georgia, South Carolina, Indiana, Pennsylvania, Texas, Virginia, and Louisiana. Additionally, we have received requests to assist with diagnosing *Neopestalotiopsis* from other countries, including Australia, Mexico, Spain, and Brazil. During the past season, we successfully developed and implemented a molecular assay for diagnosing *Verticillium dahliae*. This tool will enable rapid

detection of the pathogen in the event of its introduction into Florida, especially as the risk may increase with the growing number of transplants from California.

Objective 2

Our fungicide resistance screening of 242 isolates of *Botrytis cinerea* showed that 86%, 32%, and 7% of the tested population were moderately resistant to fludioxonil (Switch®), isofetamid (Kenja), and pydiflumetofen (Miravis® Prime), respectively (Appendix, Figure 2). From the 12 *Colletotrichum acutatum* isolates tested, 10 were resistant to strobilurin fungicides (FRAC group 11), which includes Abound and Cabrio. Among 40 isolates of *Phytophthora cactorum*, only 3 were resistant and 1 was moderately resistant to mefenoxam/metalaxyl (Ridomil Gold®). The results of our fungicide resistance monitoring are critical for developing effective disease management recommendations for the upcoming season.

Objective 3

Botrytis. Analysis of DNA sequences showed no mutations in the amplified regions of *Bos1* gene; however, different results were observed for *Mrr1* gene. The 4 sensitive isolates had no mutations, whereas at least one mutation was identified in the moderately resistant isolates (Table 1). The mutations D385Y and V575G were identified in 20-436 and 18-594, 19-472, 19-484, 20-449, and 21-155, respectively. The remaining 6 isolates had 14 to 18 mutations. Interestingly, these isolates showed higher EC₅₀ values, indicating lower sensitivity to fludioxonil.

Phytophthora. Mefenoxam-resistant isolates (both highly and moderately resistant) collected during the 2022–23, 2023–24, and 2024–25 seasons were sequenced and compared to a sensitive control isolate. DNA sequencing revealed that the six mutations previously associated with mefenoxam resistance were absent in these more recently collected isolates. In fact, their DNA sequences matched those of the sensitive controls. These findings suggest that the resistance mechanism in this emerging population may differ from those previously identified.

Summary and recommendations

We strongly encourage sample submissions to support timely and effective disease management, and continue to provide rapid turnaround thanks to our HRM assays. Based on our recent findings, we recommend reserving Switch[®], Miravis Prime[®], and Kenja[®] for periods when weather conditions are highly conducive to Botrytis fruit rot development, particularly during peak bloom, as indicated by the Strawberry Advisory System (SAS). To minimize resistance risks, the combined use of Switch[®] and Miravis[®] (both containing fludioxonil) should be limited to no more than four applications per season. Miravis[®] should be applied no more than twice per season, and the total number of Group 7 fungicide applications (including Kenja[®], Luna[®], Fontelis[®], and Merivon[®]) should not exceed four per season. In practice, this translates to a maximum of 2 applications each for Miravis[®], Switch[®], and Kenja[®]. During periods of moderate or low disease pressure, multi-site fungicides such as thiram and captan are recommended to help prevent the development of resistant strains. For diseases caused by *Colletotrichum* spp. (anthracnose fruit rot and crown rot), preventive captan applications are advised due to resistance issues with Abound[®] and other QoI fungicides. For *Phytophthora* management, early-season applications of Orondis Gold[®] or Ridomil[®] are recommended, with phosphite products used in rotation. If Ridomil[®] treatments fail to control the disease, samples should be submitted for resistance testing.

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APPENDIX

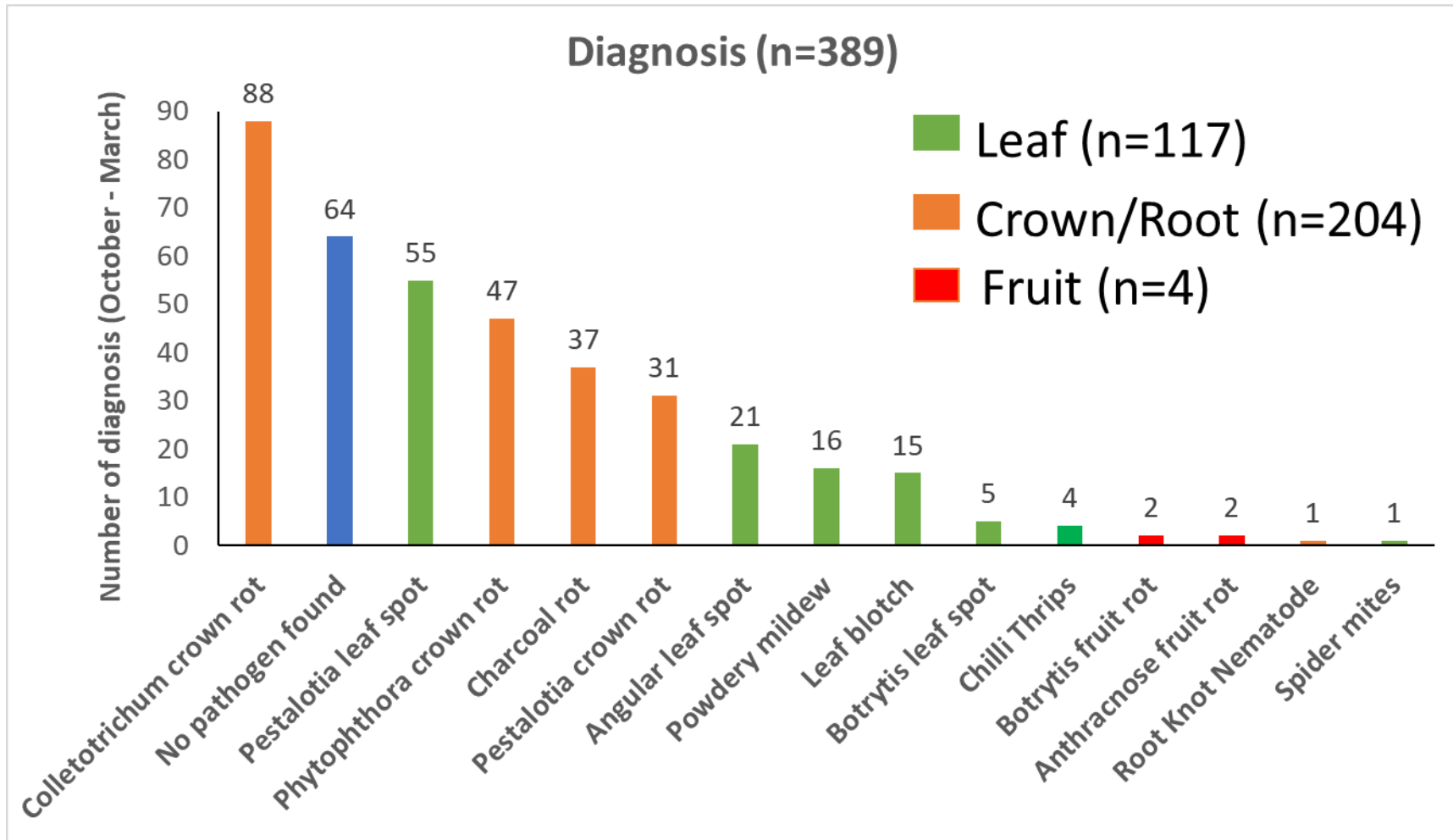


Figure 1. Overview of diagnostic results from strawberry samples (n=307) submitted by Florida growers to the diagnostic lab at GCREC during the 2024-25 season.

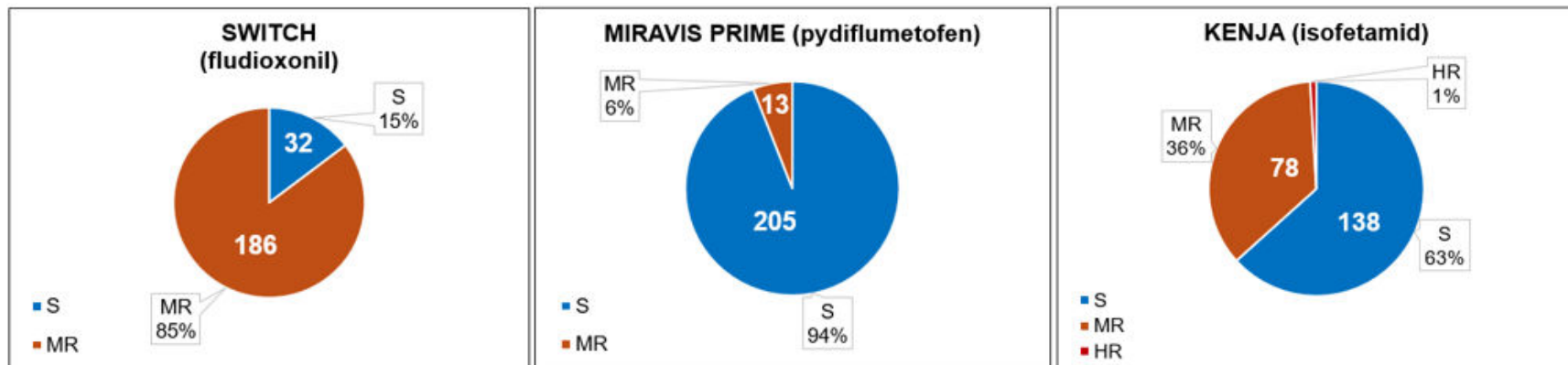


Figure 2. Frequency (%) of *Botrytis cinerea* isolates (n=242) collected during the 2024-2025 Florida strawberry season resistant to fludioxonil (in Switch and Miravis Prime), isofetamid (Kenja), and pydiflumetofen (in Miravis Prime). Isolates were classified as sensitive (S), moderately resistant (MR), and highly resistant HR.

Table 1. Identification of mutations in the *Mrr1* gene of *Botrytis cinerea* associated with resistance to fludioxonil.

Isolate ^a	Phenotype ^b	Mutations in <i>Mrr1</i> gene
19-496	S	-
20-453		-
21-148		-
21-165		-
18-575	MR	I443L, T449S, L497 deletion, V579A, E601G, G602S, R656L, A668G, G670E, C671F, C682R, S684P, G702N, G710C
18-578		M432T, I443L, L497 deletion, F568S, V579A, E601G, G602S, R634K, R656L, N666G, A668G, G670E, C671F, C682R, S684P, G702S, G710C
18-579		M432T, I443L, L497 deletion, F568S, V579A, E601G, G602S, R634K, R656L, N666G, A668G, G670E, C671F, C682R, S684P, G702S, G710C
18-594		V575G
19-472		V575G
19-484		V575G
20-436		D385Y
20-449		V575G
20-450		M432T, I443L, L497 deletion, F568S, V579A, E601G, G602S, A615V, R634K, R656L, N666G, A668G, G670E, C671F, C682R, S684P, G702S, G710C
21-154		M432T, I443L, L497 deletion, F568S, V579A, E601G, G602S, A615V, R634K, R656L, N666G, A668G, G670E, C671F, C682R, S684P, G702S, G710C
21-155		V575G

^a *Botrytis cinerea* isolates collected from commercial fields in Florida during various strawberry seasons.

^b Phenotype of *B. cinerea* isolates. S = sensitive and MR = moderately resistant.