CAPS and RPA-Based Approaches for Differentiating *Neopestalotiopsis* species affecting strawberry production

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Neopestalotiopsis disease epidemic

- Eastern North America
- First appeared in **Florida** as an aggressive disease in 2017
- **Fungal** pathogen – New species/strain of *Neopestalotiopsis* (*N.* sp.)







Baggio et al. 2021

Diagnostic Methods

- Culturing + morphology

- Molecular Methods – characterization of 3 loci
  - **Beta-tubulin (Btub),** Translation elongation factor 1 (Tef1), Internal transcribed spacer (ITS)
  - Polymerase chain reaction (PCR) +
    - Sequencing
    - Restriction digestion
    - High-resolution melting (HRM)
  - Recombinase polymerase amplification (RPA)

Kaur et al. 2023, Rebello et al. 2023

Objectives



CAPS Assay

Perform on more isolates to confirm that this is a sound method of identification



Confirm presence of new putative species of *Neopestalotiopsis* in Canada

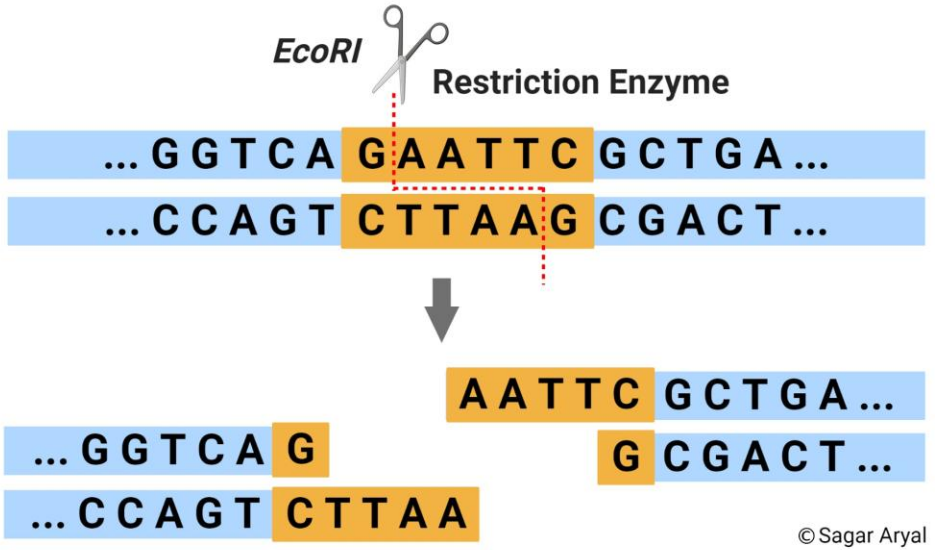


Develop RPA

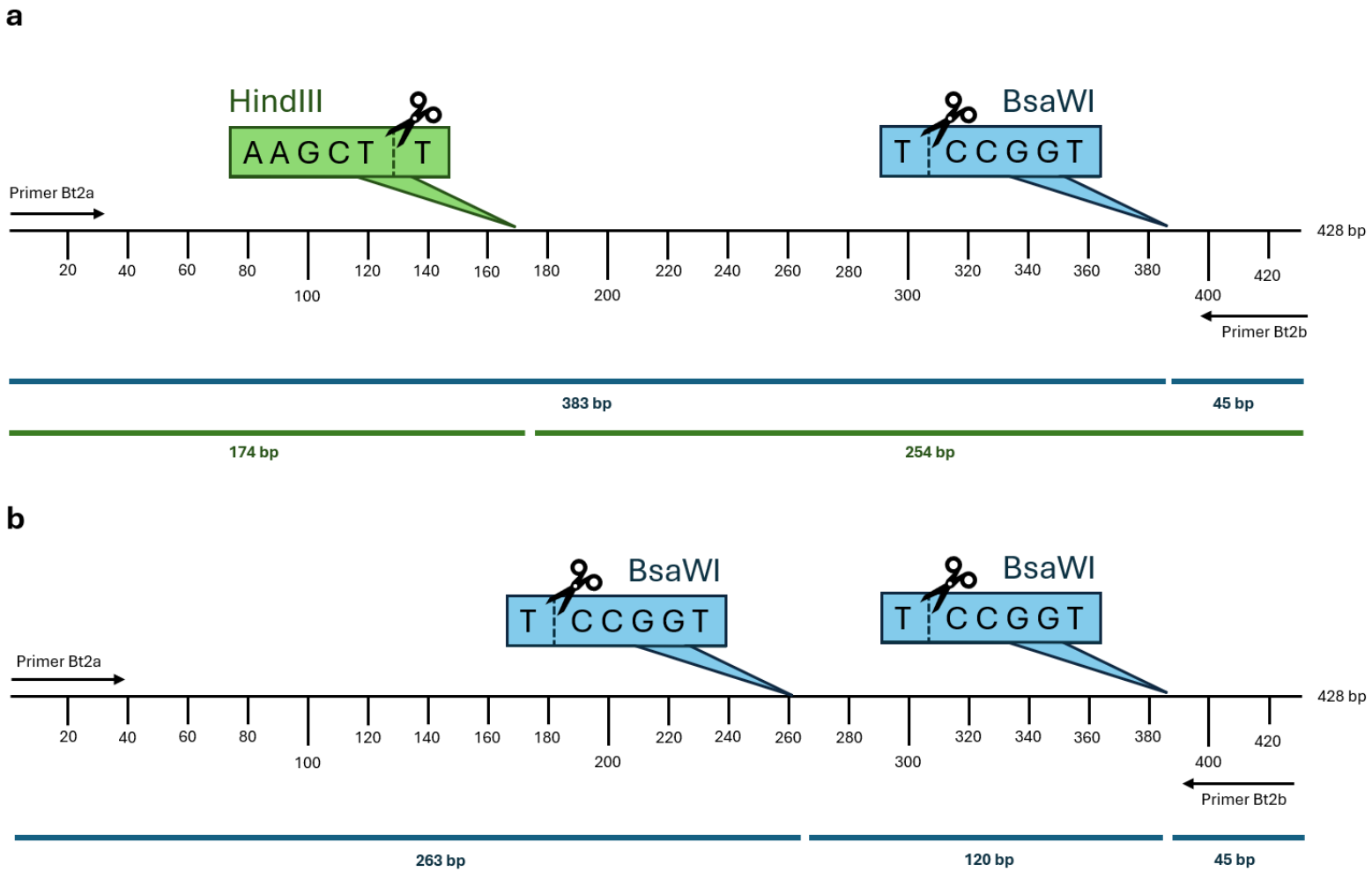
A faster, versatile method of pathogen identification

Cleaved Amplified Polymorphic Sequences (CAPS)

- SNPs allow for molecular differentiation between *Neopestalotiopsis* species
- Restriction enzymes cut PCR product
- Pattern of cutting for each species visible in gel electrophoresis
- Faster and cheaper than sequencing



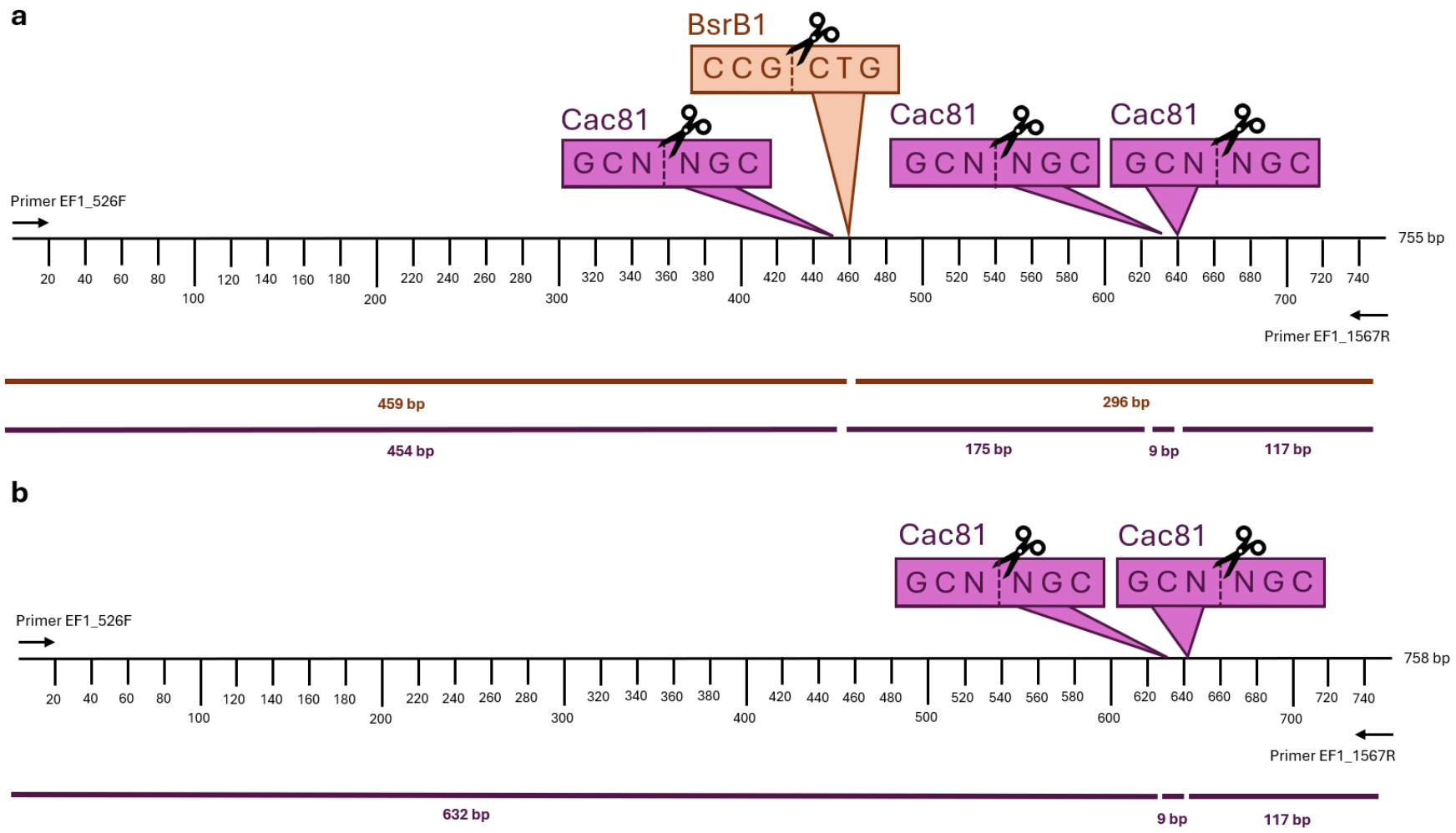
CAPS Assay: Btub



A. *N. rosae*

B. *Putative N. species & N. sp.*

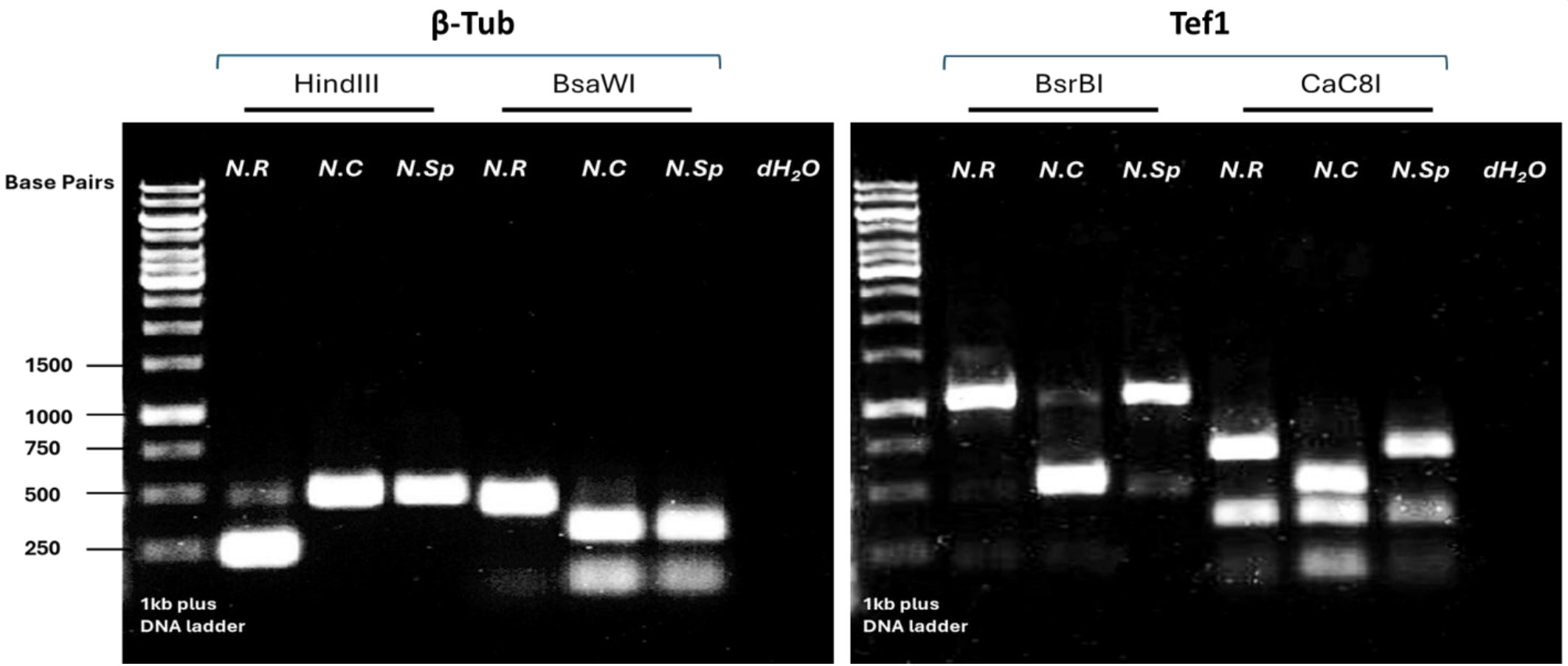
CAPS Assay: Tef1



A. *Putative N. species*

B. *N. rosae & N. sp.*

CAPS Assay



Baek et al. 2026

CAPS Assay

- Materials & Methods:
- CAPS assays performed on additional isolates of confirmed identity (sequencing):
  - 9 aggressive isolates
  - 1 putative species isolate

- Results:
- Pattern of banding was consistent with what was previously observed

Recombinase Polymerase Assay (RPA)

- Variation of PCR that uses isothermal enzyme -> no thermocycling needed
- Use of species-specific primers
  - amplification only occurs if species is present
- Faster than CAPS, less labor intensive
- Products can be run on gel electrophoresis or adapted into field kits

Primer	Sequence
F1_Nc	5′-TTACCGATCACAACTTGAGAGGTTACATGC**A**C-3′
F1_Nsp	5′-TTACCGATCACAACTTGAGAGGTTACATGC**T**C-3′

* Not real sequence

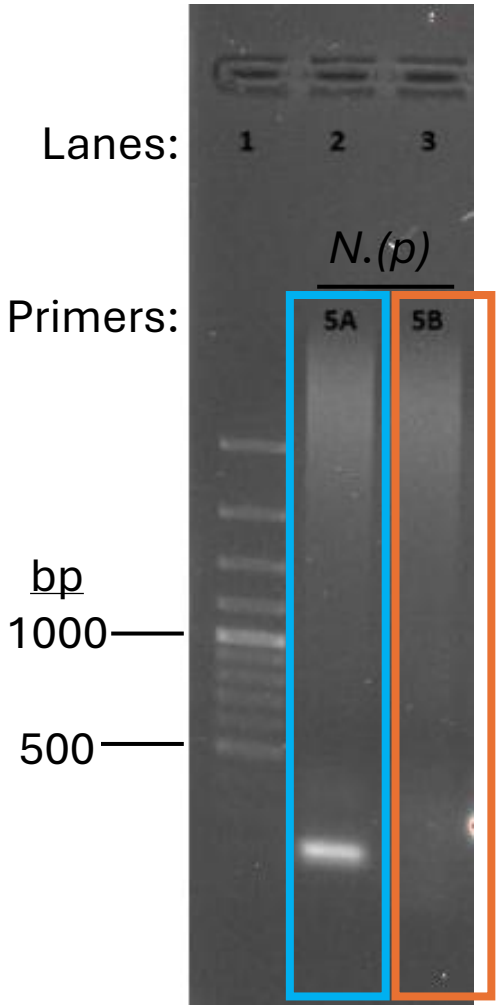
Results: Btub

- 7 primer pairs tested
- Only one (pair # 5) did not produce off-target amplification and did not amplify the non-target species

DNA = putative species

5A = primers designed for putative species / aggressive

5B = primers designed for *N. rosae*



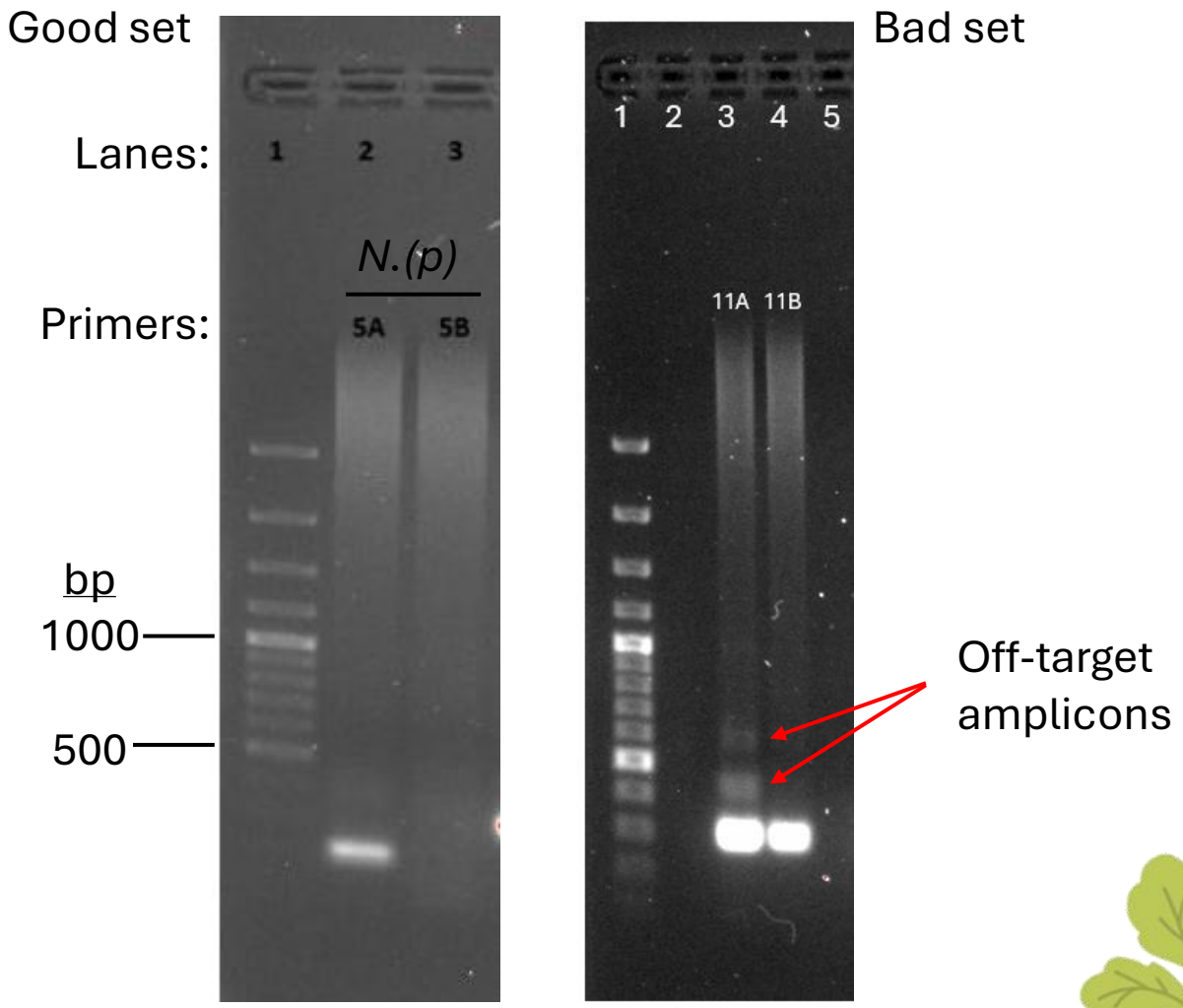
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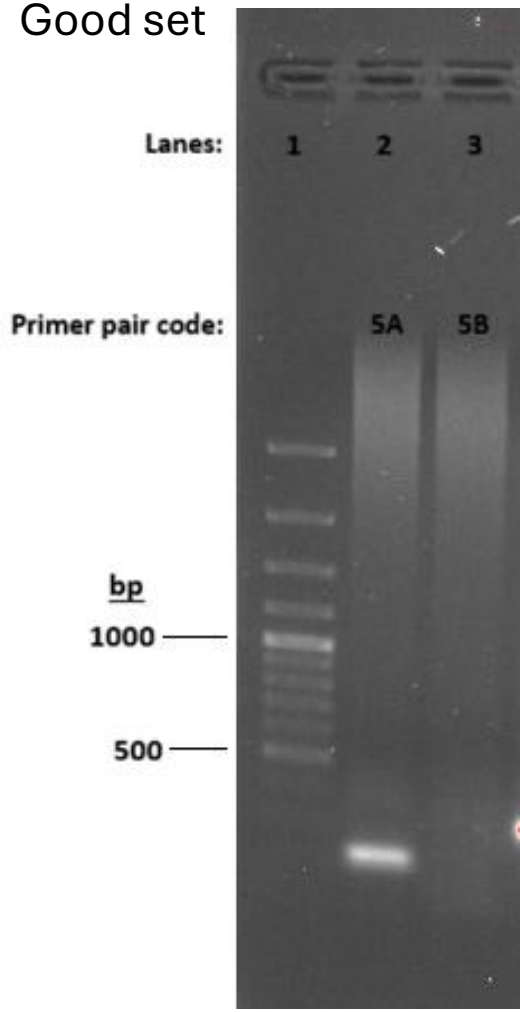
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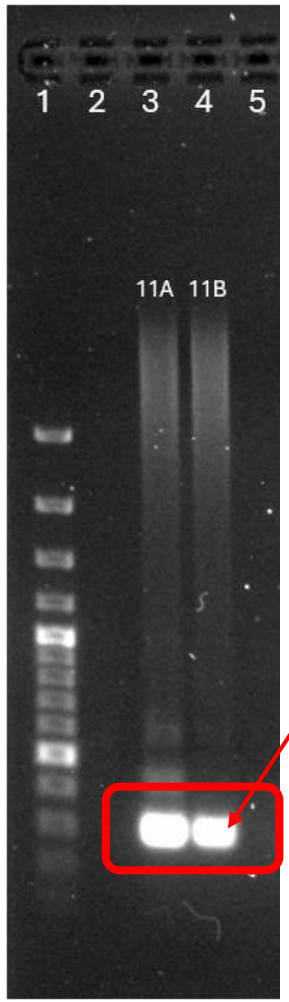
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Good set



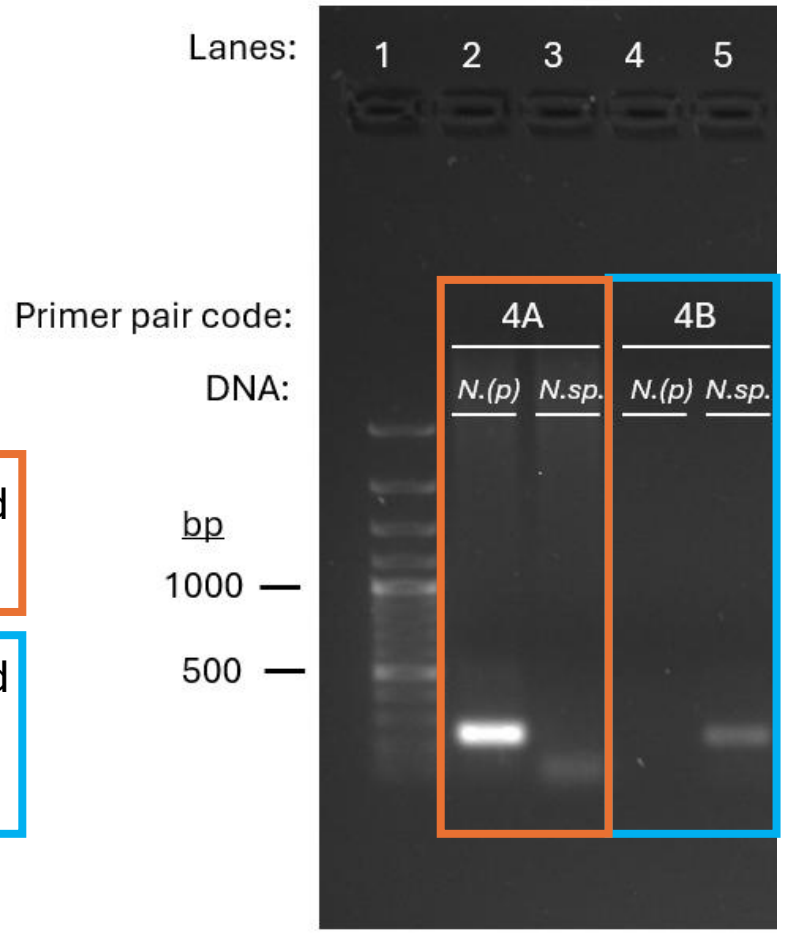
Bad set



Primers designed for *N. rosae* also amplifies same region in the putative species

Results: Tef1

- 6 primer pairs tested
- Several were species specific
- Pairs 4 and 5 were the most favorable



4A = primers designed for putative species

4B = primers designed for *N. rosae* / aggressive species

Conclusions

- CAPS
  - Sound method – cheaper, faster (than sequencing)
  - Supports existence of distinct species in Canada
- RPA
  - Primers developed which can distinguish certain species at Btub and Tef1 loci
  - Faster than CAPS
  - Can be developed into field kits
- Limitations
  - only tests for *Neopestalotiopsis* species – primarily *N.* sp. – does not provide information about other pathogens

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References

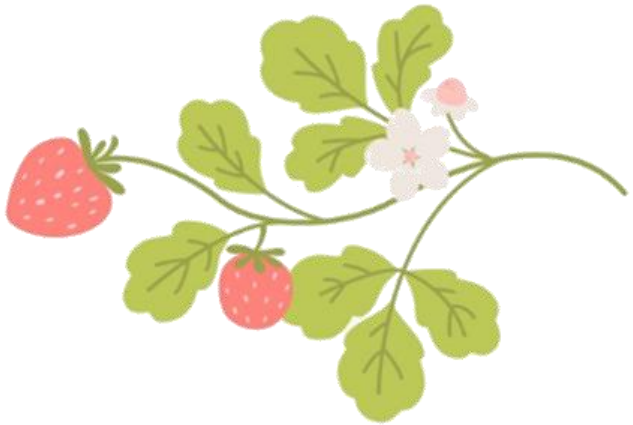
Baggio, J. S., Forcelini, B. B., Wang, N. Y., Ruschel, R. G., Mertely, J. C., & Peres, N. A. (2021). Outbreak of leaf spot and fruit rot in Florida strawberry caused by Neopestalotiopsis spp. *Plant disease*, *105*(2), 305-315.

Rebello, C. S., Wang, N. Y., Marin, M. V., Baggio, J. S., & Peres, N. A. (2023). Detection and species differentiation of Neopestalotiopsis spp. from strawberry (Fragaria× ananassa) in Florida using a high-resolution melting analysis. *PhytoFrontiers™*, *3*(1), 156-163.

Kaur, H., Gelain, J., Marin, M. V., Peres, N. A., & Schnabel, G. (2023). Development of a molecular tool for identification of a new Neopestalotiopsis sp. associated with disease outbreaks on strawberry. *Plant Disease*, *107*(5), 1544-1549.

Baek, J., Voss, A., Karki, D., Abaya, A., Pate, E., Shan, S., & Kalischuk, M. (2026). A Cleaved Amplified Polymorphic Sequence Assay to Differentiate Neopestalotiopsis Species With Differing Pathogenicity in Strawberry. *Plant Pathology*, *75*(4), e70249.

Thank you for Listening!



Questions?

