

Ongoing surveys for *Armillaria* in California: DNA-based identification, bioclimatic modeling, and information for managing *Armillaria* root disease under changing environments

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Introduction

Armillaria root disease (ARD) is among the largest causes of mortality and lost productivity in forests of the western USA (Lockman and Kearns 2016, Kim et al. 2022). In some forest areas of western North America, growth/volume losses between 16-55% have been attributed to ARD (Filip and Goheen 1984; Cruickshank 2011). Damage from ARD is expected to increase under climate change and extreme weather events (e.g., drought, temperature), because host tree stress can predispose trees to ARD (Klopfenstein et al 2009; Kim et al. 2021; Murray and Leslie 2021). Furthermore, ARD may predispose trees to beetle attack or other pathogenic fungi (e.g., Kulhavy et al. 1984, Tkacz and Schmitz 1986, Williams et al. 1986).

Studies suggest that *Armillaria* species play an important role in driving the structure and successional trajectories of California forests by reducing the abundance of tree species with higher susceptibility to ARD, while favoring more resistant or tolerant species (Hawkins and Henkel 2011). Currently, little is known about which *Armillaria* species are involved in these ecological processes in California or whether interactions occur among multiple *Armillaria* species. Wide-ranging *Armillaria* surveys using DNA-based identification methods have not been conducted in California since the early 2000s (e.g., Baumgartner and Rizzo 2001), when four *Armillaria* species were found to occur: *A. mellea*, *A. gallica*, *A. nabsnona*, and *A. altimontana* (as North American biological species X; Brazee et al. 2012). In recent decades, more reliable DNA-based methods have been developed for identifying *Armillaria* species, which can distinguish closely related *Armillaria* species, such as *A. gallica* and *A. sinapina* (Kim et al. 2006, Hanna et al. 2007, Ross-Davis et al. 2012, Klopfenstein et al. 2017).

Data from iNaturalist suggests that *Armillaria* is likely more abundant and widespread in California than previously known (Figure 1, iNaturalist 2023). However, the iNaturalist data have not been confirmed and likely contain some misidentified species. For example, iNaturalist reports list four species (*A. sinapina*, *A. ostoyae*, *A. novae-zelandiae*, and *A. borealis*) that have not yet been confirmed in scientific literature to exist in California, and few records (if any) use DNA-based identification (Figure 2, iNaturalist 2023). The report of *A. ostoyae* likely refers to *A. solidipes*, which was formerly known as North American *A. ostoyae*; however, *A. solidipes* has not yet been confirmed in California. Two other species (*A. novae-zelandiae* and *A. borealis*) have not yet been reported in North America using DNA sequence-confirmed identification, and these species would represent invasive species, if confirmed. As a complicating factor, some *Armillaria* species have been recognized as morphologically indistinguishable using microscopic and macroscopic observations. Also, for the purpose of bioclimatic modeling of *Armillaria* spp., iNaturalist data appear to have significant sampling bias associated with populated areas (Figure 3, iNaturalist 2023). Furthermore, preliminary surveys indicate that ARD is perhaps increasing in California

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(Figure 4), especially in the context of increased climate-induced stress on trees due to relatively rapid shifts in climate and extreme weather events. For these reasons, new *Armillaria* surveys are needed in California that combine accurate location information (e.g, GPS coordinates) with DNA-based identification.

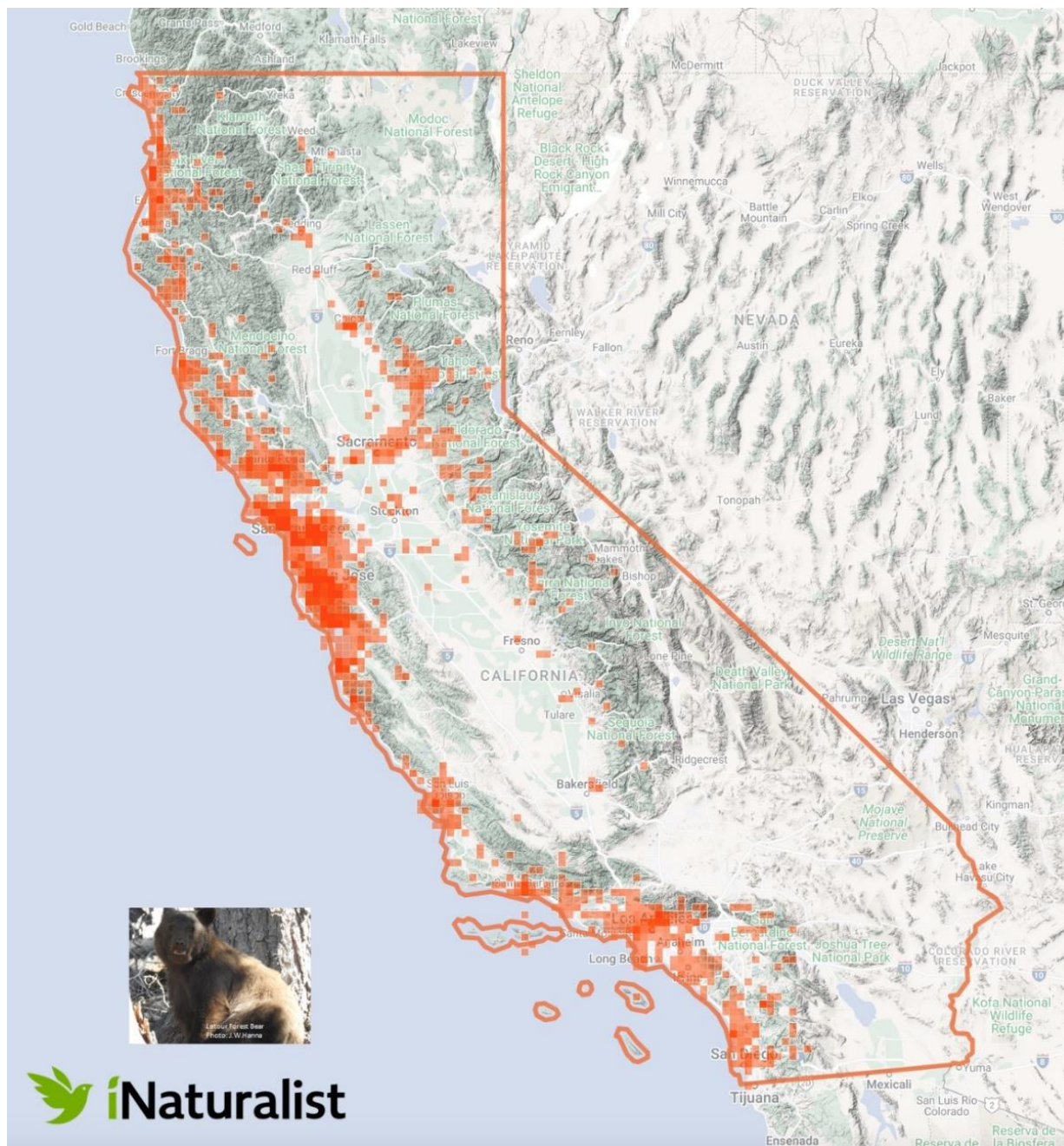
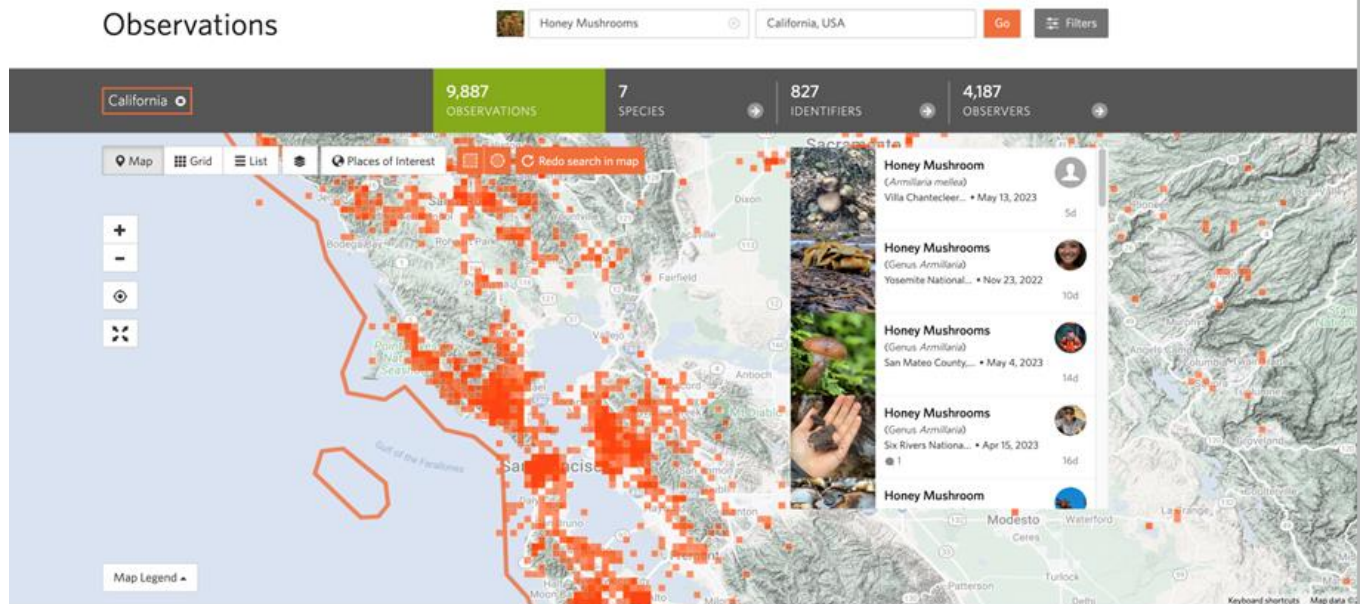


Figure 1: Range of *Armillaria* species in California based on 9,887 iNaturalist occurrence records.

Observations



Observations

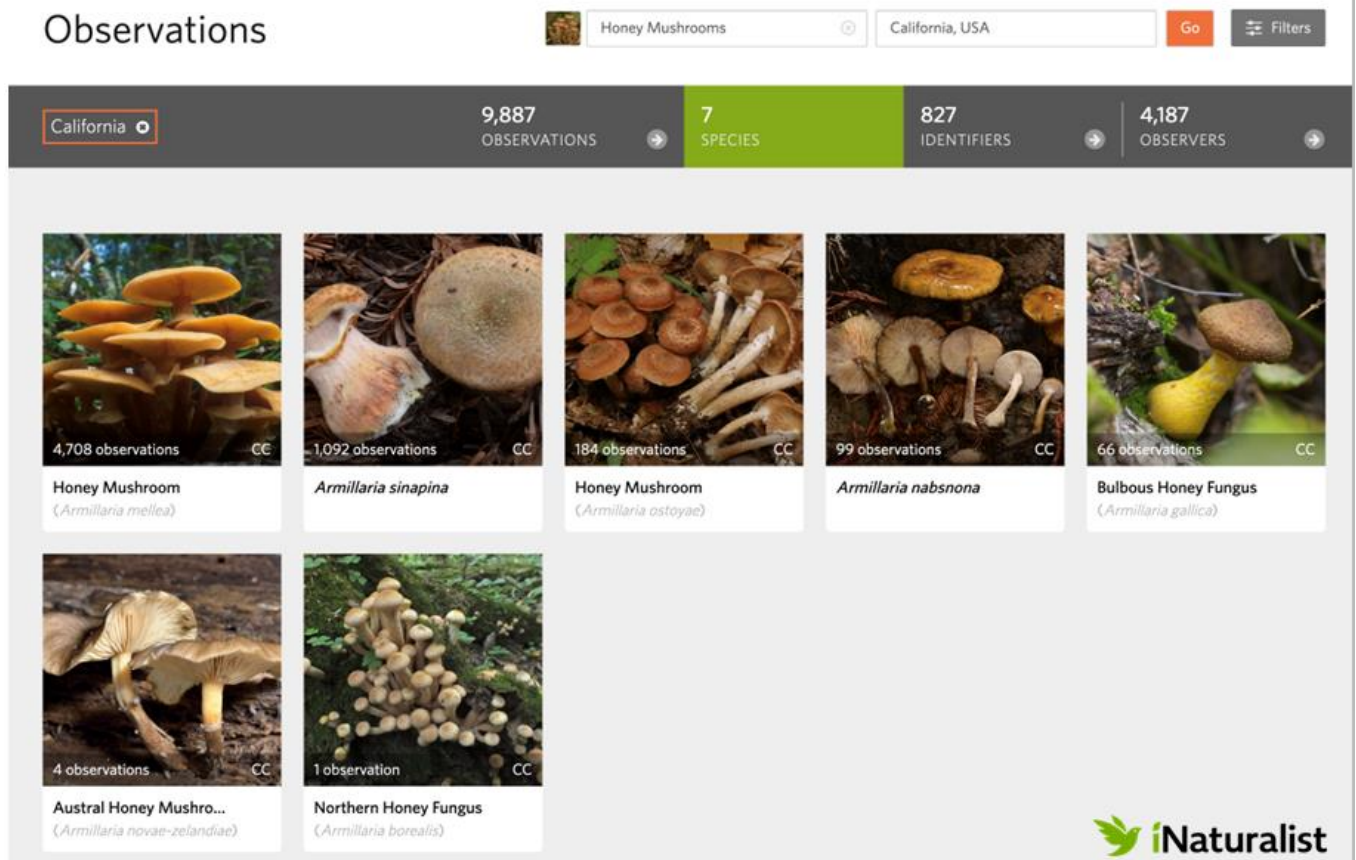


Figure 2: Example of *Armillaria* species data for California from iNaturalist.

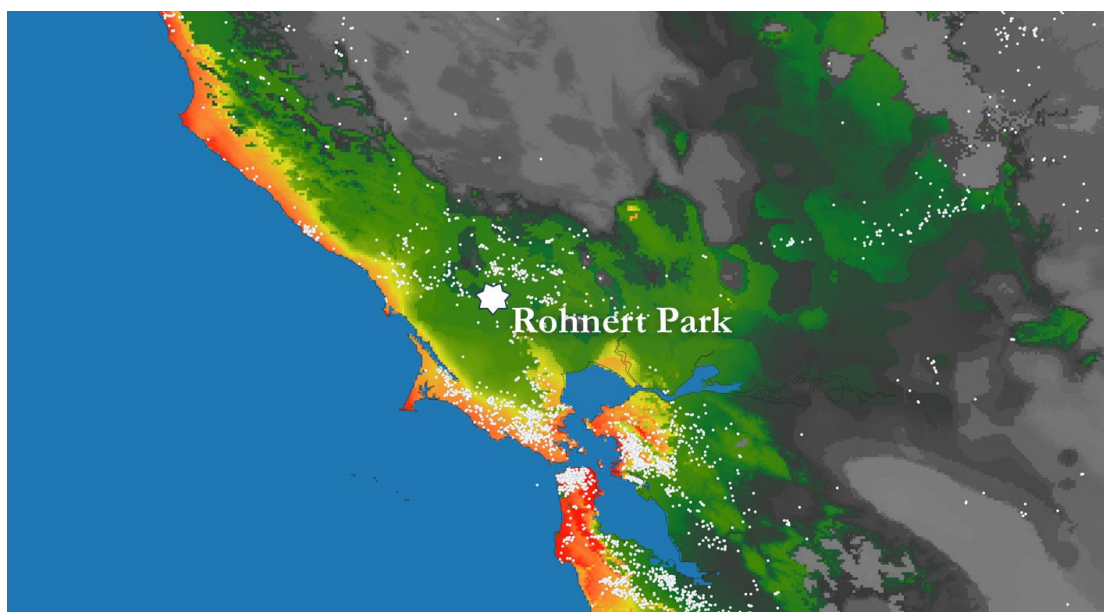


Figure 3: Example contemporary bioclimatic model for all *Armillaria* spp. centered on Rohnert Park, California, based on unconfirmed occurrence records from iNaturalist records (white dots). Darkest gray represents predicted suitable climate space, with light green, yellow, orange, and red indicating increased probabilities of climate suitability, respectively. Note the significant amount of sampling bias in populated areas.

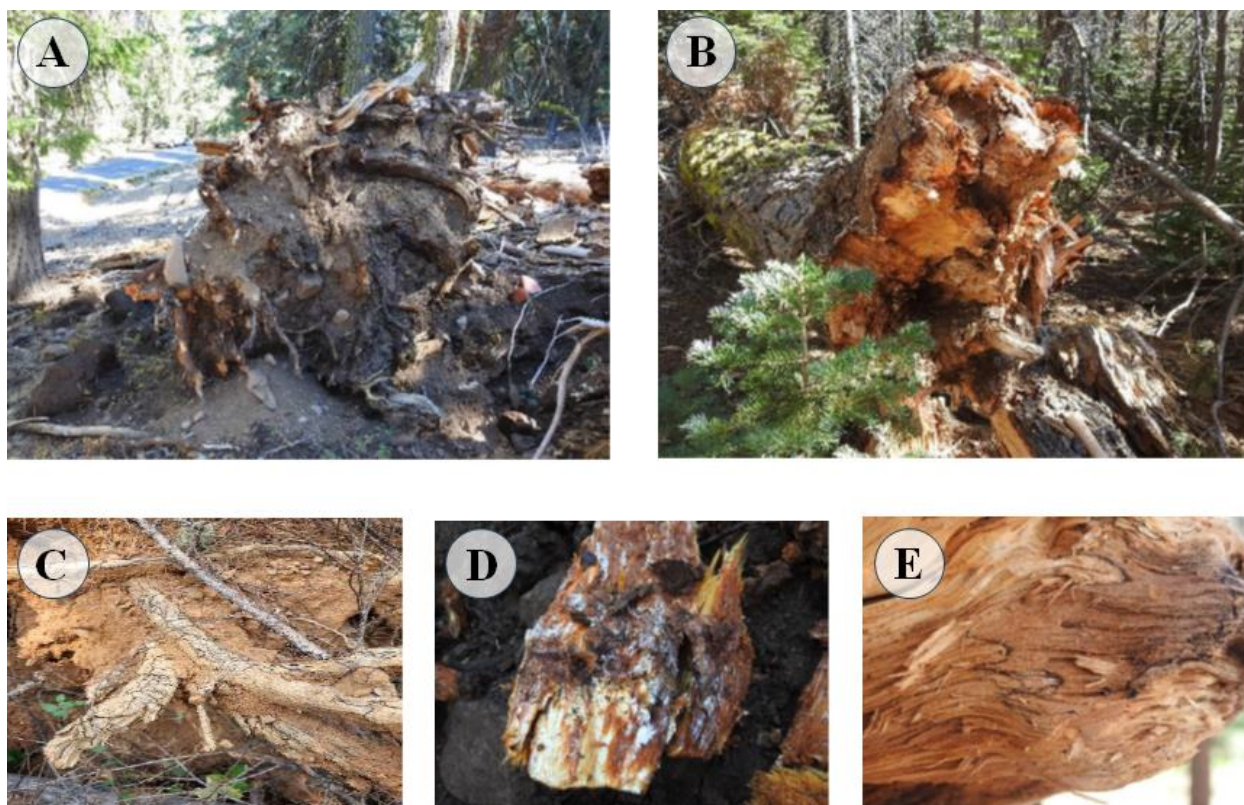


Figure 4A-E: 4A: Windthrown *Abies concolor* with *Armillaria* root disease in Klamath National Forest and 4B: Latour State Forest; 4C: Rhizomorphs from unidentified *Armillaria* spp. on windthrown tree in forest associated with the Karuk Tribe (Six Rivers National Forest) in northwestern California; 4D: Mycelial fan and rhizomorphs associated with *Armillaria* root disease of *A. concolor* in Kamath National Forest; and 4E: wood rot with zone lines associated with *Armillaria* root disease of *A. concolor* in Latour State Forest.

Objectives

The objectives of this project are to (i) conduct collections/surveys across diverse forest areas in California from which *Armillaria* will be isolated, cultured, and identified using DNA sequences; (ii) use *Armillaria* occurrence data to evaluate and refine bioclimatic models for predicting the present and future suitable climate space (the geographic area that is climatically suitable for a particular species' survival) for representative *Armillaria* species in California (if sufficient occurrence points are identified for the species); and (iii) produce predictive maps from the bioclimatic models to inform forest manager decisions in relation to ARD. These models can be compared to parallel bioclimatic models of important forest host species within the region. Any new *Armillaria* species/host combinations within California will be documented.

To supplement under-represented areas, additional *Armillaria* surveys/collections are needed across California (Figure 1). We ask collaborators to help survey for *Armillaria* within these areas and send us *Armillaria* samples (e.g., mycelial fans on live trees indicating disease activity, rhizomorphs, fruiting bodies) along with GPS information, associated host/environmental information, and photos.

Methods

DNA sequence-based diagnostics will be conducted to identify *Armillaria* species collected from diverse areas across California. Primary root systems and butts of trees and shrubs will be examined, and samples (i.e., rhizomorphs, mycelial fans, rotten wood, fruiting bodies) of *Armillaria* spp. will be collected along with precise location and associated host/environmental data. Cultured *Armillaria* isolates from each site will be identified using DNA sequencing (e.g., Kim et al. 2006, Hanna et al. 2007, Ross-Davis et al. 2012, Klopfenstein et al. 2017). Sampling locations will initially be selected based on local knowledge of areas with ARD from USDA Forest Service, other forest health professionals (e.g., CAL FIRE), and iNaturalist records of interest. Some targeted sampling will be based on preliminary models generated from surrounding regions, previous collections, and underrepresented areas that appear likely to support *Armillaria* spp.

Armillaria survey data will be integrated into a bioclimatic model to predict suitable climate space across California and adjacent regions. A bioclimatic model, such as MaxEnt (Phillips et al. 2006), will be used to determine which climatic factors are associated with the occurrence of *Armillaria* spp. across the landscape. Future distributions of suitable climate space for *Armillaria* will be predicted based on various Global Circulation Models and greenhouse gas-emission scenarios (Klopfenstein et al. 2009, Hanna et al. 2016, Kim et al. 2021, 2023), and compared with contemporary/future suitable climate spaces for major host-tree species, which have already been predicted (Rehfeldt et al. 2006). In addition, predictions will be updated with improved climate surfaces, as available. In general, potential distribution of suitable climate for ARD pathogens will be predicted, with increased risk for disease in areas where the climate is suitable for ARD pathogens to occur, but the tree host is climatically maladapted.

Expected Outcomes and Benefits

At the completion of these studies, we expect to have a much clearer picture of 1) which *Armillaria* species are present in California, 2) the present and predicted future ranges of major *Armillaria* species in California, and 3) areas in California where forest management should consider and/or address ARD. Resulting products and information from these studies will be made available to diverse groups, ranging from forest health professionals to amateur mycologists. Both contemporary and future bioclimatic model predictions will be made available via publications, presentations, on-site workshops, and/or online websites.

Acknowledgements

This project is supported by USDA-Forest Service, State and Private Forestry, Forest Health Protection - Special Technology Development Program (STDP-R5-2023-01), Rocky Mountain Research Station - Forest Woodland Ecosystems Program, and Pacific Northwest Research Station – Fire, Fuel, and Smoke Program. The findings and conclusions in this publication are those of the authors and should not be construed to represent any official USDA or U.S. Government determination or policy.

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Proceedings of the 68th Western International Forest Disease Work Conference

DoubleTree Hotel

Rohnert Park, California

June 5-9, 2023



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Papers are formatted and have minor editing for language and style but otherwise are printed as they were submitted. The authors are responsible for content.

Citation:

Brooks, R.K. (comps.) 2024. Proceedings of the 68th Western International Forest Disease Work Conference, Rohnert Park, California, 5-9 June 2023. <https://www.wifdwc.org/>

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