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 2023-24 season, we processed 223 strawberry samples, mostly showing crown, root, and 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 provide a rapid and accurate diagnosis of strawberry diseases and to develop new HRM assays for *Fusarium oxysporum f. sp. fragariae* (*Fof*) and *Phytoplasma* species.

Our HRM assay, designed to detect *Neopestalotiopsis* spp. and common crown rot pathogens in strawberries, 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 HRM assay processing and validation through standard culture procedures, with preliminary results sent within 24-48 hours. HRM assays for detecting *Fusarium oxysporum* f. sp. *fragariae* (Fof) and *Phytoplasma* spp. in strawberry samples were successfully developed to enhance our routine detection capabilities for emerging pathogens in Florida.

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

A total of 195 colonies of *Botrytis cinerea* were isolated from symptomatic fruit tissue collected from ten commercial strawberry fields. These 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). Additionally, *Colletotrichum acutatum* and *Phytophthora cactorum* isolates from samples received at the diagnostic clinic were tested with azoxystrobin (Abound) and mefenoxam (Ridomil), respectively, using mycelial growth assays.

Results

Objective 1

During the 2023-2024 season, we processed 223 strawberry samples, and many samples had more than one pathogen diagnosed. The summary of the diagnostic results is as follows: 212 cases of leaf spots, 196 instances of crown and root rot, and 21 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 center for diagnosing strawberry samples. In 2023, we received 53 strawberry samples from commercial growers and nurseries across different states, including Massachusetts, North Carolina, New York, Alabama, Georgia, South Carolina, Indiana, Pennsylvania, Texas, Virginia, and Louisiana. Additionally, we have received requests from other countries, including Australia, Mexico, Spain, and Brazil, to assist with diagnosing Neopestalotiopsis. This past season, we successfully developed and implemented a molecular assay for diagnosing *Fusarium oxysporum* f. sp. *fragariae*, which led to the unfortunate finding of this pathogen for the first time in two farms in Florida. Although an HRM assay for Phytoplasma detection was developed, no samples tested positive for this pathogen during the past season.

Objective 2
Fungicide resistance screening of 195 isolates of *Botrytis cinerea* revealed that 43%, 21%, and 6% of the isolates were at least moderately resistant to fludioxonil (Switch®), isofetamid (Kenja), and pydiflumetofen (Miravis® Prime), respectively (Appendix, Figure 2). A total of 28 isolates of *Colletotrichum acutatum* were obtained from samples showing root necrosis of Florida Medallion™ 'FL 16.30-128'. Unfortunately, 27 of 28 isolates recovered from these samples were resistant to the strobilurin fungicides (FRAC group 11), such as Abound, Cabrio, etc. Phytophthora crown rot (PhCR) represented 17% (n = 80) of the samples diagnosed in the clinic. Of the 98 Phytophthora isolates, only two were resistant, and one was moderately resistant to mefenoxam/metalaxyl (Ridomil Gold®). Results from our fungicide resistance monitoring play an essential role in disease management recommendations for the upcoming season.

Takeaways
With the fast turnaround of our HRM assays, we encourage everyone to submit samples for diagnosis to ensure effective management. Additionally, given the first detection of *Fusarium oxysporum* f. sp. *fragariae* last season and the continued increase in plant purchases from California nurseries, it is important to monitor this pathogen across strawberry fields in the upcoming season. Based on our findings, the management recommendations for Botrytis fruit rot include reserving the use of Switch®, Miravis®, and Kenja® for periods with highly favorable weather conditions for disease development, particularly during peak bloom as advised by the Strawberry Advisory System (SAS). It is important to limit the combined applications of Switch® and Miravis® (both containing fludioxonil) to no more than four per season. Additionally, Miravis® should only be applied twice per season, and no more than four applications of Group 7 fungicides (including Kenja®, Luna®, Fontelis®, and Merivon®) should be used collectively within a season. This translates to a practical limit of two applications each for Miravis, Switch, and Kenja per season. Multi-site fungicides like thiram and captan are recommended during moderate or low disease pressure periods to prevent resistant strains. For diseases caused by *Colletotrichum* spp. (anthracnose fruit rot and crown rot), preventive captan applications are recommended due to resistance issues with Abound® and other QoI fungicides. For *Phytophthora* control, early season applications of Orondis Gold® or Ridomil® are recommended, and phosphite products are recommended for rotation. If control fails after Ridomil applications, samples should be submitted for resistance testing.

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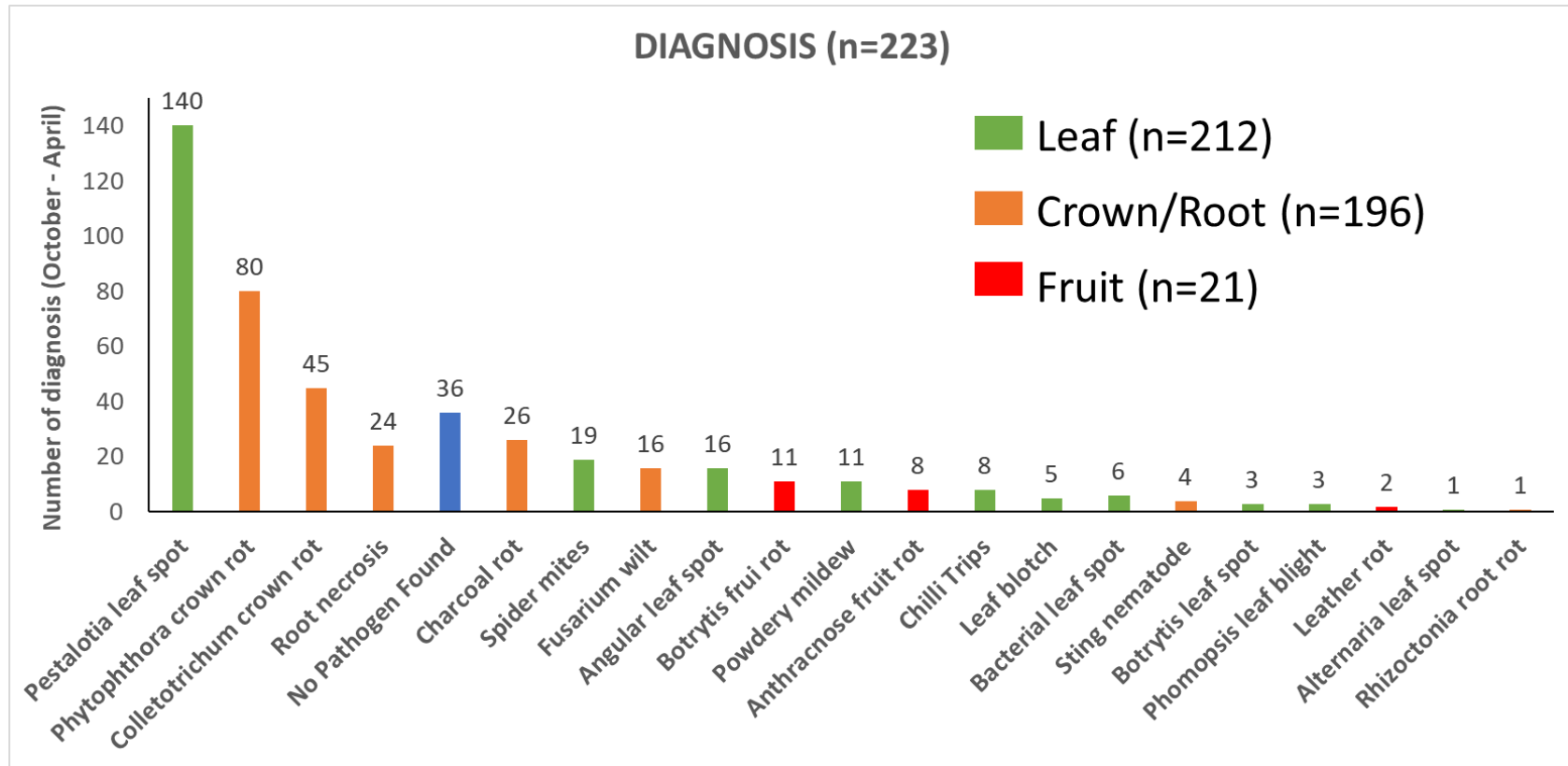E-mail: nperes@ufl.edu

Figure 1. Overview of diagnostic results from strawberry samples (n=223) submitted by Florida growers to the diagnostic lab at GCREC during the 2023-24 season.
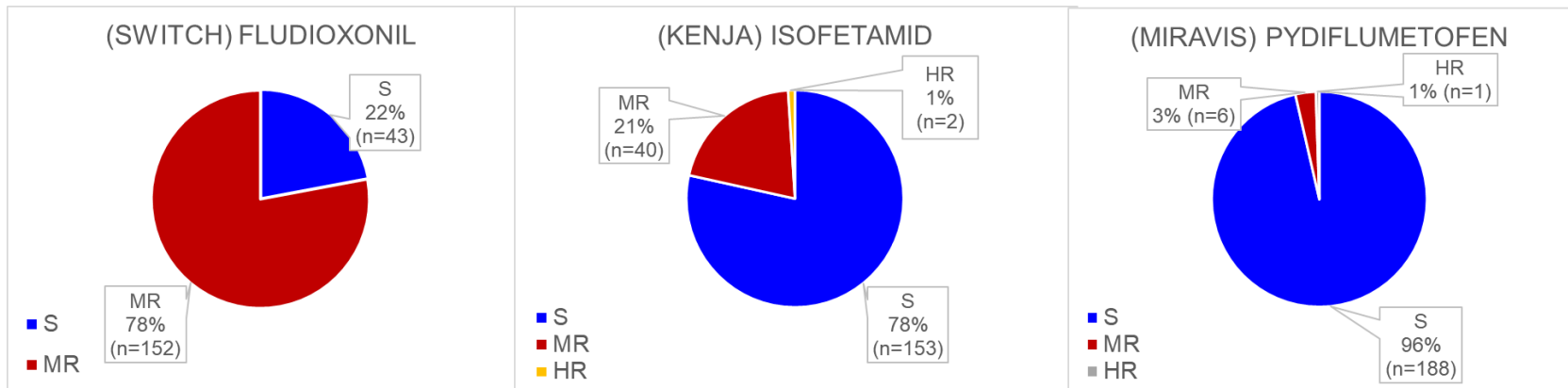
Figure 2. Frequency (%) of *Botrytis cinerea* isolates (n=195) collected during the 2023-2024 Florida strawberry season resistant to fludioxonil (in Switch and Miravis Prime), isofetamid (Kenja), and pydiflumetofen (in Miravis Prime). Isolates were classified as sensitive (S), moderately resistant (MR), and highly resistant HR.