

Field based quantification of *Clarireedia jacksoni* in bentgrass using droplet digital polymerase chain reaction (ddPCR)



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Introduction

Dollar spot caused by the fungal pathogen *Clarireedia jacksonii*, is one of the most significant diseases affecting turfgrass. Dollar spot appears as small, bleached turf patches, compromising aesthetic quality and playability. While fungicides are effective in controlling dollar spots, their use is costly and environmentally unsustainable. Developing an efficient method to quantify *Clarireedia jacksonii* can improve disease management by helping monitor inoculum load and timing fungicide application, and aiding breeders in evaluating resistant cultivars. Droplet digital polymerase chain reaction (ddPCR) has emerged as a powerful tool for pathogen quantification, offering absolute quantification without the need for standard curves and enhanced tolerance to PCR inhibitors.

Objective

- Develop and validate a ddPCR assay for the quantification of *C. jacksonii* in bentgrass samples.
- Quantify *C. jacksonii* on dollar spot susceptible and resistant bentgrass cultivars

Methods and Materials

- Internal transcribed spacer (ITS) gene region of *Clarireedia* was amplified and sequenced using ITS1 and ITS4 primers. The resulting amplicon was used to design primer and probe for this study.
- Bentgrass samples were collected from the National Turf Evaluation Program (NTEP) fairway trial at the University of Maryland.
- Three creeping bentgrass cultivars—'007XL', 'Penncross', and 'Oakley'—along with the colonial bentgrass cultivar 'Musket' were selected for clipping collection and DNA quantification.
- A cordless electric professional sheep shear was used to collect the clippings every week.
- Clippings samples were freeze-dried before DNA extraction.



Methods and Materials

- DNA extraction was conducted using the protocol provided in the QIAGEN DNeasy Plant Mini Kit.
- QX200™ Droplet Digital PCR Systems (BioRad) was used for ddPCR.
- The droplets were analyzed using QX Manager Standard Edition software.
- The seasonal disease progression of bentgrass cultivars was monitored weekly using RGB images of field plots.

Results

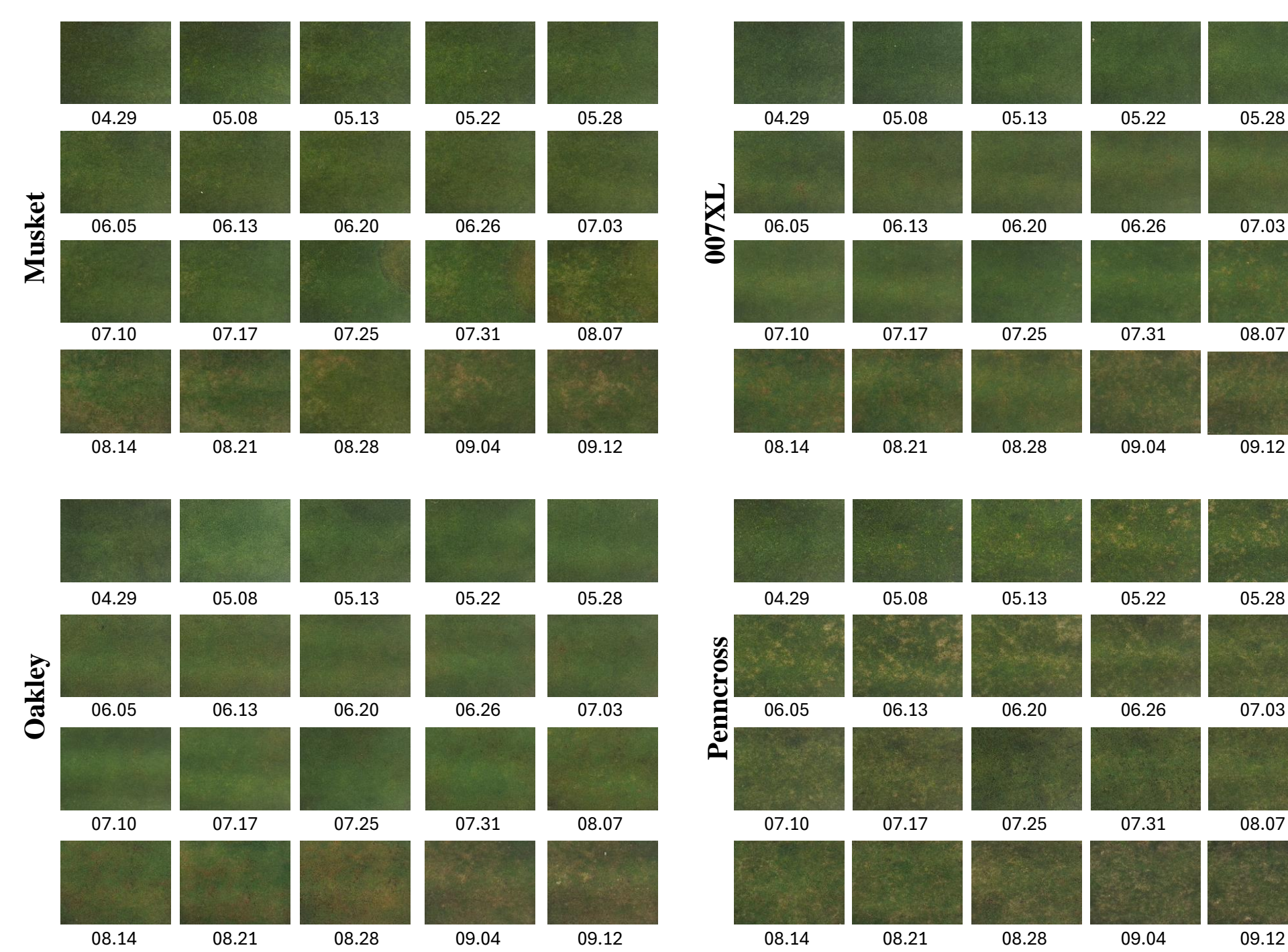


Figure 1. Field plot images showing weekly disease progression on four bentgrass cultivars (top left: Musket, top right: 007XL, bottom left: Oakley, and bottom right: Penncross).

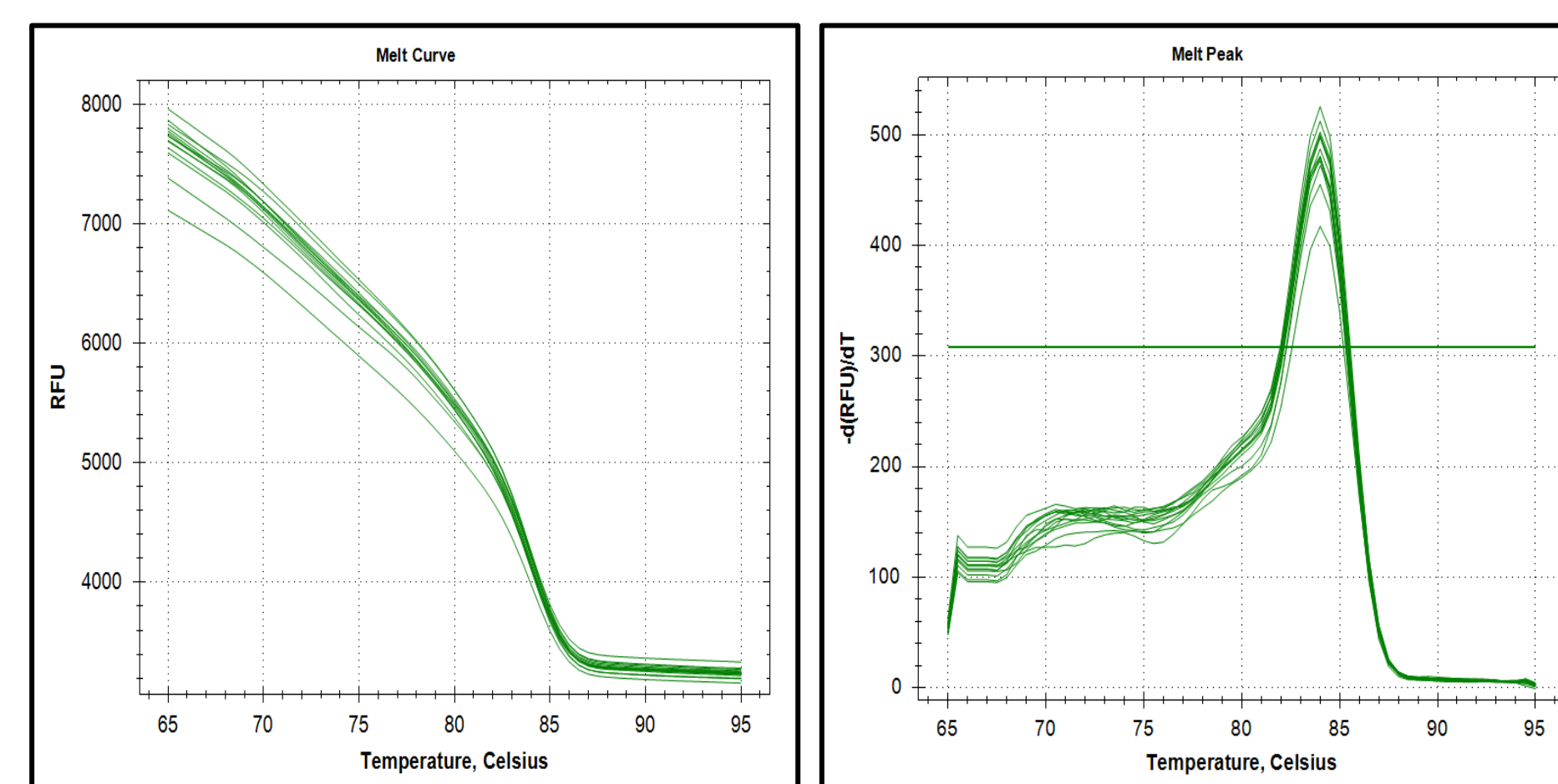


Figure 2. Melt curve (left) and melt peak (right) analysis of qPCR products of 12 random field samples along with positive control targeting *Clarireedia* DNA.

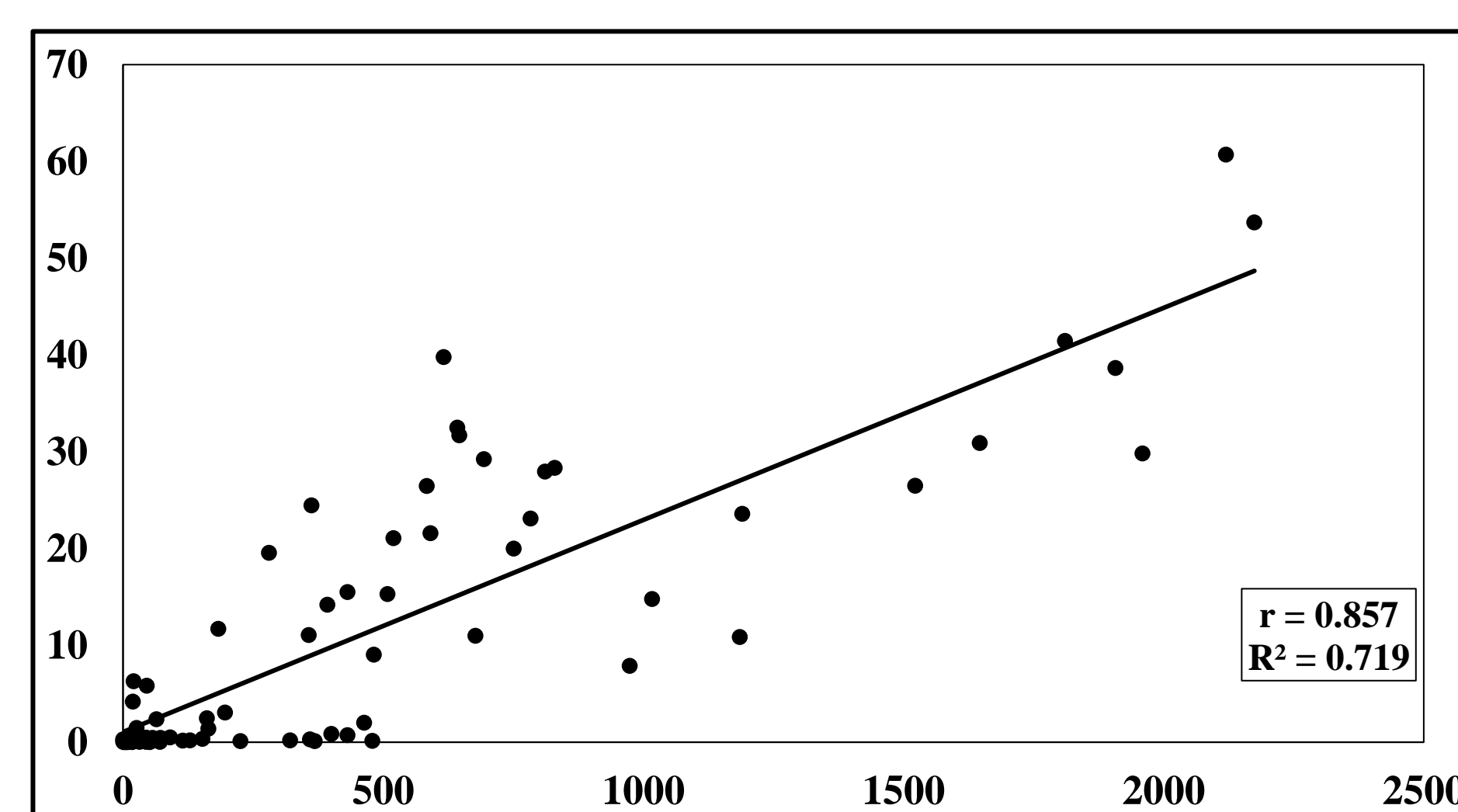


Figure 3. Scatter plot between *Clarireedia* DNA and percent disease symptoms.

Results

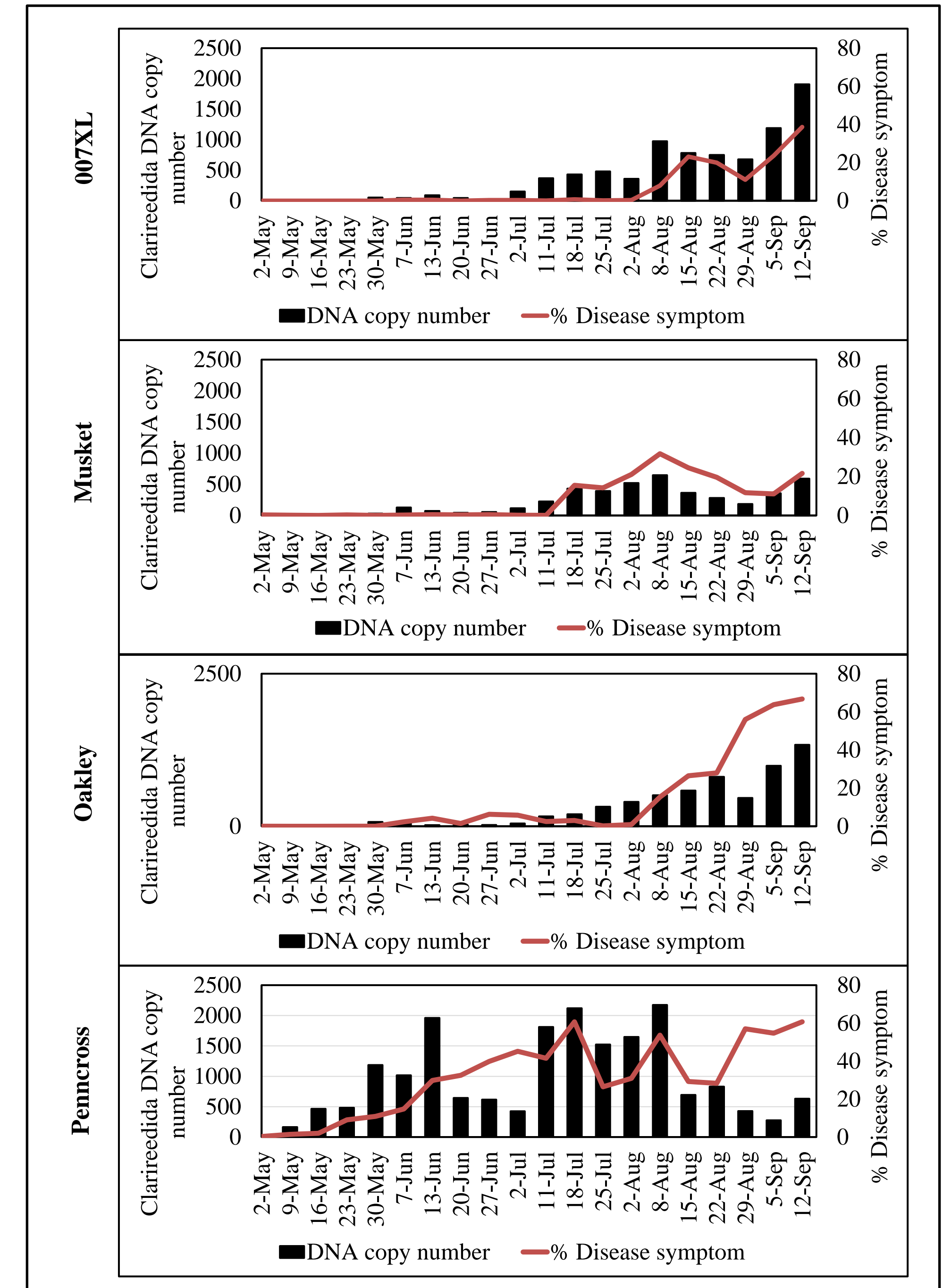


Figure 4. Temporal dynamics of *Clarireedia* DNA copies and corresponding disease symptoms across four bentgrass cultivars: 007XL, Musket, Oakley, and Penncross. DNA copy number (black bars) represents the quantification of *Clarireedia* using ddPCR while disease symptoms (orange line) are shown as percentage of disease severity observed over 14 weeks.

Summary

- We developed a highly efficient ddPCR assay for the quantification of *Clarireedia* on bentgrass field samples with a limit of quantification of 1×10^{-14} g.
- The correlation analysis ($r=0.857$ and $R^2=0.719$) suggests that *Clarireedia* DNA copy number significantly predicts disease severity.
- The resistance bentgrass cultivar can skip the early season dollar spot disease epidemic by keeping *Clarireedia* level low.
- Colonial and creeping bentgrass may have different mechanisms for disease resistance.

Acknowledgement

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