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Reaching a tipping point? A study on the roles of drought and fungal pathogens in canopy

dieback of a classic chaparral shrub, big berry manzanita (*Arctostaphylos glauca*)

A dissertation submitted in partial satisfaction of the

requirements for the degree of Doctor of Philosophy

in Ecology, Evolution, and Marine Biology

by

Laura Drake Schultheis

Committee in charge:

Professor Carla D'Antonio, Chair

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Professor Dar Roberts

Professor Hillary Young

March 2021

The Dissertation of Laura Drake Schultheis is approved.

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March 2021

Reaching a tipping point? A study on the roles of drought and fungal pathogens in canopy

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Acknowledgements

A tremendous amount of gratitude is owed to those who guided and supported me in the process of completing this dissertation. My advisor, Carla D'Antonio, has been instrumental in pushing me to ask better questions, seek answers from different angles, and cultivate the art of storytelling in science, all while investing in the pursuit of my own unique passions. I am not sure I could have chosen a better mentor. My committee members Ryoko Oono, Dar Roberts, and Hillary Young have also played invaluable roles in the development of this thesis. I am so grateful to them for lending their expertise and guidance along the way. I also could not have completed this work without generous financial support from the Schuyler Endowment, California Native Plant Society, The Coastal Fund, Cheadle Center for Biodiversity and Ecological Restoration, Earth Research Institute, and the UCSB Graduate Division and EEMB department.

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the way for my journey as an both an educator and scientist. Your support over the years has meant more than I can express, and that I now get to call you my dear friends and colleagues is honor and a dream-come-true.

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Drake-Schultheis, L., R. Oono, C. M. D'Antonio (2020). Mechanisms of severe dieback and mortality in a classically drought-tolerant shrubland species. *American Journal of Botany, 107(8)*: 1136-1147.

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Drake-Schultheis, L., D'Antonio, C. M. Landscape-level patterns of stress and dieback in *Arctostaphylos glauca* during extreme drought in southern California. *In prep.*

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- Introductory Biology lecture and lab (Spring 2021)

Teaching Assistant, University of California, Santa Barbara, CA (2013 – 2019)
- Environmental Ecology – ES (Fall 2014 - 16, 2018)
- Restoration Ecology Lab – EEMB/ES (Spring, 2014 - 2019)
- Intro to Environmental Studies – ES (Winter, 2016)
- Introduction to Plant Ecology – EEMB (Winter 2014, 2015)
- Introduction to Biology Lab – MCDB (Fall 2013)

Science Teacher, Grades 9-12, Providence Hall School, Santa Barbara, CA (2008 – 2013)
- Oversaw science and math departments as department head
- Developed curriculum for 9-12 grade science and wrote course catalogue descriptions to get all science courses approved by the CSU/UC systems and AP College Board.
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Schultheis, Laura Drake, "Drought, fungi, and death in *Arctostaphylos glauca*," Southern California Botanical Society Meeting, April 2017.

Schultheis, Laura Drake, "Reaching a tipping point in big berry manzanita?" UCSB Interdepartmental Emphasis in Environment and Society Symposium, April 2016.

Schultheis, Laura Drake, "Synergies between a fungal pathogen and exceptional drought in *Arctostaphylos glauca*," Southern California Chaparral Symposium, June 2015.

Schultheis, Laura Drake, "Reaching a tipping point in big berry manzanita? Synergies between a fungal pathogen and exceptional drought," Southern California Botanical Society Meeting, April 2015. Won award for Best Student Presentation.

RELATED MENTORSHIPS AND SERVICES

Undergraduate research advisor, UCSB (2016-present)
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- Helping develop questions and write grant proposals for student research grants
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Graduate Student Symposium Committee, UCSB (2014 – 2016)
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- Certified mentor for Santa Barbara County Teacher Induction Program
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Abstract

Reaching a tipping point? A study on the roles of drought and fungal pathogens in canopy

dieback of a classic chaparral shrub, big berry manzanita (*Arctostaphylos glauca*)

By

Laura Drake Schultheis

Extreme droughts are increasing in frequency, severity, and duration in arid and semi-

arid regions around the world due to climate change. As a result, plant species that are

typically capable of withstanding regular drought stress are exposed to conditions outside of

their normal range, rendering them susceptible to opportunistic disease-causing agents.

Theoretical frameworks describing the roles of environmental and biotic stressors in driving

plant mortality are well established. However, there is a lack of empirical data with which to

resolve how these factors interact in vivo. Furthermore, studies that document progression of

stress and dieback throughout the course of a multi-year drought event in situ are rare. In this

dissertation, I detail a series of studies aimed at understanding mechanisms of dieback and

mortality by focusing on a severe canopy dieback event in a classically drought tolerant

chaparral shrub, big berry manzanita (*Arctostaphylos glauca*) in Santa Barbara, California,

during an historic California drought. I provide strong evidence that dieback is caused by

members of the fungal Botryosphaeriaceae (*Bot.*) family in conjunction with extreme

drought, and that dieback is also related to increased drought stress along an elevational

gradient. By conducting a field survey, I identify *Neofusiccocum australe* as the most

prevalent and widely distributed fungal pathogen in *A. glauca.*, and that dieback is strongly correlated with *Bot.* infection. Using a full-factorial design in a greenhouse experiment, I provide evidence that extreme drought and infection by *N. australe* can indeed act synergistically, together driving faster and greater mortality in young (approx. one-year-old) *A. glauca* than either factor alone. Lastly, by taking measurements on water availability, dark-adapted leaf fluorescence, and photosynthesis in *A. glauca* shrubs across an elevational gradient, I provide evidence that landscape-level factors can contribute to localized variability in water stress and canopy dieback severity in *A. glauca*, and may be useful in predicting vulnerabilities during future drought. Remarkably, no new mortality was observed throughout the study, suggesting extreme resiliency in adult shrubs. However, canopy dieback alone can impact wildlife and fuel loads, even when not associated with mortality. Together, these results provide strong evidence that *A. glauca* dieback was caused synergistic effects between extreme drought and infection by *N. australe*, and that lower elevations and exposed slopes may be at greatest risk for future events.

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Chapter 4. Identifying landscape patterns of stress and dieback in big berry manzanita across a multi-year drought.

Chapter 1: Introduction

There is now strong evidence that the Earth's climate is changing due to human activities (NRC, 2010, IPCC 2013). According to the most conservative estimates, global mean annual temperatures are now outside the historic range of the last 1,300 years (IPCC 2013). Simultaneously, mean annual precipitation has declined in many parts of the Northern Hemisphere, resulting in increased drought events (Carnicer et al., 2010). Extreme climatic shifts are predicted to affect, both directly and indirectly, biogeochemical cycling, energy fluxes, wildlife habitat, and ecosystem goods and services on a global scale (Adams et al, 2010; Anderegg et al., 2012; Breshears et al., 2011). An important component in preparing for the effects of these events is to understand how (and to what extent) communities will change in response to them, making this a critical topic for ecological research (Sutherland et al., 2013).

For species to survive in dry climates, they must have evolved drought tolerance mechanisms (e.g. drought-deciduousness, seasonal canopy loss, waxy cuticles, sclerophyllous leaves, and stomatal regulation). However, extreme climate events can expose species that are typically capable of withstanding regular drought stress to conditions outside of their normal range. Furthermore, physiological responses to extreme drought can also have a negative feedback on plants' defensive abilities, rendering them susceptible to biotic attack including by insects or disease agents (Hicke and Zeppel, 2013; McDowell, 2011). Consequently, synergies between extreme climatic events and biotic attack will likely lead to more dramatic changes than would otherwise occur in historically "drought tolerant" plant communities (Breshears and Allen, 2002; Breshears et al., 2009). Future climate change is

expected to exacerbate these interactions worldwide. (Breshears and Allen, 2002; IPCC 2007, 2013; Breshears et al., 2009; Allen, 2010).

Widespread tree mortality from drought has been documented in forested systems around the world (Allen et al., 2010), and biotic attack has been associated with many of these events (Breshears et al., 2005; Logan et al., 2003; Mueller et al., 2005). However, much less focus has been given historically to understanding the consequences of extreme drought on shrubland communities like chaparral (though see Davis et al., 2002; Jacobsen et al., 2007; Paddock et al., 2013, and Venturas et al., 2016), particularly in conjunction with biotic influences. Therefore, as we face predictions of hotter, longer, and more frequent drought (IPCC 2013), it is becoming increasingly critical to hone in on the mechanisms, tipping points, and ecosystem impacts of these events. Furthermore, identifying plant mortality thresholds is of upmost importance for predicting susceptibility to extreme drought events of the future (McDowell et al, 2011; Hicke and Zeppel, 2013).

California recently experienced a record-breaking, multi-year drought from 2012-2018, estimated to be the most severe event in the last 1,000 years (Cook et al, 2015), with the 2013-2014 winter season being one of the driest on record (NOAA 2014). Drought tolerance has long been considered a common trait of shrub species in California chaparral communities where hot, rainless summers are the norm (Keeley, 1991; Meentemeyer and Moody, 2002). However, in the Santa Ynez mountain range in Santa Barbara County, the dominant and widespread big berry manzanita (*Arctostaphylos glauca*) exhibited dramatic dieback related to multi multi-year drought along with infection by opportunistic fungal pathogens in the Botryosphaeriaceae (Schultheis et al., 2018). These observations indicate that this species may be reaching a threshold in its drought resistance capabilities. Studies

have reported *Arctostaphylos* spp. to exhibit unusual scales of dieback during periods of extreme drought stress, (Brooks and Ferrin, 1994; Gitlin et al, 2006; Paddock et al., 2013), however this could be the most severe dieback event in recent history, both in terms of scale and severity. Manzanita are important members of the chaparral ecosystem, providing habitat for wildlife and food through their nectar and berries (Keeley and Hayes, 1976). Additionally, their structure makes them important components of historical chaparral fire regimes, and their fire-induced germination strategies contribute to post-fire successional trajectories (Keeley, 2006). Large-scale mortality of this species could reduce resource availability for wildlife, as well as alter fuel composition and structure in the region, resulting in an increased risk of more intense, faster burning fires. Therefore, the potential continued dieback of *A. glauca* is of great concern for both ecosystem functions and human populations alike.

Significant dieback of *A. glauca* in Santa Barbara county, California, was first observed in winter, 2014 (Drake-Schultheis et al., *in preparation*). Preliminary observations revealed patterns of dieback occurring along an elevational gradient, with effects being most pronounced at lower elevations (which are typically hotter and receive less precipitation) than at higher elevations. It was also observed that dieback was most prevalent in stands located on steep, exposed southerly-facing slopes. These observations are consistent with findings by previous studies (Gitlin, 2006; van Mantgem et al., 2006; Meng et al., 2015), Since *A. glauca* is classically drought-tolerant and able to function at very low water potentials (i.e. low water availability; Poole and Miller, 1975), it raises the question of what is driving this extreme dieback event? Could *A. glauca* be reaching a tipping point as a result of extreme drought stress, presence of a fungal pathogen, or both?

My dissertation research focuses broadly on the influence of drought and fungal pathogens on this classic, drought tolerant chaparral shrub species. Through a combination of methods, I explore the individual and interacting roles of water stress and opportunistic fungal pathogens in *A. glauca* in a major dieback event, and track the fate of individual shrubs through the progression of an historic drought. My findings are organized into three chapters based on the following questions: What are the identities and distribution of fungal pathogens associated with *A. glauca* dieback **(Chapter 2)**; How do drought stress and fungal infection interact to promote dieback and mortality in *A. glauca* **(Chapter 3)**; and How does *A. glauca* dieback progress over time during drought, and how do landscape variables and drought stress correlate with dieback **(Chapter 4)**?

In Chapter 2, I identify fungal pathogens in *A. glauca*, and discuss their distribution across the landscape in the Santa Barbara county front country region. Based on preliminary findings showing significant levels of *N. australe* in the field, I expected to find high incidence of this opportunistic pathogen in *A. glauca* across the landscape, suggesting their role in drought-related dieback. The data support this prediction, as over half of the pathogens isolated were members of the *Bot.* family, and the majority of these were identified as *N. australe,* a (presumably) novel pathogen in the region. Furthermore, *Bot.* infection was highly correlated with dieback severity, which was greatest at lower elevations. Taken together, the results show that opportunistic *Bot.* pathogens, particularly *N. australe,* are highly associated with *A. glauca* dieback across the landscape, and that lower elevations may be particularly vulnerable.

In Chapter 3, I address the hypothesis that extreme drought and *N. australe* function synergistically to promote faster and greater mortality than either factor alone. I designed a

full-factorial greenhouse experiment to identify whether *A. glauca* dieback is driven by extreme drought, infection by the fungal pathogens, or both. The results of this experiment support my hypothesis. Young (approx. one-year old) *A. glauca* inoculated with *N. australe* while simultaneously exposed to extreme water stress exhibited faster stress symptom onset, faster mortality, and overall higher morality than those subjected to either factor alone. These results provide strong evidence that the severe *A. glauca* dieback event observed during the 2012-2018 drought was the result of synergistic interactions between extreme drought *and* opportunistic pathogens, rather than the nature of the drought or particularly virulent pathogens.

In Chapter 4, I explore factors that are associated with climatic stress (elevation and aspect) in order to draw correlations between *A. glauca* stress and dieback severity. Identifying such relationships can be useful in making predictions on dieback and mortality across the landscape. By analyzing data on predawn xylem pressure potentials and net photosynthesis in shrubs along an elevational gradient, I found that patterns of water availability and physiological function both varied greatly across the landscape, and only weakly correlate with dieback severity, suggesting factors other than elevation and aspect must also be important in driving plant stress and dieback. Extreme heterogeneity across this landscape likely confounded my results, yet may also play an important role in supporting the resiliency of *A. glauca* populations as a whole. By measuring the progression of dieback in these same shrubs over time, I found that dieback severity throughout the drought increased most at lower elevations compared to high, providing evidence that shrubs at lower elevations may be particularly vulnerable. Unexpectedly, no new mortality was observed in surveyed shrubs as the drought progressed, even though many plants exhibited severe levels

of dieback throughout the study. This result shows that high levels of dieback severity do not necessarily predict morality in *A. glauca.*

In summary, my dissertation provides strong evidence that *A. glauca* dieback during the recent California drought was caused by synergistic interactions between extreme drought stress and infection by widely distributed opportunistic fungal pathogen *N. australe.* Infection and dieback severity varied considerably across the landscape, however, there is some evidence to suggest that populations at lower elevations may be at highest risk for severe dieback, either due to increased water stress, close proximity to fungal inoculum sources, or both. Additionally, shrubs located on southwest-facing slopes may also be more vulnerable due to increased sun exposure and thus environmental stress. Management efforts may want to focus on these areas when this region experiences future drought. Finally, although extreme dieback was recorded throughout the study, none of the observed shrubs succumbed to mortality. This may be the result of overall physiological resiliency, and the ability of adult shrubs to allocate resources to keep portions of the canopy alive. It could also be that the region's slightly more mesic climate offers a climatic buffer that prevented shrubs from reaching their mortality thresholds. More research is needed to identify these exact mechanisms and thresholds in *A. glauca.*

Collectively, the results of this dissertation work provide valuable knowledge on the severe dieback of an important chaparral shrub during an historic drought, with the potential for ecologically and economically costly consequences. Additionally, the data I present provide insight into the scale and progression of *A. glauca* dieback in a chaparral system, and potential patterns of future dieback in the face of predicted climate change. Future research

that seeks to further resolve landscape and environmental variables contributing to plant stress would help in identifying these patterns.

Challenges and Future directions

Heterogeneity and rugged topography across the landscape, while likely beneficial for the resilience of regional *A. glauca* populations during drought, presents significant challenges for on-the-ground monitoring. Out of necessity for safe access (particularly while conducting predawn measurements), many of the plants surveyed were located on the outer boundaries of stands, where edge effects may have been a factor. Monitoring intact, undisturbed stands using drones would yield valuable additional insight into the extent of disease deeper into stands and in stands on steep terrain or that are outside of normal visual range. The challenges of working in rugged landscapes covered in impenetrable vegetation highlight the need for using and refining remote sensing technologies, such as drone imaging, Light Detection and Ranging (LiDAR), and hyperspectral imaging as monitoring tools. Large-scale, long-term monitoring using these tools would allow researchers to retrieve data in areas that have previously been inaccessible, while also gaining a larger scale understanding of drought impacts. They ultimately will enable future studies to reveal more nuanced patterns across the landscape and between years of varying climatic conditions.

Ch. 2: Patterns and distribution of Botryosphaeriaceae fungi related to dieback in *big berry manzanita*

Abstract

Dieback and mortality in wildland plant species due to climate change has been on the rise in recent decades, and latent fungal pathogens may play a significant role in these events. During a severe multi-year drought, canopy dieback associated with latent pathogens in the Botryosphaeriaceae (*Bot*.) family was observed in stands of a dominant shrub species, big berry manzanita (*Arctostaphylos glauca*), across chaparral landscapes in California. These fungi are well-known pathogens of woody agricultural species, especially in hosts experiencing stress, yet little is known regarding their occurrence, distribution and impact in wildland systems. We conducted a field survey across an elevational gradient to identify patterns of *Bot.* infection as they relate to a) *A. glauca* dieback severity and b) landscape variables associated with plant drought stress. Three hundred individual shrubs across 30 different sites and three elevational categories (low, intermediate, and high) were sampled for occurrence of *Bot.* fungi, and each collection site was assessed for level of dieback severity. Dieback severity was positively correlated with *Bot.* isolation frequency and negatively correlated with elevation. *Neofusicoccum australe and Botryosphaeria dothidea* were the most frequently isolated species, with *N. australe* being the most common, particularly at lower elevations. However, *N. australe* isolation frequency was not correlated with dieback severity. Our results confirm the wide distribution of latent *Bot.* fungi in a wild shrubland system as an endophyte, and provide valuable insight into areas of greatest risk for future shrub dieback and mortalit*y*.

1. Introduction

Plant pest and disease outbreaks play a major role in shaping ecosystems around the world. Outbreaks can alter ecosystem structure and function, often with substantial consequences (Dale et al., 2001; Metz et al., 2011; Desprez-Loustau et al., 2014). Over the past 200 years, pest/disease outbreaks have increased due to mass exchange of biological materials from global trade (D'Antonio and Vitousek, 1992; Levine and D'Antonio, 2003; Bradley et. al., 2010) and a rise in unusual climate events resulting from global climate change (Easterling et al., 2000). Prolonged climate irregularities can subject plants to environmental stress outside of their normal resistance thresholds and make them susceptible to pests and pathogens (McDowell et al., 2008, 2011; Adams et al., 2010). For example, the increase in extreme droughts, defined here as greater in intensity and duration than historical drought regimes, has been directly linked to enhanced mortality in woody plant systems worldwide, often in association with pest/pathogen outbreaks (Allen et. al., 2010). Plant disease outbreaks are often economically costly (Alam et al., 2006; Coakley et al., 2009; Boyd et al., 2013), and can result in loss of ecosystem services in natural ecosystems. With global trade continuing to spread pests and pathogens, and global change-type drought events predicted to increase (IPCC, 2007), incidences of plant disease outbreaks are expected to increase. Understanding the role of drought and pathogens in plant dieback and mortality is therefore of critical importance.

Latent fungal pathogens are of particular concern for natural ecosystems yet their ecological roles remain poorly understood. These pathogens can live as asymptomatic endophytes within their hosts and remain undetected for long periods of time (Smith et al., 1996; Slippers et al. 2004; Burgess et al. 2004). The Botryosphaeriaceae (*Bot*.) fungi, a group

that causes considerable damage to hundreds of agricultural, ornamental, and naturally occurring host species around the world (Urbez-Torrez, 2011; Sakalidis 2013), includes many latent fungal pathogens that are difficult to detect in wild plant populations. Members of this diverse family (comprised of over 2000 taxa) can occur as endophytes, pathogens, and saprophytes on diverse woody hosts (Slippers and Wingfield, 2007, Urbez-Torrez, 2011). They are best known as pathogens that cause leaf spots, cankers, severe branch dieback, and death in economically important hosts such as grapevines (Úrbez-Torres, 2011), avocado (McDonald and Eskalen, 2011), and eucalyptus (Slippers et al., 2009). While *Bot.* fungi are rapidly becoming one of the most important agents of disease in agricultural plant hosts (Úrbez-Torres et al., 2006; McDonald and Eskalen, 2011), relatively few studies have been conducted on these pathogens in natural systems (Slippers and Wingfield, 2007; Sakalidis et. al*.,* 2011).

The *Bot.* fungi have a long history of taxonomic confusion, in part due to indistinctive morphological characteristics among species and from other fungal taxa, as well as historically poor and inconsistent descriptions early on in their discovery (Denman et al., 2000; Phillips et al., 2002; Slippers et al., 2004). Furthermore, *Bot*. host specificity and pathogenicity can vary widely among species and across geographical regions, complicating our understanding of their influence in various host species and across systems (Slippers and Wingfield, 2007). While advances in molecular sequencing and databasing have added clarity in this area (Denman et al., 2000; Slippers and Wingfield, 2007), challenges remain in understanding the diversity and pathogenicity of *Bot.* species among hosts and across regions. As a result, there is a dearth of knowledge on their ecological roles, particularly in native ecosystems.

One consistent finding is that disease outbreaks from *Bot.* fungi in agriculture are often associated with environmental stress, such as extreme heat fluctuations and drought (Schoeneweiss 1981; Lopes et al., 2016). Furthermore, studies have shown latent pathogens like *Bots* cause more damage to water-stressed hosts (Jactel et al*.,* 2012), and some *Bot.* species have been shown to grow well in water potentials much lower than what their plant hosts can tolerate (Ma et al., 2001), suggesting drought conditions increase virulence by these pathogens. Therefore, regions that have historically dry climates or experience periodic extreme drought may be especially vulnerable to disease outbreaks from latent pathogens as they are predicted to experience an increase in drought events due to climate change (Planton et al., 2008; IPCC, 2013).

Mediterranean-type climate areas are projected to be global change "hot spots" (Kerr 2008), and dry shrublands are predicted to experience some of the most rapid increases in mean temperatures (Loarie et al., 2009). Indeed, recent drought-related morality in California's semi-arid Mediterranean climate shrublands has provided support for these predictions (Jacobsen and Pratt, 2018; Venturas et al., 2016). Furthermore, the combination of dense human settlement and agricultural lands in close proximity to many natural shrubland habitats in southern California creates a likely pathway for exotic pathogen introductions and movement of pathogens from agricultural settings into wildland species. Not surprisingly, *Bot.* species have been retrieved on a variety of native chaparral shrub species in California, including *Ceanothus spp.* (Davis et al., 2002; Brooks and Ferrin, 1994), *Malosma laurina* (Aguirre *et al*., 2018), and other species of *Arctostaphylos* (Brooks and Ferrin, 1994; Swiecki and Bernhardt, 2003). Understanding the response of native species and these pathogens to extreme weather conditions will help to predict future vegetation

change and potential species losses (Desprez-Loustau et al., 2006; Slippers and Wingfield, 2007).

From 2011-2018, southern California experienced one of the most severe droughts in recorded history, with 2014 being the driest in the past 1,200 years (Griffin and Anchukaitis, 2015; Mann and Gleick, 2015). Field observations in winter 2014 identified high levels of branch dieback, and in some cases mortality, in a common ecologically important shrub, *Arctostaphylos glauca* (big berry manzanita) in coastal California. Two well-known *Bot.* species (*Neofusicoccum australe* and *Botryosphaeria dothidea*) were isolated from the symptomatic shrubs (Schultheis *et al.,* 2018). Like other members of the *Bot.* family, both *N. australe* and *B. dothidea* infect a broad range of hosts, and are known to be responsible for disease outbreaks associated environmental stress in agricultural species (Ma et al*.,* 2001; Desprez-Loustau et al., 2006; Slippers and Wingfield, 2007; Jactel et al., 2012; Van Niekerk et al., 2011). While *B. dothidea* is well established in California, with over 35 different host species having been identified (Michailides et al, 2000; Ma et al., 2001), phylogenic evidence suggests *N. australe* may be more recently introduced (Sakalidis et al., 2011; Drake-Schultheis et al., *unpublished data*). Its impact on shrublands of California has not been quantified.

Preliminary observations suggested high levels of branch dieback, and in some cases mortality, at lower elevation sites and along exposed ridges compared to higher elevations in coastal montane settings. We hypothesized that identifiable patterns would exist in the distribution of *B. dothidea* and *N. australe* across these landscapes that correlate with branch dieback and environmental variables associated with drought stress. Manzanita dieback has previously been causally associated with *Bot.* infection (Brooks and Ferrin, 1994; Swiecki

and Bernhardt, 2003; Schultheis et al., 2018). A greenhouse experiment by Drake-Schultheis et al. (2020), revealed that drought enhances onset of stress symptoms and mortality in young *A. glauca* inoculated with *N. australe* compared to shrubs subjected to drought or inoculation alone. However, to the authors' knowledge no previous quantitative studies exist on the distribution of *Bot.* species in California shrubland environments with Mediterranean climates.

To better understand the occurrence, distribution, and severity of *Bot.* infections in chaparral shrublands, we surveyed infection in *A. glauca* between April and September 2019. We also collected data on site elevation, aspect, and average percent canopy dieback at each site sampled for infection. While a variety of landscape variables are likely to influence plant stress at any given site (slope, solar insolation, and rockiness, for example), we focused on elevation because *A. glauca* already tends to occur mostly on xeric and rocky soils of exposed slopes, and therefore elevation was presumed to be the most significant factor influencing precipitation and water availability in this setting. Also, other studies have used elevation as a proxy for climate variation (Mantgem et al, 2006; Meng et al., 2015). We also recorded aspect of each sampled shrub since it influences sun exposure, temperature, and water stress.

To test our hypothesis that *Bot.* fungi and level of stress each played a role in extensive canopy dieback in *A. glauca*, the following questions were addressed: (1) What is the distribution of *Bot.* infection in *A. glauca* stands across the chaparral landscape in coastal Santa Barbara County? (2) How do levels of infection by the two Bot. fungi, *N. australe* and *B. dothidea,* compare across elevation? and (3) How do stand-level infection and elevation correlate with dieback severity? We predicted *N. australe* and *B. dothidea* to be present

across all sites and elevations, but also that *N. australe*, having been previously isolated with high frequency in the area (Schultheis et al 2018), would likewise have the greatest incidence in this study. Furthermore, we expected levels of *Bot.* infection and dieback severity to be greater at lower elevations compared to higher elevations, because lower sites typically receive less annual rainfall, thus exacerbating drought stress. This study presents the first quantitative survey summarizing the severity and distribution of *Bot.* fungi in natural shrublands, and seeks to identify important patterns of infection and dieback in *A. glauca* to predict future vulnerabilities across the landscape.

2. Methods

2.1 Study Area

The study sites were located on the generally south-facing coastal slopes of the Santa Ynez Mountains in Santa Barbara, California, USA (Fig. 1). The sites range from a lower elevation of ~550m to an upper elevation of ~1145m, and cover an area of ~47km^2 (see Supplemental Table S1). This region is characterized by a Mediterranean climate, with wet winters and hot, dry summers. Mean annual precipitation ranges from 68.4cm at lower elevations to 90.6cm at upper elevations (County of Santa Barbara Public Works, 2020). During the 2013-2014 wet season, which was two years into a multiyear drought and one of the driest years on record in California (Griffin and Anchukaitis, 2014), these areas received only 24.8cm and 31.6cm precipitation, respectively (roughly only one-third of their total means).

2.2 Site Selection

Sites were initially randomly generated from polygons drawn in the field around relatively pure stands (70% composition or greater) of *A. glauca* (Roth et al., 2015; Meerdink et al., 2019), and Drake-Schultheis, unpublished data). Polygons were then categorized according to elevation (<700m = Lower, 700-950m = Intermediate, > 950m = Upper), and numbered within their respective elevation categories. Ten sites per elevation zone were randomly selected using random number generator (Random Number Generator, 2010) for a total of 30 sites. When necessary, some randomly generated sites were substituted with nearby stands that were more accessible. Furthermore, any randomly selected sites that were discovered to be in recent fire scars (e.g. Thomas Fire, Dec. 2017) were exchanged for nearby stands that contained intact, mature *A. glauca.*

2.3 Site Data Collection

Elevation data were collected in situ using Altimeter GPS Pro (Immaginet Srl, 2020) and corroborated using Google Earth (2020). Aspect was recorded in situ in degrees, then converted to radians and transformed to linear data for analysis of "southwestness" using *cos*(aspect- 225°) according to Beers et al. (1966). This yielded aspect values ranging from -1 (northeast) to 1 (southwest), which were then used for modeling the effects of aspect on shrub dieback and *Bot.* infection.

The total percent dieback was assessed at each site as a measure of canopy health. Sites were demarcated by >50% *A. glauca* cover within a stand, as determined by visual on-the-ground assessments where the tops of the canopies could be viewed. Stand dieback was then visually estimated by two-to-three people as the percent of "non-green" vegetation

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(NGV) compared to live, green vegetation (GV) within the defined boundaries of a site (*A. glauca* are an evergreen species, thus any non-green vegetation can be concluded as suffering from some environmental or biotic stress). Categories of NGV included yellow, brown, and black leaves, and bare/defoliated canopy, and percentages were summed to reflect total NGV within a site. Total canopy cover was thus the sum of percent GV and NGV, and dieback was calculated as the total percent NGV (or 1 - % GV) to reflect the severity of canopy-level symptoms across each site.

2.4 Host plant selection and surveying

Ten individuals within each of the 30 sites were randomly selected for sampling using the random points generator feature in ArcMap (ESRI 2019. ArcGIS Desktop: *v. 10.6.1*. Redlands, CA: Environmental Systems Research Institute), for a total of 300 shrubs. Individuals were located in the field using a combination of a 1m resolution NAIP imagery base map (National Agriculture Imagery Program 2014. USDA Farm Service Agency, Washington, D.C.), a GPS device, a laser range finder, and transect tape. For stands not located within a polygon, individuals were selected either using a transect tape and a point intercept method (adapted based on the density, topography, and overall accessibility of each individual site), or haphazardly selected within the accessible confines of the stand to provide an even distribution of sampling throughout the stand. Whenever possible, individuals located more than 2m from trails and fuel breaks were selected to avoid any edge effects. Individuals with any signs of pruning or other human damage caused by humans were not selected. All individuals were sampled once between April and September 2019. Two branchlets (~2-5mm thick), each containing necrotic lesions and adjacent asymptomatic

wood tissue, were clipped per individual using sterile techniques, for a total of 600 samples. Samples were retrieved from approximately breast height and opposite sides of the shrub, whenever possible. All individuals had at least two necrotic lesions, even if no significant dieback was observed, allowing these methods to be carried out across all 300 individuals. Samples were then placed in labeled plastic bags, stored on ice, and brought back to the lab and placed in a 4°C refrigerator.

2.5 Fungal Isolation, Amplification, and Sequencing

Samples were rinsed of dirt and debris and surfaced sterilized using 100% ethyl alcohol, 0.5% bleach, and a 70% ethyl alcohol rinse. Cross sections between 1-2 mm were isolated from the advancing canker margin and plated onto half-strength potato dextrose agar (PDA) amended with streptomycin antibiotic. Cultures were incubated at room temperature until fungal colonies developed (5-14 days), and isolates of hyphal tips near the advancing margin were then re-plated onto half-strength PDA-strep to obtain pure cultures. From pure culture, any samples identified to have morphological characteristics consistent with those of *Bot.* fungi (dense, fuzzy, aerial hyphae; grey-black in color and rapid growth rate) (Slippers et al., 2004; Slippers and Wingfield, 2007) were selected for PCR. A few isolates from cultures inconsistent with *Bot.* characteristics were randomly selected from each site to amplify and sequence to verify our morphotyping method. The internal transcribed spacer region 1 (ITS1) and alpha-elongation factor-1 (EF1) genes were amplified using PCR primer pairs ITS1F and ITS4, and EF1-728F and EF1-986R, respectively, using methods modified from White et al., (1990) and Slippers et al (2004). Successfully amplified samples were sequenced using the UC Berkeley Sequencing Facility (Berkeley, CA).

2.6 Data Analysis

The severity of *Bot.* infection was calculated as the isolation frequency per site. Data were square-root transformed when necessary to meet the assumptions of normality. Differences in mean *Bot.* infection severity between elevation categories were calculated using one-way ANOVA with Tukey's HSD for post-hoc analysis with R Statistical Software (2018). Correlations between actual elevation (m) and *Bot.* infection severity were assessed using simple linear regression and ANOVA to test for significance ($\alpha = 0.05$).

Generalized linear models (GLMs) were developed to identify patterns of dieback, with dieback severity values as the response variable, and elevation (m), *Bot.* infection severity (proportion), and aspect (south-westness) as possible explanatory variables. If multiple models received substantial support ($\Delta AIC < 5$), the best model was confirmed by calculating the relative importance of each term based on the sum of their Akaike weights (MuMIn; Barton 2020). The proportion of variance explained by the models was calculated by measuring the adjusted D2 value (Guisan and Zimmerman 2000).

3. Results

3.1 Fungal isolates and sequencing

A total of 171 isolates within the *Bot.* family were obtained and sequenced from *A. glauca* cankers. All isolates produced cultures that were fast-growing (fully covering agar in 7-10 days) black or gray-black in color on the underside and black to gray-white on the top, and gray or white aerial hyphae extending upward (Figure 2). Sequences with $\geq$ 98% identities were deposited in GenBank (Accession #'s MT409741; MT416400-03; MT664119-42; MW045358-98). Of the 30 sites used for collecting samples, results from six

sites were omitted from statistical analysis relating to fungal identifications due to contamination and poor growth quality from some isolates. This resulted in a total of 24 sites (n = 8 per elevation category) and 163 *Bot.* isolates being used for these analyses. A random sampling of two-to-three isolates per site that did not exhibit morphological characteristics similar to *Bot.* fungi were also sequenced for quality control. None of these were confirmed to be *Bot.* species.

Five *Bot.* species were represented in the 163 isolates that were analyzed: *Neofusiccocum australe, Botryosphaeria dothidea, N. luteum, N. parvum,* and *Dothiorella sarmentorum.* The *Bot.* species represented slightly over half (51.1%) of total cultures that were isolated (Table 1). *N. australe* was the most common pathogen isolated (n = 104), followed by *B. dothidea* (n = 54), *N. luteum*, *N. parvum*, and *D. sarmentorum* (n = 3, 1, 1, respectively).

3.2 Infection rates and distribution

Of all *Bot.* isolates retrieved, 64.3% were from lower elevations, 51.4% from intermediate elevations, and 40.0% were from upper elevations (Table 1). Mean percent infection severity was higher for *N. australe* (32.7% $\pm$ 4.1) than for *B. dothidea* (17.3% $\pm$ 2.9), and this trend was consistent across all elevation categories (Table 2). However, this difference was only significant at the lower elevations (F_1= 6.4950, P = 0.0232; Table 2 and Fig. 3). *N. luteum*, *N. parvum*, and *D. sarmentorum* were identified mostly at upper elevation sites (Table 1).

Overall, there was a weak negative correlation between elevation and the *Bot.* infection (Fig. 4; R^2 = 0.17, $F_{1,22}$ = 4.4295, p = 0.0470). This pattern was driven mostly by

levels of *N. australe* ($R^2 = 0.19$, $F_{1,22} = 5.2191$, $P = 0.0323$), as the levels of *B. dothidea* did not correlate significantly with elevation ($R^2 = 0.05$, $F_{1,22} = 1.2095$, $P = 0.2833$).

3.3 Dieback

All 30 sites were used for evaluation of the relationship between dieback and elevation without fungal infection as a factor. Mean percent canopy dieback differed significantly between elevation categories ($F_{2,27} = 16.0446$, $P < 0.0001$), and was greatest at lower elevations (mean $= 38.5\% \pm 4.46$, Fig. 5a). There was a strong negative correlation between actual elevation (m) and dieback severity in stands across the landscape. (Table 3; Figure 5b; $F_{1,28} = 41.85$, $P < 0.0001$).

Percent canopy dieback across sites increased with severity of *Bot.* infection (Fig. 6; $R^2 = 0.24$, $F_{1,22} = 6.988$, $P = 0.0148$). Percent canopy dieback was still correlated with levels of *N. australe* alone ($R^2 = 0.21$, $F_{1,22} = 5.869$, $P = 0.024$) but not with *B. dothidea* alone ($R^2 = 0.19$, $F_{1,22} = 2.952$, $P = 0.10$). This is likely because far fewer *B. dothidea* isolates were recovered than *N. australe*, not because *B. dothidea* contributes to dieback any less than *N. australe*. Therefore, we retained level of *Bot.* infection (including all *Bot.* species) as a model parameter for testing GLMs.

The GLM that best explained canopy dieback included elevation and total *Bot.* infection severity as predictor variables (AIC $= 70.7$; Table S2). The D2 value was slightly lower for this model compared to those that included southwestness and/or an interaction term between elevation and *Bot.* infection (0.69 and 0.72, respectively), however its calculated relative importance (0.42) also suggests it to be the best model. Elevation was the most significant predictor in all models. *Bot.* infection severity was not shown to be a

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significant predictor in the best supported model (Table S3), even though it was a significant predictor using simple linear models. Still, the model containing elevation and *Bot.* infection severity calculated the highest relative importance value of 0.42, suggesting this to be a better fit model than other GLMs that were tested. Southwestness was not found to be a significant factor in any of the models for predicting percent dieback.

4. Discussion

This study provides definitive support for the hypothesis that shrub dieback, during a recent drought, and pathogen infection are strongly related in a wild shrubland setting. This is the first known quantitative support for the hypothesis that in *A. glauca*, an ecologically important shrub species in the study region, dieback is related to pathogen infection occurring along an elevational gradient. As expected, *N. australe* and *B. dothidea* were the two most frequently retrieved pathogens across all sites, however *N. australe*, the introduced pathogen, had almost twice the abundance of *B. dothidea. N. australe* is driving the correlation between elevation and *Bot.* infection, as the frequency was greater at lower elevations compared to upper elevations, while *B. dothidea* abundance did not change significantly across elevations. Level of *Bot.* infection was confirmed to be a significant predictor of stand-level dieback severity. The data also confirm that stand dieback severity is generally greater at lower elevations, which in this region experience higher temperatures and lower annual rainfall than the higher elevations sampled.

4.1 Abundance and Distribution of Bots.

While the presence of *Bot*. species has been reported previously in Santa Barbara County, this study represents the first effort (known to the authors) to understand the abundance and distribution of *Bots* occurring in natural shrublands, and the first wildland shrub survey of *Bots* across a climate gradient. The high frequency and wide distribution of *Bots* retrieved from our study sites support the hypothesis that *Bot.* species are widespread across a natural landscape, and likely contributing to the extensive dieback resulting from the recent drought. *Bot.* fungi were retrieved from nearly every site in this study (n = 27, out of 30). We could not determine *Bots.* presence from three sites due to contamination issues. The broad extent of the study area (~50km^2) suggests that infection is widespread in the region, and likely extends beyond the range of our study.

While both *N. australe* and *B. dothidea* together made up the most frequently retrieved pathogens, our data show that *N. australe* has a larger distribution and occurs in greater abundance across the study region than *B. dothidea* (Table 2). This trend was consistent across all elevations, but particularly at lower elevations (Fig. 3). One possible explanation for this is that *N. australe*, being a recently introduced pathogen, spreads more rapidly as an exotic species in *A. glauca* compared to *B. dothidea*, which has been established in California for over 150 years (Cesati and De Notaris, 1863). This hypothesis is consistent with previous studies that have shown variations in *Bot.* species abundance and virulence in Myrtaceous hosts (such as *Eucalyptus* sp.) occurring in native versus introduced ranges (Slippers et. al., 2004a, 2009; Pérez et al., 2008). However, it is difficult to evaluate the incidence of *B. dothidea* and *N. australe* in the present study in relation to historical documentation since many species in the *Bot.* complex have, until recently, been

mischaracterized (Slippers et al, 2004b, 2007; Urbez-Torrez, 2001). Only with the recent development of molecular tools have researchers begun to accurately trace phylogenetic and geographic origins of *Bot.* species. Such studies are beginning to elucidate the complex existence of *Bot.* fungi as both endophytes and pathogens around the world, and much more research is needed to understand their pathogenicity in various hosts under different conditions. Nevertheless, it remains clear from our study that *Bot.* species, particularly *N. australe,* are both abundant and widely distributed in this region, and are important pathogens in *A. glauca* shrubs.

4.2 Bot. abundance, dieback, and elevation

Because *Bot.* taxa were the most frequently retrieved pathogens and were significantly correlated with dieback, we believe that they drive *A. glauca* dieback. Further, stand dieback severity increased significantly with *Bot.* infection. This is not to say that other pathogens do not also contribute to disease symptoms, but we found no evidence of any other pathogens occurring in such high incidence as *Bot.* species. While Brooks and Ferrin (1994) identified *B. dothidea* as a likely contributor to disease and dieback in dozens of native chaparral species during an earlier drought event in southern California, and Swiecki and Bernhardt (2003) found *B. dothidea* in association with a dieback event in stands of *Arctostaphylos myrtifolia* in northern California, our study yields the most extensive results of *Bot.* infection and related dieback in a chaparral shrub species across a landscape. Further, our study resolves species identity within the *Bot.* clade and highlights the role of the recently introduced pathogen, *N. australe*.

A significant finding in this study was the relationship of *Bot.* infection and dieback with elevation. *Bot.* abundance and dieback were both found to be greatest at lower elevations, which was driven mostly by the high frequency of *N. australe* retrieved at these sites. This represents the first quantitative evidence supporting that *A. glauca* vulnerability to fungal infection is influenced by stress levels along an elevation gradient. A similar pattern was observed in northern California by Swiecki and Bernhardt (2004), who suggested that dieback in Ione manzanita (*A. myrtifolia*) infected with *B. dothidea* was greater in drier sites compared to more mesic ones, although no comparison of infection rates between sites was conducted in their study. The elevation gradient in our study was used a proxy for stress levels because annual precipitation decreases with decreasing elevation within our study region (County of Santa Barbara Public Works, 2020). Higher temperatures, which are associated with lower elevations, are also known to play an important role in drought-related mortality, as water loss from evapotranspiration is increased (Allen et al., 2015; Diffenbaugh et al., 2015). Furthermore, unpublished data for dry season predawn xylem pressure potentials on a subset of sites along the same elevational gradient revealed more negative water potentials in *A. glauca* at lower elevations compared to upper elevations as spring and summer drought sets in (Drake-Schultheis, unpublished). Thus, there is evidence that shrubs at low elevations indeed experienced the greatest water stress during the 2011-2018 drought, which predisposed them to higher levels of *Bot.* infection and enhanced dieback compared to upper elevation sites. More in-depth studies on the microbial communities and fungal loads of healthy and diseased shrubs throughout the region would help elucidate such trends.

Another possibility for the higher incidence of *Bot.* infection at lower elevations is that the lower ranges of *A. glauca* populations in Santa Barbara are often located adjacent or

in close proximity to agricultural orchards, ranches, and urban settings, which are common sources of plant pathogens, including *Bots* (Pusey, 1993; Smith et al., 1996a; Burgess et al., 2006 – from Slippers et al 2007; Mehl et al., 2017). Eucalyptus, avocado, and grapevines, which are abundant in these areas, are particularly well-known *Bot.* hosts and potential facilitators of *Bot.* introduction (Slippers et al., 2004a; Urbez-Torres, 2006, 2011; McDonald and Eskalen, 2011). Therefore, sources of inoculum from nearby populations of agricultural and horticultural hosts could be responsible for continual transmission *Bots* in wildland *A. glauca* populations, and would likely result in greater rates of infection at lower elevations. Furthermore, many of the lower sites in the survey were located near roads and/or trails, which are often subjected to additional stress from human activity like pruning and trail clearing; activities that are known to spread and promote infection by *Bot.* pathogens (Bush, 2015). While we avoided sites that showed signs of such activities in our survey, we cannot rule out the potential contributions of proximity to human encroachment to the overall higher rates of *Bot.* infection across the lower elevation zone.

It is worth noting that while our study revealed a trend of increased dieback in lower elevations, some upper elevation sites also exhibited high levels of dieback, and *Bot.* fungi (particularly *N. australe*) were retrieved from many of these sites. Upper elevations also experienced significant stress during the 2011-2018 drought, and water-related microsite variables outside the scope of this study like slope, solar incidence, soil composition, and summer fog patterns factors likely contributed to increased stress and subsequent dieback. Additionally, *N. luteum, N. parvum, and D. sarmentorum* were isolated primarily from upper sites. Host plants in these sites may serve as potential reservoirs for disease because the milder climate conditions promote greater host survival and thus pathogen persistence as

endophytes. This serves as an important reminder that continued global change-type drought may eventually jeopardize susceptible species populations even at the upper boundary of their range.

4.3 Possible mechanisms promoting dieback

Our results are consistent with well-known theoretical models describing the relationship between environmental stress and biotic infection, which generally ascribe extreme drought stress as a mechanism for plant predisposition to disease (Schoeneweiss...., Manion, 1991; McDowell et al., 2008, 2011; McDowell, 2011). These frameworks illustrate dynamic interactions between environmental stress, plant hydraulic functioning and carbon balance, and biotic attack, and a growing body of research has focused on understanding the roles of these factors in driving plant mortality, especially during extreme drought (Wullschleger et al., 2004; Desprez-Loustau et al., 2006; Oliva et al., 2014; Camarero et al., 2015). While the data collected in this study do not directly address the specific mechanisms leading to *Bot.* infection and dieback in *A. glauca,* our results can be discussed in the context of how life histories and physiological adaptions elicit differential responses to drought in woody plants, particularly in chaparral shrubs. For example, shallow-rooted, obligate seeder shrubs (those that reproduce only by seed following fire disturbance) like *A. glauca* have been shown to be more susceptible to drought-induced mortality during acute, high intensity drought than deep-rooted, resprouter shrubs (Paddock et al., 2013; Venturas et al., 2016; Jacobsen and Pratt, 2018). This supports our observations of pronounced *A. glauca* decline during an historic California drought compared to nearby resprouter species like chamise (*Adenostoma fasciculatum*), and laurel sumac (*Malosma laurina*).

Additionally, physiological mechanisms related to drought tolerance may further explain predisposition to disease in *A. glauca*. For example, high resistance to cavitation is a common trait associated with more dehydration-tolerant species like *A. glauca* that maintain hydraulic conductivity during seasonal drought (Tyree and Sperry, 1989; Davis et al., 1999; Pratt et al., 2007; McDowell et al. 2008; Venturas et al., 2016). While cavitation resistance is thought to assist in the continuation of photosynthetic activity even at very low seasonal water potentials (Pratt et al., 2007; Pausas and Keely, 2014), it has also been associated with greater mortality rates during high intensity drought in a variety of woody plant systems including mediterranean shrublands (Jacobsen et al., 2007; Paddock et al., 2013; Venturas et al., 2016), temperate deciduous forests (Hoffman et. al., 2001) and eucalyptus forests (Rice et. al., 2004). High resistance to cavitation requires heavy carbon investment for stronger and denser stem xylem tissue (Jacobsen et. al., 2007; McDowell et al. 2008), which can result in limited carbon for investment in defense against pathogens like *Bot.* fungi. Furthermore, colonization of pathogens during drought may further disrupt the carbon balance of plants as it influences defense and repair, creating a feedback loop that can drive plants toward a mortality tipping point (Desprez-Loustau et al., 2006; Oliva et al., 2014). Thus, while dehydration tolerance may be important during typical seasonal drought conditions (and possibly advantageous in seedlings that must germinate after fire under low water conditions (Paddock et. al., 2013; Venturas et al., 2016)), it may be a much riskier strategy and lead to greater mortality during global-change type drought, especially in the presence of pathogens. These frameworks are consistent with our findings and provide further evidence that *A. glauca* experiencing acute levels of drought stress are highly predisposed to *Bot.* infection particularly at lower elevations that experience heightened levels of water stress.

4.4 Applications

The results of this study provide strong evidence that *A. glauca* in the study region are vulnerable to *Bot.* disease and dieback, and possibly eventual mortality, related to acute drought. This is consistent with Venturas et al. (2016), who found that acute drought in 2014 led to reduced abundance in *A. glauca* and other obligate seeder chaparral species and even type-conversion in the Santa Monica Mountains of southern California, USA. A review by Jacobsen and Pratt (2018) found similar consistencies among shallow-rooted, obligated seeding shrubs. Clearly, there is strong support that *A. glauca* populations are at risk for future dieback, and thus should be the focus of more intense studies aimed at understanding the possible mechanisms driving such events. Manzanita are important members of the chaparral ecosystem (Borchert et al., 2004; Keeley 2006) and large-scale dieback and mortality of this species could reduce resource availability for wildlife (Keeley and Hays, 1976), as well as increase the risk of more intense, fires in an ecosystem already associated with increasingly frequent fire activity.

Additionally, our study provides valuable insight into areas of greatest risk for dieback and mortality, which are predominantly in lower elevations. These are important factors to consider when predicting vulnerabilities and potential impacts of future extreme drought events (IPCC, 2007). Mediterranean shrublands like those in southern California already considered high risk for global-change type drought, (Kerr, 2008; Loarie et al., 2009), and research suggests a general trend of upwards-shifting ranges in southern California chaparral species driven by changes in climate (Kelly and Goulden, 2008).

Therefore, populations of *A. glauca* occurring at the lower edge of their natural range are at high risk for dieback and mortality, and should be the focus of management efforts.

Lastly, while studies on the various physiological mechanisms for plant survival during drought are critical for predicting differential responses to stress, there is an increased emphasis on the importance of understanding the diverse role of pathogens in order to accurately model species vulnerabilities to climate change (Jactel et al., 2012; Oliva et. al., 2014). Studies that incorporate the impact of pathogens help inform new integrative approaches to protecting plants against drought and biotic infection, rather than treating these influences separately. Examples include Jactel et al., (2012), whose meta-analysis showed the significant effects of water stress on symptom severity in plants infected with latent pathogens like *Bots,* and experiments like Drake-Schultheis et al. (2020), who found interactive effects between drought stress and infection from *N. australe* in driving symptoms of stress and increasing mortality rates in *A. glauca*. The results of our study align with these frameworks, and provide additional evidence that as climate change models are predicting more intense and frequent drought events, our need to understand the role of latent pathogens in at-risk natural systems is becoming more critical.

5. Conclusion

There is now strong evidence to suggest that extreme drought increases the vulnerability of *A. glauca* shrubs to infection and associated dieback from latent *Bot.* pathogens. Our hypothesis predicted that plants at lower elevations experiencing greater environmental stress are more susceptible to pathogen infection and subsequent dieback. The strong correlations between elevation, *Bot.* infection, and dieback that were found in this

study provide further evidence of the importance of these interactions, and these results have implications for significant ecosystem impacts of future drought events, particularly at lower elevations. Overall, our results confirm that *Bot.* fungi, especially *N. australe*, are widely distributed across the landscape in this region of Santa Barbara, and that lower elevations are the most vulnerable to disease outbreak and dieback resulting from acute drought. We therefore suggest that the presence of fungal pathogens is an important factor to consider in studies that reveal high mortality rates among plants with high resistance to cavitation during global-change type drought.
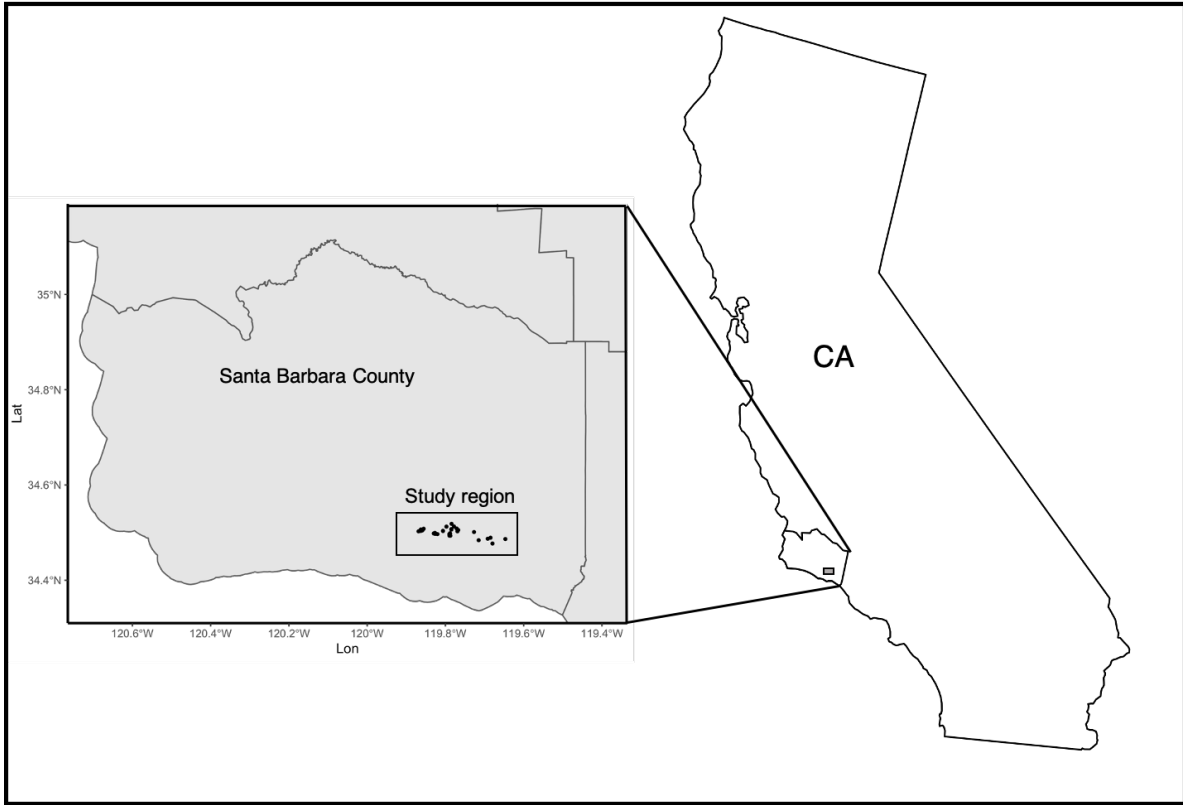
Fig 1. Location of study sites. The study region was located in the front country of Santa Ynez Mountains in Santa Barbara County (California, USA). Black dots are locations of 30 study sites.
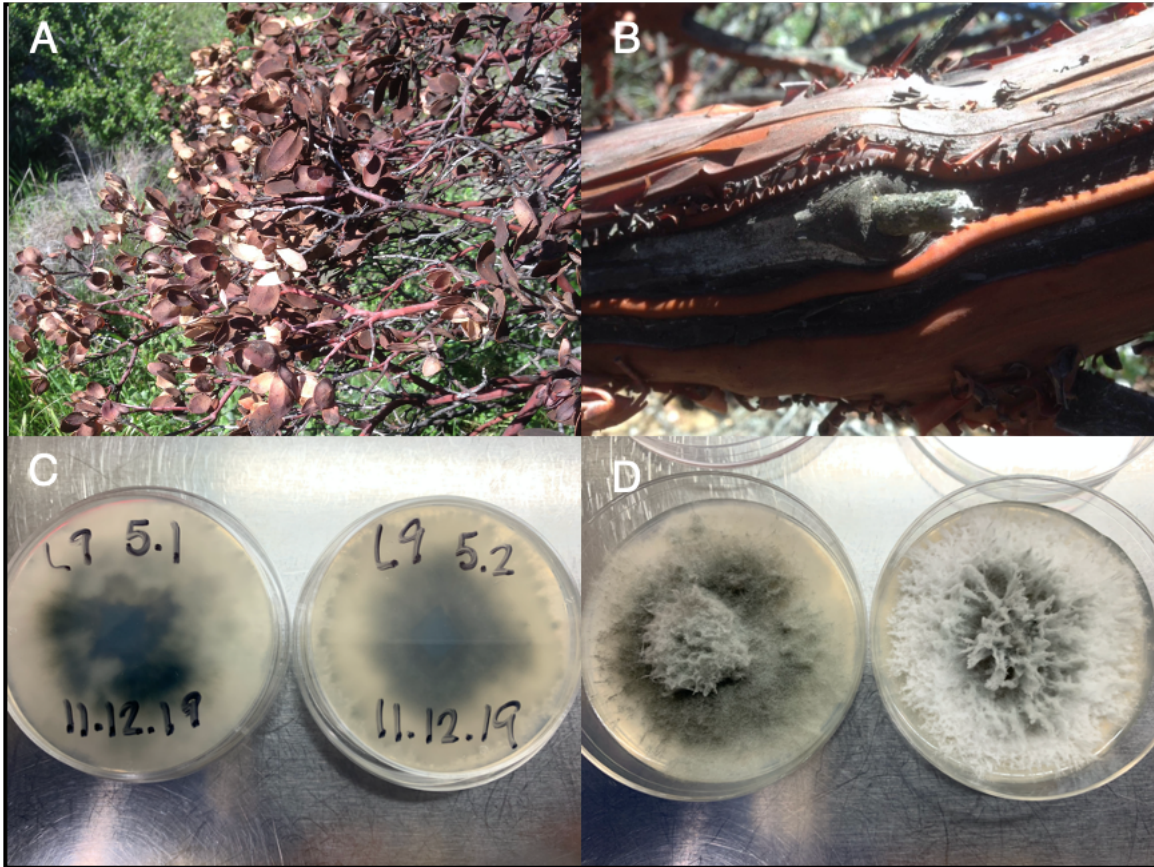
Fig 2. Symptoms of *Bot* infection on *A. glauca* include A) leaf discoloration and branch dieback and B) branch cankers. (C) and (D) cultures of isolates retrieved from *A. glauca* branch cankers were used to preliminarily identify *Bots* based on colors and growth rate of (C) the underside of plates and (D) aerial hyphae.

Table 1: Incidence[a] of *Botryosphaeriaceae* fungi in *Arctostaphylos glauca* across elevation categories in Santa Barbara, California, USA

Site	Site Total[b]	No (%) Bot.[c]	N. australe	B. dothidea	N. luteum	N. parvum	D. sarmentorum
			Number (%) of cultures yielding				
Lower Elev.							
L1	13	12 (92.3)	6 (46.2)	6 (46.2)	0	0	0
L2	10	10 (100)	7 (70.0)	2 (20.0)	1 (10.0)	0	0
L3	12	6 (50)	5 (41.7)	1 (8.3)	0	0	0
L4	19	10 (52.6)	6 (31.6)	4 (21.1)	0	0	0
L6	17	3 (17.6)	3 (17.6)	0	0	0	0
L8	12	5 (41.7)	5 (41.7)	0	0	0	0
L9	15	14 (93.3)	11 (73.3)	3 (20.0)	0	0	0
L10	12	8 (66.7)	3 (25.0)	5 (41.7)	0	0	0
Total	110	68(64.3)	46(43.4)	21(19.7)	1(1.3)	0	0
Intermediate Elev.							
I1	12	2 (16.7)	1 (8.3)	1 (8.3)	0	0	0
I2	17	4 (23.5)	2 (11.8)	2 (11.8)	0	0	0
I4	12	9 (75)	5 (41.7)	4 (33.3)	0	0	0
I5	13	11 (84.6)	8 (61.5)	3 (23.1)	0	0	0
I7	18	7 (38.9)	5 (27.8)	2 (11.1)	0	0	0
I8	17	12 (70.6)	6 (35.3)	6 (35.3)	0	0	0
I9	11	9 (81.8)	7 (63.6)	2 (18.2)	0	0	0
I10	10	2 (20)	1 (10.0)	1 (10.0)	0	0	0
Total	110	56(51.4)	35(32.5)	21(18.9)	0	0	0

[a] Presence of *Bots.* as determined by positive identification of cultures grown from cankers

[b] Total number of cultures grown from each site

[c] Number of cultures (and percentage of total cultures grown per site) yielding *Bot.* fungi

Table 1: Incidence[a] of *Botryosphaeriaceae* fungi in *Arctostaphylos glauca* across elevation categories in Santa Barbara, California, USA

Site	Site Total[b]	No (%) *Bot.*[c]	Number (%) of cultures yielding				
			N. australe	*B. dothidea*	*N. luteum*	*N. parvum*	*D. sarmentorum*
Upper Elev.							
U1	9	3 (33.3)	2 (22.2)	1 (11.1)	0	0	0
U2	14	6 (42.9)	5 (35.7)	0	0	0	1 (7.1)
U3	14	0	0	0	0	0	0
U4	11	6 (54.5)	2 (18.2)	4 (36.4)	0	0	0
U6	9	3 (33.3)	2 (22.2)	1 (11.1)	0	0	0
U7	11	6 (54.5)	1 (9.1)	4 (36.4)	1 (9.1)	0	0
U8	14	4 (28.6)	4 (28.6)	0	0	0	0
U9	17	11 (64.7)	7 (41.2)	2 (11.8)	1 (5.9)	1 (5.9)	0
Total	99	39(40.0)	23(22.2)	12(13.4)	2(1.9)	1(0.7)	1(0.9)
Total Survey	319	163(51.5)	104(32.7)	54(17.3)	3(1.0)	1(0.2)	1(0.3)

[a] Presence of *Bots.* as determined by positive identification of cultures grown from cankers

[b] Total number of cultures grown from each site

[c] Number of cultures (and percentage of total cultures grown per site) yielding *Bot.* fungi

Table 2: Mean retrieval of *Botryosphaeriaceae* fungi in *Arctostaphylos glauca* across elevation categories in Santa Barbara, California, USA

	n	*B. dothidea*	*N. australe*	F_1	Prob > F
Lower	110	19.7 ± 6.1	43.4 ± 7.0	6.495	**0.0232**
Inter.	110	18.9 ± 3.8	32.4 ± 7.8	2.1715	0.1627
Upper	99	13.4 ± 5.2	22.2 ± 4.8	1.5087	0.2396

Means ($\pm$ SE) of *B. dothidea* and *N. australe* retrieved from Lower, Intermediate, and Upper elevations, expressed as percent of total cultures grown from cankers. P-values in **bold** indicate significant differences between species according to a one-way ANOVA.
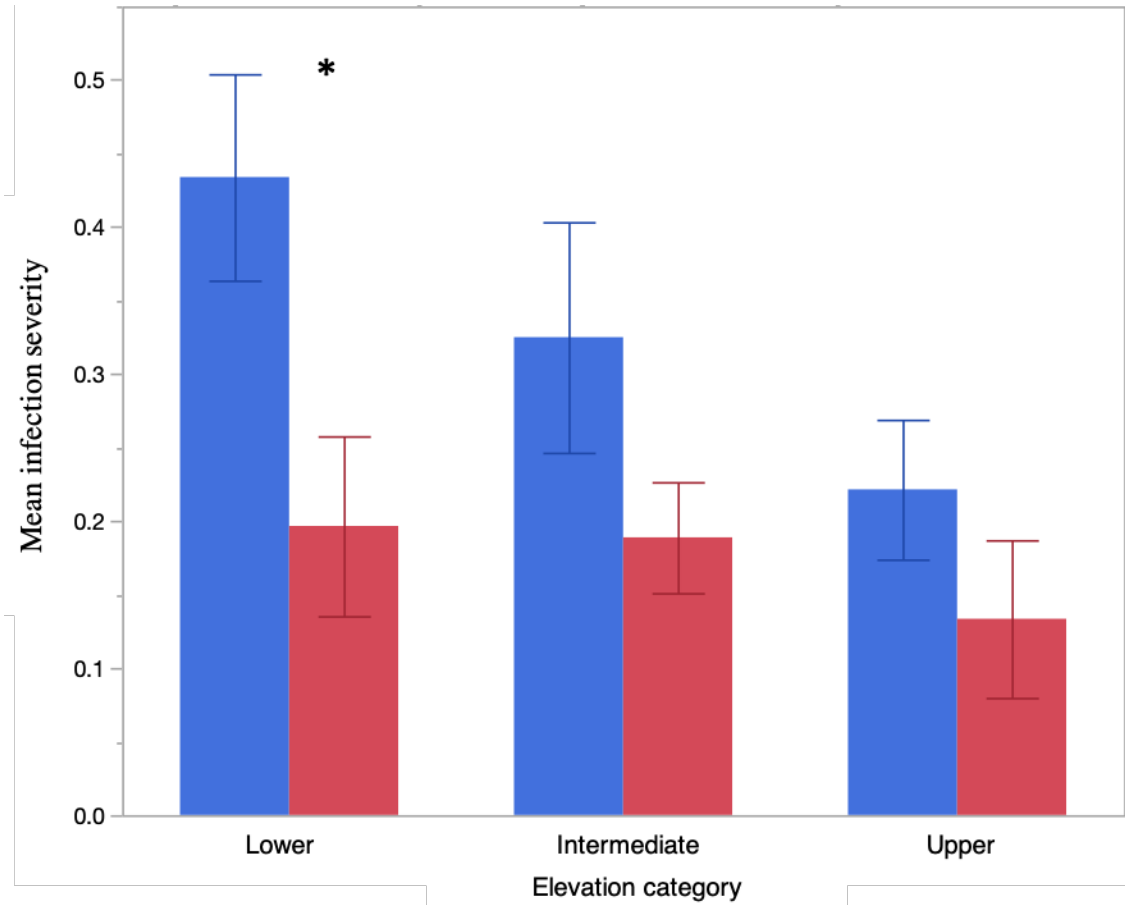
Figure 3. Infection severity (expressed as mean proportions of the total number of cultures per elevation category) of *N. australe* (blue bars) and *B. dothidea* (red bars) isolated from culture of *A. glauca* cankers across elevation categories. Error bars represent ± standard error. ANOVA with Tukey's HSD were performed for infection severity between *N. australe* and *B. dothidea* at each elevation level (* = p < 0.05).
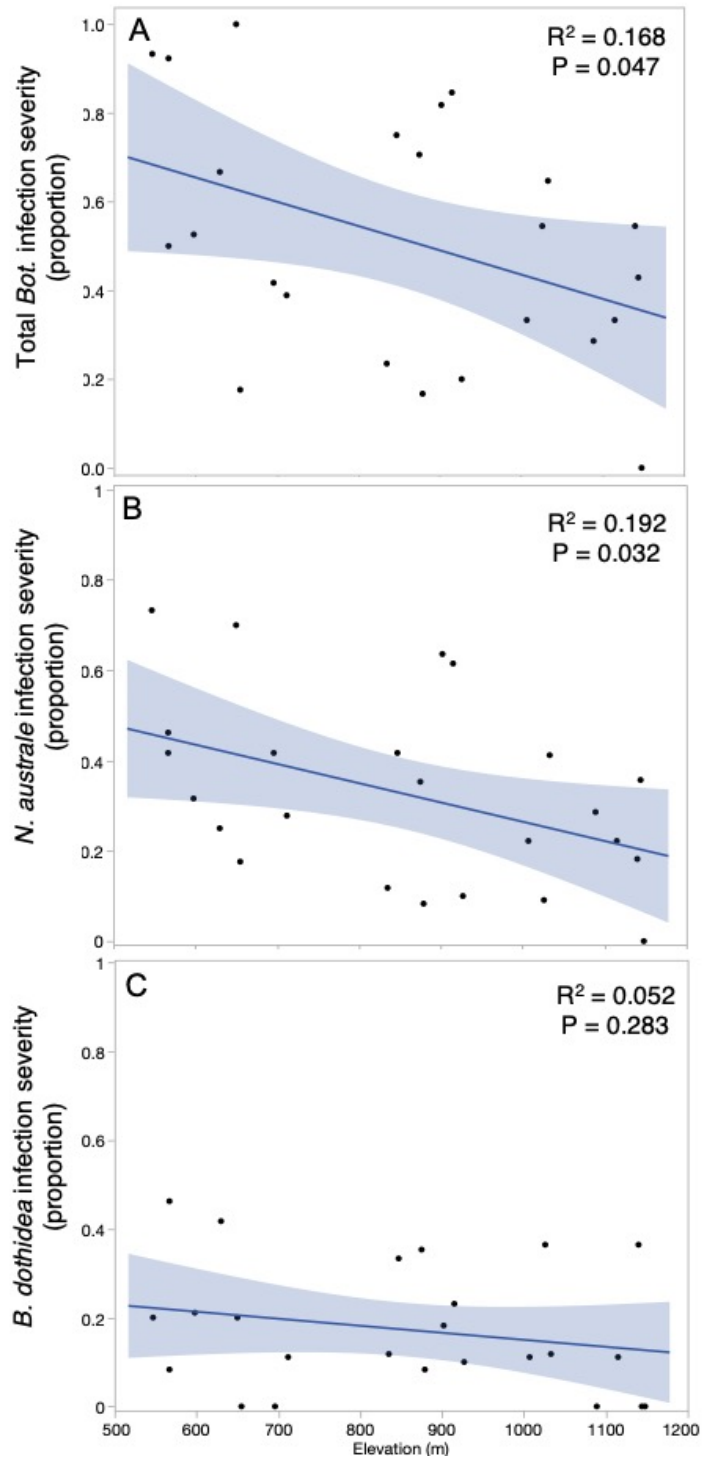
Figure 4. The relationship between elevation and a) total *Bot.* b) *N. australe*, and c) *B. dothidea* infection (expressed as a proportion of the total number of cultures per site, *n* = 24). Blue solid lines represent best fit linear regression and shaded areas = 95% confidence intervals.

Table 3. Results of simple linear regression for percent dieback*.

Parameters[a]	Estimate	Std. Error	t value	Prob > t	R^2
Elevation	-0.0063	0.0009	-7.068	< 0.0001	0.64
Infection$_{Bots}$	2.9288	1.1080	2.643	0.0148	0.24
Infection$_{Na}$	3.7531	1.5492	2.423	0.0241	0.21
Infection$_{Bd}$	3.954	2.302	1.718	0.0998	0.12
Southwestness	-0.8278	0.5091	-1.626	0.115	0.09

* Percent dieback data were square root-transformed to meet assumptions of normality

[a] Infection$_x$ = Infection severity based on the proportion of *Bot.* species, *Bots*; *Neofusiccocum australe* only, *Na*; and *Botryosphaeria dothidea* only, *Bd*.
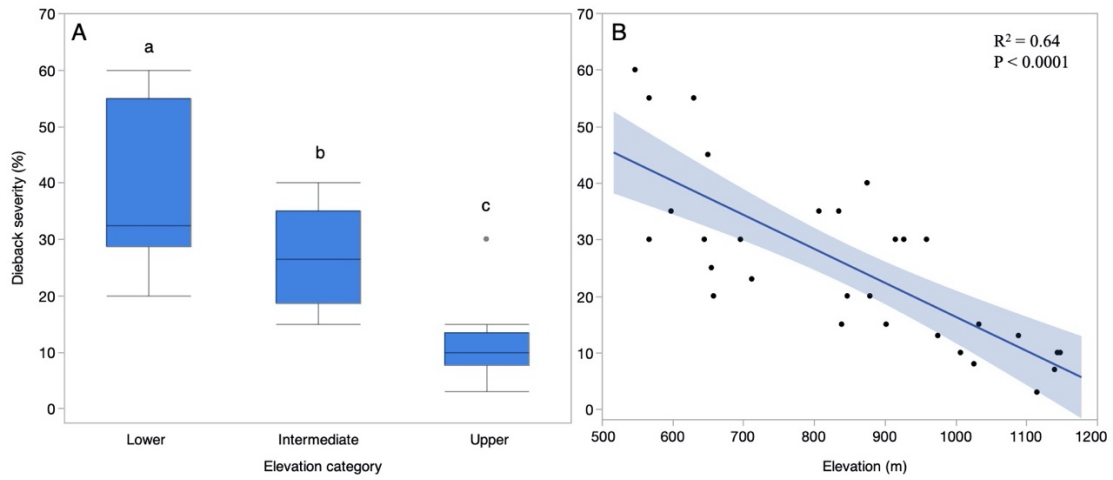
38

Fig. 5. Relationship between elevation and percent dieback. Percent dieback by (a) elevation category (n = 10 per category), and (b) actual elevation (n = 30). Different letters in panel (a) denote significant differences between categories.
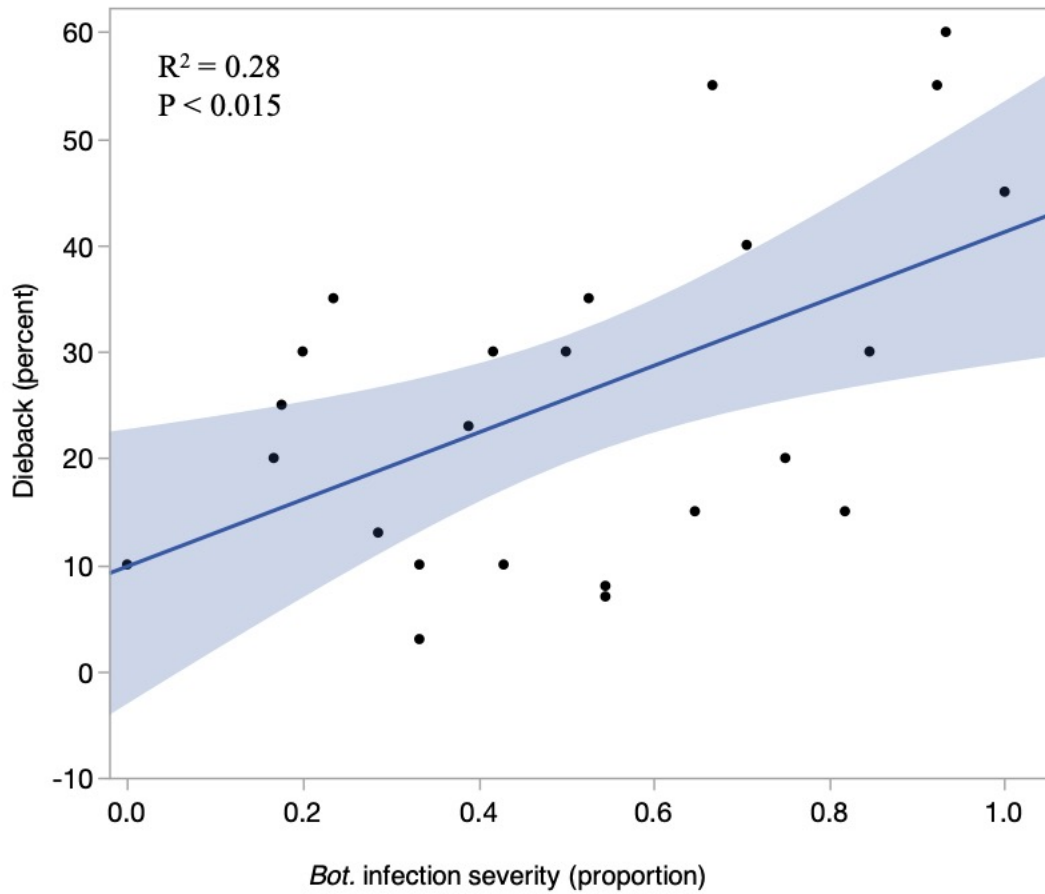
Fig. 6. Simple linear regression showing the relationship between *Bot*. infection severity (proportion of *Bots* retrieved per site) and percent dieback (n = 24).

S1 Table. Site locations and characteristics. Data collected are elevation category, actual elevation, coordinates, and aspect.

Site ID	Elev. Cat	Act. Elev.(m)	Lat.	Lon.	Aspect (degrees)
L1	Lower	567	34.493394	-119.78755	107
L2	Lower	650	34.497203	-119.826255	165
L3	Lower	567	34.503705	-119.806381	135
L4	Lower	598	34.493489	-119.789724	110
L5	Lower	645	34.498125	-119.788078	195
L6	Lower	655	34.4985	-119.788387	206
L7	Lower	658	34.496888	-119.789949	118
L8	Lower	696	34.499418	-119.826965	165
L9	Lower	547	34.503351	-119.806164	117
L10	Lower	630	34.496853	-119.820499	180
I1	Intermediate	879	34.50359	-119.863103	157
I2	Intermediate	835	34.506332	-119.784795	230
I3	Intermediate	807	34.477119	-119.679767	270
I4	Intermediate	847	34.512595	-119.79742	60
I5	Intermediate	915	34.502692	-119.868698	225
I6	Intermediate	839	34.507346	-119.784926	240
I7	Intermediate	712	34.49826	-119.829739	168
I8	Intermediate	875	34.504667	-119.858971	200
I9	Intermediate	902	34.506605	-119.862795	139
I10	Intermediate	927	34.508184	-119.85571	178
U1	Upper	1007	34.512942	-119.778215	225
U2	Upper	1144	34.501234	-119.726975	170
U3	Upper	1148	34.484016	-119.715133	200
U4	Upper	1140	34.487176	-119.691879	218
U5	Upper	959	34.518463	-119.784337	153
U6	Upper	1115	34.486556	-119.646882	165
U7	Upper	1026	34.48927	-119.684753	230
U8	Upper	1089	34.508638	-119.771441	177
U9	Upper	1033	34.50547	-119.767276	139
U10	Upper	975	34.502707	-119.769306	225

Supplemental Table S2. Generalized linear models (GLMs) describing percent dieback. The models that best describes percent dieback, i.e. those with the lowest Akaike Information Criterion (AIC) scores, are marked in bold.

Factors[a]	AIC	D2	GLM Weight
E	87.2	0.61	-
SW	113.6	0.07	-
PB	92.0	0.19	-
E, PB	**70.7**	**0.69**	**0.42**
E, PB, SW	**70.8**	**0.72**	**0.38**
E, PB, SW, E*PB[b]	**72.2**	**0.72**	**0.19**

[a] Factor codes: elevation, E; southwestness, SW; percent *Bots*, PB
[b] Interaction between factors

Supplemental Table S3. Parameter estimates of the best fit GLM describing variation in percent dieback by elevation (E) and proportion of *Bots* (PB). Significant effects ($\alpha = 0.05$) are in bold.

Term	Estimate	SE	t-value	Prob > t
Intercept	2.665	0.2468	10.798	**< 0.0001**
E	-0.0014	0.0002	-6.206	**< 0.0001**
PB	0.1393	0.1698	0.821	0.421

Chapter 3. Interactions between drought and opportunistic pathogens as drivers of mortality in big berry manzanita.

Abstract

Mortality events involving drought and pathogens in natural plant systems are on the rise due to global climate change. In Santa Barbara, California, USA, big berry manzanita (*Arctostaphylos glauca*) have experienced canopy dieback related to both a multi-year drought, and infection from fungal pathogens in the *Botryosphaeriaceae* family. A greenhouse experiment was conducted using *N. australe* to test the specific influences of drought and fungal infection on *A. glauca*. A full factorial design was used to compare four treatment groups (drought + inoculation; drought – inoculation; watering + inoculation; and control: watering – inoculation). Data were collected for 10 weeks on stress symptoms, changes in leaf fluorescence and photosynthesis, and mortality. Results indicated significant effects of watering and inoculation treatments on net photosynthesis, dark-adapted fluorescence, and disease symptom severity ($P < 0.05$), and a strong correlation was found between physiological decline and visible stress ($P < 0.0001$). Mortality differed between treatments, with all groups except for the control experiencing mortality (43% mortality in drought – inoculation, 83% in watering – inoculation, and 100% in drought + inoculation). A Kaplan-Meier survival analysis showed drought + inoculation to have the least estimated survivorship compared to all other treatment groups. In addition to a possible synergistic interaction between drought and fungal infection in disease onset and mortality rates in *A. glauca*, these results indicate that young, non-drought stressed plants are susceptible to mortality from *N. australe* infection, with important implications for the future of wildland shrub communities.

1. Introduction

Reports of large-scale, drought-associated mortality events in forest and woodland systems have been on the rise in recent decades (Allen et al., 2010). These reports have spanned across biomes, including in classically drought-tolerant species across Europe (Markalas, 1992; Peñuelas et al., 2001; Tsopelas et al., 2004; Gadbin-Henry et al., 2007;Sarris et al., 2007; Vennetier et al., 2008), Australia (Fensham and Holman, 1999; Fensham et al., 2009), Africa (El Abidine, 2003; Bentouati, 2008), and the United States (Breshears et al., 2005; Gitlin et al., 2006; Venturas et al., 2016). As a result, interest has been growing in understanding how species that are typically capable of withstanding periodic drought stress may become susceptible to drought and experience significant dieback and even large-scale mortality when exposed to acute (i.e., sudden and often hotter or drier than normal) or prolonged chronic drought (Breshears et al., 2009; McDowell et al., 2013; Venturas et al., 2016). These droughts of unusual extremes are referred to as "global-change-type drought" (Breshears et al., 2005) and are becoming more common as the climate warms (IPCC, 2013).

While the exact physiological mechanisms leading to dieback and mortality during such events are variable across species and conditions, drought is generally hypothesized to promote physiological decline either via loss of hydraulic functioning or carbon starvation (McDowell et al., 2008; McDowell, 2011) or a combination of both (McDowell et al., 2013). In the case of hydraulic failure, plants with insufficient soil water experience xylem cavitation (air-filled cavities), which can ultimately lead to cellular death. Alternatively, plants that avoid drought by closing their stomata to reduce water loss subsequently suffer insufficient carbon supply to meet other metabolic demands. In either scenario, the stress that

drought places on a plant is likely to cause measurable decreases in physiological functions that may be irreversible (McDowell et al, 2008; Anderegg et al., 2012; Hartmann et al., 2013; Feller, 2016).

An additional factor that can play a significant role in drought-related dieback and mortality is the presence or introduction of biotic agents. Indeed, introduced plant pathogens have been well documented to cause canopy dieback and dramatically alter community structure in a variety of forested systems (Richens, 1983; Dinoor and Eshed, 1984; Jarosz and Davelos, 1994; Weste, 1994; La Porta et al., 2008). Some well-known examples in the United States include Dutch elm disease (Gibbs et al., 1975; Brasier, 2001), chestnut blight (Anagnostakis, 1987), white pine blister rust (Maloy, 1997), and sudden oak death (Rizzo et al., 2005). Significant pathogen events have also impacted the landscape in wildland shrub communities including sclerophyll shrub woodlands in Australia (Weste, 1981) and salt desert scrub in the western United States (although in this case pathogens were one of a few possible causes of die-off) (Nelson et al., 1990). However, large-scale dieback of shrubs has been less documented than their arboreal counterparts, despite evidence of disease from fungal species being abundant in many scrubland systems including southern California chaparral (Davis et al., 2002), northern California foothill shrublands (Swiecki and Bernhardt, 2003), and South African fynbos (Jacobsen et al., 2012). Such studies, along with expectations of increasing threats from pathogens due to climate change and accelerating trade/movement of biological materials globally (e.g., Bradley et al., 2010; Diez et al., 2012), have led scientists and land managers alike to anticipate introduced pathogens as important contributors to future changes in wildland communities.

While both global-change-type drought and pathogens are likely important contributors to plant dieback and mortality, current research suggests that these two factors are not mutually exclusive (McDowell et al., 2013). Rather, canopy dieback and mortality may result from the combined influences of environmental stress and biotic agents, and theoretical frameworks describing these influences have been put forth (Schoeneweiss, 1975, 1981; Boyer, 1995; Desprez-Loustau et al., 2006; McDowell et al., 2008; Jactel et al., 2012; Oliva et al., 2014). These frameworks incorporate biotic agents into the drought-hydraulics complex described above, whereby pathogens and insects may amplify or be amplified by drought-associated hydraulic failure or carbon starvation (McDowell et al., 2008). Amplification can occur when biotic agents damage host tissue—by defoliation or blocking transportive vessels, for example—to the extent that the effects of drought are greatly exacerbated (Larsson et al., 1983; Wullschleger et al., 2004; Jacobsen et al., 2012). Alternatively, physiological responses to extreme environmental stress can have negative effects on plant defense systems, rendering them susceptible to mortality through biotic infection (Crist and Schoeneweiss, 1975; Ma et al., 2001; Hicke and Zeppel, 2013). In both scenarios, the effects of biotic agents and drought stress are strongly linked, and these interactions have been well documented in drought-tolerant systems such as South African fynbos (Jacobsen et al., 2012), red pine forests (Blodgett et al., 1994), eucalyptus forests (Smith and Wingfield, 1994; Barradas et al., 2018), and California chaparral (Swiecki and Bernhardt, 2003; Brooks and Ferrin, 2004; Aguirre et al., 2018). Latent or secondary pathogens (those that take advantage of environmental stress) are particularly likely to be involved with dieback and mortality events in these systems, as they are known to increase damage in hosts experiencing drought stress (Desprez-Loustau et al., 2006; McDowell et al.,

2008; Jactel et al., 2012). Therefore, while drought events alone are expected to play an important role in reshaping ecosystems as the climate changes, in some cases, synergies between environmental stress and biotic influences might lead to shifts in plant community structure and composition, and thus ecosystems as a whole.

In the Santa Ynez Mountains in Santa Barbara County, California, United States, big berry manzanita (*Arctostaphylos glauca*) began exhibiting dramatic canopy dieback (defined here as significant canopy death) during the 2011–2018 drought (L. Drake-Schultheis, unpublished data). Shrubs in the genus *Arctostaphylos* are common in Mediterranean shrub communities extending from southwest Oregon to northern Baja California (Vasey et al., 2012; Kauffman et al., 2015). They may occur in monospecific stands or in alliances with other important community members like chamise (*Adenostoma* spp.) and *Ceanothus* spp. (Borchert et al., 2004; Kauffman et al., 2015). Within these alliances, *Arctostaphylos* spp. frequently occupy >50% average cover (Borchert et al., 2004), which along with their nutritious and prolific fruits, and fire-induced regeneration strategies, make them one of the most important members of the chaparral community (Keeley 2006). In the southern California chaparral ecosystem where hot, dry summers with high vapor pressure deficit are the norm (Keeley, 2002; Meentenmeyer and Moody, 2002), seasonal drought tolerance has long been considered a common strategy among dominant plant species, including *A. glauca*. However, the severity of recent canopy dieback observed suggests that this species is reaching a threshold in its drought-resistance capability. Concurrent with observations of canopy dieback, visible symptoms of fungal infection were observed (Schultheis et al., 2018) including wood cankers and leaf discoloration (green to brown and gray-black), both of

which progress during prolonged drought stress, suggesting that multiple driving forces contribute to manzanita dieback.

Molecular sequencing identified the dominant fungal pathogen found on symptomatic *A. glauca* in this area to be *Neofusicoccum australe*, a member of the well-known pathogenic Botryosphaeriaceae (*Bot.*) family (Schultheis et al., 2018). Members of this family are most commonly associated with disease in plant species experiencing severe environmental stress (Schoeneweiss, 1981; Desprez-Loustau et al., 2006; Slippers and Wingfield, 2007), including *Arctostaphylos* spp. (Brooks and Ferrin, 1993; Swiecki and Bernhardt, 2003). They are also known to play a variety of functionally diverse roles, from asymptomatic endophytes (Fisher et al., 1993; Burgess et al., 2005; Taylor et al., 2005; Dakin et al., 2010) to obligate pathogens (Sakalidis et al*.,* 2011; Phillips et al., 2013). Yet, while *N. australe* has been described around the world (Armengol et al., 2008; Amponsah et al., 2009; Espinoza et al., 2009), relatively few studies have been conducted on its specific interactions with host species, as it was only fairly recently described (Slippers et al., 2004; Phillips et al., 2013). Historically, *Bot.* pathogens have most frequently been studied in agricultural host species (Ma et al., 2001; Slippers and Wingfield 2007; Amponsah et al., 2011), and little is known regarding their ecological role in wildland ecosystems (Slippers and Wingfield, 2007; Sakalidis et al., 2011), especially with regards to chaparral shrubland systems (but see Davis et al., 2002; Swiecki and Bernhardt, 2003). The present study was aimed at identifying the possible role of *N. australe* in *A. glauca* dieback in Santa Barbara County, particularly in combination with extreme drought. Because this pathogen has only recently been reported on wild shrub species in California (Schultheis et al., 2018) and is thought to be an introduced

species native to Western Australia (Sakalidis et al., 2011), this outbreak represents a new and undescribed threat to these wildland plant assemblages.

This study addresses the following questions: (1) How does *A. glauca* respond physiologically to drought and fungal infection, separately and together? (2) Are these responses correlated with visual signs of stress, specifically leaf health? (3) Can drought and fungal presence interact to increase or accelerate plant mortality compared to drought or fungi alone in *A. glauca*? To address these questions, a greenhouse experiment was conducted in November 2016 through February 2017 manipulating both drought and fungal infection and observing trends in plant stress symptoms, physiological function, and mortality. We predicted that both drought stress and fungal infection would lead to declines in physiological function compared to the control and that these declines would be strongly correlated with increases in stress severity. Furthermore, we expected that those individuals experiencing both drought stress and fungal infection would die sooner than those in all other treatment groups. This experimental study elucidated the potential of the interaction between drought stress and introduced pathogens to significantly impact chaparral shrub health and important implications for the future of these shrubs faced with increasingly frequent global-change-type droughts.

2. Methods

2.1 Growing A. glauca

Thirty *A. glauca* plants were purchased in 2015 as 1-year-old propagules cut from on-site adult trees at Las Pilitas nursery in Santa Margarita, California, United States. No signs of disease on adults or propagules were reported by the nursery. Plants were immediately

transplanted to 10-L pots and kept outside, watered regularly, and allowed to grow for 18 months before being transferred into a greenhouse bay. Plants were observed regularly during this time to ensure no signs of disease developed. Of the 30 plants, five did not survive, either due to transplant stress or infection by insects, reducing the total number of plants 25.

2.2. Treatments

A completely randomized full-factorial design was used to organize the individuals into four treatment groups: droughted and inoculated with *N. australe* (D+; $n = 7$), droughted and not inoculated (D–; $n = 6$), watered and inoculated with *N. australe* (W+; $n = 7$) and a control; watered and not inoculated (W–; $n = 5$). Data were collected for ~90 days to track declines in health and mortality rates among the different treatments.

Drought-treated plants received 1 L of water on the day of inoculation (Day 0 of experiment) and another 0.5 L on day 38. Those with no drought treatment received 0.5 to 1.0 L of water by hand once per week depending on soil moisture, which was monitored regularly using a TDR machine from Soil Moisture Co. (Santa Barbara, CA, USA). Soil moisture for non-drought plants was maintained between 15–25% moisture for the entire experiment. Plants were carefully watered to eliminate risk of contamination via water splash.

Cultures for inoculations were made from re-isolations of field samples that were collected in January 2016 and positively identified to be *N. australe* (GenBank accessions MH754931 and MH777004; Schultheis et al., 2018). Inoculations took place on 3 November 2016 (Day 0 of experiment), using methods adapted from Michailides (1991) and Swiecki

and Bernhardt (2003). Mycelial plugs were made from 8-d-old cultures growing on half-strength potato dextrose agar amended with streptomycin to prevent bacterial contamination. Plants were first sprayed with 70% isopropyl alcohol to sterilize the surfaces and surrounding areas. Mycelial plugs were taken from the advancing margin of *N. australe* cultures (using a 3-mm cork borer) and placed on strips of Parafilm (mycelial side up) using sterile petroleum jelly for adhesion. Plugs were then placed (mycelial side down) to superficial wounds made on the main stem (also using a sterile 3-mm cork borer). The Parafilm strips were then gently wrapped 2–3 times around the stem to keep the plugs in place and prevent contamination. Those plants not receiving fungal inoculation received a control inoculation with uncultured potato dextrose agar (also half-strength and amended with streptomycin) using the same techniques.

To confirm Koch's postulates, the standard criteria to determine the agent causing a disease (Manion, 1991), we reisolated fungi from stem tissue at least 2-cm above the point of inoculation (POI) in harvested plants, amplified using primer pairs ITS1F/ITS4 for the ITS and EF1-728F/986R for alpha-elongation factor-1 (EF1). They were sequenced using the protocol described by Schultheis et al. (2018).

2.3 Greenhouse conditions

Greenhouse conditions were set to reflect summer conditions in Santa Barbara. Daily temperatures were maintained between 18–30°C during the day and 10–15°C at night. Humidity maintained at 50%. Photosynthetically active radiation (PAR) lights were set to provide 14 h of daylight per day and a maximum of 2000 μmol. Plant positions were

randomized weekly using a random number sequence generator (Random Sequence

Generator, 2010) to eliminate any microclimate effects in the greenhouse.

2.4 Data collection

2.4.1. Plant structure

Before inoculations, each plant was measured for basal circumference, number of

branches, plant height, and canopy volume. Volume was determined by measuring the width

of the canopy in two perpendicular directions and calculated as the volume of an elliptical

cone: $V = (\pi \cdot \text{Width 1} \cdot \text{Width 2} \cdot \text{Height})/3$.

2.4.2. Plant physiology

Physiological stress due to drought and pathogen infection was inferred from weekly

measurements of net photosynthesis (net CO_2 assimilation rate; A_{net}) and dark-adapted

chlorophyll fluorescence (maximum potential PSII efficiency; F_v/F_m) using a LI-COR

6400XT (LI-COR, Lincoln, NE, USA) and Hansatech FMS2 system fluorometer (Hansatech

Instruments, Norfolk, UK), respectively. Leaves were dark-adapted using leaf clips for 20–30

min before measuring fluorescence. One healthy (green), fully expanded leaf per plant, or on

one healthy and one stressed/diseased leaf per plant if symptom onset had begun (to reflect

overall canopy health). All data were collected between 10:00 hours and 16:00 hours to

capture peak values for the day, with the majority of measurements taken between 10:00

hours and 12:00 hours. Due to mechanical issues, chlorophyll fluorescence was not measured

on 4 and 11 November and on 20 December 2016. Net photosynthesis and dark-adapted

fluorescence were chosen as proxies for plant health, as lower values correlate strongly with

higher levels of drought stress (Cornic and Massacci, 1996; Vilagrosa et al., 2010). Optimum

values for F_v/F_m are 0.80–0.83 in C_3 plants (Björkman et al., 1987).

2.4.3. Stress index

A stress index was created for assessing and quantifying drought and fungal

symptoms. These symptoms included changes in leaf color from green to yellow, brown, or

black, the appearance of black leaf veins indicating spread of the fungus, and in rare cases,

presence of fungal cirrhi exuding from host tissue. Symptoms were rated according to the

percentage of canopy affected (Table 1).

2.4.4. Assessment of mortality and harvesting

Mortality of an individual was assessed by using the stress index, inspecting the

texture of the leaves, and evaluating gas exchange and dark-adapted fluorescence values. An

individual was determined dead and harvested immediately if it had a stress index score of

five or higher, leaves were crispy instead of flexible, *and* if at least two leaves (on different

branches, where applicable) measured A_{net} and F_v/F_m values of less than 0.5 µmol CO_2·m^{-2}·s^{-1}

and less than 0.300, respectively. The dates each plant was harvested and structural data at

harvest (described in the methods above) were recorded.

Stems of harvested plants were checked for lesions by scraping the bark away from

the POI and looking for darkened, necrotic tissue extending upward from the POI. Lesion

length was measured in centimeters from the POI to the farthest advancing margin of the

lesion.

2.5 Contamination prevention

To prevent sporulation and possible contamination among plants, inoculated plants were regularly inspected for signs of sporulation (e.g., growth of cirrhi on leaves and branches) and sprayed with 70% ethanol as needed, according to methods of Brown-Rytelewski and McManus (2000) and Slippers and Wingfield (2007). All inoculated plants were harvested, bagged, and stored at 4°C.

2.6 Data analyses

Soil moisture, plant structure, physiological data, and disease severity were statistically compared using ANOVA in JMP, version 14 Pro (SAS Institute, Cary, NC, USA), and post hoc analyses of means were performed using Wilcoxon signed rank test. Two-way factorial ANOVAs were conducted on the influence of watering regime (watered or droughted), inoculation treatment (inoculated or non-inoculated), and interaction effects between watering regime and inoculation treatment on plant A_{net}, F_v/F_m, and disease severity. Correlations between disease severity and physiological stress responses (A_{net} and F_v/F_m) were also examined in JMP using a linear regression analysis to determine maximum fit. Survivorship of each treatment group was estimated using the Kaplan–Meier survival analysis with the survival package (v. 2.43-3; Therneau, 2018) in R v. 3.5.1 (R Core Team, 2018). A Cox proportional-hazards model was followed by a Peto and Peto post hoc test to test for statistical significance of Kaplan–Meier survivorship. Due to the small sample size of individuals available for the experiment, all reported results for survival were based on a 90% confidence level, and P-values above 0.05 but below 0.1 were considered significant trends. All other tests were conducted using a 95% confidence level for significance.

3. Results

3.1 Drought treatment

The watering regimes produced significant differences in mean percentage soil moisture values between watering treatments (drought and watered) after Week 2 of the experiment (Fig. 1). While the watered plants averaged 15.9% ± 0.46 throughout the experiment with very little fluctuation, the droughted plants declined steadily after each watering event (Weeks 0 and 4). Mean soil moisture at the end of the experiment for drought-treated plants was 1.18% ± 0.39 and 15.96% ± 1.60 ($P < 0.0001$). Inoculation treatments had no effect on soil moisture ($P = 0.503$).

3.2 Effect of treatments plant physiology

Mean A_{net} and F_v/F_m declined throughout the experiment in all treatment groups except for the control (Fig. 2). By Week 10, mean values in the control group were significantly higher than all other treatment groups for both A_{net} ($F_{3, 21} = 8.089$, $P = 0.0009$) and F_v/F_m ($F_{3, 21} = 13.735$, $P < 0.0001$). A significant interaction was found between watering regime and inoculation treatment on both A_{net} and F_v/F_m (Table 3; $P = 0.0456$ and 0.008, respectively).

3.3 Stress onset/severity

Discoloration of leaves occurred on individuals experiencing both drought- and fungal-related stress (Fig. 3). Leaves of drought-treated plants turned from green to brown as drought stress progressed, and leaves from inoculated plants (both drought-treated and watered) turned brown or dark grey/black. No fungal symptoms appeared in the D– group

throughout the experiment; therefore, all stress symptoms in this group were presumed to be drought-related.

There was a significant interaction effect between watering regime and inoculation treatment on stress severity in Week 10 (Table 3; $P = 0.0058$). Stress symptoms appeared most rapidly in the D+ and W+ treatment groups, and by Week 5 both of these groups had greater stress symptom severity than the control plants (Figure 4, ($F_{3, 21} = 5.81$, $P = 0.0047$)). The D+ treatment group had greater mean stress severity than the D– group but was not significantly different from the W+ group. By Week 10, the interaction between watering and inoculation had strengthened and although all individuals in the D+ treatment group had the highest levels of disease severity (mean $= 6.0 \pm 0.0$ SE), all treatment groups exhibited higher mean disease index scores than the control group ($F_{3, 21} = 15.91$, $P < 0.0001$, Figure 4).

3.4 Correlations between stress severity and A_{net}, F_v/F_m

Across all treatment groups, both A_{net} and F_v/F_{m} were significantly correlated with stress severity in simple linear and polynomial regression; however, polynomial models resulted in best fits for the data and are thus reported here ($F_{2, 271} = 230.05$, $P < 0.0001$; $F_{3, 195} = 560.18$, $P < 0.0001$, respectively). F_v/F_{m} ($R^2 = 0.90$) exhibited a stronger correlation than A_{net} ($R^2 = 0.63$), but A_{net} began to decline at lower stress severity levels than F_v/F_{m} (Fig. 5; Appendix S1, see Supplementary Data with this article). Within inoculation treatments, A_{net} was more strongly correlated with disease severity in inoculated plants ($R^2 = 0.74$, $P < 0.0001$) than non-inoculated groups ($R^2 = 0.43$, $P < 0.0001$), regardless of drought treatment.

$F_\mathrm{v}/F_\mathrm{m}$ correlated strongly with disease severity in both inoculated ($R^2 = 0.91$, $P < 0.0001$) and non-inoculated ($R^2 = 0.84$, $P < 0.0001$) plants.

3.5 Mortality

Survivorship at the end of the experiment significantly differed between treatment groups ($F_{3, 21} = 25.44$, $P < 0.0001$), with the D+ group experiencing the greatest mortality (zero survivorship; Fig. 6). Mortality was next highest in the W+ group (17% survivorship), followed by the D– group (57% survivorship). All individuals in the control group (W–) survived. A Kaplan–Meier product-limit approach for survival probability confirmed that the likelihood of survival was significantly less for the D+ group than for W+, D–, and W– ($P = 0.087$, $P = 0.003$, and $P = 0.009$, respectively).

There was no significant correlation between plant canopy volume and survival ($R^2 = 0.006$, $P = 0.91$) or stem diameter and survival ($R^2 = 0.009$, $P = 0.66$), indicating that pre-treatment plant size was not a confounding variable in this experiment.

4. Discussion

The results of this study support the hypothesis that drought stress reduces resistance to pathogens in *A. glauca*, and fungal infection enhances plant mortality compared to drought alone. As predicted, both physiological metrics showed declines as drought and disease progressed, suggesting rapid plant responses to both stresses. Furthermore, a strong correlation was found between declining physiological function (A_net and $F_\mathrm{v}/F_\mathrm{m}$) and increases in stress severity index, suggesting that visible signs of stress may be used to assess physiological decline and reveal more severe underlying problems in the field. Finally,

although mortality rates for inoculated groups (both droughted and watered) were similar, drought-stressed *A. glauca* shrubs infected with *N. australe* trended toward faster and greater mortality than in any other treatment group.

4.1 Correlations between visible and physiological stress

Both chlorophyll fluorescence and net photosynthesis declined as hosts were exposed to drought and fungal infection. Each of these factors caused measurable physiological stress in *A. glauca* individually; however, in combination, stress symptoms showed up earlier and more strongly (Fig. 4A). Additionally, an important result was the relationship between visible stress symptom severity and physiological function. Both A_{net} and F_v/F_m were found to be highly correlated with visible signs of stress that ultimately led to plant mortality. This is consistent with previous studies that have found that F_v/F_m correlates strongly with eventual mortality, and therefore, can be an indicator of drought-related mortality risk in natural systems (Valladeras and Sánchez-Gómez, 2006; Vilagrosa et al., 2010; Venturas et al., 2016). Furthermore, A_{net} was shown to decline even with very low levels of visible stress, suggesting it may be valuable as an early detector of plant stress even before major visible symptoms appear. While A_{net} and F_v/F_m can be useful tools for measuring physiological stress, they are expensive and difficult to measure on the ground at large scales. Therefore, using visible stress severity indices may be a promising and cost-effective method with which to quickly carry out large surveys aimed at predicting drought- and fungus-related mortality in the field.

4.2. Synergy and predisposition to disease

High mortality was observed in all inoculated plants regardless of drought treatment, indicating that *N. australe* may act as an obligate pathogen on *A. glauca*, at least in young, small individuals as were used in this study. However, mortality occurred much faster in the D+ (drought + inoculated) group. Additionally, some individuals in the D– and W+ group at Week 10 survived well beyond the termination of experiment (*n* = 3 and *n* = 1, respectively), suggesting the ability of *A. glauca* to allocate sufficient resources for defense against drought stress, and in some cases, infection by *N. australe*, but a greater vulnerability in the simultaneous presence of both factors. Therefore, it appears that a synergistic interaction does exist whereby exposure to both drought and infection by *N. australe* yields more accelerated decline than either factor alone.

It is likely that *A. glauca* susceptibility, or "predisposition" to disease (Schoeneweiss, 1975), is due to the interactive roles of water and carbon availability in plant defenses against drought stress and biotic invaders, as modeled by McDowell et al. (2011) and Oliva et al. (2014). Their framework describes a system in which plant hosts are able to allocate resources to either survive extreme environmental stress *or* defend against biotic invasion, but may succumb via depleted carbon resources when exposed to multiple stressors. For example, hosts like *A. glauca* can persist through drought with high resistance to cavitation (Venturas et al., 2016). They can also divert carbon resources to block the spread of pathogens (Oliva et al., 2012, 2014*)*. However, the combination of global-change-type drought and infection by pathogens like *N. australe* may leave these hosts vulnerable when they no longer possess the resources needed to simultaneously resist cavitation and invasion by the pathogen. Furthermore, extreme drought conditions can enhance optimal conditions

for the growth of pathogens like *Botryosphaeriaceae* fungi that thrive in more negative water potentials than the host can withstand (Ma et al., 2001). These factors combined can push the host beyond a threshold, increasing branch dieback and ultimately increasing the likelihood of whole plant mortality.

Understanding the role of pathogens and drought stress in native vegetation canopy loss has long been of great interest to ecologists, though research involving such systems has yielded varying results regarding these interactions. For example, a meta-analysis by Jactel et al. (2011) found that in studies on the effects of pathogens and insects on forest plant hosts during drought, damage to hosts varied greatly based on the feeding habits and substrate of the pathogen (e.g., primary vs. secondary agents, and leaf vs. wood-dwelling agents) and severity of the water stress. In the case of secondary agents (e.g., *Bot.* fungi), more damage occurred on hosts experiencing water stress compared to non-stressed controls, and damage severity increased with increasing water stress. These findings are consistent with the results of the present study and support the hypothesis that drought stress predisposes hosts to pathogen impacts. Other studies have found similar results regarding secondary pathogens in drought-tolerant plant systems, including red pine forests (Blodgett et al., 1997), eucalyptus forests (Barradas et al., 2018), and chaparral shrublands (Brooks and Ferrin, 1994; Swieki and Bernhardt, 2003; Aguirre et al., 2018). By contrast, Davis et al. (2002) concluded that drought-induced cavitation alone, not infection by *Bot.* pathogens, caused canopy dieback of southern California *Ceanothus* sp. during drought, suggesting that secondary agents do not always benefit from drought-related predisposition.

Clearly, while secondary pathogens are known to become pathogenic in hosts experiencing environmental stress (Slippers and Wingfield, 2007), the mechanisms driving

this relationship in different plant hosts are not fully understood. Although field studies have been conducted on the presence of fungal pathogens on shrubland species during drought (Davis et al., 2002; Jacobsen et al., 2012; Aguirre et. al., 2018), controlled experiments manipulating both drought and fungal treatments in naturally occurring species are rare and typically involve tree systems (Crist and Schoeneweiss, 1975; Guyon et al., 1996; Blodgett et al., 1997; Solla and Gil, 2002, Barradas et. al., 2018) rather than wildland shrub species (although see Brooks and Ferrin, 1994, and Swiecki and Bernhardt, 2003). To the authors' knowledge, this is the first experiment to investigate the influences of drought and infection by *N. australe* on *A. glauca* by manipulating both factors.

4.3 Relevance to the field

The results of this experiment, along with the identification of *N. australe* and other *Bot.* species in the region (Schultheis et al., 2018), suggest that the severe canopy dieback of *A. glauca* observed in Santa Barbara County between 2012 and 2016 is likely the result of global-change-type drought combined with the presence of opportunistic fungal pathogens like *N. australe*. While there is evidence to suggest that acute drought alone may cause some mortality in *A. glauca* (Paddock et al., 2013; Venturas et al., 2016), the presence of *N. australe* and other pathogens likely exacerbates stress and accelerates mortality in these hosts. Furthermore, *N. australe* has long been reported in avocado orchards in Santa Barbara County (Halma and Zentmeyer, 1953; McDonald et al., 2009); however, there are no known reports or indications of major disease and dieback of *A. glauca* in surrounding chaparral shrubland system until recently, during the especially dry winters of 2013 and 2014 (C. D'Antonio and L. Drake-Schultheis, personal observations). Thus, we suspect that while *N.*

australe has likely been present on *A. glauca* hosts (possibly for decades), the drought of 2011–2018 was the most severe in the region in the past 1200 years (Mann and Gleick, 2015) and may have been significant enough to push adult *A. glauca* past a tipping point of defensibility against *N. australe*.

It should be noted that results of experiments on young plants, which may be highly susceptible to drought and drought-related mortality due to limited carbon reserves, may not scale directly to large, mature individuals in the field (He et al., 2005). This study showed high mortality in 2-year-old *A. glauca* exposed to a fungal pathogen with and without drought, in contrast with field observations of diseased, large adults (>1.5–3 m height) exhibit severe canopy dieback (>50%) and are ridden with fungal cankers, yet still survive (L. Drake-Schultheis, unpublished data). Previous studies have yielded similar results: for instance, photosynthesis was shown to be greatly reduced in oak seedlings compared to adults in drought years compared to wet years (Cavender-Bares and Bazzaz, 2000), and He et al. (2005) reported that responses of red maple and paper birch saplings to a 1995 drought were significantly different than those of mature adults. Similarly, since hosts are often able to allocate carbon reserves to compartmentalize canker-causing agents like *N. australe* within carbon-rich barriers (Oliva et al., 2014), larger individuals with more biomass and greater carbon stores are able to utilize and direct more resources to defense than younger, smaller individuals. Thus, mature plants can better persist through biotic attack during environmental stress than their younger counterparts and experience various levels of canopy dieback rather than full mortality. *Arctostaphylos glauca* are obligate seeders, meaning they are killed by fire and must maintain populations by individuals recruiting from seed rather than

resprouting from their base. Therefore, young, small individuals may be of greatest concern for future populations of this species.

Because current research is predicting more frequent and extreme drought events (IPCC, 2013), more exotic pathogens (Bradley et al., 2010), and more frequent fire in these southern California shrublands (Safford and Van de Water, 2014), populations of *A. glauca* could decline because small individuals may be highly susceptible to disease and mortality. A valuable next step for understanding these risks and predicting future shifts in vulnerable chaparral communities would be to monitor young recruiting populations of *A. glauca* for *N. australe* for signs of stress, infection, and mortality in the wild.

5. Conclusion

In the face of rapid climate change, it is increasingly important to understand the abiotic and biotic mechanisms driving ecological landscape change. Large plant dieback events can produce major ecological consequences, including changes in vegetation cover (Allen and Breshears, 1998; Mueller et al., 2005), increased fire risk (Hanson et al., 2000; Bigler and Veblen, 2001), and changes in hydrology (Redding et al., 2008; Winkler et al., 2009), all of which affect ecosystem structure and functioning (Anderegg et al., 2013). Furthermore, the loss of even a few species can trigger effects on the local food web structure (Carnicer et al., 2001; Borer et al., 2005; Bascompte et al., 2007), and increase risk of invasion (Davis et al., 2000; Kane et al., 2010). The results of this study suggest that small individuals of *A. glauca*, one of the most common and widespread species the southern California chaparral community, are at high risk of disease and dieback due to opportunistic pathogens and extreme drought. The potential for dieback of *Arctostaphylos* spp., which

provide food for animals such as mice, rabbits, and coyotes (Keeley and Hays, 1976) and are an important component of post-fire woody regeneration in chaparral, raises concerns regarding changes to ecosystem structure and functioning in the coming decades.

Many ecosystems today are facing unprecedented drought (IPCC, 2013); yet, the interactions of drought and pathogens in wildland settings are difficult to study because the multitude of confounding variables and the challenges of manipulating both the pathogens themselves and climate. Thus, greenhouse studies such as this one are increasingly essential to understand the influences of drought and pathogens as they relate to dieback events, as well as to understand the relationship between stress and shrub/tree ontogeny (McDowell et al., 2013). Critical questions remain regarding the relative tipping points for large-scale dieback among historically drought-tolerant species such as *A. glauca* that today are facing the combination of extreme drought and novel (introduced) pathogens. These pathogens may not express themselves until there is drought, highlighting the need for broader field surveys and long-term monitoring of wildland ecosystems. An important step to understanding the role of disease in contributing to vegetation change is also to isolate pathogens and test their pathogenicity under varying controlled conditions. This study provides one such step for what appears to now be a widespread, opportunistic introduced pathogen in an important native California chaparral shrub.

Table 1. The stress index used to quantify canopy stress severity according to percentage canopy affected on individual *Arctostaphylos glauca* plants.

Rating	Percentage range (amount of canopy affected[a])
0	Symptom not seen
1	<2.5
2	2.5 to <20
3	20 to <50
4	50 to <80
5	80 to <97.5
6	97.5 to 100

[a] Stress severity was assigned by estimating the percentage of total canopy with drought stress, fungal infection, or both.
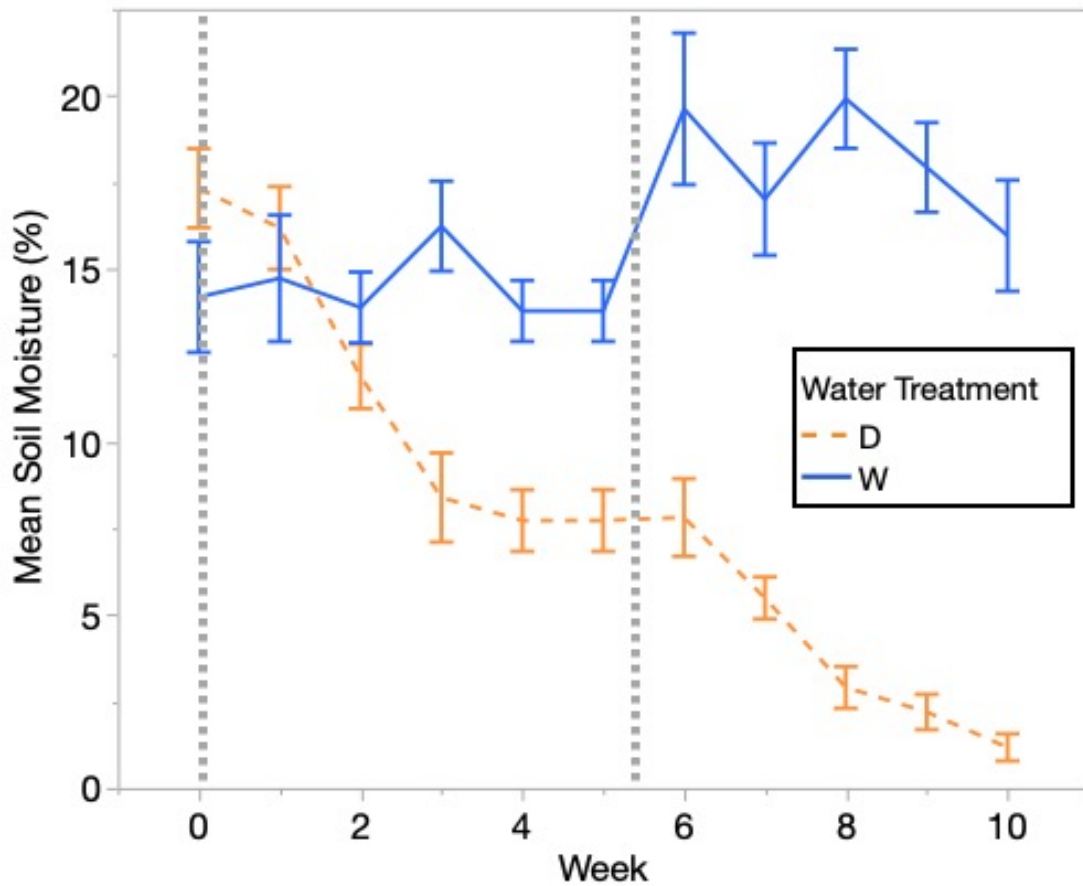
Figure 1. Mean percentage soil moisture between watering regimes: drought, D; water, W. Gray, dotted lines mark when droughted plant received water. On the day of inoculations (Week 0), all plants were watered to soil saturation. Individuals were then either not watered at all (D) or watered regularly with 0.5 to 1.0 L of water to maintain a target soil moisture range of 15–20%. During Week 5, drought-treated plants received 0.5 L of water, then no water for the remainder of the experiment. Values are means of each watering regime (n = 7–14) $\pm$ SE.
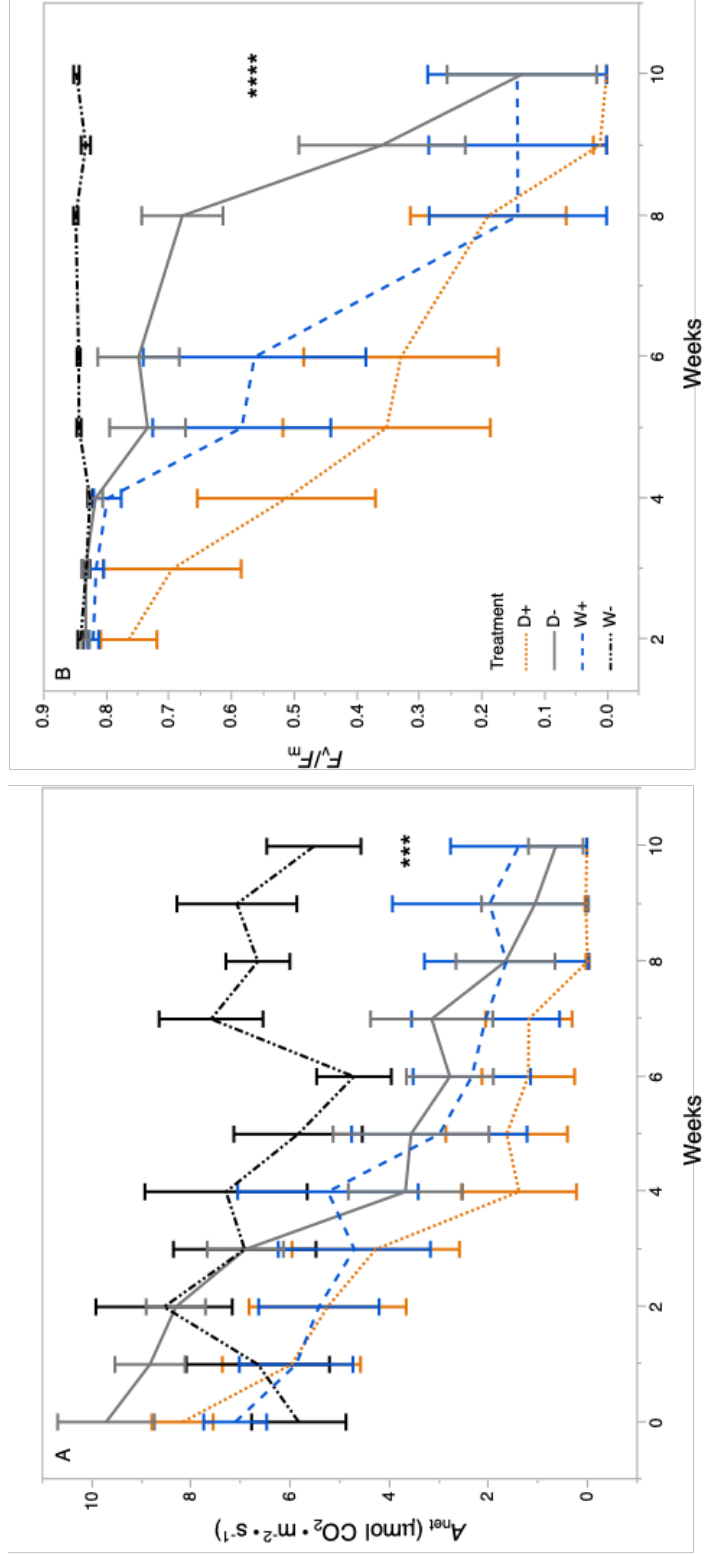
Figure 2. Mean (A) photosynthesis, A_{net}, and (B) dark-adapted fluorescence, F_v/F_m, by experiment week for big berry manzanita (*Arctostaphylos glauca*) subjected to drought and fungal inoculation, D+; drought without inoculation, D−; watering and inoculation, W+; and watering without inoculation, W−; control. F_v/F_m measurements did not begin until Week 2 of the experiment. Bars denote means ± SE, $n = 5$–7. ANOVA significance levels comparing means at Week 10 between treatment groups: ***$P < 0.001$; ****$P < 0.0001$

Table 2. Means for net photosynthesis, A_{net}, and dark-adapted chlorophyll fluorescence, F_v/F_m, and mortality among treatment groups of *Arctostaphylos glauca* in Week 10.

Treatment	Symbol	A_{net} (µmol $CO_2 \cdot m^{-2} \cdot s^{-1}$)	F_v/F_m	Mortality (%)
Drought + Inoc	D+	0.00**	0.00**	100
Drought – Inoc	D–	0.63 $\pm$ 0.55*	0.135 $\pm$ 0.11*	43
Water + Inoc	W+	1.38 $\pm$ 1.38a*	0.143 $\pm$ 0.14*	83
Water – Inoc (Control)	W–	5.51 $\pm$ 0.95	0.847 $\pm$ 0.004	0

Notes: Means ($\pm$SE) in each column are averages for all individuals within a treatment group (n = 5–7). Means within a column followed by asterisks differed significantly from the control according to a Dunn method comparison, (*$P < 0.05$, **$P < 0.01$).

Table 3. Two-way ANOVA for effects of watering regime (Water) and inoculation treatment (Inoc.) on photosynthesis (A_{net}), dark-adapted fluorescence (F_v/F_m), and stress index (SI) *Arctostaphylos glauca* at Week 10.

Response variable	Treatment	df	F	P > F
A_{net}				
	Water	1	14.3852	0.0011
	Inoc.	1	8.327	0.0088
	Water × Inoc.	1	4.5166	0.0456
F_v/F_m				
	Water	1	19.3904	0.0002
	Inoc.	1	18.7113	0.0003
	Water × Inoc.	1	8.5912	0.008
SI				
	Water	1	20.6157	0.0002
	Inoc.	1	24.0342	<0.0001
	Water × Inoc.	1	9.4454	0.0058

Figure 3. Examples of the range of symptom severity levels used in the stress index to assess percent canopy affected in *Arctostaphylos glauca*. (A) Rating level = 0 (0% affected). (B) Rating level = 2 (2.5 to <20% affected). (C) Rating level = 4 (50 to <80% affected). (D) Rating level = 6 (97.5 to 100% affected).
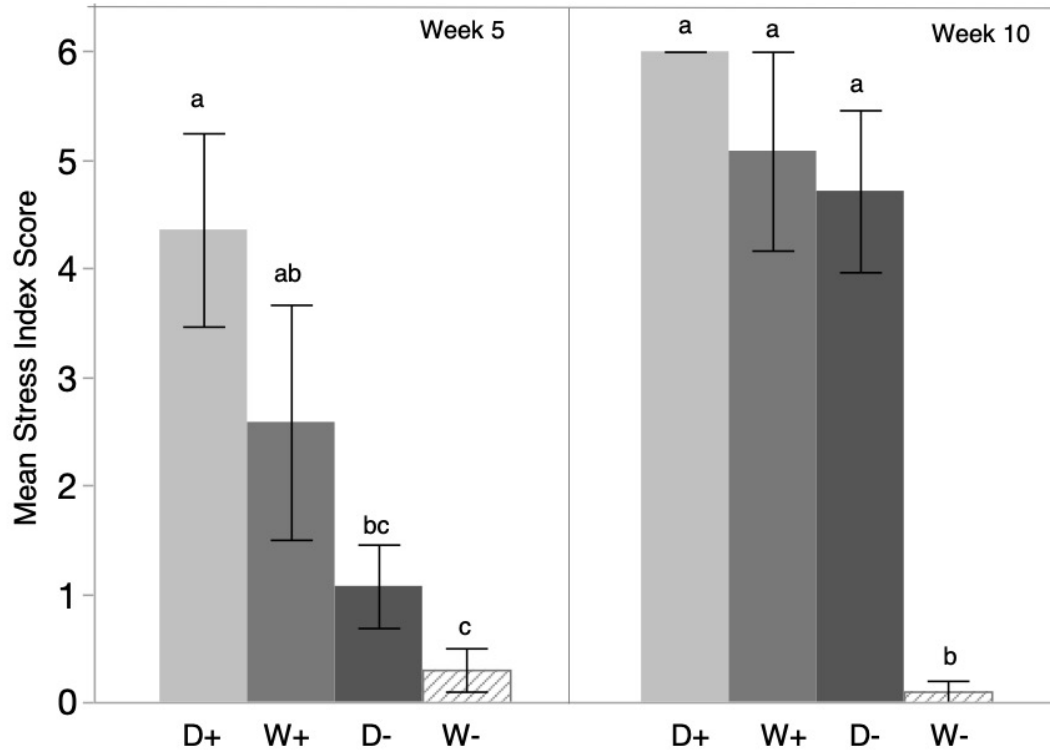
Figure 4. Mean stress severity scores for each treatment group of *Arctostaphylos glauca* at Week 5 and Week 10 of experiment. Bars are mean stress severity scores (±SE) averaged for all individuals within a treatment group (*n* = 5–7). Bars represented by the same letter are not significantly different according to a Wilcoxon test, $\alpha < 0.05$). General plant stress, including an aphid outbreak early on in the experiment, caused some low levels of foliar discoloration on a few of the control plants. However, as it was impossible to know the exact source of the discoloration during the experiment, all symptoms were recorded as stress-related, resulting in the scores seen in the control group in the data. All plants in the control group (W–) remained asymptomatic of any fungal disease for the entire duration of the experiment.
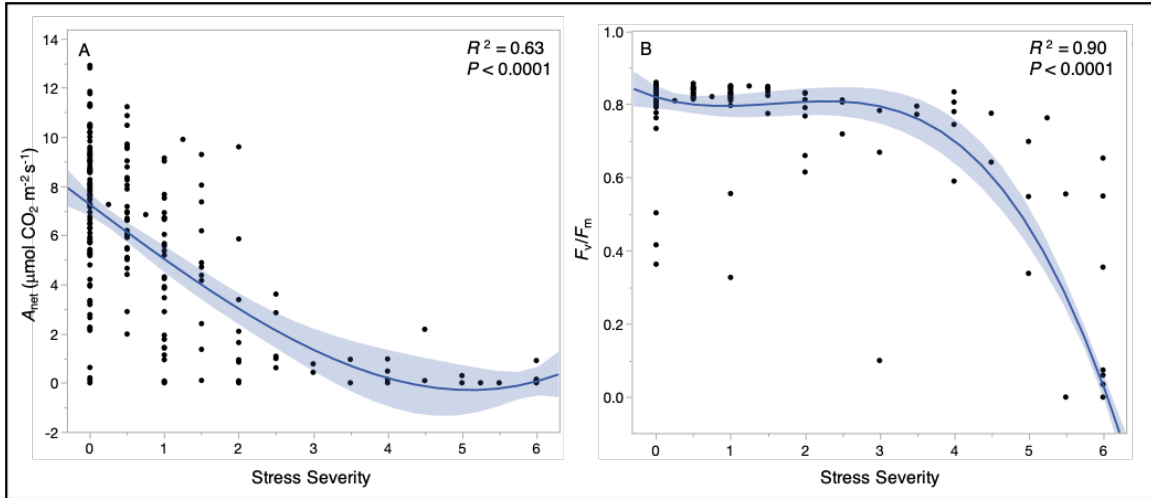
Figure 5. Correlations between stress severity and (A) net photosynthesis, A_{net}, and (B**)** dark-adapted fluorescence, F_v/F_m, across all treatment groups (D+, D–, W+, W–). Blue lines represent the best fit of each polynomial regression with 95% confidence intervals (gray-shaded regions).
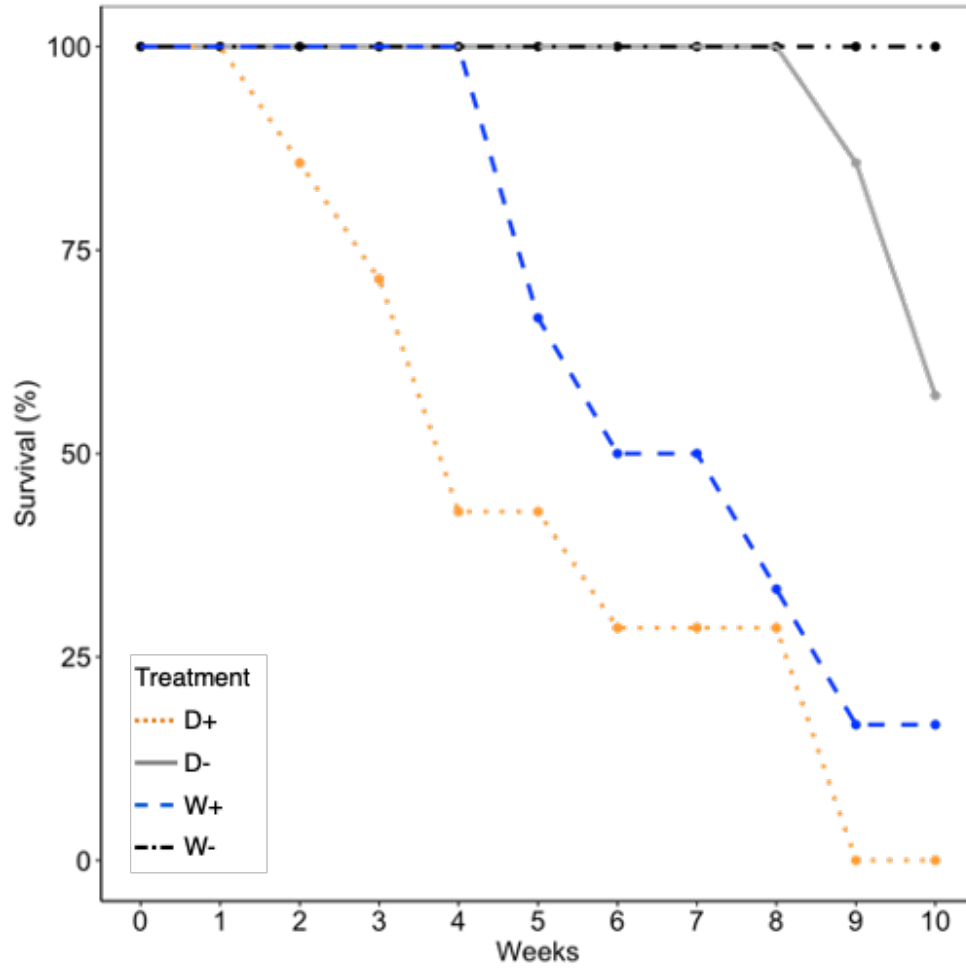
Figure 6. Percentage survival during 10 weeks of experiment for all treatment groups, D+, D–, W+, W–. *n* = 5–7 per treatment. Survival was determined each week as the total number of individuals still alive from the original number on Day 1 of the experiment.

Appendix B

Supplemental Table S1. Summary of regression model statistics best describing variation in A_{net} and F_v/F_m by stress severity (X) in *Arctostaphylos glauca*. Results are across all treatment groups. Significant effects ($\alpha = 0.05$) are in bold.

Response Variable	Term	Estimate	SE	t-ratio	Prob > t
A_{net}	Intercept	7.3058	0.2166	33.73	**<.0001**
	X	-2.639	0.3006	-8.78	**<.0001**
	X^2	0.2371	0.0486	4.88	**<.0001**
	R^2: 0.629				
	Adj. R^2: 0.627				
F_v/F_m	Intercept	0.8193	0.0153	53.47	**<.0001**
	X	-0.0609	0.0346	-1.76	0.0797
	X^2	-0.0486	0.0166	2.83	**<0.0052**
	X^3	-0.0097	0.0019	-5.04	**<.0001**
	R^2: 0.896				
	Adj. R^2: 0.894				

Chapter 4. Identifying landscape patterns of stress and dieback in big berry manzanita across a multi-year drought.

Abstract

Drought events worldwide are increasing in frequency, duration and intensity. In plant assemblages such as Mediterranean-type shrublands that are typically considered to be drought tolerant, the extent to which extreme droughts cause dieback of dominant plant species is poorly known. Here, we measured water availability, dark-adapted fluorescence, photosynthesis and canopy dieback of a dominant and classically drought tolerant Mediterranean-climate shrub, *Arctostaphylos glauca*, during an historic drought in southern California. We hypothesized that dieback severity was related to variations in water availability due to elevation and aspect as measured by xylem pressure potentials (XPP), We found that aspect and elevation were both correlated with XPP, with XPP being lowest at lower elevation and in plants with a more SW aspect, but that this was dependent on season. Canopy dieback increased across most sites over the study period, with the greatest increase seen in lower elevation sites. Aspect was significantly related to dieback across all years, though elevation was also strongly correlated in the highest dieback year. Surprisingly, no new whole plant mortality was observed, suggesting high resiliency of individual shrubs during this prolonged drought. However, our results also indicate that A. glauca are susceptible to losing a large percentage of their canopy, and that populations at lower elevations and on southwest-facing slopes are particularly vulnerable to such dieback. Given the importance of A. glauca in fruit provisioning to wildlife and fuel load in chaparral

shrublands, canopy dieback – even when not associated with shrub mortality – will likely have significant consequences on chaparral foodwebs and fire regimes.

1. Introduction

Extreme drought events from climate change have produced immediate and dramatic effects in recent years, with costs often exceeding $1 billion due to their widespread economic and ecological impacts (Smith and Katz, 2013; NOAA, 2014). Among the ecological consequences is widespread tree mortality, (Allen et al, 2010; Carnicer et al., 2011), event within plant systems that have historically been considered drought-tolerant (Breshears et al., 2005; Allen et al., 2010; Clifford et al., 2013). While seasonal droughts are known to be a natural and regular occurrence in arid and semi-arid regions, the increased frequency, duration, and intensity with which they have occurred in recent years is highly unusual (Allen et al, 2010; Clifford et al, 2013). Such extreme droughts, referred to as "global-change type drought" (Breshears et al., 2005), are predicted to continue, and even become the norm, as a result of human-induced climate change (IPCC 2013; Franco et al., 2014). Consequently, species that are typically capable of withstanding regular drought stress may be susceptible to canopy dieback, and mortality, as a result of shifts in drought regimes (Breshears and Allen 2002; Breshears et al, 2009; McDowell et al., 2008).

One such plant community that may be vulnerable to extreme climatic change is chaparral. Chaparral shrublands, which occupy approximately 7 million acres throughout California (Quinn and Keeley, 2006), are a dominant vegetation community in southern California, composed primarily of evergreen, drought tolerant shrubs and subshrub species including manzanita (*Arctostaphylos* spp), ceanothus (*Ceanothus* spp), and chamise (*Adenostoma fasciculatum*). These species are well adapted to the seasonal variations in

temperature and precipitation typical of mediterranean climates where hot, rainless summers are the norm (Mooney, 1982; Keeley, 1991; Meentemeyer and Mooney, 2002). However, mediterranean-type regions like southern California are predicted to experience rapid increases in temperature (Loarie et al., 2008), and increased drought occurrence and severity (Planton et al (2008); IPCC, 2013), resulting from human-caused climate change. These regions have thus been designated as worldwide global change "hot spots" (Kelly and Goulden 2008, Kerr 2008). Indeed, recent studies have reported extensive mortality of chaparral shrub species resulting from global-change type drought throughout southern California (Davis et al., 2002; Paddock et al., 2013; Coates et al., 2015 Jacobsen and Pratt, 2018). Thus, climate change represents a significant threat to native plant community persistence in this region.

A critical topic for ecological research is understanding where, how, and to what extent plant communities will change as a result of increased drought (Sutherland et al, 2013). Studies aimed at understanding the physiological mechanisms behind drought-related plant mortality – and why some plants suffer mortality from drought while others survive - have elucidated a variety of complex mechanisms of plant mortality (McDowell et al, 2008). These include loss of hydraulic conductance (hydraulic failure), exhausted carbon reserves (carbon starvation), and susceptibility to pests and pathogens due to being in a weakened state from drought (Breshears et al., 2009; McDowell et al., 2011; Hicke and Zeppel, 2013; Oliva et al., 2014).

Measuring xylem pressure potential (XPP) can be a useful index of soil water availability (Poole and Miller, 1981), and dark-adapted fluorescence (Fv/Fm) can be a quick and accurate indicator of plant stress, as values drop significantly in water-stressed plants,

(Cornic and Massacci, 1996; Vilagrosa et al., 2010). Together, these may be useful tools for predicting plant vulnerabilities to drought and biotic invasion. Landscape variables such as elevation, slope, and aspect have also been shown to correlate with plant water stress (Poole and Miller, 1981; Gitlin et al, 2006; van Mantgem et al, 2006; van Mantgem and Stephenson, 2007; Meng et al., 2015) and mortality (Koepke et al, 2010; Baguskas et al, 2014), and can be useful for predicting vulnerabilities during drought. However, major knowledge gaps still remain, and studies combining field mortality patterns with physiological data on plant water stress are rare (McDowell et al., 2008; Breshears et al., 2009).

Plants employ a variety of complex strategies to cope with drought stress, but generally fall along a continuum of "drought avoiders" or "drought tolerators". Drought avoidance, also known as "isohydry", refers to plants that regulate stomatal conductance (G_s) to maintain high minimum water potentials as soil dries out (Tyree and Sperry, 1989; Tardieu, 1993; Choat et al., 2012). While this strategy reduces the risk of xylem cavitation and subsequent hydraulic failure, it may increase the likelihood of carbon starvation, as C assimilation is greatly reduced (McDowell et al., 2008). Conversely, drought tolerant (anisohydric) plants maintain higher G_s, even at very low water potentials, which allows for continued C assimilation but with greater risk of xylem cavitation (Tyree and Sperry, 1988; McDowell, 2008). These different strategies can have significant implications for ecosystem-level consequences of severe drought (Breshears et al., 2009; Roman et al., 2015); indeed, recent studies have linked anisohydry with greater levels of mortality in chaparral systems (Venturas et al., 2016; Jacobsen et al., 2018).

An historic drought in southern California provided an opportunity to simultaneously measure physiological stress and dieback severity along an elevational gradient in a

classically drought-tolerant evergreen chaparral shrub, big berry manzanita (*Arctostaphylos glauca*). *A. glauca* is one of the largest and most widely-spread members in a genus consisting of nearly 100 species. Its range extends as far north as the Cascade mountains and south into Baja California, though it is most dominant in southern California shrublands (Hickman, 1993). They frequently occur on exposed ridges and rocky outcroppings). In the chaparral shrublands of Santa Barbara County, it occurs from elevations of about 500-1200m. *A. glauca* are obligate seeders, and must recruit from the seedbank following fire (Keeley, 1977, 1998; Keeley and Davis 2007). Compared to resprouters, which regenerate from a carbohydrate-rich burl at their base following fire, seeders tend to be fairly shallow-rooted (< 1m), and are thus less able to access deep water sources (Hellmers et al., 1955; Miller and Ng, 1977; Miller and Poole, 1979; Thomas and Davis, 1989). Seeders are generally considered to be more tolerant of seasonal drought than resprouters (Poole and Miller, 1975; Davis et al, 1999; Paddock et al., 2013), possibly a mechanism for shallow-rooted seedlings to survive summer drought in an open post-fire environment following germination (Jacobsen et al., 2007; Pratt et al., 2012). However, this strategy has also been linked to higher mortality during extreme drought (Jacobsen et al., 2007; Pratt et al., 2013; Venturas et al., 2016). *A. glauca* are also known to exhibit anisohydric mechanisms of drought tolerance (Poole and Miller, 1975; Paddock et al., 2013), and can exhibit extremely low water potentials ($\leq$ -6.0MPa) and high resistance to cavitation during seasonal drought (Poole and Miller, 1975; Jacobsen et al., 2007; Venturas et al., 2016).

In 2014, we observed sudden and dramatic dieback in *A. glauca* in the Santa Ynez mountain range of Santa Barbara, California during an historic drought (Griffin and Anchukaitis, 2014). The drought that lasted from 2012 to 2018 in southern California was the

most severe to hit the region in 1,200 years (Griffin and Anchukaitis, 2014), with 2014 being

the driest year on record (Mann and Gleick, 2015). Preliminary field observations indicated

greater levels of canopy dieback at lower elevation stands compared to higher elevations.

Dieback also seemed to be more prevalent on exposed and southwest-facing slopes, which in

this region experience direct sunlight for most of the day. Other studies have reported

significant *Arctostaphylos* spp. dieback (Brooks and Ferrin, 1994) and even mortality (Gitlin

et al, 2006; Paddock et al., 2013; Venturas et al., 2016; Jacobsen and Pratt, 2018) during

periods of extreme drought stress, further suggesting species in this genus are vulnerable to

drought-related mortality.

Additionally, we observed widespread symptoms of fungal infection – including

branch cankers and brown/black leaf discoloration – later identified as members of the

opportunistic *Botryosphaeriaceae* family (Schultheis et al., 2018), suggesting multiple

factors may be driving canopy dieback in this species. Drought-related mortality has

previously been associated with opportunistic fungal pathogens in *A. glauca* and other

chaparral shrubs (Brooks and Ferrin, 2004; Aguirre at al., 2018), yet few studies have sought

to understand the relative levels of drought stress incurred by plants infected with these

pathogens, or how stress is related to canopy dieback and/or mortality.

A. glauca shrubs are important members of the chaparral ecosystem, providing

habitat and food for wildlife through their nectar and berries (Keeley and Hayes, 1976). Their

structure and fire-induced germination strategies also make them significant components of

the chaparral fire regime and post-fire successional trajectories (Keeley, 2006). Large-scale

mortality of this species could reduce resource availability for wildlife, as well as alter fuel

composition and structure in the region, resulting in an increased risk of more intense, faster

burning fires. Therefore, the potential continued dieback of *A. glauca* is of great concern for both ecosystem functioning and human populations alike. Yet because of the heterogeneity of landscapes in this rugged region, it is possible that portions of the landscape will act as refugia for drought-susceptible species.

We hypothesized that *A. glauca* dieback severity is associated with areas of increased water stress across the landscape. To better understand the patterns and trajectory of *A. glauca* stress and dieback across a topographically diverse region of coastal California, we asked the following specific questions: (1) How severe is drought-related stress and dieback in this region? (2) How do plant stress and dieback severity vary with elevation and aspect across the landscape? (3) How does dieback change across the landscape as a multi-year drought progresses? We chose xylem pressure potential (*XPP*) as an indicator of plant water availability, and measured dark-adapted fluorescence and net photosynthesis as proxies for drought-related plant stress and physiological function. To address Question 1, we conducted an initial survey measuring general levels of canopy dieback, shrub water availability, and stress in the region. To address Questions 2 and 3, we conducted a more in-depth study of how shrub water relations and dieback vary with aspect and along an elevational gradient, and tracked changes in dieback severity for the four final years of the seven-year drought. We expected to find areas of *low XPP* (suggestive of higher water stress) correlated with greater physiological stress responses, and more severe dieback in lower elevation sites and on southwest aspects. Alternatively, shrub stress and dieback may be dependent on a wider variety of variables, particularly in a landscape as heterogeneous as this. Additionally, we predicted that dieback severity and individual shrub death would increase over time in lower elevations and exposed slopes compared to upper elevations and more mesic slopes.

2. Materials and Methods

2.1 Research area

The specific area chosen for this study is located in the Santa Ynez mountains of Los Padres National Forest (Table 1). Stands of *A. glauca* occur from approximately 400m to 1200m elevation, and are frequently mixed with other co-dominate woody evergreen shrub species including *Adenostoma fasciculatum*, *Ceanothus megacarpus* and, at lower elevations, *Malosma laurina*. The landscape of this region is extremely heterogeneous, with unstable terrain composed largely of sandstone rock outcroppings and sandstone-derived soils (Supplemental Table S1), and steep slopes and ridges that are interrupted by deep canyons (Meentemeyer and Moody, 2002). These dramatic features, while common habitat for chaparral plant communities, were a limitation in our ability to choose field sites. Thus, we relied heavily on accessibility by road and trail in finding sites. The climate in this region is of a mediterranean-type, with cool moist winters and a hot, dry summer season. The majority of rainfall typically occurs from November to April, and mean annual rainfall, based on a 120-year average, is 47cm (Santa Barbara County Public Works Hydrology Division, 2021).

2.2 Precipitation data

Three weather stations, equipped with real-time, self-recording data loggers and maintained by the Santa Barbara County Public Works Hydrology Division, were chosen to retrieve precipitation data during the drought based on proximity and similar elevation to study sites. The Trout Club (369m), San Marcos Pass (671m), and El Deseo Ranch (1006m) stations represented low, intermediate, and high elevations, respectively. Data from these stations were retrieved from the Santa Barbara County Public Works Hydrology website.

Average rainfall at these stations, based on 54-69 year means, increases with elevation from 68.3cm to 90.4cm (Table 2). Annual rainfall data for this study are presented in "rainfall years" from November 1 of one year to October 31 of the next, to reflect the seasonal wet period preceding each sampling period. Consistent with these historical trends, annual rainfall at Trout Club was lowest between the 2014-15 and 2018-19 water years (Fig. 1). However, during this same time period, rainfall totals were generally lower at El Deseo Ranch (the highest elevation) compared to San Marcos Pass (intermediate elevation).

2.3. Preliminary assessment of site dieback and shrub stress

2.3.1 Site selection

In 2015, an initial survey was conducted to assess dieback, as well as shrub water demand and physiological stress as the summer dry season progressed during the drought. Five sites were chosen for this survey (Sites B-F; Table 1) representing variable elevation, slope, and aspect, but were also limited by access, safety and proximity to roads. Sites were defined as being composed of greater than 50% *A. glauca* cover, except for site C, which had lower than 50% *A. glauca* cover but favorable access. Boundaries were delineated using a combination of on-the-ground visual assessment and polygons drawn using 1m National Agriculture Imagery Program (NAIP) imagery (U.S. Department of Agriculture, 2014) within ArcGIS® (ESRI Corp, Redlands, CA). Later, they were refined using a Phantom 4 Pro Drone (Shenzhen DJI Sciences and Technologies Ltd, Shenzhen, Guangdong, China).

2.3.2 Stand Dieback

Each site was initially assessed for *A. glauca* stand dieback severity and site mortality in winter, before new summer leaf-out occurred (Table 1). Stand dieback severity was defined as the percent of non-green or defoliated canopy cover within the boundaries of the

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site, and was estimated by the collective valuation of two-to-three people viewing the stand from different angles. If stands were not completely pure *A. glauca* (which was common), we did not include canopies of other species in our estimation of average percent dieback. Data on stand mortality were collected by counting the number of dead individuals within site boundaries using ArcGIS. Number of dead individuals per site was also recorded.

2.3.3 Shrub selection

To measure shrub water availability and physiological stress through the summer dry season, twenty individuals per site were selected based on similar size (2 to 3m in height), accessibility, and representing different levels of health along a continuum. From these twenty, ten individuals were randomly chosen using a random number generator and tagged for collecting repeated data on stress and dieback as the dry season progressed. At one site (site E) only nine individuals fell within the size criteria and were readily accessible, therefore the sample size for this site was nine, for a total of 49 shrubs used in this initial survey. All selected individuals were measured for height and canopy volume. Measurements included basal diameter (measured where the trunk met the ground), height, and canopy width in two directions. Total plant volume was calculated as the volume of a pyramid height and the two canopy widths (*volume = [height x canopy width 1 x canopy width 2]/3*).

2.3.4 Water availability and drought stress

From May through September of 2015, pre-dawn *XPP* and dark-adapted fluorescence (F_v/F_m) were measured on each individual every four to six weeks as indices for water

availability and physiological stress, respectively. Sites were visited between 3-5am, and only two-to-three sites were visited per night to ensure measurement in similar time windows. F_v/F_m was measured using the FMS 2 system (Hansatech Instruments Ltd). In most cases, F_v/F_m was measured on two leaves per plant under dark conditions (red light settings only were used on headlamps), and values were averaged later. If F_v/F_m values on a single individual were greater than 0.100 units apart, a third measurement was taken. After collecting F_v/F_m measurements, three samples per individual were selected for measuring *XPP* data. Samples were clipped and bagged, and immediately placed on ice to be transported back to the lab and measured using a Scholander Pressure Chamber (PMS Instrument Company; Albany, Oregon, USA). The average time from when plant samples were clipped to when they were put in the chamber was 90 minutes. Typically, two of the three samples were pressure bombed and averaged, although the third sample was used if recorded values were greater than 1.0 MPa apart, or if a sample was damaged. Values for all 49 shrubs were then averaged across the entire population for each sampling period to track drought stress through the dry season.

2.4. *Correlations between plant stress, landscape variables and dieback progression*

2.4.1 *Site selection*

In 2016, we began a survey focused on understanding the relationships between landscape variables associated with drought stress and plant physiological function and dieback. A sixth site (Site A) was added at a lower elevation to extend the elevational gradient for this study, and was chosen based on similar shrub and site characteristics to the other five sites (Table 1). We selected shrubs using the same criteria and methods used in

2015. Sites B-F, along with previously selected shrubs at these sites, remained the same. Collectively, sites A-F represented a 330m meter elevational gradient.

2.4.2 Shrub Dieback

Data on canopy dieback for each individual were collected in fall 2016. Dieback was assessed as the actual percent of "non-green" vegetation, defined as yellow, brown, and black/gray leaves, as well as bare/defoliated stems within the canopy. Percent dieback of each canopy was estimated by two-to-three researchers viewing multiple angles of each shrub, and final estimates were determined after thorough consultation. Site dieback was then calculated as the mean of all selected shrubs within a site. Entire stand dieback was also estimated using a combination of ground-level assessments and, when available, aerial drone photographs. These were used to confirm that canopy dieback of individual shrubs were collectively representative of whole stand dieback.

2.4.3 Elevation and Aspect

Elevation data for individual shrubs were collected in situ using Altimeter GPS Pro (Immaginet Srl, 2020) and corroborated using Google Earth (2020). Aspect was recorded in situ in degrees, then converted to radians and transformed to linear data for analysis of "southwestness" using *cos*(aspect- 225°) according to Beers et al. (1966). This yielded aspect values ranging from -1 (northeast) to 1 (southwest), which were then used for modeling the effects of aspect on shrub function and dieback.

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2.4.4   Water availability and physiological function

Data were again collected approximately every 4-6 weeks throughout the summer dry season, this time from June through October. *XPP* measurements were taken using the same methods as described above. Additionally, we measured daytime gas exchange (net photosynthesis, A_{net}) to gain a better understanding of plant function throughout the dry season and as water availability declined. A_{net} was measured using a Licor 6400XT (Lincoln, Nebrasksa, USA). CO_2 was set to 400ppm, and photosynthetically active radiation (PAR) was set between 1400-1800, to reflect maximum photosynthesis conditions at peak daytime hours. All measurements were taken between 9am and 11am DST, typically on the morning after the predawn measurements were made. A single fully-expanded leaf was chosen per plant, and two readings were recorded and averaged later during data processing. Leaves were traced in the field, and tracings were brought back to the lab and scanned to calculate leaf area within the chamber (maximum areas = 2 x 3cm) using ImageJ software (Schneider et al., 2012). Gas exchange data were then adjusted by calculated leaf areas. Our FMS 2 system broke in August, and we were therefore unable to collect any F_v/F_m data after the July fieldwork.

2.5   Change in dieback during the drought

We continued surveying the plants from sites A-F every fall through the duration of the drought until 2019, using the same methods as above. In *A. glauca*, new leaf growth occurs annually in the spring/summer, therefore it was possible for dieback to decrease from one year to the next. In November 2019, the Cave Fire burned through much of our study area, including all of site A and major portions of site B.

2.6   Data analysis

Differences in means between sites for plant physiology and dieback were compared

using one-way ANOVA with Tukey's HSD *post hoc* analysis for means separations. Dieback

data were square-root transformed for normality. Linear regression models were used to

identify correlations of elevation and aspect between plant drought stress and dieback.

Multiple regression models were developed using landscape factors and plant physiology as

predictors for canopy dieback, and AIC values were calculated for choosing the best fit

model. A repeated measures ANOVA was performed to compare dieback levels between

sites and across four years of the study (fall 2016 – 2019). Statistical analyses were

performed using JMP Pro Statistical Software (*v.* 15.0.0) and R Studio (*v.* 1.1.456).

3. Results:

3.1 Preliminary assessment of site dieback and shrub stress (2015)

3.1.1   Stand dieback and mortality

All sites exhibited severe levels of dieback (> 80% of the stand exhibiting some level

of canopy dieback) except site F, in which only 20% of the stand was affected (Table 1).

Average canopy dieback was generally greater at lower sites compared to high, except for

site E, which exhibited the highest severity. Mortality was greatest at sites A and E, each

with six dead individuals identified.

3.1.2   Water relations

Total annual rainfall across all sites prior to our 2015 survey (2014-15 water year)

was 46.4cm, with the majority of precipitation occurring in December 2014, and the last rain

event occurring in June (Fig. 2). From May to September, mean XPP and F_v/F_m generally declined, though there was a slight increase in F_v/F_m in early July. Mean XPP decreased substantially, from 0.67 ± 0.07 MPa in May, to -5.57 ± 0.25 MPa in September. The decrease in mean F_v/F_m between May and September tracked with that of XPP, indicating both high water demand and physiological stress among shrubs by the end of the summer dry season during this drought year.

3.2. Correlations between shrub stress, landscape variables and dieback progression (2016)

3.2.1. Water relations

In the 2015-16 water year, average rainfall was nearly the same as 2014-15 (47.6cm), however the majority of rain occurred from January to March, with the last significant rain falling in April. (Fig. 3) As in 2015, XPP values declined throughout the summer dry season of 2016 (Fig. 3). June XPP values were -0.78 MPa ± 0.04, and mean A_{net} was 5.6 ± 0.28 µmol CO_2 m^{-2} • s^{-1}, suggesting the shrubs were experiencing very little water stress. There were significant differences in mean XPP between sites ($F_{5,53} = 4.127$, P = 0.0031), with the lowest elevation site (A) being the lowest (most negative) in June. Elevation and aspect were both found to be correlated with XPP in June, and while these relationships were weak ($R^2 = 0.19$ and 0.12, respectively) they were significant (p < 0.05, Table 3. Unlike XPP, there was no significant variation in A_{net} across sites in June.

By October, XPP values had decreased across all sites (Fig. 4). Mean XPP was -7.25 MPa ± 0.18, and a total of 18 individuals measured mean XPP values of -8.0 MPa or below. Photosynthesis values were also much lower than previously measured (mean $A_{net} = 1.59 \pm$

0.23 μmol CO_2 m^{-2} • s^{-1}). Interestingly, however, only sites A-D exhibited significant declines in fall A_{net} compared to their early summer values; sites E and F showed no significant change from June to October, despite dramatic changes in *XPP*. There were no significant differences in predawn *XPP* between sites in October, as means were all equally low ($F_{5,53}$ = 1.518, P = 0.200), yet mean A_{net} between sites varied considerably (Fig. 4, P < 0.0001), and a linear regression showed that A_{net} significantly declined with elevation (Table 3; R^2 = 0.28, P < 0.0001). There was no significant relationship between September A_{net} and aspect.

3.2.2. *Shrub Dieback*

Mean canopy dieback differed by site in fall 2016 (Fig. 5; $F_{5,53}$ = 2.44, P = 0.0463), however, there were no discernable patterns between dieback and elevation in this year (Table 4; $F_{1,57}$ = 0.504; P = 0.481). Aspect was weakly correlated with dieback, with southwestness being associated with greater levels of dieback ($F_{1,57}$ = 6.08, P = 0.017, R^2 = 0.10). There was no correlation between plant volume and dieback.

Fall dieback correlated inversely with both *XPP* and leaf-level A_{net} ($F_{1,57}$ = 8.06; P = 0.0063, and $F_{1,57}$ = 11.91; P = 0.0011, respectively)*,* though these relationships were weak (Fig. 6; R^2 = 0.12 and 0.17, respectively). Linear models were developed to test the effects of physiological traits (*XPP* and A_{net}) and landscape characteristics (elevation, aspect) with late-dry season dieback as potential drivers of mortality. The best model (lowest AICc) consisted of A_{net} and aspect (AICc = 262.7; Table 5). Fall *XPP,* while significantly correlated with dieback in a simple linear regression*,* was not found to be a significant contributor to the best

fit multiple regression models. This is likely due to collinearity, as fall XPP and A_{net} were highly correlated ($R^2 = 0.51$, $F_{1,57} = 59.32$, $P < 0.0001$).

3.3 Dieback over time (2016-2019)

Repeated measures ANOVA for the 2015-16 through 2018-19 water years showed that dieback varied over time and across sites ($F_{4,44} = 3.06$; $P = 0.026$; time $F_{3,42} = 6.21$; $P = 0.0014$; Table 6). Site D was omitted from this analysis as three individuals were destroyed by fire fighters during fire prevention efforts in 2017. Differences between sites were not consistent across years ($F_{12,111.4} = 2.16$; $P = 0.0182$; Fig. 7). Sites differed from one another in fall 2016 and 2019 ($P = 0.0463$ and 0.0114, respectively), but not in fall 2017 (which was a wetter than average year) and 2018 (a drier than average year). Elevation did not appear to drive these differences, as it was negatively correlated with dieback only in 2019, when dieback severity was greatest across most sites. There was, however, a positive correlation between SW aspect and dieback that was significant across all years (Table 4): the greater the SW orientation, the more severe the dieback.

While differences in dieback severity between sites were not always significant, some patterns in dieback across sites were consistent over time. From fall 2016 to 2019, sites A and E continued to exhibit the greatest mean dieback, while site F continued to have the lowest amount of dieback (Fig. 7). In 2018-19, sites A and E both had mean dieback of nearly 50% ($51.5\% \pm 9.19$ and $45.0\% \pm 10.1$, respectively) while mean dieback at site F was $17.51\% \pm 8.59$.

Overall, mean dieback increased from 2016 to 2019 (Fig. 8). Dieback increased the most at sites A and B $\Delta_{dieback} = 15\% \pm 6.54$ and $20.3\% \pm 7.22$, respectively), while sites C, E,

and F only increased by 6.2% $\pm$ 7.2, 3.9% $\pm$ 10.5, and 2.2 $\pm$ 2.3%, respectively. Contrary to our predictions, there was no drought-related mortality observed in the plants that were surveyed during this study. A total of 15 individuals across all six sites exhibited $\geq$ 80% canopy dieback between 2014 and 2019 yet continued to survive and, in some cases, decreased in their percent canopy dieback as new branches and foliage were produced. The distribution of shrubs with this level of dieback appeared to be random across sites (Supplemental Table S2).

4. Discussion

This study sought to understand spatial patterns of *A. glauca* canopy dieback across the landscape during an unprecedented drought, and track the progression of dieback and eventual mortality in this classically drought-tolerant shrub. Our data support the hypothesis that dieback is related to water stress from drought, and that this varied across the landscape, but there was considerable variation between sites and across time. While aspect was significantly correlated with dieback across all years, we found no consistent significant effect of elevation on dieback until 2019, late in drought period. Further, while dieback generally increased across all sites from 2016 to 2019, we observed no new incidences of mortality during this study, suggesting that in this landscape *A. glauca* individuals are resilient to this punctuated but overall prolonged drought.

4.1 Patterns in Water relations

Consistent with our predictions, elevation and aspect appeared to both have significant correlations with *XPP* and A_{net}. While these correlations were weak, their

significance lends support to our hypothesis that plant stress is related to these landscape variables. These relationships also provide evidence that elevation and southwestness may be used as tools for identifying areas where plant canopies are more vulnerable drought.

The low correlation coefficients may be due to extreme landscape heterogeneity in the study region including heterogeneity in substrate rockiness, soil accumulation and topographic concavity, confounded by a relatively small sample size within each site. There are many microclimate variables that were not included in this study and that may contribute to variations in water stress, including site temperature, vapor pressure deficit, and fog patterns. Additionally, though we did not find plant size to be correlated with drought stress or dieback, we believe this is due to our efforts to select even-aged individuals for monitoring, while previous studies have found significant effects of plant canopy diameter on dieback and mortality (Venturas et al., 2016). Also, because *A. glauca* recruit from seed after fire, individuals within a stand tend to be of similar size and age. Therefore, we recommend that future studies include greater variation in stand age so that relationships between age/size class and dieback can be better evaluated.

4.2 Dieback progression

Elevation was not shown to be highly correlated with dieback in all years of the study, and there did not appear to be a correlation between dieback annual precipitation. Yet, some important landscape patterns were revealed. Our hypothesis that dieback severity is related to landscape variables associated with water availability was most strongly supported in fall 2019, when dieback increased significantly with decreased elevation and increased southwestness. Aspect was correlated with dieback severity across all years, suggesting this

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could be a significant variable to consider when generating models for future drought risk. Moreover, it was found that the increase in dieback severity ($\partial_{\text{dieback}}$) from 2016-2019 was greatest at lower elevations compared to high. In other words, while most sites experienced an overall increase in dieback, the increase at lower elevations was much more dramatic. Collectively, these results suggest that while populations of *A. glauca* across the landscape in Santa Barbara county are susceptible to extreme drought, those occurring on exposed, southwest-facing slopes and/or at the lower edge of their range may be less capable of recovery, particularly during prolonged drought. This is consistent with studies showing that low elevations and exposed slopes can correlate with plant water stress (Gitlin et al, 2006; Poole and Miller, 1981; Mantgem and Stephenson, 2007; Meng et al., 2015) and mortality (Baguskas et al, 2014).

Interestingly, the dieback severity was greatest in fall 2019, despite the region experiencing above-average rainfall in both the 2016-17 and 2018-19 water years (Fig. 1). Thus, a single year of above-average rainfall was not sufficient to restore shrubs to pre-drought canopy health levels. One possible explanation for this is that 2011-2016 and the 2017-18 wet seasons were extremely dry, pushing *A. glauca* individuals towards a threshold of drought resistance that could not be counteracted by one or two wet years. Venturas et al. (2016) found *A. glauca* to have high levels of hydraulic failure, dieback, and whole shrub mortality in Malibu County during the drought year of 2014, and a study by Paddock et al. (2013) from an intense drought year in California (2003-2004) yielded similarly high mortality for this species. This supports the significance of intense drought years that preceded higher rainfall years in our study. Additionally, Gill and Mahall (1986) and Mahall et al. (2010) found evidence that some chaparral shrubs do not respond to surplus water, and

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thus high-rainfall years (particularly those in which the majority of rainfall occurs in a few major events) may not be accurate predictors of shrub recovery. Shallow-rooted species like *A. glauca* may only be able to benefit from soil water availability near the surface, and/or they may be relying on water availability in rocky outcrops that is more dependent on outcrop cavity structure than on individual rain event totals. Therefore, short-term excessive rainfall may be irrelevant for the recovery of these shrubs.

Another hypothesis is that opportunistic fungi, as identified in prior work (Schultheis et al., 2018; Drake-Schultheis et al., 2020), may have played a significant role in the sudden development and continuation of dieback. Biotic agents are known to exacerbate drought effects by amplifying hydraulic failure and/or carbon starvation (McDowell et al., 2008; Oliva et al., 2014). *A. glauca*, already weakened by drought, may have been further impacted by fungal pathogens emerging from their latency between 2012 and 2016. Drake-Schultheis et al. (2020) have documented synergistic interactions of drought and latent fungal pathogens on *A. glauca* in a greenhouse setting. However, more research is required to identify the exact role of latent fungal pathogens in *A. glauca* in the field. Interestingly, Mahall et al. (2010) report detailed phenological measurements of *A. glauca* individuals in a nearby population in the 1980s following individual leaves for up to three years. They do not report dieback or evidence of fungal disease in the canopy of any individuals despite detailed descriptions of leaf condition. Persona communication with Mahall and Thwing likewise confirm the absence of evidence for pathogenic fungi in their study plants. Drake-Schultheis et al. (unpublished) have evidence for the very recent introduction of the most virulent pathogen in this system, *Neofusicoccum australe*. While the Mahall et al. study was conducted during the drought of the late 1980s and this drought was similar in magnitude, it

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may be cumulative drought and a new fungal pathogen together that are today causing canopy dieback.

4.3 Mortality

A surprising result of this study was that while mortality among *A. glauca* was noted across the landscape at the beginning of this study, no new mortality was observed over the following four years of drought, despite some very low *XPP* measurements in 2016. Indeed, the *XPP* obtained in Fall 2016 are almost twice as negative as those measured in *A. glauca* shrubs in this same region (-3.0 to -4.0 MPa) during an earlier drought in the late 1980s (Mahall et al. 2010). Furthermore, a significant number of shrubs across sites reached extreme levels of canopy dieback ($\geq$ 80%) yet survived and in some cases even showed evidence of recovery. These observations are indicative of an impressive resilience to drought stress in this chaparral shrub species. As previously reported, *A. glauca* are typically identified as anisohydric, exhibiting continued gas exchange during drought and high resistance to cavitation (Paddock et al., 2013; Venturas et al., 2016). Our data showing the continuation of gas exchange well into the dry season (and at extremely low water potentials) further supports this strategy, although A_{net} did shut down in the lower elevation populations.

The Venturas et al. and Paddock et al. studies also found greater mortality in *A. glauca* than was recorded in our study, and in other chaparral shrubs with high cavitation resistance– a relationship suggested to be the result of greater susceptibility to high intensity drought. Therefore, why high mortality was not also observed in our study is unclear. It may be a result of Santa Barbara experiencing a slightly milder climate, compared to those of the aforementioned studies, which both took place in more arid regions of southern California, including in a chaparral-desert ecotone. Summer fog, a normal occurrence in Santa Barbara

County, may also play an important role in reducing vapor pressure deficit, thus providing critical drought stress relief during the summer months. Furthermore, our study areas were dominated by stands of relatively large, mature plants, which have been shown to exhibit greater survival rates during drought than smaller individuals (Schlesinger and Gill, 1980; Venturas et al., 2016). It is possible that this contributed to the overall resiliency of local *A. glauca* populations. Future studies which directly compare climatic variables and mortality of *A. glauca* – of various ages – across ecoregions during extreme drought would therefore be of great value, and may elucidate a better understanding of the driving factors and relative vulnerabilities of plants in high-risk ecosystems.

4.4   Importance of landscape heterogeneity

Consistent with our hypothesis, plants located at lower elevations generally experienced more physiological stress and (at least by the end of our study) greater dieback severity than those at higher elevations. However, there was also considerable variability within sites and along the elevational gradient. We hypothesize this is due to the extreme heterogeneity of the Santa Ynez mountain landscape, and that certain landscape features outside the scope of this study may act as refugia for *A. glauca* resilience. Since *A. glauca* is a relatively shallowly-rooted species, variables such as slope, aspect, slope angle, concavity, and rockiness – all of which vary greatly across the region and influence temperature, water availability, or both – likely impact shrub functioning and vulnerability to drought on a very local scale. In considering long-term predictions, it is possible that while some stands of *A. glauca* may suffer high levels of dieback and even mortality during extreme drought, populations as a whole may be resilient because of microsite and landscape heterogeneity.

Although higher elevations in our study area historically record greater rainfall, we found this trend to be slightly disrupted during our study period: rainfall at the highest elevation site was *lower* than at the intermediate site from 2015-16 to 2018-19 (Table 2). Furthermore, while elevation is known to influence temperature such that lower elevations are generally hotter than higher ones within a region, human-induced climate warming has been shown in some cases to occur more rapidly at higher than at lower elevations. For instance, Giambelluca et al. (2008) found more rapid warming at elevations above 800m in Hawaii, and a review by Pepin et al. (2015) found enhanced warming with elevation in high mountain regions globally (though the results in Pepin et al. varied greatly by region and season). Therefore, higher elevation sites in our study may also be experiencing more warming. We do not have temperature records for our six sites so we cannot separate temperature trends or anomalies from other elevational impacts. Nonetheless, the lack of a consistent elevation effect could be due not only to the confounding effects of other topographic features (slope angle, aspect, rockiness), but also to lower rainfall and increased warming in some higher elevation sites.

From the early studies of Whittaker (1956, 1960) ecologists have long recognized the influence of aspect on plant composition and vegetation dynamics in montane environments. Yet rarely have investigators tied performance or mortality of individuals within a species population to subtle differences in aspect that occur within a single population (though see Ogle et al., 2000; Gitlin et al, 2006; Baguskas et al, 2014; and Paz-Kagan et al., 2017). Here we found that aspect was the strongest predictor of dieback over time, with plants in SW aspects showing greater dieback than plants on more north-facing aspects. This influence of aspect may be the result of direct negative effects of sun exposure and higher temperatures in

a semi-arid environment (Ogle et al., 2000; Nevo, 2001; Bennie et al., 2008) or maybe indirect whereby influences of aspect are mediated through aspect-effects on soil and microbes. For example, Gilliam et al. 2015 demonstrated greater soil organic matter accumulation and different microbial community composition in N facing compared to S facing slopes. Regardless of mechanism, the finding that SW aspect is detrimental to these plants could be important for restoration practitioners seeking to identify promising sites on the landscape where restoration of individual species may be more successful (Questad et al. 2014).

Another source of variability both within the landscape region we studied, and across Southern California chaparral, which was not directly measured in this study, is the occurrence of fog. The Santa Barbara region experiences predictable fog in May and June, and late summer cloud shading and fog events have been demonstrated to reduce vapor pressure deficit (Williams et al., 2008), slow plant seasonal water loss (Emery et al. 2018) and provide direct foliar uptake of water (Emery, 2016). We observed significant variation in fog intensity between sites, particularly at night during the early summer months (June and July), even between sites on a given night. Sites C and D in particular often experienced heavy fog drip, while other sites remained dry. We found plants saturated by fog to exhibit higher water potentials on such nights, similar to findings in Mahall et al. (2010), suggesting that fog drip may be sufficient enough to percolate to shallow roots. As mentioned previously, *A. glauca* is considered to be a relatively shallowly rooted chaparral shrub species and has been shown to be sensitive to even small changes in rainfall (Mahall et al. 2010, Lesage et al, in preparation). Additionally, some coastal California shrub species have been shown to exhibit foliar water uptake from fog (Emery, 2016; Berry et al., 2019), though

this has not yet been demonstrated for *A. glauca*. Regardless, variations in summer fog patterns have previously been shown to influence late-season water potentials in other *Arctostaphylos* spp. (Vasey et al., 2012), possibly causing populations with greater exposure to fog to have higher "safety margins" with which to avoid cavitation (Jacobsen et al., 2007). Similarly, Emery et al. (2018) have shown that fog can reduce the rate of water loss in coastal shrublands in California. Mortality of *A. glauca* during drought documented in other studies (Paddock et al., 2013) occurred in more interior sites, with less fog influence. Thus, we believe the role of local and regional variation in fog occurrence in *A. glauca* drought resistance warrants further research.

5. Conclusions

While dieback severity increased over time, the fact that no new plant-level mortality was observed during the study supports previous findings that *A. glauca* are resilient to prolonged drought. The relatively more mesic climate of Santa Barbara may have contributed to resiliency in our study, compared to studies in more xeric shrubland regions that reported high mortality of *A. glauca* during the same drought (Paddock et al., 2013; Jacobsen et al. 2015). Landscape heterogeneity, while likely confounding some of our results, may also be advantageous in creating differential water availability across the landscape, thus providing refuges that can ultimately allow resilience of *A. glauca* populations in a region. However, severe and widespread dieback, like that which we observed, still represents a threat to healthy ecosystem functioning, and has implications for fuel management in regions that are already fire-prone. Lower elevations and exposed, southwesterly slopes may continue to see

the highest levels of dieback during future drought events, and thus should be identified as

high-risk and potential focus areas for management.

Table 1: General Site Characteristics of six sampling sites along an elevational gradient during initial study (2015 for sites B-F; Site A was added in 2016).

Site	Latitude (deg N)	Longitude (deg W)	Elevation (m)	General Aspect (deg)	Stand affected[a] (%)	Canopy dieback[b] (%)	Mortality[c] (# individuals)
A	34.493	-119.789	595	100	90	65	6
B	34.497	-119.788	650	114	90	60	1
C	34.504	-119.786	791	230	80	40	0
D	34.513	-119.797	862	81	95	50	2
E	34.518	-119.784	960	173	85	80	6
F	34.515	-119.779	986	226	30	20	3

[a] Estimation of percent stand affected by dieback, mortality, or both during initial survey
[b] Estimation of mean percent canopy dieback across the stand during initial survey
[c] Number of dead individuals located at each site during initial survey.

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Table 2. Location information and historical averages[a], and total precipitation (cm) per water-year (Nov. 1 – Oct. 31) during the study (2014-15 to 2018-19) at weather station sites in this local area and maintained by Santa Barbara County along an elevation gradient

Data Logger Site	Lat	Lon	Elev. (m)	Precipitation (cm)							
				2014-15	2015-16	2016-17	2017-18	2018-19	Historical average
Trout club	34.49064	-119.79861	365.9	**41.63[b]**	**43.41**	88.42	**33.88**	101.24	68.3
San Marcos Pass	34.51181	-119.82335	681.4	**47.9**	**52.27**	112.34	**46.74**	121.49	86.3
El Deseo Ranch	34.49055	-119.69492	1006.1	**49.73**	**46.96**	103.15	**44.37**	115.54	90.4

[a] Based on 69-year record (Trout Club), 55-year record (San Marcos pass), and 54-year record (El Deseo Ranch)
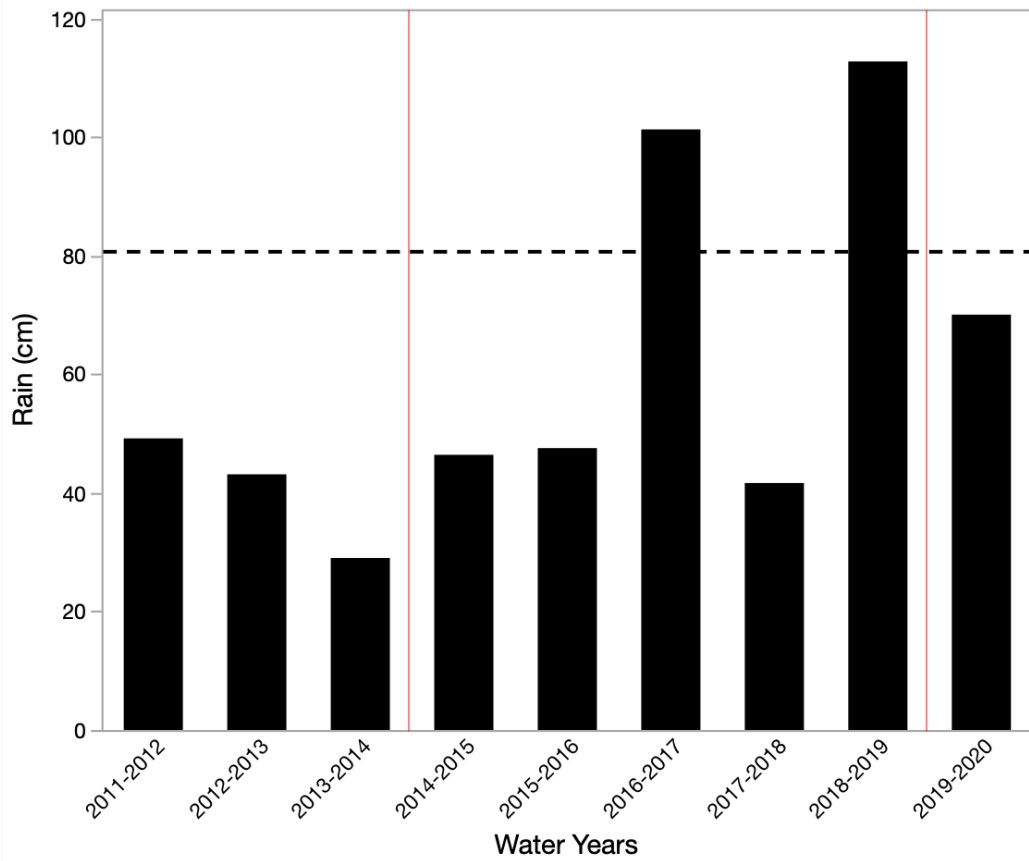[b] Values in **bold** represent below-average rainfall totals

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Fig. 1. Mean annual rainfall (2011-2020) for Trout Club, San Marco Pass, and El Deseo Ranch sites combined. Dashed line indicates the historical mean annual rainfall (based on 69-, 55-, and 54-year means, respectively; 81.7cm). Bars falling withing the red vertical lines indicate study period. Water years are 1 September to 31 August.
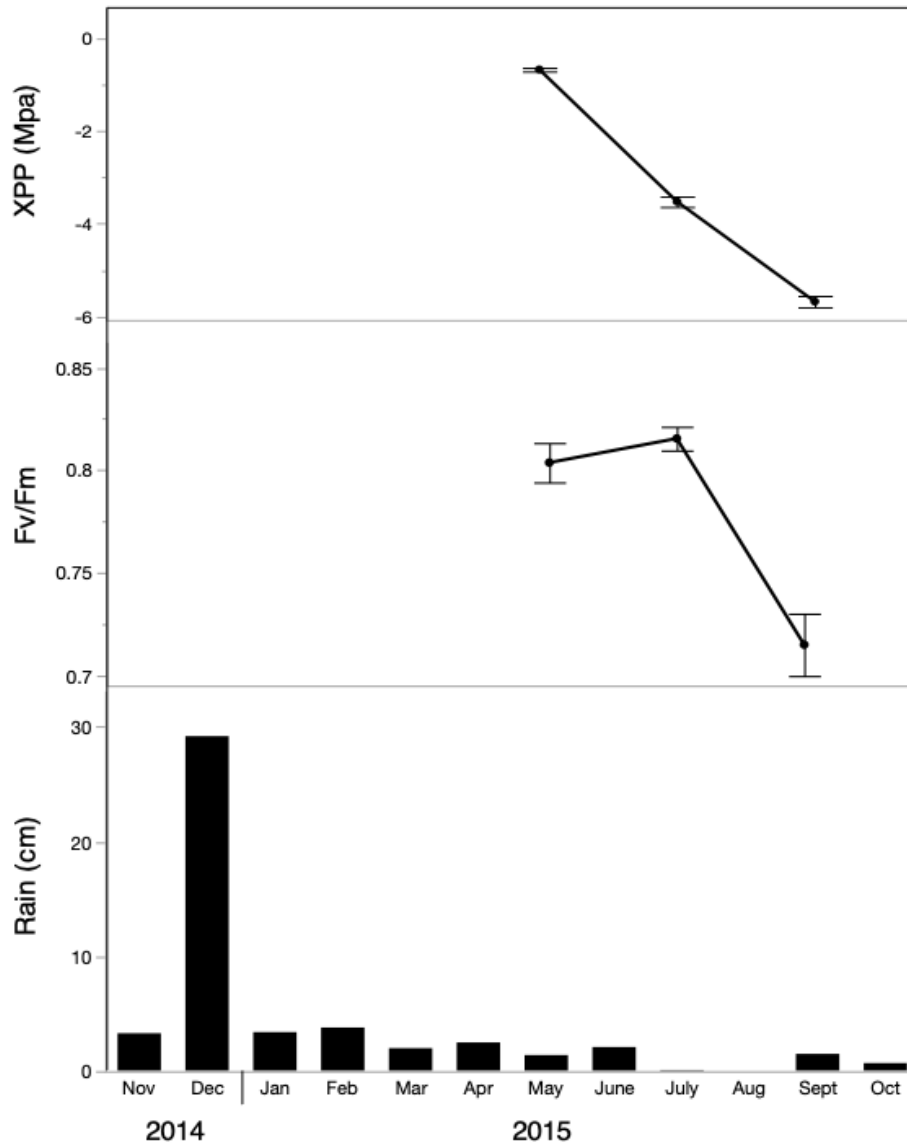
Fig. 2. Average rainfall at Trout Club, San Marcos, and El Deseo Ranch, and dark-adapted fluorescence (Fv/Fm), and pre-dawn xylem potentials (XPP) of *A. glauca* at study sites B-F in May, July, and September during the 2014-15 water year.
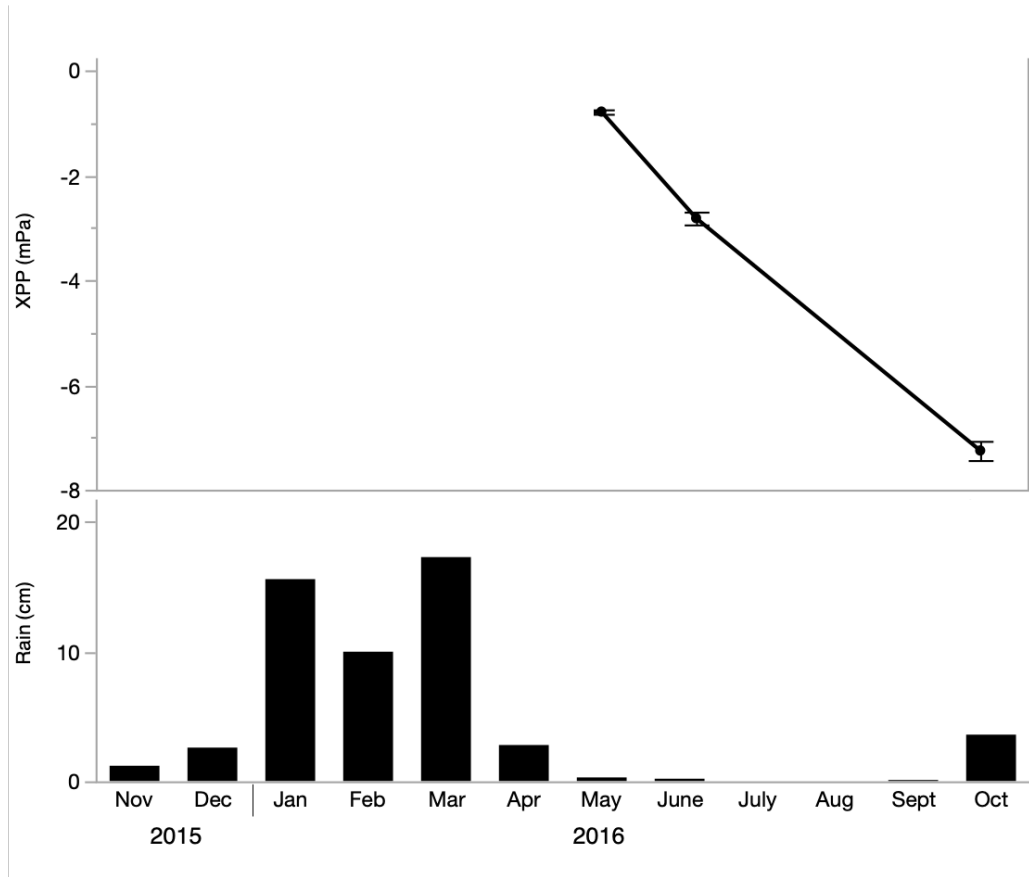
Fig. 3. Average monthly rainfall at Trout Club, San Marcos, and El Deseo Ranch, and pre-dawn xylem potentials (XPP) of *A. glauca* at study sites A-F in May, June, and October during the 2015-16 water year.

Table 3. Parameter estimates and ANOVA result for effects of elevation and aspect (southwestness; *SW*) on xylem pressure potential (*XPP*) and net photosynthesis (A_{net}) in June and October of 2016.

Month	Effect	XPP						A_{net}					
		Estimate	df	SS	F	Prob > F	R²	Estimate	df	SS	F	Prob > F	R²
June	Elev. (m)	0.0009	1	1.19	13.51	**0.0005**	0.19	-0.003	1	10.75	2.55	0.1162	0.04
	Aspect (SW)	-0.172	1	0.76	7.88	**0.0068**	0.12	-0.889	1	20.35	5.02	**0.0290**	0.08
Oct.	Elev. (m)	0.0017	1	3.90	1.80	0.1630	0.03	0.006	1	49.89	21.81	**<0.001**	0.28
	Aspect (SW)	-0.472	1	5.75	2.30	0.0888	0.05	-0.466	1	5.59	1.82	0.1822	0.031

Results of simple linear regression with elevation and aspect as predictor variables and XPP and A_{net} as responses. Values in bold indicate statistical significance based on ANOVA ($\alpha = 0.05$).
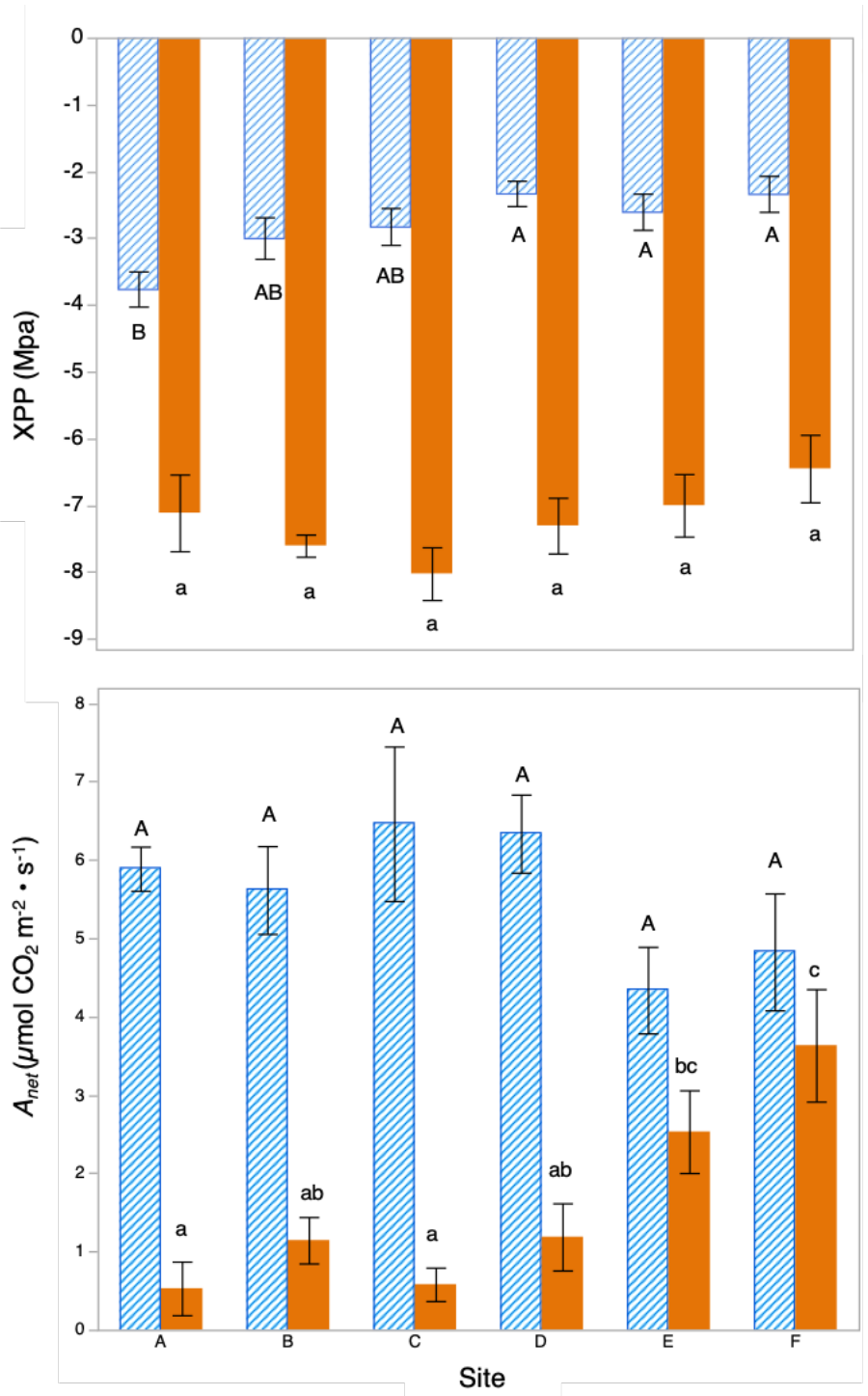
Fig. 4. Mean xylem pressure potentials (*XPP*) and net photosynthesis (*A*ₙₑₜ) at each site in June (slanted blue lines) and October (solid orange) 2016 (n = 9 – 10). Different letters between sites within a given date represent statistical differences based on one-way ANOVA and Tukey's HSD post hoc analysis using α = 0.05 (uppercase letters correspond to June values, and lowercase correspond to September values). Means ± SE.
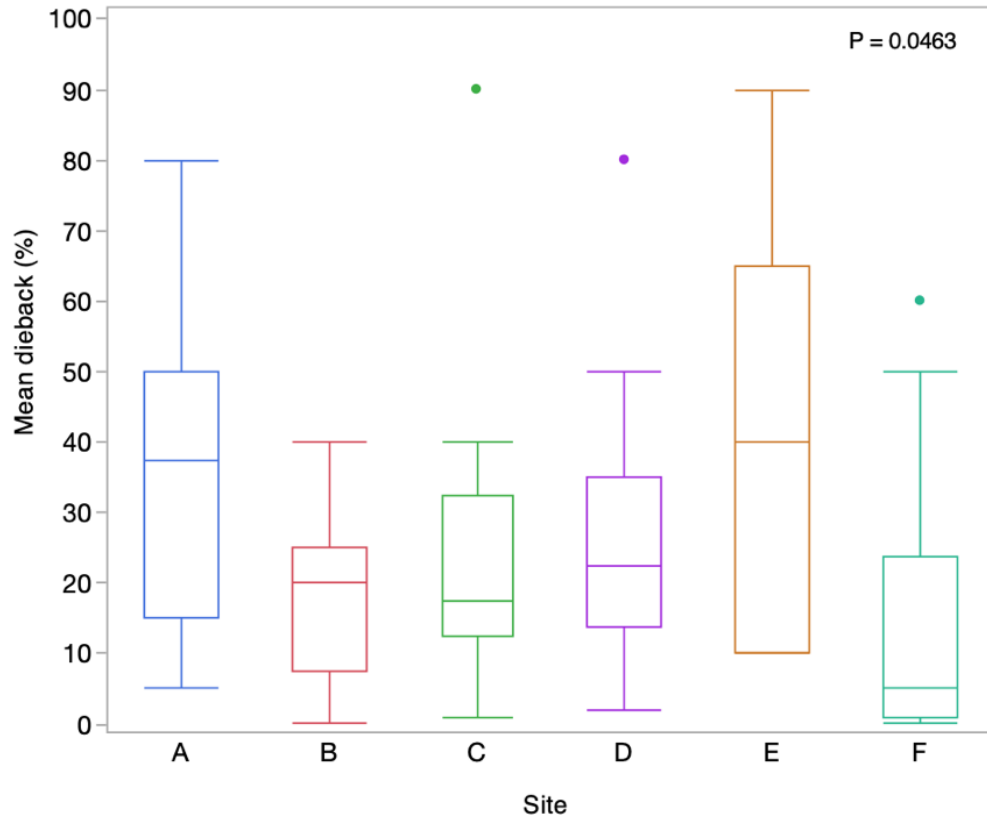
Fig. 5. Mean dieback for each site in fall 2016. Sites listed in order of increasing elevation. Data were square-root transformed and a one-way ANOVA was performed to test for statistical significance ($\alpha = 0.05$). Error bars are $\pm$ SE.

Table 4. Parameter estimates and ANOVA results[a] for effects of elevation and aspect (southwestness; *SW*) on percent dieback from 2015-16 to 2018-19.

Effect	Year	Estimate	df	SS	F	Prob > F[b]	R^2
Elev. (m)	2015-16	-0.001	1	2.91	0.50	0.4807	0.01
	2016-17	-0.003	1	13.16	2.09	0.1539	0.04
	2017-18	-0.002	1	6.18	1.11	0.2976	0.02
	2018-19	-0.005	1	36.10	6.41	**0.0143**	0.11
Aspect (SW)	2015-16	1.114	1	32.01	6.08	**0.0167**	0.10
	2016-17	1.48	1	51.26	9.18	**0.0038**	0.15
	2017-18	1.239	1	35.81	7.11	**0.0101**	0.12
	2018-19	1.675	1	65.49	12.87	**0.0007**	0.19

[a]Dieback values were square root transformed for analysis.
[b]Values in bold indicate statistical significance based on ANOVA ($\alpha = 0.05$).
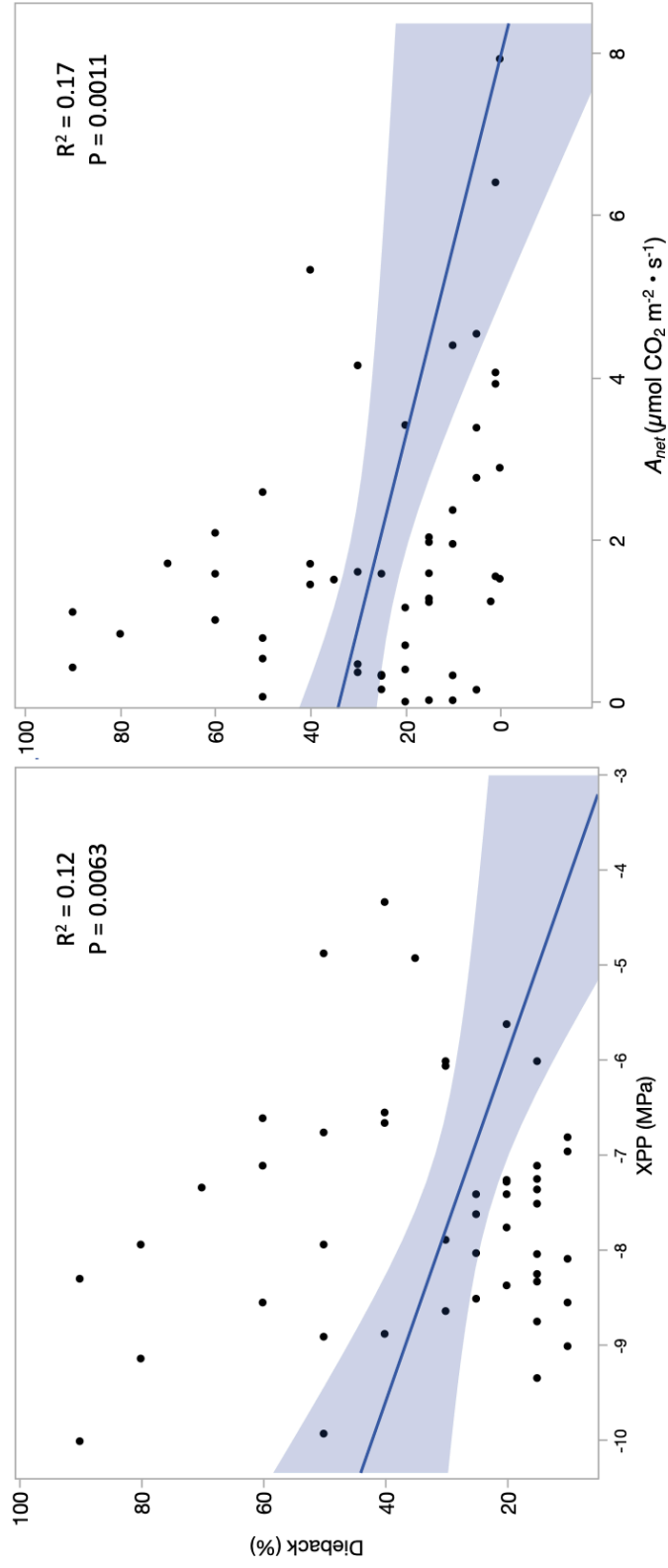
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Fig. 6. Relationship between dieback and A) xylem pressure potential and B) net photosynthesis in late summer 2016 (n = 59). Dieback data were square root transformed, and a one-way ANOVA was performed to test for significance (α = 0.05).

Table 5. Results of multiple regression model testing the effects of photosynthesis (A_{net}), and aspect (southwestness; SW) on percent dieback in 2016.

Term	Estimate	Std Error	t	Prob>$|t|$
Intercept	5.659	0.381	14.87	<0.0001
A_{net}	-0.506	0.162	-3.13	**0.0028***
SW	0.889	0.427	2.06	**0.0445***

* values in bold are significant, $\alpha = 0.05$

Table 6. Results for repeated measures ANOVA on effects of site and time on dieback (2016-2019)

Effect	df	Error	F	Prob > F
Site	4	44	3.057	**0.0263**
Time	3	42	6.209	**0.0014**
Time*Site (Wilk's Lambda)	12	111.41	2.162	**0.0182**

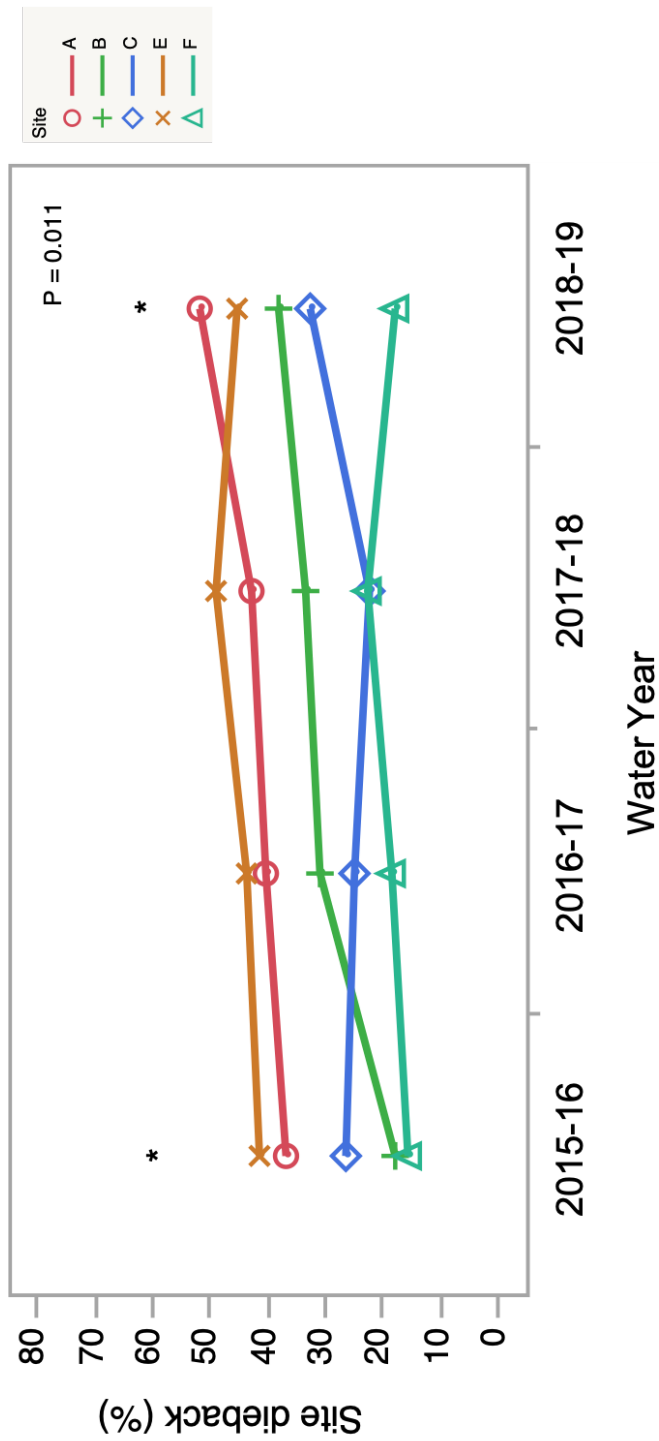Values in bold indicate statistical significance based on ANOVA ($\alpha = 0.05$)

Fig. 7. Mean fall percent dieback at each site per water year (2015-16 to 2018-19). Black upward-facing triangles indicate above-average rainfall, and downward-facing triangles indicate lower than average rainfall. 2014-15 is not included as Site A had not yet been identified, and Site D is excluded as three trees were destroyed between 2016 and 2017. Significant differences between sites were
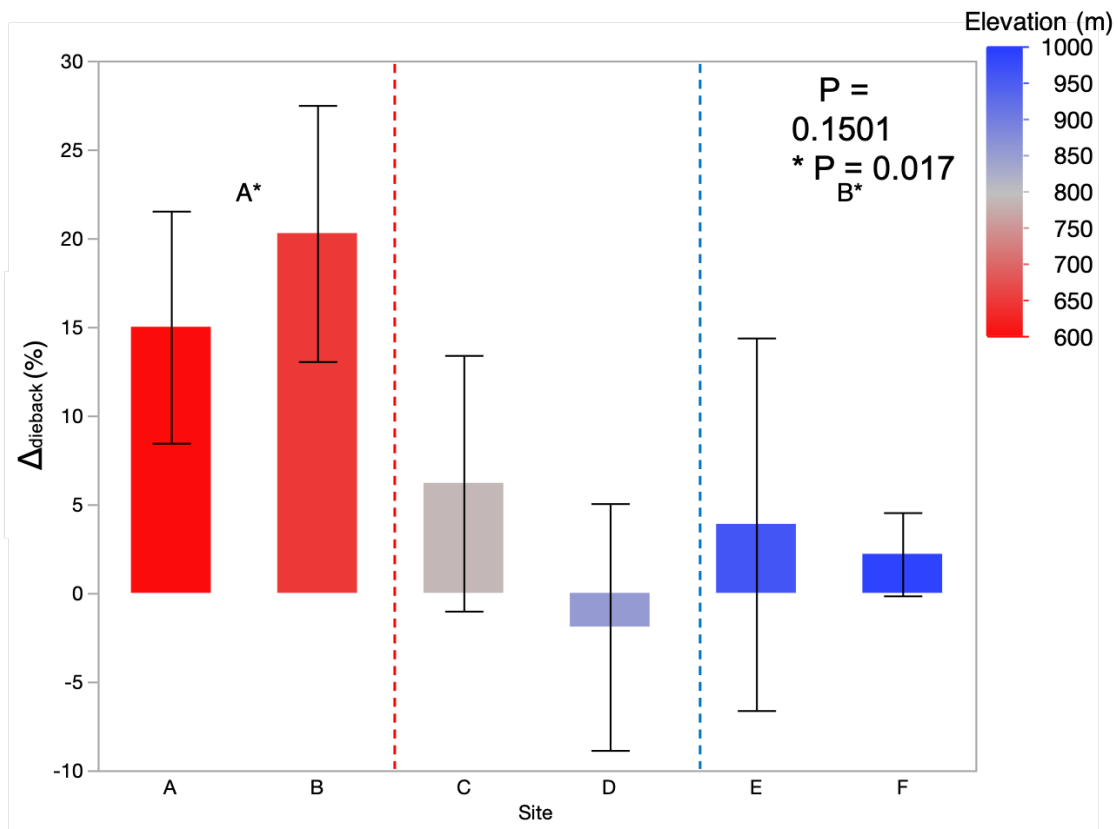
Fig. 8. Change in mean percent dieback (∂dieback) at each site from 2015-16 to 2018-19 water years. Error bars ± SE. P = results of ANOVA between all sites, whereas *P = results of ANOVA between the two lowest and two highest sites (indicated by the red and blue dashed lines, respectively), with intermediate sites removed.

Appendix C

Supplemental Table S1. Soil types and years since disturbance for six study sites

	Soil type	Year of last disturbance
Site A	Rock-outcrop[a]-Maymen[b] complex (Rb)	Painted Cave Fire (1990)
Site B	Rock-outcrop-Maymen complex (Rb)	Coyote Fire (1964)
Site C	Rock-outcrop-Maymen complex (Rb)	Unknown
Site D	Lodo[c] rock outcrop (LbG)	Unknown[d]
Site E	Maymen stony fine sandy loam (MaG)	Coyote Fire (1964)
Site F	Rock-outcrop-Maymen complex (Rb)	Oso Canyon Fire (1923)

[a]Rock-outcrop: 0-60in unweathered bedrock; Parent material: sedimentary rock

[b]Maymen: 0-4in stony fine sandy loam, 4-14in loam, 14-18in bedrock; Parent material: residuum from shale, conglomerate and/or sandstone

[c] Lodo: 0-11in gravelly clay loam, 11-59in unweathered bedrock; Parent material: residuum weathered from sandstone and shale

[d] no fire disturbance has occurred at this site in recent recorded history, however it is situated along a regularly-cleared fuel break. It is unknown when the site was last cleared prior to the study, however we estimate the shrubs to be approximately 40-50+ years old based on size comparison with stands of known ages.

Supplemental Table S2. Number of shrubs per site that exhibited $\geq$ 80% canopy dieback between 2015 and 2019.

Site	$\geq$ 80% dieback (#[a])
A	4
B	2
C	1
D	2
E	4
F	2

[a] Each shrub was only counted once

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