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# In Vitro Antifungal Effect of Essential Oil-Based Biopesticides and Synthetic Fungicides against the Avocado Scab Pathogen *Elsinoe perseae*

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## Abstract

Avocado scab, caused by the fungus *Elsinoe perseae*, is a major disease in most avocado producing regions with warm and humid climate<sup>1</sup>. Scab causes aesthetic damage to the peel of the fruits leading to significant post-harvest losses due to fruit rejection at the packing houses<sup>2</sup>. Although this pathogen causes economic losses and restricts the cultivation of sought-after but highly susceptible cultivars<sup>3,4</sup>, there are few available management approaches and almost none for organic growers. Our objective was to evaluate the in vitro antifungal activity of three essential oils (thyme oil, cinnamon oil, and tea tree oil) and three chemical fungicides (copper hydroxide, propiconazole, and fluazinam) against *E. perseae*. Six fungal isolates were obtained from infected tissues of the avocado cultivars Lula (n=2), Peterson, and Lima Late. Sensitivity to essential oils was assessed using an amended agar media assay with six concentrations of each essential oil. Colony diameter was used to determine EC50 values and minimal inhibitory concentrations within the tested isolates. Using an amended agar media assay, we determined that thyme oil (250 µl/l) and cinnamon oil (125 µl/l) inhibited *E. perseae* colony growth completely while tea tree oil only reduced *E. perseae* growth by 14% at the highest concentration tested (2000 µl/l). Sensitivity tests using synthetic fungicides showed that propiconazole (134 mg/l) completely inhibited the growth of the pathogen while copper hydroxide and fluazinam reduced the radial growth at 84% and 56%, respectively.

## Materials & Methods

**In vitro assays:** determination of (EC50), fungistatic and fungitoxic activity.

Six *Elsinoe perseae* isolates, three essential oils, and three synthetic fungicides.

Compounds	Brand name	Concentrations of active ingredient tested
	<b>(Active Ingredient)</b>	<b>tested</b>
Thyme oil	ThymeGuard® (23% a.i.)	62.5- 125- 250-500- 1000- and 2000 µl/l
Tea tree oil	TIMOREX <sup>ACT</sup> (12.5% a.i.)	62.5- 125- 250-500- 1000- and 2000 µl/l
Cinnamon oil	Cinnerate (60% a.i.)	62.5- 125- 250-500- 1000- and 2000 µl/l
Copper hydroxide	Kocide® 2000 (53.8% a.i.)	3.87 mg/l
Propiconazole	Tilt® (41.8% a.i.)	134 mg/l
Fluazinam	Omega®500F (40% a.i.)	465 mg/l



Fig 1. In vitro screening- Methodology summary.

## Results - Fungicide sensitivity test –Essential oils

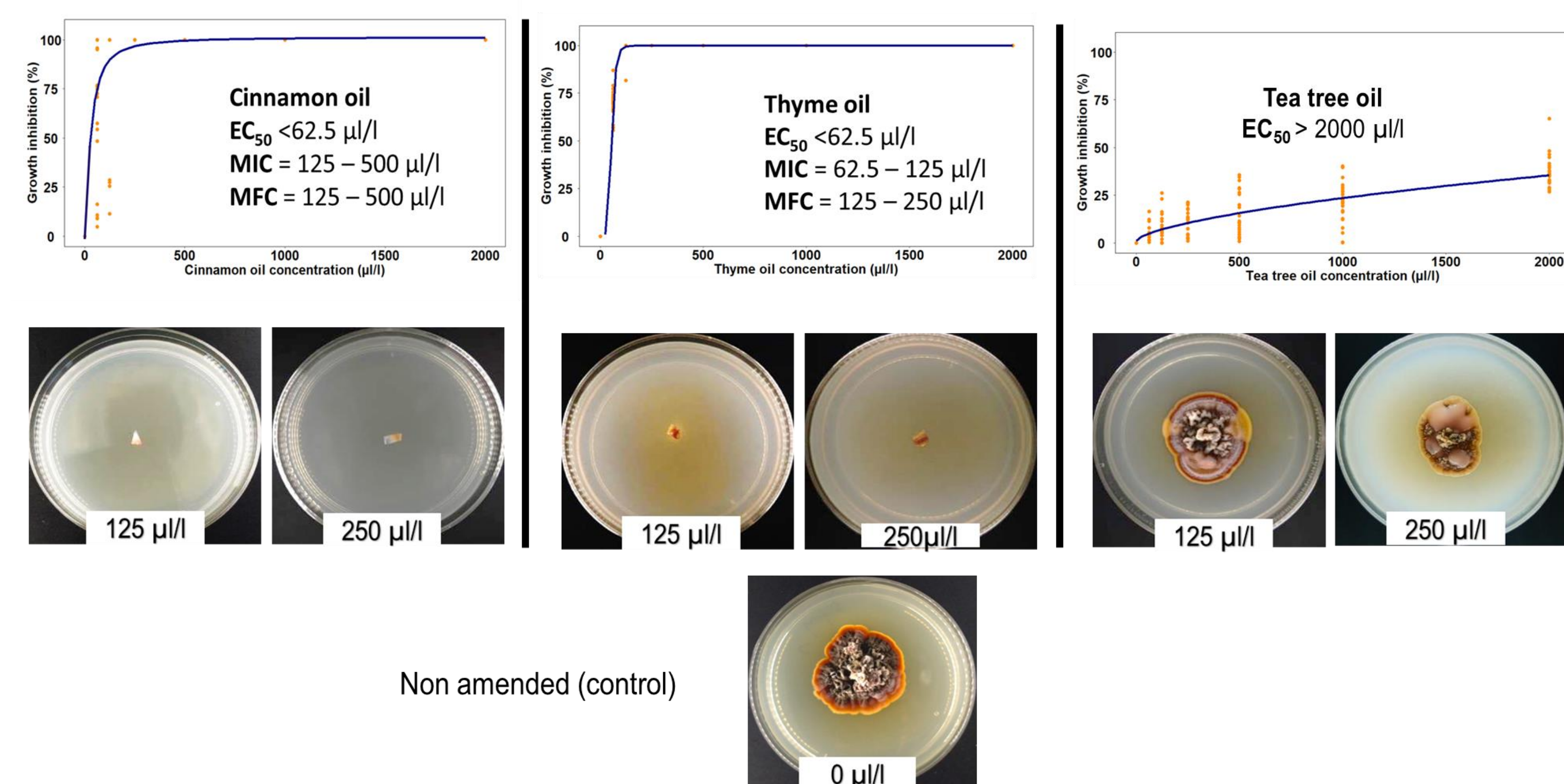
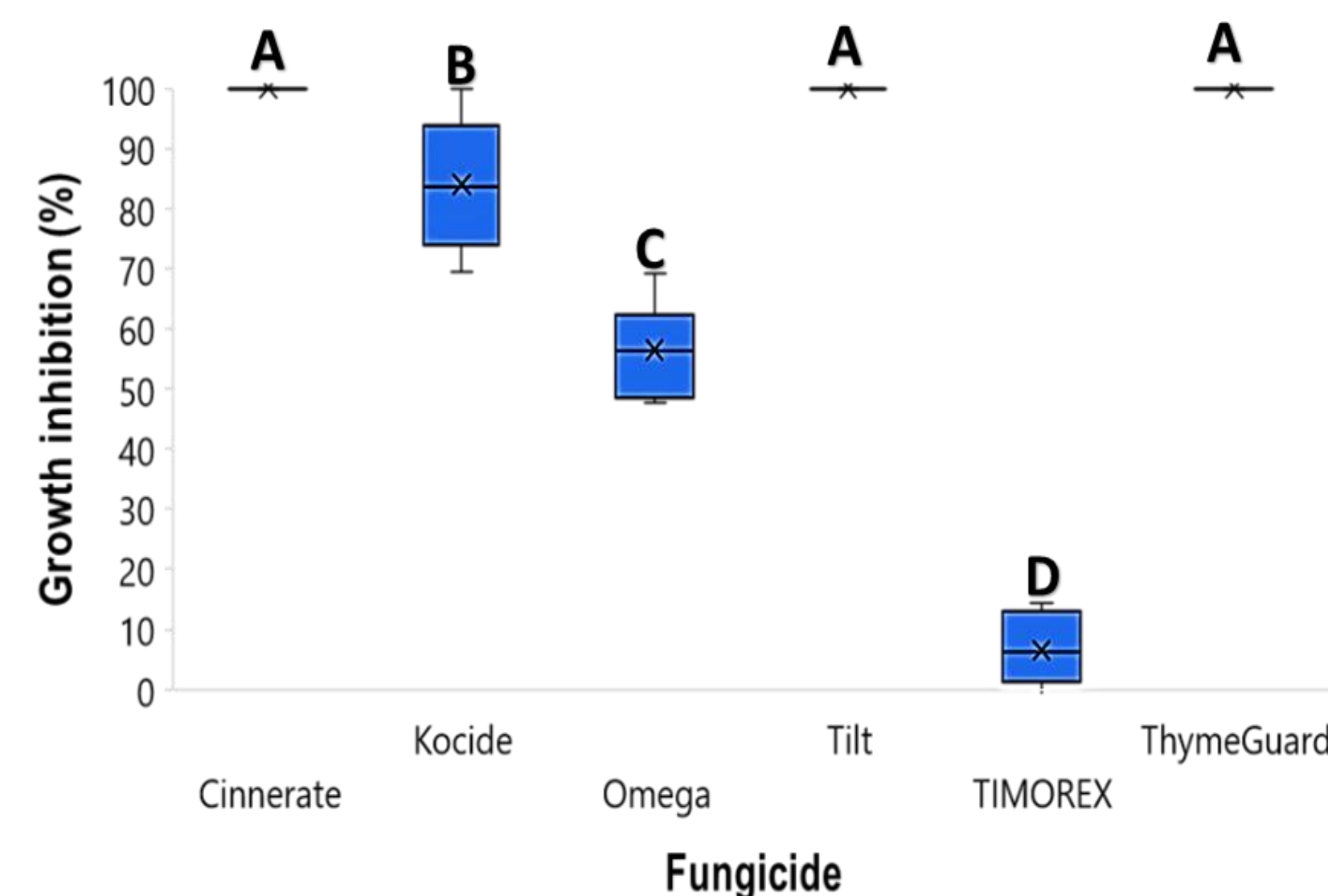
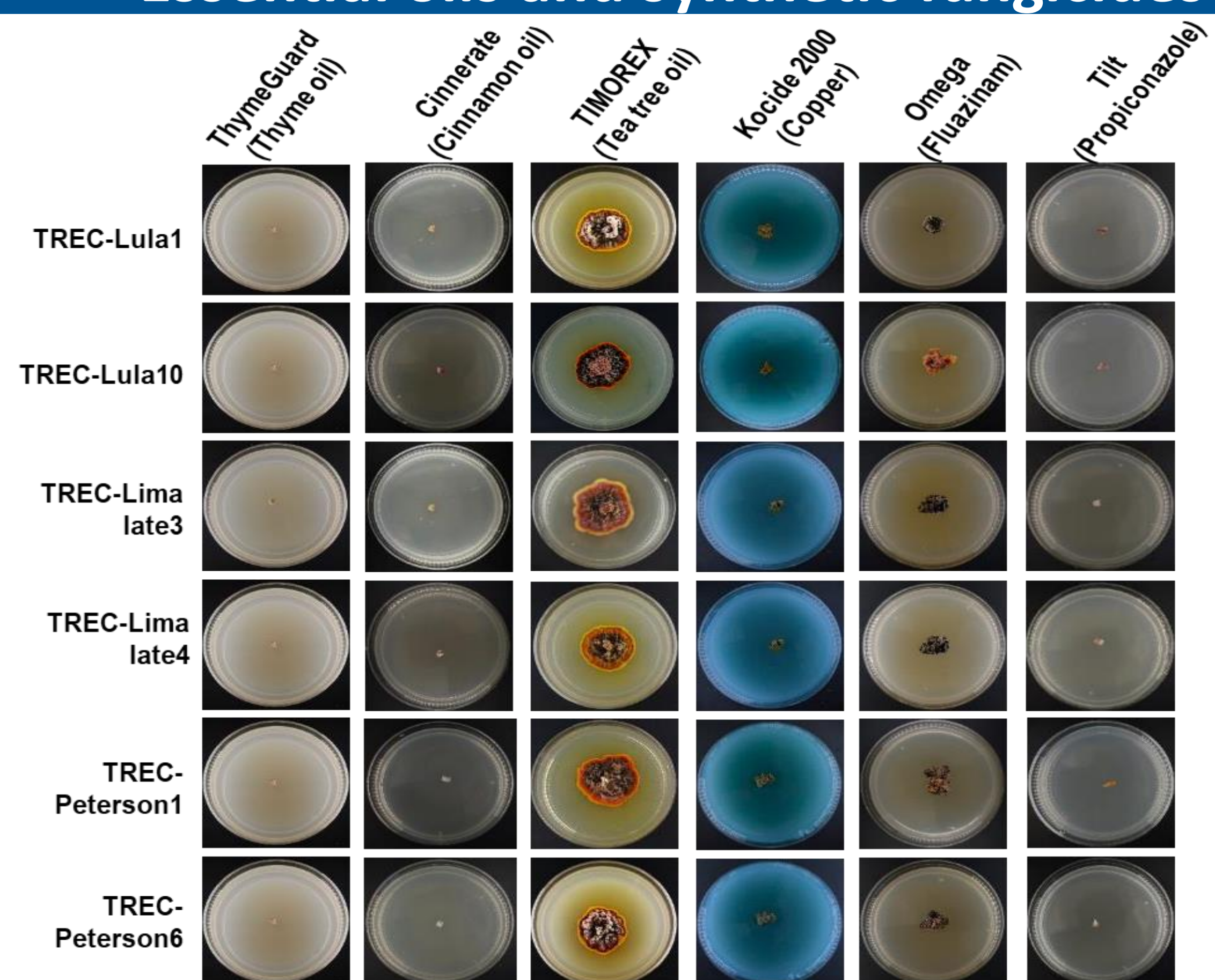


Fig 2. Antifungal activity of thyme oil, cinnamon oil and tea tree oil

- Thyme oil has fungistatic activity at ≥ 125 µl/l and fungicidal activity at ≥250 µl/l.
- Cinnamon oil has fungicidal activity at ≥ 125 µl/l.
- Tea tree oil did not inhibit completely (100%) or had fungicide activity against any of the six *E. perseae* isolates even at the highest concentrations tested (≤2000 µl/l).

## Fungicide sensitivity: Essential oils and synthetic fungicides



Field label rates of ThymeGuard (23 % thyme oil), Cinnerate (60% cinnamon oil) and Tilt (41.8% propiconazole) completely inhibited (100%) the growth of *E. perseae*.

Fig 3. Antifungal activity of essential oils and synthetic fungicides at label rates of commercial products.

## Future Work

- Formulations containing the compounds used in this study should be tested in the field as an alternative strategy for managing avocado scab.
- Future field trials are needed to evaluate the efficacy of propiconazole formulations applied as spray for controlling *E. perseae* in avocado
- Data from this study will provide guidelines to avocado producers who expect to adopt organic practices including plant-derived essential oil biopesticides.
- Testing sensitivity to synthetic fungicides at different concentrations to estimate EC50 values, including Abound (22.9% azoxystrobin)

## References

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# Generating Classical and Molecular Resources for Accurate Identification of Avocado Scab (*Elsinoe perseae*)

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## Abstract

Avocado scab, caused by the fungus *Elsinoe perseae*, is an important disease of avocado in most humid, tropical, and subtropical regions where avocados are commercially grown<sup>1,2</sup>. Scab causes aesthetic damage to the peel of avocado fruit causing significant post-harvest losses due to fruit rejection at packing houses<sup>3</sup>. Rapid and accurate disease diagnosis is critical for the early implementation of adequate and effective management practices that can mitigate their negative impact on crop production. Unfortunately, scab damage on avocado fruit, resulting from infections of *E. perseae*, is often confused with similar damage (e.g., scarring) resulting from non-fungal related causes such as thrips, beetles, and mechanical injuries. Avocado scab symptoms on fruits appear as blackish/brownish oval and raised forms of cork scab and are scattered on the skin of the fruit. Symptoms resulting from mechanical damage are localized to a specific part of the fruit while those caused by insects resemble brown leather that usually cover the entire surface of the fruit. We have developed classical resources to facilitate the identification of scab in the field, as well as to facilitate isolation, identification, and storage of the causal agent in the laboratory (from cultures). We also generated visual disease rating scales (leaf and fruit) to facilitate the monitoring of scab incidence and severity so that researchers and growers can make informed decisions on disease management practices (i.e., spray timing). This scale was used to assess the susceptibility of twenty-three cultivars grown in south Florida, resulting in the update of previous data published in 2019 for major green-skin cultivars<sup>4</sup>. The resources intended for the identification of the pathogen have been validated with molecular-based techniques. Among three commercially available DNA extraction methods tested, the genomic DNA extracted from samples (i.e., fruit and leaves) resembling typical symptomatology of scab using the Qiagen-QIAamp Fast DNA Stool kit allowed isolation of DNA from avocado host tissue that resulted in positive detection of *E. perseae*. The PCR-based assay protocol, which takes less than 24 hours for the detection of *E. perseae*, can be easily implemented across plant diagnostic clinics, allowing producers to make timely management decisions. Finally, our direct diagnostic protocol, standardized disease rating scales, and novel laboratory resources on the avocado scab pathogen, can be used to further research on this disease and expand our knowledge on cultivar susceptibility, fungicide efficacy, and strain virulence.

## Early symptoms from leaf and fruit tissue are ideal for scab recovery

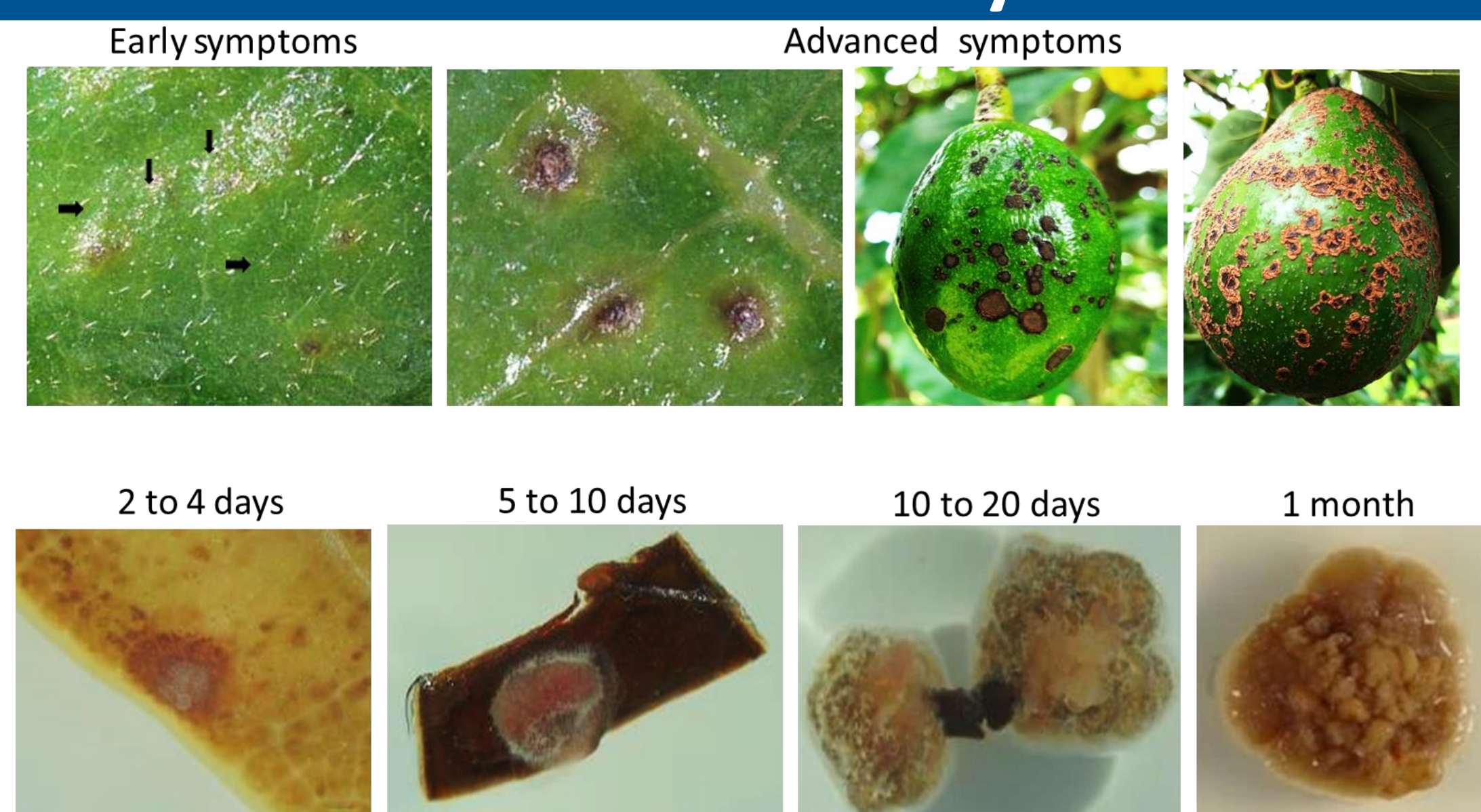


Fig 1. Symptomatic avocado specimens and isolates on PDA.

## Colony morphology varied based on isolate and media type

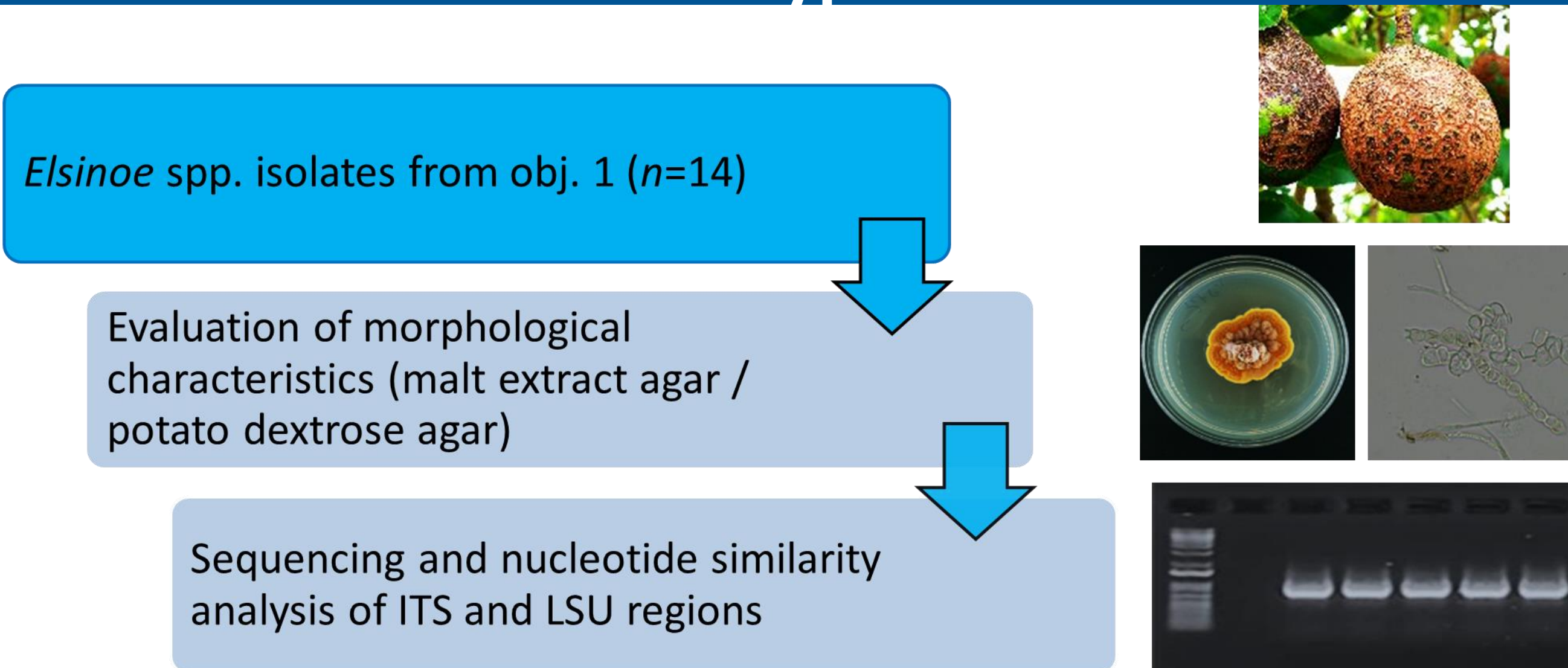


Fig 2. Methodology for morphological and genetic characterization.

## Based on ITS and LSU, two groups were found

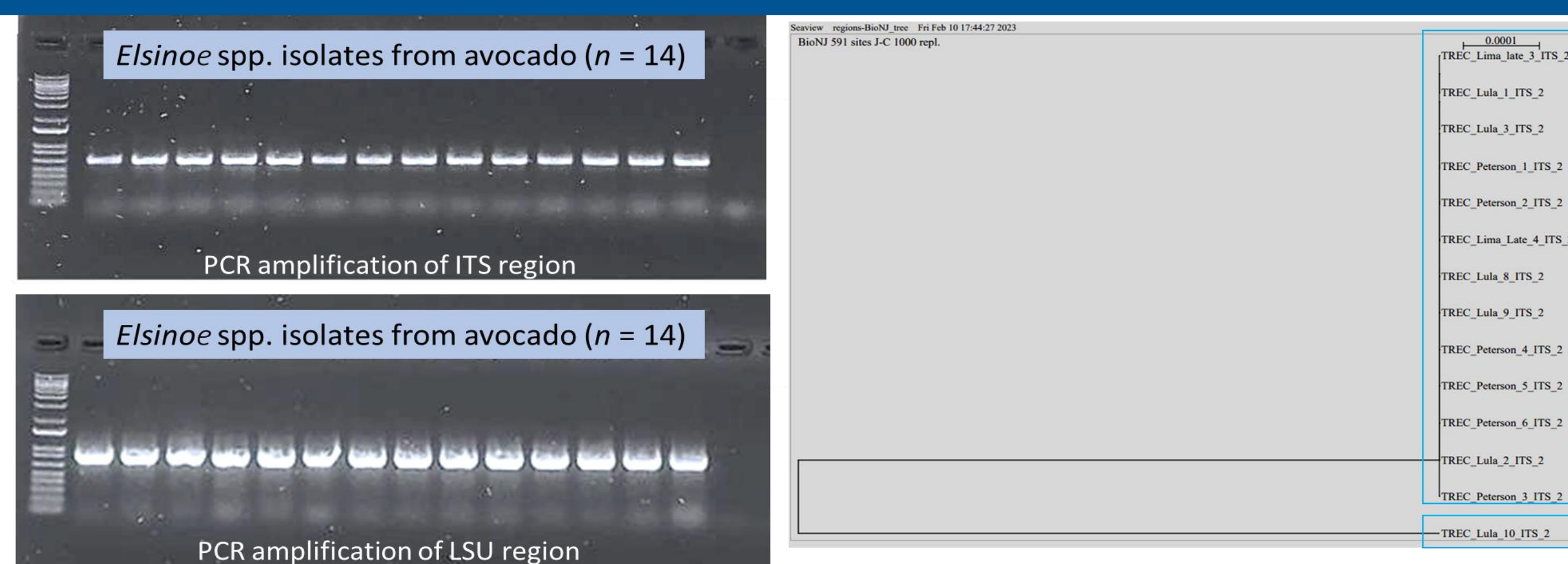


Fig 3. PCR amplification of ITS and LSU regions.

## Quick and accurate diagnostic protocol to differentiate mechanical damage from scab lesions



Figure 4. PCR amplification of the species-specific primers SpF5 and SpR6 using DNA from host tissue .

## Development of visual rating scale

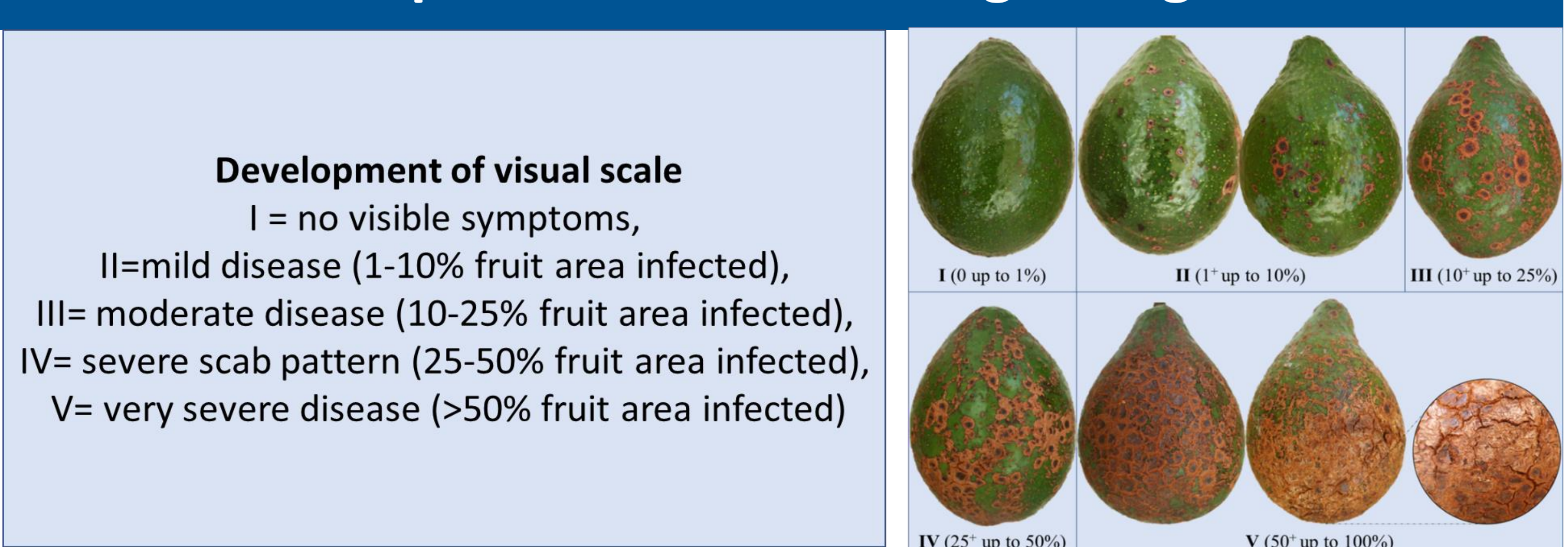


Figure 5. Disease severity rating scale used to score the level of scab damage on avocado fruit.

## Conclusions

This study

- Validated and optimized protocols for the isolation, accurate identification maintenance, storage
- Validated and optimized a species-specific DNA-based detection protocol to detect the pathogen directly from infected tissue
- Updated cultivar susceptibility grown in south Florida

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