

# Elucidating the Causes of *Opuntia littoralis* Decay in Native Nurseries and Restoration Sites in Southern California

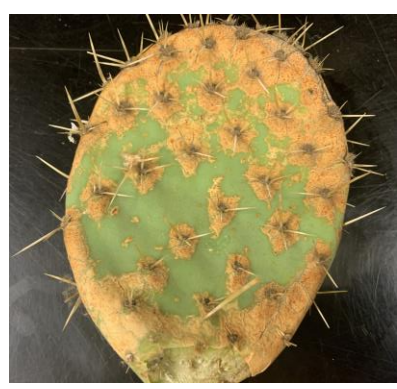
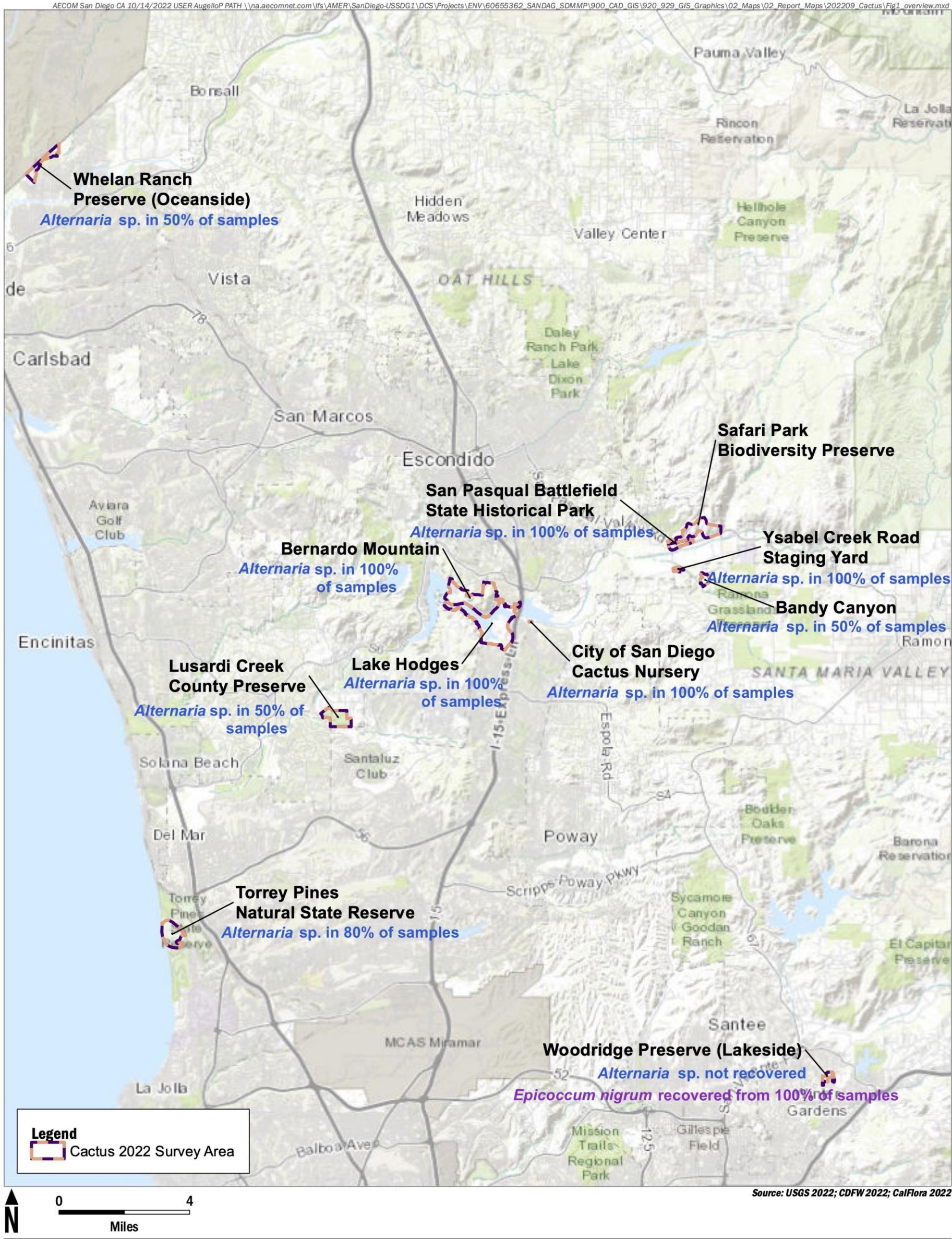
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## Challenge

- In 2021, *Opuntia littoralis* (Coastal prickly-pear) plants at a restoration nursery in San Diego County were observed with circular yellow, red, and gray dry lesions surrounding their spines
- In 2022, further field surveys across 10 restoration sites in San Diego County showed affected plants with similar decay symptoms, at 5-25% prevalence
- Cacti decay observed in restoration nurseries and in native habitats created concern about pathogen introductions into ecosystems that support coastal cactus wrens (a species of concern in CA)

## Pathogen Survey

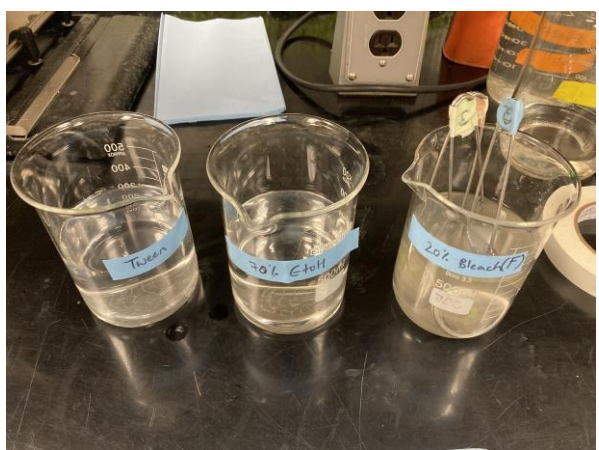
- Field surveying and sampling:** AECOM ecologists walked meandering through 10 restoration sites transects in San Diego County, collecting symptomatic cacti



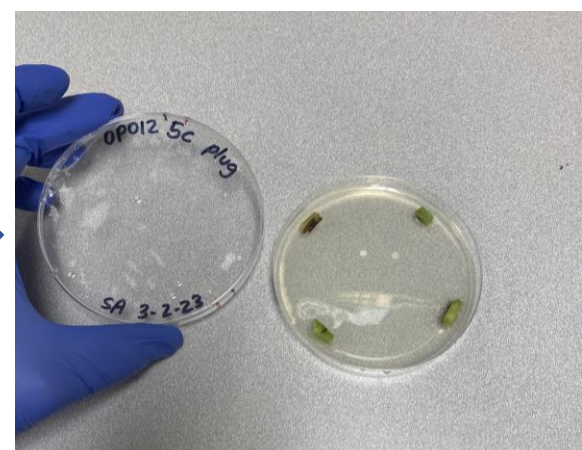
Above: Cladode sample in the field and in the lab

Left: 10 sampling locations with respective *Alternaria* sp. recovery rates

- Fungal isolation and identification:**



Symptomatic sections of cladodes were surfaced disinfested and margins of the lesions were placed on fungal selective medium (1/10 PDA + tetracycline)



Fungal pure cultures were recovered from plant tissue isolations



Molecular identification: DNA extraction from 130 individual samples followed by amplification and Sanger sequencing of ITS and ATPase regions

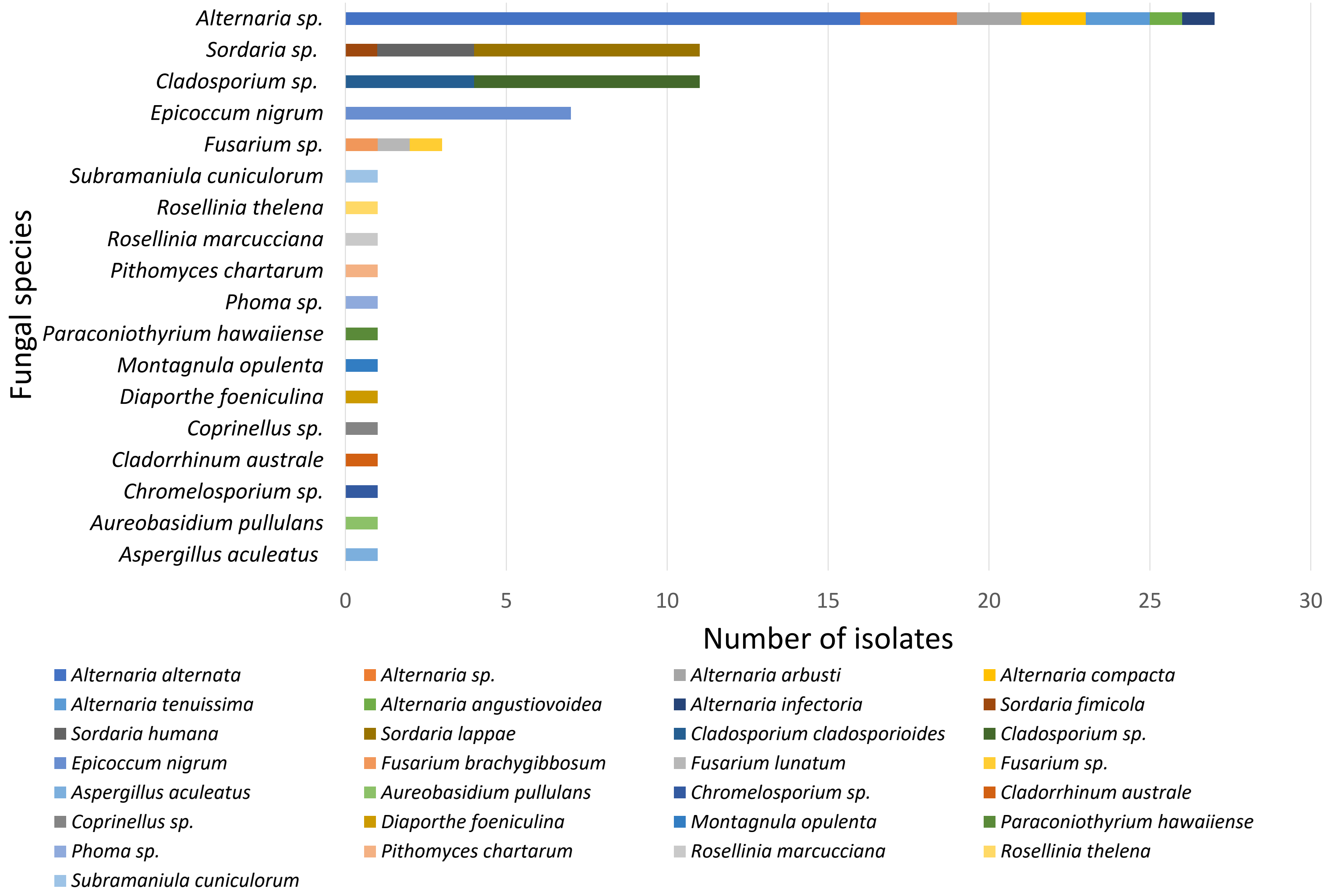
## References

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- Calflora: Information on California plants for education, research and conservation, with data contributed by public and private institutions and individuals, including the [Consortium of California Herbaria](https://www.calflora.org/app/taxon?crn=5940). [web application]. 2022. Berkeley, California: The Calflora Database [a non-profit organization]. Available: <https://www.calflora.org/app/taxon?crn=5940>
- Infante, N.B., da Silva, G.C.S., Feijó, F.M. et al. *Alternaria* species associated with cladode brown spot in cactus prickly pear (*Nopalea cochenillifera*). *Eur J Plant Pathol* **160**, 215–226 (2021). <https://doi.org/10.1007/s10658-021-02236-5>

## Objectives

- Determine what fungal species are associated with *Opuntia* decay
- Conduct pathogenicity trials to determine if the most prevalent fungi recovered are the causal agents of the observed symptoms
- Use our findings to inform advisors and growers and to further develop and implement best management practices to prevent pathogen spread

## Survey Results



- Alternaria* species were recovered from 81% of 21 samples collected at 9 out of 10 locations. Species included *A. alternata* (59%), *Alternaria* sp. (11.1%), *A. tenuissima* (7.4%)
- Cladosporium* sp. (48%), *Epicoccum nigrum* (33%), and *Sordaria* sp. (23.8%) were also recovered at high percentages of samples, but they have not been reported as plant pathogens

## Conclusion

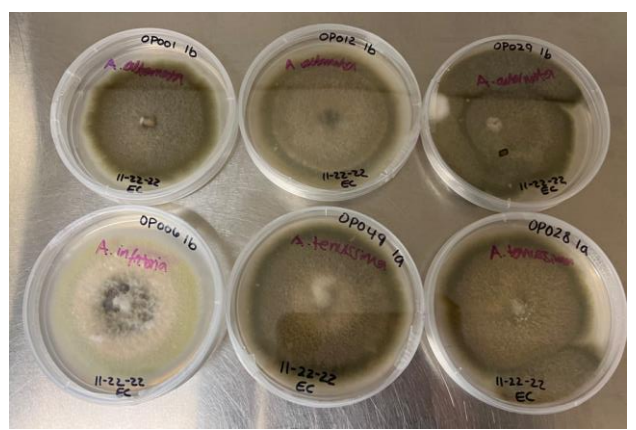
- Alternaria* species were the most frequently recovered (81%) fungi from symptomatic *Opuntia littoralis* from San Diego County surveys
- Inoculating healthy cladodes with *Alternaria* isolates (that were recovered from the initial survey) in the laboratory confirmed that these isolates cause similar symptoms to those observed in the field
- Both inoculation methods caused symptoms on healthy cladodes, but lesions caused by spore suspensions were less variable in size than plugs

## Current and Future Work

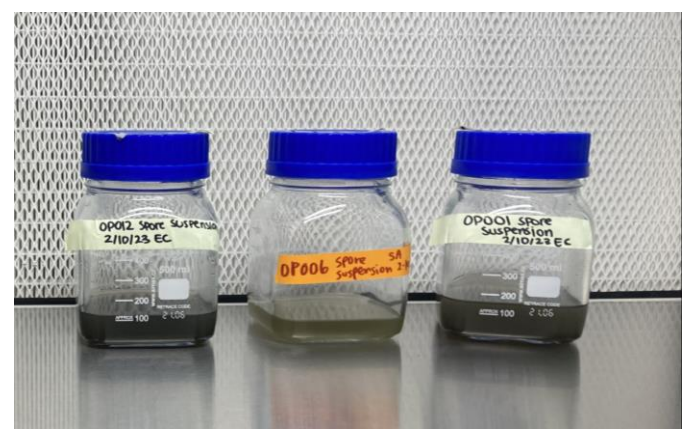
- To complete Koch's postulates:
  - We are reisolating *Alternaria* sp. from lesions on inoculated cladodes
  - We are conducting 2 additional replicates of the pathogenicity trial
- We will conduct greenhouse experiments to evaluate the effect of environmental factors—humidity and water availability—on disease severity
- Ultimately, we will be able to inform the development of best management practices based on our findings

## Pathogenicity Trial

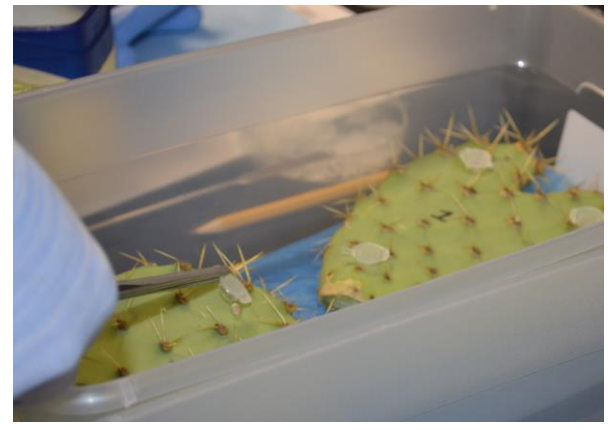
- A pathogenicity trial was conducted on healthy cladodes harvested from the initial sampling locations
- Healthy cladodes were inoculated with three frequently recovered *Alternaria* sp. (from samples collected at different locations)
- Two inoculation methods were evaluated: spore suspensions and infested plugs
- Lesion development was measured weekly starting a month after inoculation



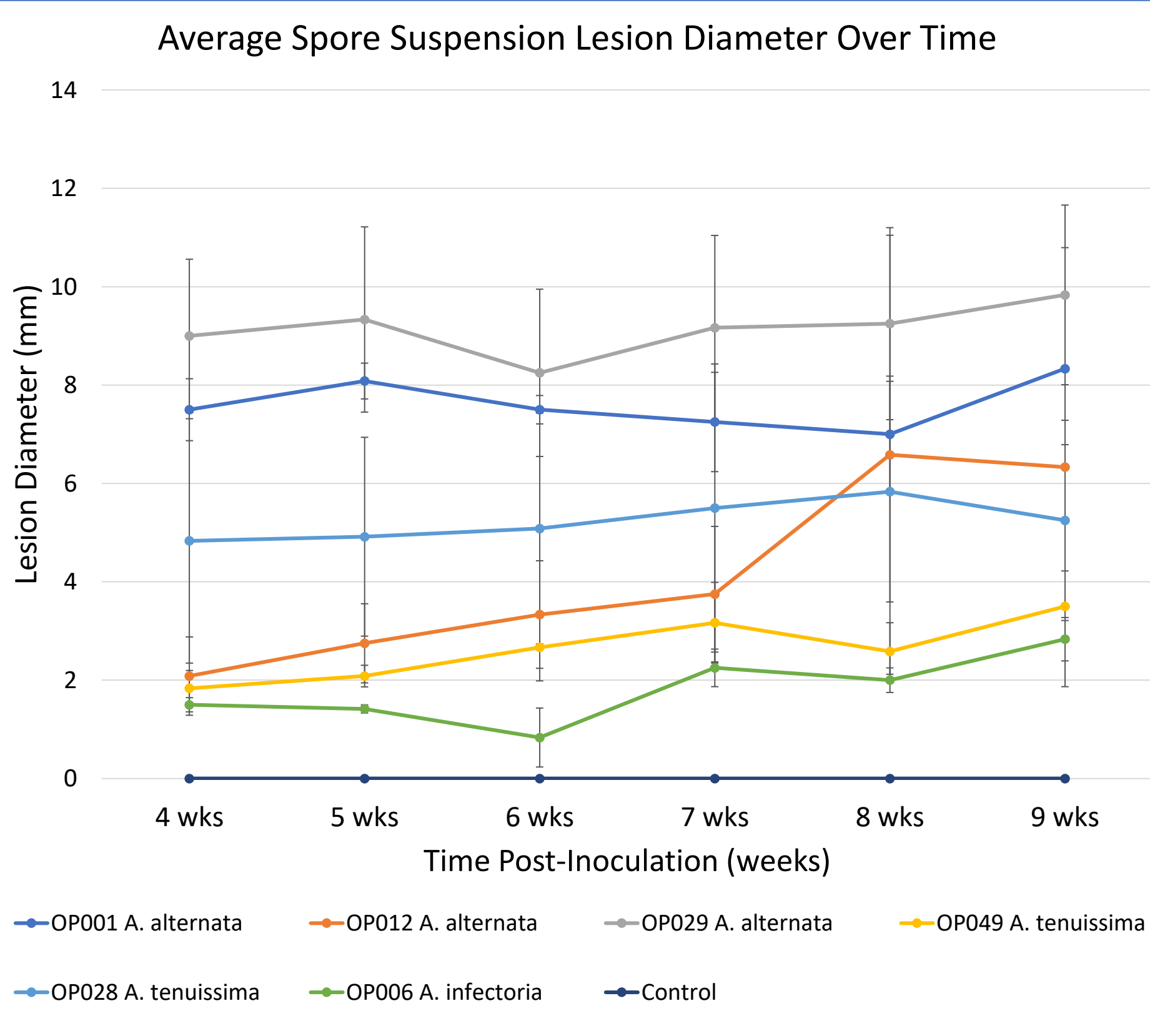
*Alternaria* isolates grown in 1/10 PDA + Tetracycline media



*Alternaria* sp. spore suspensions created

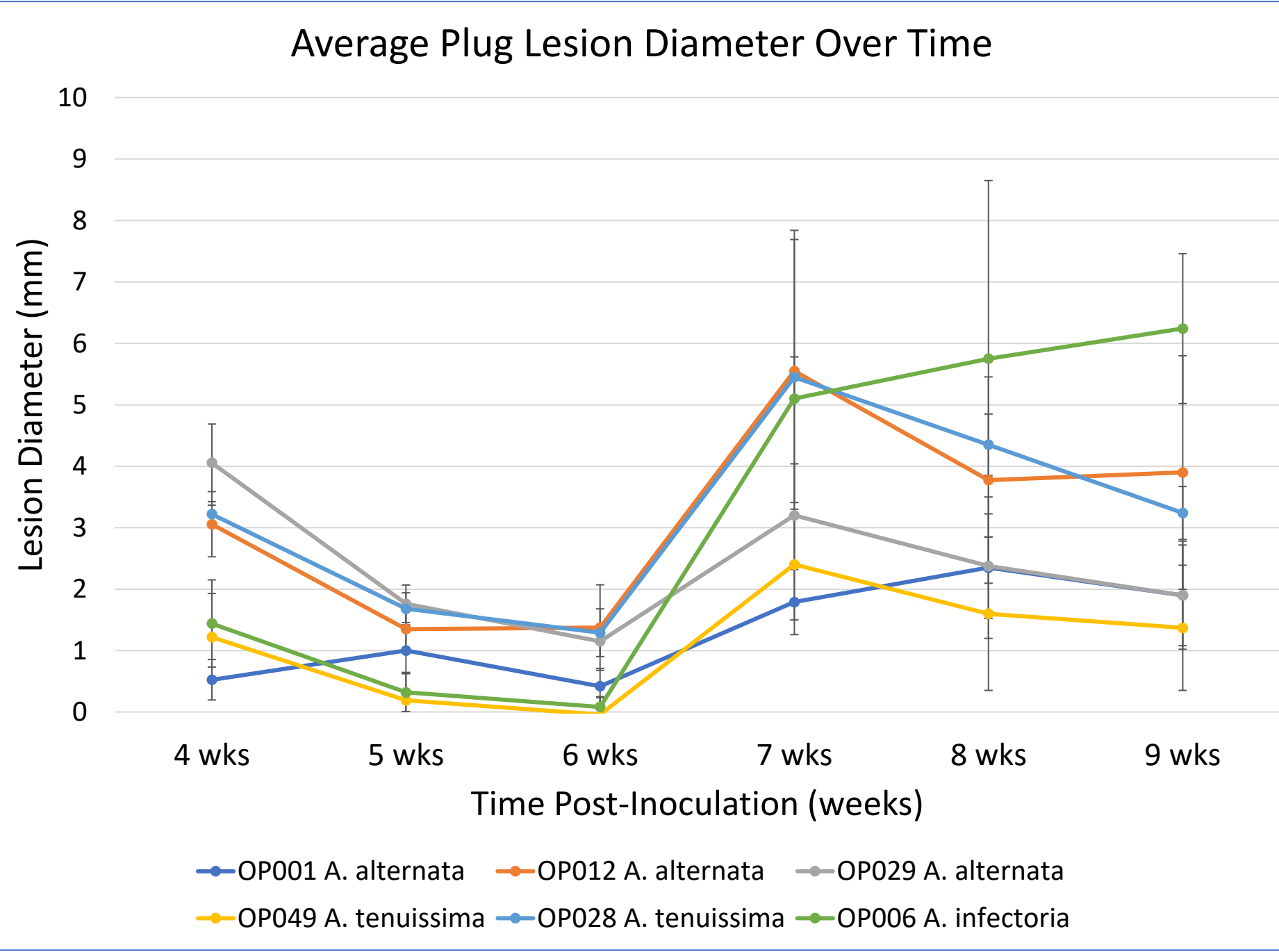


Inoculation of healthy cladodes



Healthy cladodes inoculated with *Alternaria* sp. using spore suspensions developed lesions ranging from 2.8 mm to 9.8 mm in 9 weeks

*Alternaria alternata* isolates were the most virulent



Healthy cladodes inoculated with *Alternaria* sp. using infested plugs developed lesions ranging (normalized) from 1.4 mm to 6.2 mm in 9 weeks

We observed minor lesions in the mock control for plug inoculated healthy cladodes, so this data was normalized to have the control as a baseline

## Acknowledgements

- We thank all members of the Del Castillo Greenhouse and Nursery Pathology Lab
- We thank AECOM for their collaboration and SANDAG for funding the project