UNIVERSITY OF CALIFORNIA
SANTA CRUZ

ECOLOGICAL AND EVOLUTIONARY DRIVERS OF LOCAL AND REGIONAL SPREAD IN *DITTRICHIA GRAVEOLENS*, AN INVASIVE ANNUAL PLANT

A dissertation submitted in partial satisfaction
of the requirements for the degree of

DOCTOR OF PHILOSOPHY

in

ECOLOGY AND EVOLUTIONARY BIOLOGY

by

Miranda Kathleen Melen

December 2024

The Dissertation of Miranda Kathleen Melen is approved:

Professor Ingrid M. Parker, chair

Professor Kathleen M. Kay

Professor Karen D. Holl

Professor Virginia Matzek

Peter Biehl
Vice Provost and Dean of Graduate Studies

Table of Contents

List of Figures ……………………………………………………………………..iv

Abstract ………………………………………………………………………………v

Acknowledgments ………………………………………………………………….. vii

Introduction ……………………………………………………………………...1

Chapter 1: Invasion away from roadsides was not driven by adaptation to grassland habitats in *Dittrichia graveolens* (stinkwort) ……………………………………..11

Chapter 2: Low competitive ability explains *Dittrichia graveolens'* association with roadsides ………………………………………………………………………… 48

Chapter 3: Seed bank behavior of an invasive annual Aster (*Dittrichia graveolens*). 75

Conclusions ………………………………………………………………… 99

Appendix 1 ……………………………………………………………………107

Appendix 2 ……………………………………………………………………113

Appendix 3 ……………………………………………………………………116

References ……………………………………………………………………117

List of Figures

Chapter 1

Figure 1 42
Figure 2 43
Figure 3 44
Figure 4 45
Figure 5 46
Figure 6 47

Chapter 2

Figure 1 69
Figure 2 70
Figure 3 71
Figure 4 72
Figure 5 73
Figure 6 74

Chapter 3

Figure 1 93
Figure 2 94
Figure 3 95
Figure 4 96
Figure 5 97
Figure 6 98

Abstract

ECOLOGICAL AND EVOLUTIONARY DRIVERS OF LOCAL AND REGIONAL SPREAD IN *DITTRICHIA GRAVEOLENS*, AN INVASIVE ANNUAL PLANT

Miranda K. Melen

Invasive plants significantly threaten ecosystems, especially when they expand away from anthropogenic environments into natural habitats. This dissertation investigates the ecological and evolutionary drivers of invasion in *Dittrichia graveolens* (stinkwort), a recent California invader primarily associated with roadsides but showing signs of spread into adjacent vegetated areas. Chapter 1 examines whether adaptive differentiation facilitates this shift. We found no evidence that *D. graveolens* populations have evolved traits favoring performance in grassland habitats, suggesting that roadside populations are not yet adapted to compete in established vegetation. Chapter 2 reveals that the roadside association of *D. graveolens* is likely due to its poor competitive ability. Resident grassland species severely limited *D. graveolens* growth in both greenhouse and field experiments. This highlights the interacting roles of competition and disturbance in driving invasion patterns in this species. Chapter 3 explores the dormancy and germination traits of *D. graveolens*, seeking evidence for the evolution of reduced dormancy in edge populations. Contrary to prediction, seed bank behavior was similar between core and edge populations. Most seeds germinated in the first year immediately after the first rain, but burial strongly affected germination. Together, these chapters deepen our understanding of the constraints and drivers of *D. graveolens* invasion. Effective management practices should prioritize limiting soil disturbance, promoting competition to restrict *D. graveolens* spread, and strategically manipulating seed burial in conjunction with control efforts.

Acknowledgments

I would not be here, acknowledging the people and supporters in my life, if it were not for one person who took a chance on me and invited me into her life. I am deeply grateful for the mentorship and research training that Dr. Ingrid Parker has generously shared with me. Her guidance, knowledge, and friendship have nurtured me into the scientist I am today. Thank you, Ingrid, for your endless encouragement and for the incredible written and verbal feedback on my writing, teaching, and presentations. Your model of mentorship, teaching expertise, and shared research experiences inspire me to keep learning and stay forever curious.

Thank you to my committee members. Dr. Kathleen Kay, it has been a pleasure to serve as your teaching assistant and to learn from you about plant families, active learning techniques, and how to navigate both lectures and labs. Thank you for the opportunity to co-teach with Julia Harenčár as a teaching assistant and as a university fellow. Dr. Karen Holl, I first met you in Costa Rica during my undergraduate studies, where you introduced me to tropical restoration. Your explanation of your graduate students' work left a lasting impression, and I came away with a newfound appreciation for ecological restoration. Dr. Virginia Matzek, thank you for taking me on that transformational trip to Costa Rica to learn about tropical biology. Your teaching and mentorship during my undergraduate career fostered my love of environmental science. You three women have been incredible role models, and I hold you in the highest regard.

Thank you, Nicky Lustenhouwer, for supporting me throughout the early stages of my graduate career and reminding me to take a breath once in a while. Your guidance and statistical help have been invaluable during the second half of my research, and I am so grateful for your time and energy.

Thank you to the support staff in EEB, the UCSC greenhouse, and Blue Oak Ranch Reserve. Dr. Judy Straub, Stephanie Zakarian, and Eva Chudnow, thank you for your continued support of our graduate program—we are incredibly lucky to have you in our department. I am grateful to the UC Santa Cruz Greenhouse staff for their facilities and plant care, especially Sylvie Childress and Laura Palmer. Sylvie, not only do I appreciate you keeping my plants alive through storms, fires, and disease, but I am also immensely thankful for your detailed notes on the greenhouse and incubation chamber settings! I am grateful to Dr. Zac Harlow and Zac Tuthill from Blue Oak Ranch Reserve for their knowledge, assistance, and the use of facilities and research space.

I am thankful for my friends and the incredible team of undergraduates who supported me with lab, greenhouse, and fieldwork throughout my graduate career. Special thanks to Cole Jower, Eric Lynch, Matt Hinshaw, and Kaili Hovind for field support and experimental setup. I deeply appreciate the creativity and ingenuity of my senior thesis students: Emma Snyder, Andrew Lopez, and Amy Chattaway. My gratitude goes to undergraduate researchers and Team Dittrichia members: Michael Fernandez, Kyle Dahilig, Josie Borden, Emma Gloudeman, Kaytie Hensley, Arly

Orona, Carolina Saucedo, Daphne Smith, Dillon Hadden, Erica Frederickson, Jackson Phillips, Tania Cooley, Tom Chaubet, Ryan Chiriboga, Ian Budesa, Kenton Kiser, Rachael Bradley, Trevor Jensen, Becky Tapia, Toni Jaroszewska, Veronica Ness, Xinwen Hu, Colby Cole, and Dante Park. You all made my graduate experience wonderful and fulfilling!

I am forever grateful to have been part of the “Fantastic Year” Cohort in EEB. Your friendship, guidance, creativity, and energy pulled me through tough times and kept me going. Thank you, Matt Kustra, for your endless coding support, Ryan Salladay, for planning social events and creating a sense of community, and Sushmita Poudel, for your encouragement and shared coding journey. I extend my gratitude to Julia Harenčár and Laura Goetz, who were instrumental in helping me find my place in the graduate community. I cherish our friendship and the memories of writing and coding together. Thank you for pulling me in, giving me hugs, and never letting go.

I thank the permitting agencies that allowed me to collect seeds for this study: the Santa Clara Valley Water District and the Don Edwards San Francisco Bay National Wildlife Refuge. This research was funded by the United States Department of Agriculture, National Institute of Food and Agriculture, Agriculture and Food Research Initiative Grant 2020-67013-31856 to I.M.P. Financial support was also provided by the UC Santa Cruz Department of Ecology and Evolutionary Biology, the Center to Advance Mentored Inquiry-Based Opportunities (CAMINO), the UC Santa Cruz Doris Duke Conservation Scholars Program, the UC Santa Cruz Plant

Sciences Fund, the California Native Plant Society Marin County Chapter, and the Jean H. Langenheim Graduate Fellowship.

I respectfully acknowledge the Ramaytush and Tamien Ohlone peoples, the original inhabitants of the lands where I collected seeds and conducted field experiments. I also acknowledge with gratitude the Awaswas-speaking Uypi Tribe for the use of their ancestral land in conducting greenhouse experiments.

Finally, my deepest gratitude goes to my family. To my husband, Steven, for his unwavering support throughout this journey—your understanding and encouragement have allowed me to focus on my research and professional development while navigating parenthood. To my daughter, Marina, for her irresistible laughter, boundless curiosity, and daily reminders to have fun. My love and appreciation also go to my parents, who have stood by me through everything. To my mother, Shirley, who taught me to write and shares my love of plants and nature, and to my father, Randy, who has always cheered me on and reminded me that I can achieve anything I set my mind.

Truly, I stand on the shoulders of giants, but I am also lifted by wings of love.

Chapter 1 is a lightly edited version of the following publication:

Melen MK, Snyder ED, Fernandez M, Lopez A, Lustenhouwer N, Parker IM. 2024. Invasion away from roadsides was not driven by adaptation to grassland habitats in Dittrichia graveolens (stinkwort). Biological Invasions, 26: 2923-2939. https://doi.org/10.1007/s10530-024-03359-6

Introduction

Biological invasions allow us to study the interplay of ecology and evolution in the context of rapid global change. Rapid environmental changes and human activities are linked to biological invasions as a global phenomenon (Moran and Alexander 2014), causing significant ecological, economic, and human health impacts (Vitousek et al. 1997; Mack et al. 2000; Pyšek and Richardson 2010; Paini et al. 2016). Diagne et al. (2021) estimate that between 1970 and 2017, the total minimum reported costs of biological invasions globally was US$1.288 trillion (2017 US dollars), an average of US$26.8 billion annually. Biological invasions create natural experiments where ecological processes and evolutionary responses intersect, and through this lens, we can assess how species respond to novel environments. Climate change, habitat disturbance, and globalization are accelerating the spread of invasive species (Hulme 2009; Poland et al. 2021). Understanding the relationship between invasive species ecology and evolution is critical for forecasting and mitigating impacts. An integrative approach that considers both ecological pressures and evolutionary potential is an important tool for managing invasive species in our changing world.

Rapid evolution can enable invasives to exploit new niches, adapt to competitive pressures, enhance dispersal, or lose seed bank dormancy to confer greater fitness. Adaptive evolution in response to ecological change or novel conditions is now recognized as a common feature of populations in both wild and managed systems (Thompson 1998; Hairston et al. 2005; Phillips and Shine 2006; Prentis et al. 2008).

Non-native species have provided many of our best examples of this evolutionary change in response to ecological conditions (Thompson 1998; van Kleunen and Fischer 2008; Lande 2015; Rollins et al. 2015; Reznick et al. 2019), and there is mounting evidence that rapid evolution may play a prominent role in invasions (Phillips and Shine 2006; Rollins et al. 2015). In plants, this has included the evolution of life history (Maron et al. 2004; Dlugosch and Parker 2008b), reduced defense (Turner et al. 2014; Ethridge et al. 2023), and phenological adaptation (Buswell et al. 2011; Colautti and Barrett 2013). While many non-native plants show evidence of adaptive evolution in their introduced range, it is still unclear how often evolution directly affects the invasion or exacerbates the invader's impact (Zenni et al. 2014; Colautti and Lau 2015). Rapid evolution is a concern for management and policy because it adds uncertainty to risk assessment and the prediction of invasions.

In actively spreading populations, invasion fronts experience strong selection for traits promoting spread, such as high dispersal, high fecundity, and short generation time (Travis et al. 2009). Theory predicts that this selection leads to increased dispersal and fecundity (Travis and Dytham 2002) and reduced seed dormancy, ultimately accelerating invasion rates (Williams et al. 2016). Colonizers thriving along roadsides are often adapted to disturbance (Frenkel 1977), capable of tolerating some physical stress, and they tend to grow best under low interspecific competition (“ruderal” *sensu* Grime 1977). These species typically exhibit traits associated with “r-selected” life history strategies, including early maturation, high seed output, and rapid generation times (Oka 1983; Bazzaz 1986). However, as individuals disperse away from roadsides through secondary spread, they encounter more stable, less disturbed environments with higher interspecific competition from established vegetation (Johnston and Johnston 2004; Ward et al. 2020). In such conditions, natural selection may favor traits for rapid resource acquisition, supporting their persistence in these communities (Grace 1990).

Roadsides, transportation corridors, and other disturbed environments facilitate primary spread of non-native species (Baker 1974; Tyser and Worley 1992; McDougall et al. 2018). The movement of species away from roadside edges and into established vegetation (secondary spread) is a critical phase in the invasion process (Ward et al. 2020). Habitat associations reflect ecological trade-offs, where species thrive in certain conditions but face limitations from biotic interactions with nearby resident species. Ruderal species are often the first to colonize roadsides and bare ground but are typically poor competitors in undisturbed environments (Frenkel 1977; Grime 1977, 2006). Disturbance creates heterogeneity and reduces competition by freeing space and resources, enabling colonizers to exploit these opportunities (Hobbs and Huenneke 1992; Greipsson 2011; Catford et al. 2012). This process underscores the vital role of disturbance in facilitating the invasion of many non-native species (Pimm 1989; Hobbs and Huenneke 1992; Tyser and Worley 1992; Minchinton and Bertness 2003; McDougall et al. 2018).

Seed dormancy is a critical phase of the plant life cycle and affects population dynamics and the maintenance of plant diversity (Gioria et al. 2012; Funk et al. 2020). Seed banks are a bet-hedging strategy for annual plants (Cohen 1966; Philippi and Seger 1989; Simons 2011; Lennon et al. 2021). A long-lived seed bank may allow invasive species to persist in variable environments; at the same time, in colonizing species under selection to spread rapidly, dormancy may be selected against. Long-lived seed banks are an essential part of the life history of many weedy species (Gioria et al. 2012), and seed banks provide a persistent, recurring source of invasive plants (Roberts and Boddrell 1984; Fletcher et al. 2015; Moravcová et al. 2018). Understanding the seed bank is a critical research need for managers (Funk et al. 2020). Long-term germination behavior is challenging to study, which is probably why it is so poorly understood despite its ecological and evolutionary importance. Response to seed germination cues can influence cohort dynamics, and management response must adapt to control flare-ups and recurring sources of invasion.

Dittrichia graveolens (L.) Greuter (stinkwort) is a fall-flowering annual in the Asteraceae family, characterized by yellow radiate flowers and wind-dispersed fruits (Brownsey 2012; USDA 2013). It germinates during the rainy season (Brownsey et al. 2013a) and spends several months as a rosette before bolting in June. During its rosette stage, the plant often grows inconspicuously under other vegetation and delays root development until May and June, when bolting begins. At this point, it develops a large taproot, enabling access to nutrients and water deeper in the rhizosphere than many other annual species (DiTomaso and Brownsey 2013; Brownsey et al. 2014). Buds appear in late July and August, with flowering occurring from September to December and fruiting and seed dispersal from October through December (Brownsey et al. 2013b). Plants often exhibit buds, flowers, and seeds simultaneously, complicating control efforts. The wind-dispersed fruits feature barbed pappus bristles that readily attach to hair, fur, and clothing (DiTomaso and Brownsey 2013). The stems and fully developed leaves are covered in glandular hairs that secrete a sticky oil, giving the plant a camphor-like scent (Harzallah-Skhiri et al. 2005; Aghel et al. 2011) and the reason for its common name "stinkwort". This stickiness facilitates propagule movement by adhering to machinery and vehicle undercarriages (DiTomaso and Brownsey 2013).

Native to the Mediterranean region of Europe, *D. graveolens* has successfully invaded other continents such as Australia, New Zealand, South Africa, and North and South America (Parsons and Cuthbertson 2001; Brownsey 2012; Brownsey et al. 2013b; Santilli et al. 2021). In Europe, the species has expanded its range and evolved to flower earlier in its northern limits, demonstrating its capacity for rapid evolution (Lustenhouwer et al. 2018). In California, *D. graveolens* was first recorded in 1984 (Preston 1997) and has since been listed as a high-risk invasive species by the USDA due to its rapid spread and significant impact potential (Brownsey et al. 2013b; USDA 2013). The barbed pappus makes it harmful to livestock when ingested

(Meadly 1965; Philbey and Morton 2000) and can cause milk tainting (Meadly 1965), while contact with its oils can trigger dermatitis in humans (Thong et al. 2007).

While *D. graveolens* is thought to thrive primarily along roads and disturbed soils in California (Preston 1997; DiTomaso and Brownsey 2013), there is a lack of empirical evidence for whether *D. graveolens* can and will invade undisturbed lands and rangelands (USDA 2013). Researchers have hypothesized that due to its slow root growth (Brownsey et al. 2013a), *D. graveolens* may be a poor competitor. It readily invades riparian corridors and wetlands (Parsons and Cuthbertson 2001; DiTomaso and Brownsey 2013). More recently, observations of *D. graveolens* by land managers and community members in California (Calflora 2024) have included reports of the species in rangelands(California Rangeland Conservation Coalition 2014; Melen et al. 2024), and it is common in rangelands in Australia (Meadly 1965; Parsons and Cuthbertson 2001). Populations of *D. graveolens* that have spread away from roadsides will experience selection for more efficient resource use and may adapt to better compete with resident plant communities.

In **Chapter 1**, we tested for adaptive differentiation between *D. graveolens* populations on the roadside and in vegetated habitats. We collected seeds from eight pairs of vegetated sites and their nearest (presumed progenitor) roadside population. We assessed germination behavior in the lab; germination rates were slightly reduced in seeds from vegetated sites, which may indicate lower seed viability. Otherwise, seeds did not show consistent differences between the two habitat types. In a randomized block design greenhouse experiment, we assessed differences in the response of genotypes from roadside and vegetated sites to competition with *Bromus hordeaceus* and with *Festuca perennis*. We also tested for increased performance in vegetated habitat with a grassland field experiment using a randomized block design with a control and a neighbor removal treatment. The control was an unmanipulated grassland plant community dominated by European annual grasses, whereas the neighbor removal treatment had above- and belowground competition removed by hoeing plots before planting *D. graveolens* and then maintaining the plots with weekly weeding. Competition strongly reduced performance of *D. graveolens* in both the greenhouse and the field, but plants originating from vegetated sites did not show enhanced competitive ability. Our findings show no evidence of adaptive differentiation between *D. graveolens* populations from roadside and vegetated habitats to date, suggesting that invasiveness in grasslands has not been enhanced by rapid evolution in the 40+ years since this species was introduced to California. Evolutionary constraints or potentially high levels of gene flow at this small scale may limit adaptation to novel habitats along roadsides.

In **Chapter 2**, we tested whether *D. graveolens* grows best along roadsides due to a preference for soil conditions or is limited to these disturbed environments by plant competition. We compared the response of both seeds and plants to field topsoil and engineered fill as a proxy for the physical microenvironment of roadside soils. Using seeds collected from eight sites in the County of Santa Clara, we compared the germination behavior of seeds on the two substrates (field topsoil and engineered fill) in a laboratory experiment. There was no germination advantage in engineered fill. Competition with two annual grasses (*Bromus hordeaceus* and *Festuca perennis*) showed strong growth suppression of *D. graveolens*. However, soil type greatly influenced the effect of competition, which was stronger in field soil than in engineered fill. Engineered fill limited growth for all species, suggesting that roadside soils provide a refuge from competition.

We implemented multiple field experiments to study competition in the field. In Year 1, we quantified *D. graveolens* response to four competition treatments: (1) control, the grassland including the year's plant growth as well as the previous year's thatch; (2) thatch removal, which involved raking and removing the previous year's thatch; (3) aboveground removal, where we used a string trimmer to trim grassland vegetation to 8 - 13 cm above the ground; (4) and above+below removal, where we tilled the soil thoroughly to remove above- and belowground biomass. In Year 2, we replicated our test of the relative effect of above- and belowground competition on the survival of *D. graveolens*, with belowground competitor removal and background vegetation clipping. We further explored aboveground interactions on *D. graveolens* using a shading experiment. Finally, to separate the effects of belowground interactions, we used a two-factor trenching experiment with weed cloth and water and water + nutrient additions. Protection from belowground competition with weed cloth substantially increased survival rates, but the water and nutrient treatments were not significantly different from control plots. While the most important competition mechanism remains unresolved, removing belowground competition significantly improved *D. graveolens* survival and growth, suggesting that competition confines *D. graveolens* to disturbed roadsides. Management should prioritize reducing bare, disturbed areas and enhancing plant competition in areas vulnerable to invasion.

In **Chapter 3**, we investigated the ecological and evolutionary factors influencing seed dormancy and germination of *D. graveolens*. To explore evolution with invasive spread, we compared four long-established populations from California's core region with four recently established populations at the invasion edge in the foothills of the Sierra Nevada Mountains. Our research included three experimental approaches: (1) a mesocosm study to assess germination of surface-sown seeds under outdoor conditions over three years, (2) a laboratory experiment to evaluate the effect of burial depth, and (3) a field dormancy experiment tracking seed survival in buried mesh bags over three years. Overall, we found no significant difference in dormancy or germination between core and edge populations. Most seeds (83.9 %) germinated in the first year, with only a small percentage germinating in later years. Burial depth significantly reduced germination, showing a sharp decline below 5 mm. Finally, viability persisted through the first (65.9 %) and second (59.9 %) years in the field dormancy study, but dropped sharply by year three (29.6 %). These findings suggest that *D. graveolens* dormancy and germination behavior have not evolved on the spreading edge. They respond strongly to rainfall seasonality and burial, with most seeds germinating with the first moisture cues, unless buried. This study highlights potential management strategies to limit *D. graveolens* spread in California, including monitoring and control after the first rains and strategic burial to suppress germination.

Chapter 1

Invasion away from roadsides was not driven by adaptation to grassland habitats in *Dittrichia graveolens* (stinkwort)

Abstract

Invasive plants along transportation corridors can significantly threaten ecosystems and biodiversity if they spread beyond anthropogenic environments. Rapid evolution may increase the ability of invading plant populations to establish in resident plant communities over time, posing a challenge to invasion risk assessment. We tested for adaptive differentiation in *Dittrichia graveolens* (stinkwort), an invasive species of ruderal habitat in California that is increasingly spreading away from roadsides into more established vegetation. We collected seeds from eight pairs of vegetated sites and their nearest (presumed progenitor) roadside population. We assessed differentiation between populations in roadside and vegetated habitat for germination behavior and for response to competition in a greenhouse experiment. We also tested for increased performance in vegetated habitat with a grassland field experiment including a neighbor removal treatment. Germination rates were slightly reduced in seeds from vegetated sites, which may indicate lower seed viability. Otherwise, plants did not show consistent differences between the two habitat types. Competition strongly reduced performance of *D. graveolens* in both the greenhouse and in the field, but plants originating from vegetated sites did not show enhanced competitive ability. Our findings show no evidence of adaptive differentiation between *D. graveolens* populations from roadside and vegetated habitats to date, suggesting that invasiveness in grasslands has not been enhanced by rapid evolution in the 40+ years since this species was introduced to California. Evolutionary constraints or potentially high levels of gene flow at this small scale may limit adaptation to novel habitats along roadsides.

Introduction

Only a small proportion of introduced species will become invasive and have substantial ecological impacts (Williamson and Fitter 1996; Blackburn et al. 2011). Resource managers must allocate limited resources to management and eradication efforts focused on the most problematic species. Assessing the potential risk of newly introduced species is essential for prioritizing these efforts (Robinson et al. 2017). Such risk assessment includes evaluating which habitats are vulnerable to invasion by a species, and evaluating traits that make that species likely to invade those habitats (Diez et al. 2012; El-Barougy et al. 2021). Yet traits may evolve. In fact, introduced species have provided many classic examples of rapid evolution (Thompson 1998; Reznick et al. 2019). Rapid evolution of key traits may play a prominent role in promoting invasions (Maron et al. 2004; Buswell et al. 2011; Colautti and Barrett 2013; Turner et al. 2014). Evolutionary change is a key source of uncertainty in risk assessment for introduced species (Whitney and Gabler 2008; Clements and Ditommaso 2011), and there is a strong need for studies that will lead to a more comprehensive and nuanced understanding of where and when adaptive evolution promotes invasion.

Transportation corridors play an essential role in the early stages of invasion of introduced plants (Follak et al. 2018; Hogan et al. 2022). Vehicle traffic facilitates spread by moving plant propagules along roadways, accelerating dispersal rates, and establishing new roadside populations (Hansen and Clevenger 2005; Von Der Lippe and Kowarik 2007). Road construction and maintenance result in roadside soil compaction and erosion (Lázaro-Lobo and Ervin 2019; Mills et al. 2020). Runoff from roads increases salinity, chemical and heavy metal contaminants, and further contributes to soil erosion (Trombulak and Frissell 2000; Lázaro-Lobo and Ervin 2019). These roadside soil conditions provide ideal corridors for many stress- and disturbance-tolerant invasive plant species to take up residence and disperse because plant cover is lower (Mills et al. 2020) and plant competition pressures are reduced (Greenberg et al. 1997). However, an introduced plant must spread away from these anthropogenic environments to be considered a problematic invader. Here, roadside populations act as a source of propagule dispersal into adjacent plant communities (Hansen and Clevenger 2005; Kalwij et al. 2008; McDougall et al. 2011).

Life history theory and adaptive strategies could contribute to our understanding of the emergence of invasive species (Guo et al. 2018, 2022). Species that grow along roadsides exemplify the classic ruderal life history, with high fecundity, short generation time, and long-distance dispersal traits (Frenkel 1977; Dietz and Edwards 2006; Travis et al. 2009). Life history theory predicts that because of inherent evolutionary tradeoffs, ruderal species will be poor competitors in highly competitive habitats (Grime 1977; Burton et al. 2010; Pierce et al. 2017). In fact, Guo et al. (2022) found that species categorized as invasive were more associated with “competitor” traits while species categorized as naturalized but not invasive were associated with the “ruderal” traits. Yet individuals dispersing from a roadside population into more ecologically stable, vegetated areas will experience strong selection associated with greater competition and other environmental conditions such as higher soil fertility, differences in moisture availability, soil microbes(McDougall et al. 2011, 2018), and increased biotic interactions (e.g., herbivory) (Trombulak and Frissell 2000; Leblond et al. 2013; Muñoz et al. 2015). Evolution of traits conferring greater fitness in these vegetated habitats could increase invasiveness and exacerbate impact on competing resident species. For rapid evolution to promote invasion, however, selection would need to overcome those life history tradeoffs underlying adaptive strategies; we do not yet know how easily or how often this may occur. The first step is to look for evidence of divergence between populations actively spreading away from transportation corridors and their ruderal progenitors.

We used *Dittrichia graveolens* (L.) Greuter (stinkwort) as a model to investigate adaptive evolution's role in promoting invasion away from roadside habitats. Introduced to California in the early 1980s, this herbaceous member of the Asteraceae was originally found in disturbed areas along railroad tracks and roads (Preston 1997; Brownsey et al. 2013a). Native to the Mediterranean Basin in Europe, *D. graveolens* grows in bare, disturbed habitats, including roadsides, crop and fallow land, stony riverbanks, and ruderal zones associated with annual or biennial weeds (Brullo and de Marco 2000; Rameau et al. 2008). It is a fall-flowering annual producing yellow radiate flowers and wind-dispersed fruits (Rameau et al. 2008). In California, *D. graveolens* germinates during the winter rainy season (Brownsey et al. 2013a) and spends several months growing vegetatively as a rosette before bolting in June. Flowering from September to December, *D. graveolens* sets seed and disperses from October through December (Brownsey 2012). Between its first observance in 1984 and 2012, *D. graveolens* spread to 62% of California counties (Brownsey et al. 2013a), reaching 79% of counties (46 out of 58) by 2020. It is now spreading east into the Sierra Nevada Mountains along transportation corridors (Calflora 2020).

More recently, *D. graveolens* in California has been observed spreading into areas with established vegetation (Brownsey et al. 2013a), including wildlands and rangelands (i.e., areas of natural vegetation grazed by livestock or wild herbivores). This calls attention to the potential invasion risk of *D. graveolens*. The USDA lists *D. graveolens* as a high-risk invasive species based on its high impact potential and ability to rapidly spread (USDA 2013). The plant is dangerous to livestock (Meadly 1965; Philbey and Morton 2000; Ponticelli et al. 2022) and causes contact dermatitis in humans (Thong et al. 2007; Ponticelli et al. 2022). In the County of Santa Clara, where the species was first observed, populations of *D. graveolens* can grow away from roadsides and co-occur with grassland species in established vegetative areas.

The introduction and spread of *D. graveolens* in California provides a unique opportunity to test the role of adaptive evolution in its spread away from roadsides. Earlier studies demonstrated rapid evolution in *D. graveolens* as it expanded its native range from the Mediterranean into higher latitudes (Lustenhouwer et al. 2018); in a common garden in the Netherlands, populations from the northern range edge flowered earlier, which increased fitness in the shorter growing season. In addition, niche modeling suggests that the species has expanded its climate niche since the mid-20th century, consistent with rapid evolutionary change (Lustenhouwer and Parker 2022). Similar to California, roadsides played a major role as transportation vectors during *D. graveolens'* native range expansion from the Mediterranean region to northern and central Europe (e.g., Brandes 2009; Frajman and Kaligarič 2009).

Here we studied whether *D. graveolens* populations in California have undergone evolution throughout their spread away from roads into more vegetated areas. We tested for phenotypic differences between paired populations: a population colonizing a vegetated area and its closest roadside, presumed progenitor, population. We quantified differences in germination behavior and used a greenhouse experiment to test for the response to competition in field soils. Finally, we used a field experiment in an established grassland to compare genotypes from roadside and vegetated sites in their phenology and response to release from competition.

Methods and Materials

Study sites

The County of Santa Clara (37.36°N 121.97°W) is located at the southern end of the San Francisco Bay. The County encompasses the Santa Clara Valley, which is bounded by the Diablo Range to the east, Santa Cruz Mountains to the southwest, and San Francisco Bay salt marshes to the northwest. Due to its proximity to the Pacific Ocean and the moderating effects of the San Francisco Bay, the valley experiences a mild Mediterranean climate with warm, dry weather much of the year (Grossinger et al. 2007). The rainy season is predominantly from November to April and only yields about 375 mm of annual precipitation with a standard deviation of 125 mm (McKee et al. 2003).

Plant community survey

In the summer of 2020, we identified *D. graveolens* populations in the County of Santa Clara within a 25-mile (~40 km) distance of the Alviso railway location where the species was originally found (Preston 1997). In collaboration with local resource managers and using online sources (e.g., CalFlora and Google Maps), we generated a list of populations where *D. graveolens* was growing in plant communities (vegetated habitat) at least 40 m from roadways. These vegetated habitats were not landscaped and generally associated with public parks or accessways that were dominated by common non-native annual species in the Poaceae and Asteraceae. Of an original list of 15 populations, our final study included 8 that were publicly accessible by foot and had not been eradicated before September 2020. For each population, we then located the nearest *D. graveolens* population along a roadside. We found roadside populations by walking away from the vegetated population along sidewalks and paths on the nearest hardened road. Each pair of populations in vegetated and roadside habitats is called a "site" (Figure 1). We selected roads for this study that were hardened with an asphalt surface with speeds of 40.2 – 72.4 kph. The substrate of the roadside habitat was composed of engineered fill used in the construction of the roadbed. The two habitats (roadside and vegetated) within a site show strong spatial autocorrelation in many environmental characteristics (Table S1). Road density within a 3.14 km^2 sampling area of each habitat ranged between 1 – 14 km/km^2. Habitat elevation ranged between 3 – 210 m above mean sea level. Because primary spread of *D.* graveolens is along roads, and dispersal away from roadsides is a secondary process, we make the assumption that the nearest roadside population is the likely source of invasion for each vegetated population. The fact that the separate sites are far away from each other ensures that the populations in vegetated habitat are much more likely to be related to their nearest roadside than they are to each other.

Between July 1st and August 14th, 2020, we conducted plant community surveys at all 16 populations (Table S2). We walked the perimeter for each population of *D. graveolens* and placed pin flags around the edge. We then laid a 50 m transect tape along the longest axis (for roadsides, transects were always parallel to the road) and placed a 0.5 × 0.5 m quadrat at three equidistant points along the axis. We visually estimated percent cover within each quadrat for *D. graveolens*, other vegetation, and bare ground (sum equaling 100%). For each population, we identified species within the three quadrats and then walked the area to search for additional rare species. Taxa were identified to species when possible using The Jepson Manual: Vascular Plants of California (Second Edition).

In September and October of 2020, we sampled seeds from each of the 16 populations. We collected from at least 10 individuals, 3 m apart, for each population, along a randomly-placed transect. We combined seeds from all individuals in a population.

Seed behavior

In the summer of 2021, we compared germination behavior of seeds from roadside and vegetated habitat types. We did three studies on different substrates: one on moist filter paper, one on engineered fill, and one on field topsoil collected from a site on the UC Santa Cruz campus. For this experiment, we used filter paper as a control to test seed behavior in ideal germination conditions, engineered fill as a proxy for roadside soils manufactured for roadbed construction, and field topsoil as a proxy for soils from plant communities. We germinated 50 seeds from each population in Petri dishes (80 Petri dishes; 5 replicates with 10 seeds each) for each substrate (filter paper, engineered fill, and field topsoil). Seeds were visually inspected beforehand to ensure that only fully developed seeds were used for all experiments. Petri dishes were sealed with Parafilm M™ and placed in a randomized block design in an incubation chamber with a daytime temperature of 23 ºC from 0900 - 0100 h and a nighttime temperature of 19 ºC from 0100 - 0900 h. We scored germination daily until no further germination was observed, then 7 more days (a total of 23 d on filter paper, 12 d on engineered fill, and 11 d on field topsoil). Signs of germination included the first emergence of the root radical or the cotyledon. Petri dishes were misted with DI water, and germinated seeds were removed once scored. We also took one homogenized sample of 30 seeds from each of the 16 populations and weighed them to the closest 0.001 g.

Plant growth response to competition

To assess the response of *D. graveolens* to competition, and how it might have evolved during the invasion of vegetated sites, we exposed plants originating from roadside and vegetated habitats to a competition treatment in both a greenhouse and a field setting. The aim of the greenhouse experiment was to uncover genetic differentiation between roadside and vegetated habitats at high replication and highly controlled conditions. The field experiment (see *Relative fitness in a field setting*) was designed to look for adaptive differentiation under more realistic conditions.

We quantified response to competition in a greenhouse experiment with three treatments: *D. graveolens* grown alone (1 plant per pot), and *D. graveolens* with *Bromus hordeaceus* or with *Festuca perennis* (2 plants per pot). These non-native European annual grasses were selected because they are commonly found in California's annual grasslands (Seabloom et al. 2003; Dawson et al. 2007; HilleRisLambers et al. 2010) and were observed at or near the eight sites. We collected *B. hordeaceus* seeds from Blue Oak Ranch Reserve and *F. perennis* seeds from the Terrace Lands of Younger Lagoon Reserve on the UC Santa Cruz Coastal Science Campus.

We germinated *D. graveolens* seeds in the conditions described above. We germinated grasses in trays with potting mix and placed them under fluorescent light banks for 16-hour length days and 8-hour length nights. Once radicles and cotyledons emerged, seedlings were transplanted in sets of three (one for each treatment). We grew plants in D16 Deepots (5 cm diameter, 18 cm height) in the greenhouse using field topsoil collected from a UC Santa Cruz campus site. Pots were then randomized into a blocked design with each block consisting of one *D. graveolens* seedling from each of the 16 populations for each of the three competition treatments, N = 48 per block × 8 blocks (384 total).

After 4 months, we harvested *D. graveolens* aboveground biomass at the crown and dried it in a 60 ºC oven for 3 days before weighing it.

Relative fitness in a field setting

The field experiment was conducted at Blue Oak Ranch Reserve, part of the University of California Natural Reserve System. Blue Oak Ranch Reserve is located within the County of Santa Clara on the western slopes of Mount Hamilton in the Diablo Range, just east of San Jose, California, United States (37°22'54.89"N, 121°44'10.55"W). Blue Oak Ranch Reserve supported cattle grazing until 1972. This former rangeland represents a key habitat type threatened by the invasion of *D. graveolens*.

The experimental site is in a non-native grassland with a mixture of annual grasses and forbs (Table S3). Land managers mow the site in the spring. At the site, common herbivores include deer, rabbits, California ground squirrels, and wild pigs. We protected the experiment with hog fencing and reduced herbivory pressure from deer and rabbits. Although not near the experimental site, *D. graveolens* actively invades Blue Oak Ranch Reserve.

At Blue Oak Ranch Reserve, we tested whether rapid evolution during invasion into vegetated sites has enhanced fitness in the presence of grassland competitors. We established a 10 m × 26 m fenced field site and used a randomized block design with 10 blocks of 1.5 m^2 plots. The data presented here are a subset of a larger ecological study elucidating *D. graveolens* response to different disturbance mechanisms. Here we focus on the response of plant genotypes and include only two treatments: grassland control (high competition) and complete competitor removal (no competition). We left the previous year’s thatch for the grassland control treatment and allowed resident vegetation (including the two species from our greenhouse experiment, *Bromus hordeaceus* and *Festuca perennis*, as well as 15 other plant species; Table S3) to grow throughout the experiment. For the competitor removal treatment, we tilled the soil to completely remove below and aboveground biomass in December 2020 and then weeded to remove aboveground biomass throughout the growing season.

In January 2021, we germinated seeds in Petri dishes in incubation chambers before transplanting them into soil collected in late December 2020 from Blue Oak Ranch Reserve. Seedlings grew in the greenhouse for about eight weeks until all plants had their first two true leaves emerge and lengthen. Seeds could not be sown directly into the field due to biosafety concerns.

We planted seedlings into each plot from February 27 - March 24, 2021 (20 plots total). Each plot included one *D. graveolens* individual from each of the 16 populations, in a 4 × 4 grid centered on the plot. Plants were separated by 33 cm, with a 25 cm buffer. During the first month of growth, we replaced any *D. graveolens* that died. We surveyed plants weekly to assess *D. graveolens* survival and bud initiation until all plants had either produced buds or perished.

We terminated plants at the first sign of budding to prevent reproduction of a noxious weed. As proxies for reproductive output, we measured height and biomass. We harvested aboveground biomass by cutting at the root crown and drying in a 60 ºC oven for three days before weighing. Height and biomass were strongly correlated (r = 0.74, N = 157), and results for the two response variables were similar. Therefore we present only the results for final biomass.

Data analysis

We used R version 4.2.2 (2022-10-31; R Core Team 2022) for all statistical analyses. Our general approach for each response variable (except the plant community survey) was to run mixed effects models with, at minimum, a fixed effect for habitat (roadside vs. vegetated) and a random effect for site. The site random effect takes into account the genetic similarity between the two nearby populations within a site, and captures landscape-scale variation between sites in, for example, elevation and roadside density.

Plant community survey

We calculated the average percent cover of bare ground, *D. graveolens*, and other vegetation per population by taking the mean of the three quadrats along each transect. Species richness was the total number of species found at a population (the three quadrats + surrounding rare species survey). We evaluated differences in percent cover and species richness between source habitats (roadside and vegetated) using paired t-tests (N = 8 sites with pairs of roadside and vegetated populations at each site).

Seed behavior

We analyzed the germination rate on each of the three substrates (filter paper, engineered fill, and field topsoil) using a mixed-effects Cox proportional hazards model (coxme and survival packages; Therneau 2022a, b), with source habitat as a fixed effect and site, population, and dish number as nested random effects. We evaluated the main effect of source habitat using a Type II partial-likelihood-ratio test (car package; Fox and Weisberg 2019). We calculated average seed mass for each source habitat using a Welch Two Sample t-test.

Plant growth response to competition

We calculated response to competition as the log response ratio (LRR) of the aboveground biomass, LRR = ln(biomass with competitor / biomass alone), on a per-block basis (N = 8 blocks) for each of the 16 seed origins (vegetated or roadside habitat at each of the 8 sites). Therefore, each seed origin had 8 replicate LRR estimates for each competitor grass (*Bromus hordeaceus* and *Festuca perennis*). We fit a linear mixed effects model for each competitor with LRR as the response variable, source habitat as a fixed effect, and random effects for population nested in site, and block (lme4 package; Bates et al. 2015). Block was removed from the *B. hordeaceus* model because it did not explain sufficient variance, causing a singular fit. We tested for differences between source habitats using Type II Wald F-tests with

Kenward-Rogers degrees of freedom (car package; Fox and Weisberg 2019). To evaluate whether each competitor grass affected the biomass of *D. graveolens*, we tested whether the LRR intercept was significantly different from zero using t-tests with Kenward-Rogers degrees of freedom (pbkrtest and lmerTest packages; Halekoh and Højsgaard 2014; Kuznetsova et al. 2017).

Relative fitness in a field setting

The field experiment had four response variables: survival (assessed both as total proportion surviving and time to death), final biomass at budding, and phenology (the survey date buds first appeared). We used a similar statistical approach for all response variables, fitting mixed effects models with source habitat, competition treatment, and their interaction as fixed effects; and initially including random effects for site, population nested in site, and block. Random effects that explained very low amounts of variance, causing singular fits, were removed. When interaction terms were not significant, they were removed and models were re-run with main effects only. Here we describe the structures of the final models.

We compared total survival to budding with a generalized linear mixed model using a binomial family with a logit link function; fixed effects were source habitat and competition treatment, and random effects were population nested in site, and block (glmmTMB package; Brooks et al. 2017). We evaluated the main effect of source habitat using a Type II Wald Chi-Square test (car package; Fox and Weisberg 2019). Second, we analyzed survival using a mixed-effects Cox proportional hazards model (coxme and survival packages; Therneau 2022a, b); fixed effects were source habitat and competition treatment, and random effects were population nested in site, and block. We evaluated the main effects of source habitat and competition treatment using likelihood ratio tests.

We analyzed final biomass at the time of bud production using a linear mixed effects model (lme4 package; Bates et al. 2015); fixed effects were source habitat, competition treatment, and their interaction, and the only remaining random effect was site. We evaluated the main and interaction effects using Type II Wald F-tests with Kenward-Rogers degrees of freedom (car package; Fox and Weisberg 2019). We used a log transformation of the biomass data to improve homoscedasticity.

To assess changes in phenology, we compared the timing to bud for those plants that reached the reproductive state, using a mixed-effects Cox proportional hazards model (coxme and survival packages; Therneau 2022a, b); fixed effects were source habitat and competition treatment, and random effects were population nested in site, and block. We evaluated the main effects using likelihood ratio tests.

Results

Plant community survey

Roadside habitats had higher amounts of bare ground than vegetated habitats (mean difference 41.9%, 95% CI [4.2 , 80], paired $t_7 = 2.63$, $P = 0.034$; Figure 2a). Roadside habitats appeared to have substantially less resident plant cover (not including *D. graveolens*) on average than vegetated habitats (Figure 2b), but this difference was not significant (mean difference -28%, 95% CI [-67, 11], $t_7 = -1.70$, $P = 0.13$). Species richness was not significantly different (mean difference -1.75, 95% CI [-5, 1.5], $t_7 = 1.26$, $P = 0.25$). Average species richness was 4.13 ± 2.59 SD at roadsides and 5.88 ± 3.27 SD at vegetated sites. Resident plant species at all sites were predominantly non-native annuals (Table S2).

Seed behavior

Seeds originating from vegetated habitats consistently had a slightly reduced probability of germination compared to seeds originating from roadside habitats (22% on filter paper, 11% on engineered fill, and 11% on field topsoil). This difference was significant on filter paper (relative risk of 0.78 ± 0.18 SE; $X^2_1 = 85.60$, $P < 0.001$; Figure 3), engineered fill (relative risk of 0.89 ± 0.09 SE; $X^2_1 = 80.86$, $P < 0.001$), and field topsoil (relative risk of 0.89 ± 0.12 SE; $X^2_1 = 30.6$, $P < 0.001$). Average seed mass varied from 0.243 to 0.333 and did not differ between source habitats (roadside = 2.26 mg, vegetated = 2.37 mg; $t_{12.11}$ = -1.18, P = 0.259; Table S4).

Plant growth response to competition

The growth of *D. graveolens* was strongly affected by competition with non-native grasses (Figure 4). Both *Bromus hordeaceu*s (intercept = -2.94 ± 0.22 SE, $t_{11.48}$ = -13.64, P <0.001) and *Festuca perennis* (intercept = -4.47 ± 0.13 SE, $t_{10.62}$ = -33.32, P < 0.001) strongly reduced the growth of *D. graveolens*. Plants from vegetated sites did not show evidence of a more robust response to competition: LRR did not differ between source habitats when *D. graveolens* was grown with either *B. hordeaceus* ($F_{1,7}$ = 0.032, P = 0.86) or *F. perennis* ($F_{1,7}$ = 0.37, P = 0.56).

Relative fitness in a field setting

We found no significant interactions between source habitat and treatment for any of the response variables (overall survival: X^2_1 = 0.069, P = 0.79; survival analysis: X^2_1 = 0.018, P = 0.89; biomass: $F_{1,152.81}$ = 2.34, P = 0.13, phenology: X^2_1 = 2.18, P = 0.14), indicating there was no differentiation between source habitats in their response to competition. Therefore the interactions were removed from the models.

We evaluated survival to reproduction in two ways. First, overall survival to reproduction was not affected by source habitat ($X^2_1 = 0.069$, $P = 0.79$), but was strongly affected by treatment ($X^2_1 = 78.84$, $P < 0.001$), with 53% greater survival to reproduction (absolute difference) in the competitor removal treatment compared to the grassland control. Second, consistent with the results for overall survival, our survival analysis showed that the competitor removal treatment reduced the mortality risk by 82% ($X^2_1 = 99.09$, $P < 0.001$; Figure 5a). There was no significant difference between source habitats ($X^2_1 = 0.0001$, $P = 0.99$).

When we assessed aboveground biomass, we found that plants in the competitor removal treatment were significantly larger than those in the grassland control ($F_{1,152.42} = 241.24$, $P < 0.0001$; Figure 5b). Similarly to survival, we found no significant difference between source habitats ($F_{1,151.85} = 0.11$, $P = 0.74$).

In terms of phenology, plants in the grassland control treatment initially started reproducing sooner, but by the end of the growing season, plants in the competitor removal treatment reproduced sooner on average than plants in the grassland control ($X^2_1 = 56.13$, $P < 0.0001$; Figure 6). We found no significant difference between source habitats ($X^2_1 = 0.29$, $P = 0.59$).

Discussion

Roads are vectors of invasion, as introduced species often spread along transportation corridors (Hansen and Clevenger 2005; Kalwij et al. 2008). However, to be considered invasive, a species must not just persist in ruderal populations but also spread aggressively away from roadsides, requiring traits that allow it to compete with resident plants. Evolution in introduced species can be an essential driver of invasion (Maron et al. 2004; Buswell et al. 2011; Colautti and Barrett 2013; Turner et al. 2014). *Dittrichia graveolens* is rapidly spreading along roads in California, and more recently has been observed establishing populations in vegetated areas away from roads. Does rapid evolution of competitive ability and other traits associated with surviving in vegetated habitats contribute to its invasiveness?

Our study found little evidence that populations of *D. graveolens* spreading away from roadsides into plant communities have evolved greater competitive ability. Response to competition for plant growth, survival, and reproduction did not differ between roadside and vegetated source habitats, and this was true in both greenhouse and field studies. Several factors could contribute to a lack of measurable adaptive differentiation between roadside versus vegetated populations. First, it may be that there has not been enough time for rapid evolution to occur. Introduced to California likely in the early 1980's or late 1970's, the annual *D. graveolen*s has spent around 40 generations in the County of Santa Clara where we studied it, although populations spreading away from roads may have experienced fewer generations in the competitive environment of the vegetated sites. Populations in California may ultimately evolve adaptations to vegetated environments away from roads in the future, but none have been detected so far. In contrast to our study, others have observed rapid evolution within a few decades of introduction. For example, Ethridge et al. (2023) found that *Setaria faberi* evolved larger leaf area within 34 generations as a result of agricultural selection pressure, and Dlugosch and Parker (2008b) found increased growth in *Hypericum canariense* from sites where introductions were < 25 generations. Moreover, Lustenhouwer et al. (2018) found rapid evolution of phenology in populations of our study species *D. graveolens* in the Netherlands within 2 decades of arrival in the country. These previous studies suggest that adaptive evolution in *D. graveolens* should have been possible within the time frame of its invasion in central California. However, evolutionary patterns can differ between traits of interest; Fletcher et al. (2023) found strong differentiation in biomass, height, and phenology between invasive populations of Johnsongrass (*Sorghum halepense*), but no difference in their response to competition as evaluated by growth on bare ground vs background vegetation.

Second, novel selection pressures may have been weak; selection may not differ substantially between the two habitat types. Our vegetated sites were often somewhat disturbed, and some were mowed; species composition was similar on and off the roadside (Figure S1), and plant diversity was low overall. Thus our vegetated sites might share some environmental conditions with roadsides in this suburban setting. However, roadside sites did show less resident plant cover and substantially more bare ground than vegetated sites (Figure 2). We found that *D. graveolens* was strongly suppressed by competition in both the field experiments and the greenhouse experiment, and field experiments regardless of the competitor identity, suggesting competition should represent a strong selection pressure. In the greenhouse experiment, we saw that *D. graveolens* grew poorly in the presence of *B. hordeaceus* and *F. perennis*. This pattern was echoed in our field plot at Blue Oak Ranch Reserve, which was similar in structure and species composition to many of the 8 vegetated sites from which seeds were collected. The field experiment showed strong effects of competition from resident plants in the grassland on *D. graveolens* survival, phenology, and growth. Therefore, it is likely that selection on competitive ability does differ between roadsides and intact grasslands.

Variation among roadside locations and among vegetated locations would limit our ability to detect adaptive responses at individual sites, and this could contribute to the lack of consistent differences between habitats in our results. For example, an NMDS analysis showed substantial variation across the sites for plant composition (Figure S1). Perhaps most importantly, vegetated habitats varied substantially for overall resident plant cover and amount of bare ground across the sites (Figure 2). At the time of our survey, two sites (Oakridge Pond and South San Jose VTA) showed over 80% cover of resident vegetation, while two sites (Parkway Lakes RV and Penitencia

Creek Trail) had very low cover. In our greenhouse experiment, those two populations from vegetated habitats with high cover did show the predicted pattern of stronger competitive ability than their paired roadside populations. However, this anecdotal evidence was not supported by general trends.

Third, there may be a lack of heritable genetic variation for relevant traits, particularly for traits that increase competitive ability (Nei et al. 1975; Amos and Harwood 1998). The introduction of *D. graveolens* to California may have involved a significant reduction in genetic variation through a strong founder effect. The first observation of *D. graveolens* was in Alviso (San Jose) in 1984 (Preston 1997). This area, near the railway tracks, was also likely the first invasion point, with subsequent spread throughout the County of Santa Clara and eventually to much of California. Founder effects during invasion often reduce variation in invasive species (reviewed in Dlugosch and Parker 2008a; Dlugosch et al. 2015). However, many studies have shown evolutionary change despite reduced variation (Blows and Hoffmann 2005; Dlugosch and Parker 2008b; Estoup et al. 2016).

Finally, evolutionary divergence could be limited by gene flow from roadside to vegetated habitats (Ureta et al. 2008; Bagavathiannan et al. 2011). Gene flow is one of the primary factors counteracting local adaptation, and it is expected to have strong maladaptive (or swamping) effects in the relatively small populations of expanding range edges (May et al. 1975; Lenormand 2002; Anderson and Song 2020).

Population pairs in our study ranged in distance from 540 m to as little as 40 m apart. Flowers can self-fertilize in *D. graveolens,* although flowers are also insect-pollinated in the native range (Rameau et al. 2008; Albaba 2015). Pollen dispersal distances and outcrossing rates have not been measured; however, McEvoy et al. (2023) found that heterozygosity is low across the genome, consistent with a highly self-fertilizing mating system. In contrast, seed dispersal is expected to be considerable in this wind-dispersed species with pappus-bearing seeds, suggesting high gene flow between populations is possible via seeds. Even with *D. graveolens*' highly selfing mating system, gene flow over short distances could easily be why we did not observe adaptive evolution away from roadsides. This contrasts with other studies showing the evolutionary divergence of introduced species over more considerable distances (Colautti et al. 2009; Buswell et al. 2011; Clark 2018; Alexander and Levine 2019). Nonetheless, Fletcher et al. (2023) studied range-wide differentiation in invasive *Sorghum halepense* and still found no differentiation in competitive ability on the continental scale.

The only significant difference between roadside and vegetated source habitats was for germination success, which was lower overall in seeds from vegetated source habitats (Figure 3). The higher proportion of ungerminated seeds from vegetated sites could indicate either lower seed viability or higher dormancy rates. Lower seed viability may reflect a poorer maternal environment or an increase in inbreeding and inbreeding depression in these nascent populations (Nei et al. 1975; Barrett and

Husband 1990). Higher dormancy rates could be adaptive in a variable environment (Venable and Brown 1988; Satterthwaite 2010). Brownsey et al. (2013b) found no evidence for primary dormancy in California populations of *D. graveolen*s, and we found in other germination experiments with California populations that only dead seeds did not germinate under incubation conditions like those reported here. However, germination experiments in the native range showed higher levels of viable ungerminated seeds, closer to 20% (Lustenhouwer et al. 2018). Ongoing studies in our group will provide new insights into seed bank dynamics in the future.

We quantified differentiation between populations in roadside and vegetated habitats using a multi-faceted approach to maximize our chances of observing adaptive differences if there were any. The germination and greenhouse studies under controlled conditions allowed us to minimize other sources of variance and maximize sample size. In contrast, the field study subjected *D. graveolens* plants to realistic environmental conditions with high competition and mortality. Our field site was similar to the vegetated areas where *D. graveolens* is actively invading, including dominant species shared with the vegetated source sites (Tables S4 and S2, respectively). Therefore, we expected that adaptive differences between the source populations should have been revealed under the field conditions. However, it is impossible to eliminate the possibility that adaptive differentiation could be exposed under different environmental conditions.

We looked for population differentiation for plant phenology and did not find any; nor did we find differentiation in the phenology response to stress. Plant competition can lead to physiological stress if resources are limited, and physiological stress can strongly affect plant phenology (Aragón et al. 2007). Competition can initiate stress-induced flowering in some Mediterranean plant species (Takeno 2016). Development time may respond to stress by advancing or delaying reproduction (Fox 1990); such phenotypic plasticity is not necessarily adaptive, but it can be (Anderson et al. 2012). Previous work suggests that flowering time in *D. graveolens* can evolve; plants from the expanding northern edge in Europe flowered earlier in a common garden (Lustenhouwer et al. 2018). Such rapid adaptation in flowering time is commonly seen in response to shifts in latitude in invasive plants (e.g., Leger and Rice 2007; Colautti and Barrett 2013; van Boheemen et al. 2019). Changing phenology can have strong fitness effects on invasive plants (Colautti and Barrett 2013) and may increase competitive effects on other plant species (Alexander and Levine 2019). We did find differences between field treatments affecting time to flowering; initially, some plants began reproducing sooner in the grassland control plots, which could be explained by stress-induced flowering, although overall plants flowered earlier in the competitor-removal plots. While we observed marked phenotypic plasticity in phenology, we did not find evidence for adaptive divergence between roadside and vegetated sites for either phenology or phenotypic plasticity in phenology.

We did not control for the maternal environment of seeds in our study. Environmental variation for field-collected seeds can influence the results of common garden studies with invasive plants (e.g., Turner et al. 2014). Ideally, we would have replicated the entire experiment with a second set of seeds generated in the greenhouse. Unfortunately, we could not delay our experiments for the 12 months required to grow this extra generation. A standard indicator of variability in the quality of the maternal environment is seed size; seed weights in our sample did not show consistent differences between collections from roadside and vegetated sites (Table S4), indicating that overall seed quality was similar. However, the seed germination rate was lower in vegetated sites, which could reflect lower seed viability. We predicted that evolution would result in greater competitive ability in populations in vegetated sites, and our data did not support our prediction. If patterns of maternal provisioning or epigenetics in the vegetated sites systematically reduced survival, biomass, and response to competition relative to roadside sites, then maternal effects could have masked adaptive differentiation.

Some *D. graveolens* populations are spreading away from roadsides and successfully invading plant communities; our results suggest that rather than locally adapted populations, these plants in vegetated communities are able to grow there when they can get a foothold because of phenotypic plasticity. Baker’s (1965) concept of the “general purpose genotype” of colonizing species proposed that phenotypic or developmental plasticity underlies the success of many weedy invaders (Parker et al.

2003). Although we saw that plants of all origins were negatively impacted by competition in the greenhouse (Figure 4) and in the field (Figure 5), plants in the field experiment were still able to flower with an adjustment in phenology (Figure 6). These plants, persisting in low numbers in suboptimal conditions, may be able to take advantage of localized or periodically large disturbances such as fires or intensive management activities involving soil disturbance (Hansen and Clevenger 2005).

The evolution of competitive ability in invasive species has been a significant research focus for nearly 30 years, generally in the context of reallocating resources with escape from specialized natural enemies (Blossey and Nötzold 1995). Many studies have compared traits related to competitive ability between populations from the native and introduced ranges (Bakker and Wilson 2001; van Kleunen and Schmid 2003; Bossdorf et al. 2005; Felker-Quinn et al. 2013; Yuan et al. 2013; Callaway et al. 2022). Studies exploring the evolution of competitive ability with expansion into new habitats within the introduced range are less common (but see Fletcher et al. 2023). According to life history theory, tradeoffs exist between traits that increase fitness in highly competitive environments and dispersal and reproductive traits that favor a ruderal lifestyle in highly disturbed, more open environments (Grime 1977; Pierce et al. 2017). In invasive species, selection for dispersal and reproduction at the invasion front may lead to declines in competitive ability (Burton et al. 2010). In its native range, *D. graveolens* thrives in disturbed soils and is commonly found along roadsides (Brownsey et al. 2013a). This is common in introduced plants, and in fact, ruderal traits may be selected for as introduced plants spread along transportation corridors. Our results suggest that even strong selection in less disturbed, more competitive environments may not result in the rapid evolution of invasive ability as plants spread away from roads. Opposing selection pressures on roads and away from roads, with gene flow linking close populations, may represent an insurmountable barrier to the evolution of increased competitive ability in invasive plants. To the extent that these barriers to adaptation persist over time, evolution will not represent an urgent threat to management activities or risk assessments.

Figures

Figure 1. In September and October 2020, we collected *D. graveolens* seeds from eight sites in the County of Santa Clara. Each site had two paired populations: a population in a vegetated habitat and the closest roadside population. Map created using QGIS [3.32.0-Lima] (QGIS Development Team 2023).

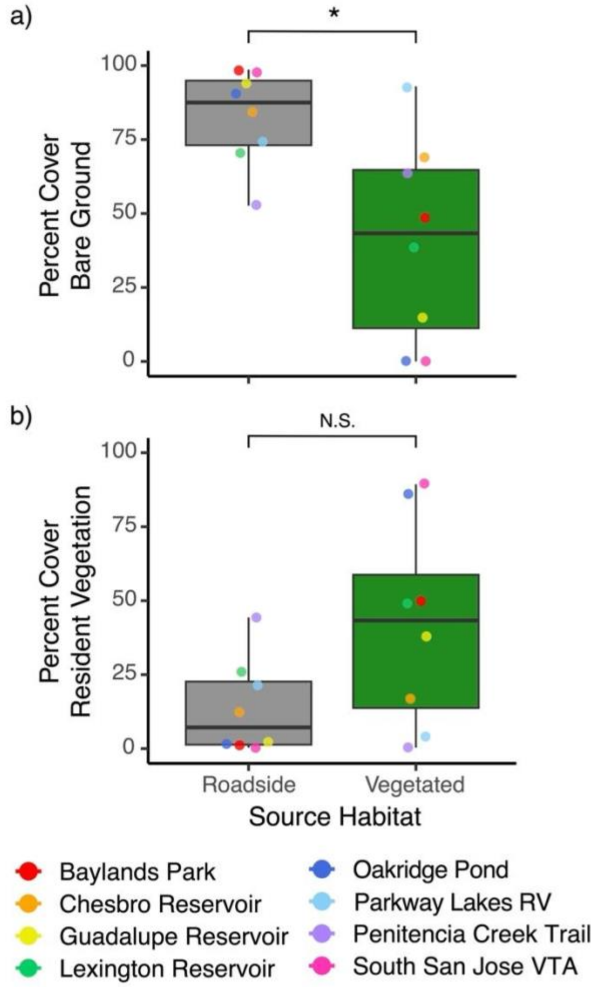


Figure 2. Differences in percent cover of bare ground (a) and resident vegetation (b) between roadside and vegetated sites (points indicate N = 8 sites per category). Boxes correspond to the median, first and third quartiles, and whiskers extend to the furthest value within 1.5 × the inter-quartile range. Star indicates significance of paired t-test.

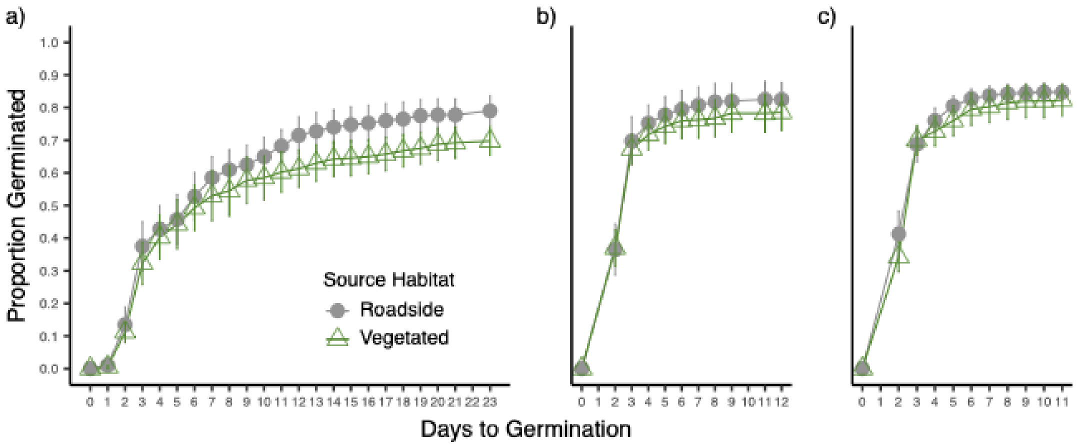


Figure 3. Cumulative proportion germinating per day of *D. graveolens* seeds collected from roadside (filled gray circles) and vegetated (open green triangles) source habitats. Seeds were germinated on a) filter paper, b) engineered fill, and c) field topsoil. Values shown are means ± 1 SE of 8 sites, after first estimating site means from 5 dishes (proportion germinated out of 10 seeds each).

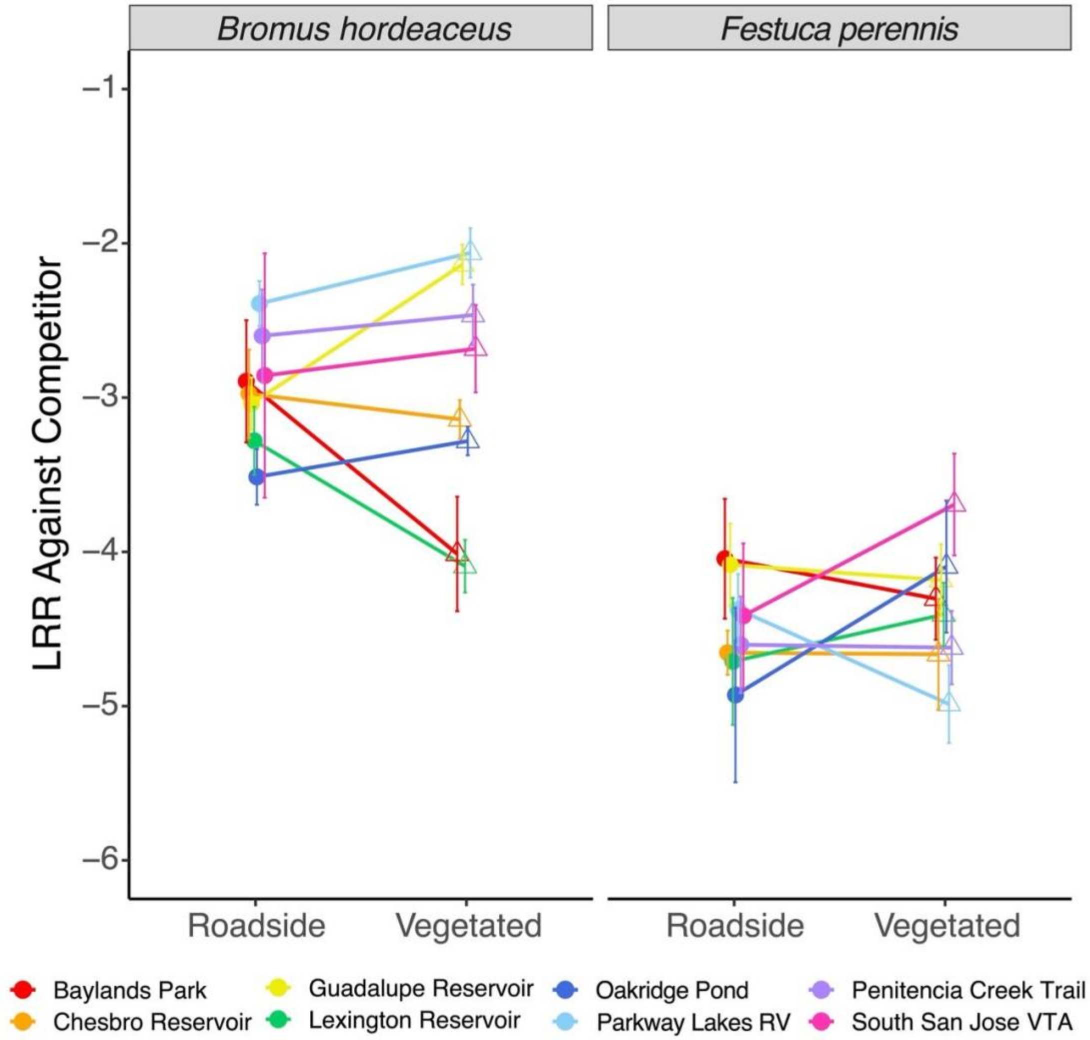


Figure 4. The log response ratio of biomass against each grass competitor (*B. hordeaceus* and *F. perennis*), calculated as the mean ± 1 SE across 8 replicate blocks for each seed origin. Lines of the same color connect seeds originating from paired roadside and vegetated habitats. Filled circles signify roadside habitats and open triangles signify vegetated habitats. We found that *D. graveolens* is a poor competitor, regardless of the source habitat.

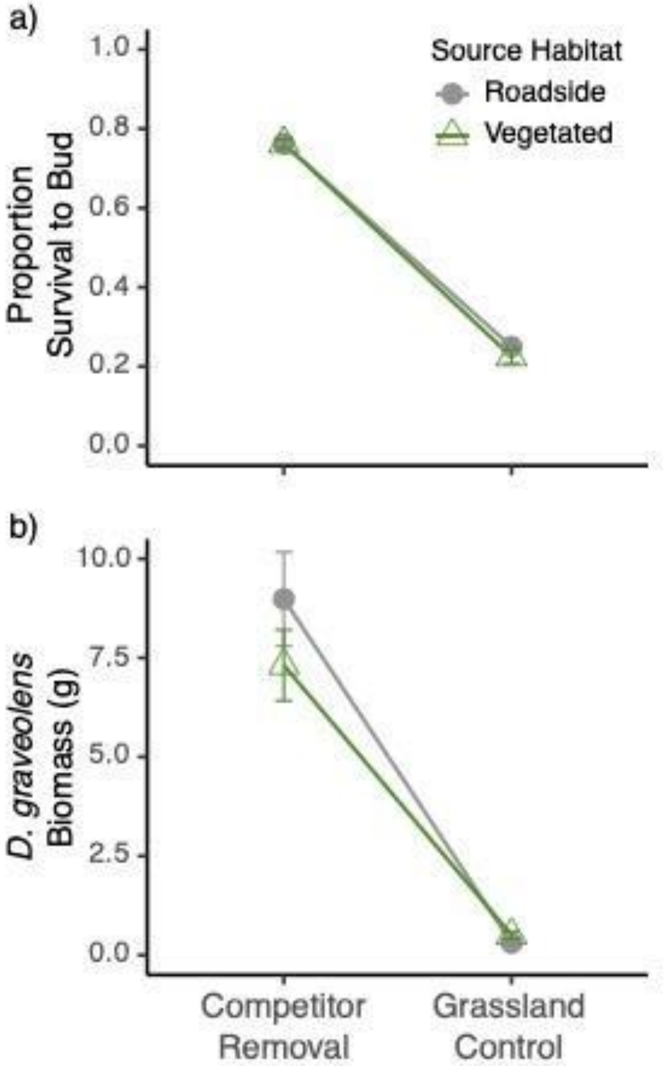


Figure 5. Plants from roadside and vegetated source habitats did not differ in fitness proxies survival and biomass between field treatments competitor removal and grassland (control) (Type II Wald Chi-Square test). (a) The proportion of *D. graveolens* that survived to produce buds (means ± 1 SE across 8 sites). (b) Aboveground biomass (g) of *D. graveolens* (means ± 1 SE of 8 sites, after first estimating site means from 10 plants). Filled gray circles signify roadside habitats and open green triangles signify vegetated habitats.

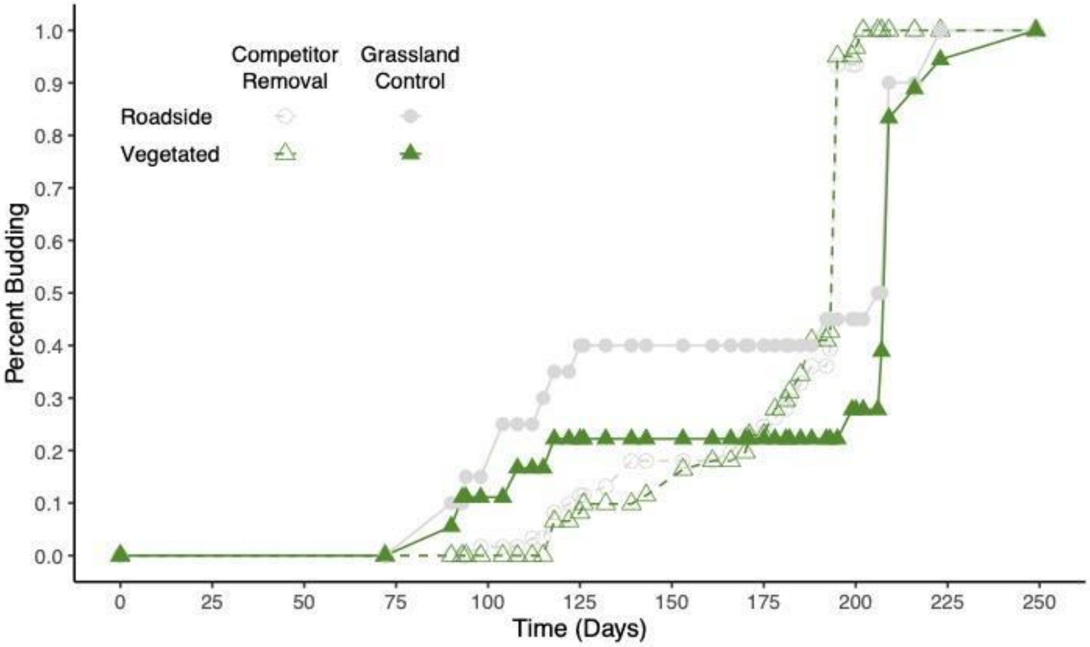


Figure 6. Flowering phenology (the percent of *D. graveolens* budding over time) showing roadside and vegetated source habitats for each treatment (competitor removal and grassland control). Gray lines signify roadside populations and green lines signify vegetated populations. Open symbols indicate competitor removal treatments and closed symbols indicate grassland control treatments.

Chapter 2

Low competitive ability explains *Dittrichia graveolens'* association with roadsides

Abstract

Invasive plants commonly establish along roadsides, highlighting the importance of understanding the mechanisms underlying this pattern for effective management. *Dittrichia graveolens* (stinkwort), a recent California invader, primarily inhabits roadsides but is beginning to spread into nearby plant communities. We tested whether *D. graveolens* grows best along roadsides due to a preference for soil conditions or is limited to these disturbed environments by plant competition. Lab and greenhouse experiments showed no germination advantage in engineered fill (simulating roadside soils), and *D. graveolens* grew slightly better in field topsoil. Competition trials with two annual grasses (*Bromus hordeaceus* and *Festuca perennis*) in both soil types (engineered fill and field topsoil) showed strong growth suppression of *D. graveolens* by both grasses, with engineered fill limiting growth for all species, suggesting that roadside soils provide a competitive refuge. In field experiments, we applied four treatments to examine disturbance effects: control, thatch removal, aboveground biomass removal, and complete biomass removal. Removing belowground competition significantly improved *D. graveolens* survival

and growth. Our findings indicate that competition confines *D. graveolens* to disturbed roadsides, so management should prioritize reducing bare, disturbed areas and enhancing plant competition in areas vulnerable to invasion.

Introduction

Disturbance is a critical process in many ecosystems, providing heterogeneity and influencing diversity at both the patch-level and landscape scale (Hobbs and Huenneke 1992; Greipsson 2011). Disturbance reduces competition and frees up space and resources, which colonizing species can exploit (Hobbs and Huenneke 1992; Catford et al. 2012). Thus, disturbance also plays an important role in the invasion process for many non-native species (Pimm 1989; Hobbs and Huenneke 1992; Minchinton and Bertness 2003; Catford et al. 2012). Roadsides, transportation corridors, and other disturbed environments facilitate primary spread of non-native species (Baker 1974; Tyser and Worley 1992; McDougall et al. 2018). The movement of species away from roadside edges and into established vegetation (secondary spread) is a critical phase in the invasion process (Ward et al. 2020).

Roadside soil is often disturbed from the initial construction and ongoing road maintenance, characterized by increased compaction and erosion (Lázaro-Lobo and Ervin 2019; Mills et al. 2020). Road runoff increases soil salinity and can contain vehicle contaminants (Trombulak and Frissell 2000; Lázaro-Lobo and Ervin 2019), and roadsides often have lower nutrient availability (Liu et al. 2021). Due to maintenance regimes and physical impacts from vehicle traffic, roadsides have high rates of disturbance and lower plant cover (Christen and Matlack 2006).

A classic question in ecology is whether distributions reflect an affinity for certain environmental conditions or rather competitive exclusion from higher-quality habitats. Some plant species are more successful in specific abiotic conditions (Wamelink et al. 2018; Gioria et al. 2023) leading to strong patterns of association with these soils or environmental conditions. In contrast, species may be widely distributed, but biotic interactions with more competitive plant species, herbivores, or pathogens may limit their range (Gioria et al. 2023). Understanding the nature of an association with roadsides is crucial for effective management. If a species has an affinity for disturbed soils, then land managers should focus early detection on those areas. If biotic interactions, such as escape from competitive pressure, limit the distribution of a species then land managers can focus on reducing disturbance and promoting competition.

We used *Dittrichia graveolens* (L.) Greuter (stinkwort; Asteraceae) to investigate whether a recently introduced plant currently associated with roadsides grows best along roadsides due to abiotic soil conditions or is limited to these disturbed environments by plant competition. This annual herb was introduced to California in the early 1980s and was originally found in disturbed areas along railroad tracks and roads in the County of Santa Clara (Preston 1997; Brownsey et al. 2013a). Native to the Mediterranean Basin in Europe, *D. graveolens* is often found in bare, disturbed habitats, including roadsides, agricultural lands, gravel riparian areas, and ruderal zones associated with annual or biennial weeds (Brullo and de Marco 2000; Rameau et al. 2008). Since its initial detection in 1984, *D. graveolens* has spread across California, now occupying over 83% of counties, with a range extending > 400 km north, > 200 km east, and > 690 km south.

In California, *D. graveolens* has been observed spreading into wildlands and rangelands away from roads (Brownsey et al. 2013a; Melen et al. 2024). These observations highlight the spreading potential of *D. graveolens* and the invasion risk this species poses. However, little is known about this invasion process and what conditions enable *D. graveolens* to spread away from roadsides into more intact plant communities. Here we studied whether *D. graveolens* populations preferentially grow along roadsides or if they are limited to growing along roadsides due to competition. We tested for differences in germination and growth in two soil types: engineered fill and field topsoil. Finally, we used field experiments manipulating disturbance in an established grassland to determine the effects of above- and belowground competition.

Methods and Materials

Study sites

The County of Santa Clara (37.36°N 121.97°W) is located at the southern end of the San Francisco Bay in California, USA. The county encompasses the Santa Clara Valley, boarded by the Diablo Mountain Range to the east, the Santa Cruz Mountains to the southwest, and San Francisco Bay to the northwest. The valley experiences a mild Mediterranean climate with warm, dry weather much of the year (Grossinger et al. 2007) due to its proximity to the moderating effects of the San Francisco Bay. The rainy season is predominantly from November to April with 375 ± 125 mm SD of annual precipitation (McKee et al. 2003), and the average daily mean temperature in the San Jose region ranges from 27.9°C to below-freezing (Hanson et al. 2004).

Soil sources

We compared the response of both seeds and plants to field topsoil and to engineered fill as a proxy for the physical microenvironment of roadside soils. Field topsoil was collected from a woodland site, and engineered fill was taken from a nearby construction project at UC Santa Cruz; several cubic tons of these soils were in storage at a central campus location, and we subsampled several buckets of each soil type for our experiments (Figure 1).

Seed behavior in roadside and field soil

In September and October 2020, we collected *D. graveolens* seeds from 16 populations in the County of Santa Clara. For each population, we collected from ≳10 individuals at 3 m intervals along a randomly-placed transect, and combined seeds into a single sample.

In June and July 2021, we compared germination behavior of seeds on the two substrates; including 10 seeds in each of 80 Petri dishes for each substrate (engineered fill and field topsoil). We visually inspected each seed beforehand to ensure we used only fully developed seeds. Petri dishes were sealed with Parafilm M™ and placed in a randomized block design in an incubation chamber with a 16-hour day and 23 ºC/19 ºC day/night temperature cycle. Each day we scored germination until no further germination was observed, then for 7 more days (engineered fill = 12 d, field topsoil = 11 d). When scoring for germination, we looked for the first emergence of the root radical or the cotyledon and removed any germinated seeds. We kept soil moist by misting with DI water.

We used R version 4.3.1 (2023-06-16; R Core Team 2023) for all statistical analyses. Our general approach for each response variable was to run mixed effects models with, at minimum, fixed effects for soil and competition and block as a random effect.

We analyzed the germination rate on two substrates (engineered fill and field topsoil) using a mixed-effects Cox proportional hazards model (coxme and survival packages; Therneau 2022a, 2023), with soil type as a fixed effect, and dish (N = 5) nested within population (N = 16) as random effects. We evaluated the main effect of soil type using a Type II partial-likelihood-ratio test (car package; Fox and Weisberg 2019).

Plant growth response to disturbed soil

We assessed the response of D. graveolens to competition and abiotic soil conditions in the same two substrates with three competition treatments: *D. graveolens* grown alone, with *Bromus hordeaceus*, or with *Festuca perennis*. These non-native European annual grasses were selected because they are commonly found in California's annual grasslands (Seabloom et al. 2003; Dawson et al. 2007; HilleRisLambers et al. 2010), including at our field site described below (Melen et al. 2024). We collected *B. hordeaceus* seeds from Blue Oak Ranch Reserve (37.38° N 121.74°W) and *F. perennis* seeds from Younger Lagoon Reserve (36.96°N 122.07°W) on the UC Santa Cruz Coastal Science Campus.

We germinated *D. graveolens* seeds in the conditions described above (see *Seed behavior*). We germinated grasses in potting mix trays under fluorescent light banks with 16-hour days. We filled D16 Deepots (5 cm diameter, 18 cm height) with engineered fill and field topsoil and then transplanted seedlings in sets of three (one for each treatment) after radicles and cotyledons emerged. We randomized pots into a blocked design with each block consisting of 2 *D. graveolens* seedlings from each of the 16 seed-source sites for each of the 3 competition treatments, N = 96 per block × 8 blocks (768 total). We grew plants in a greenhouse for 4 months before harvesting. We clipped *D. graveolens* aboveground biomass at the crown and dried it in a 60 ºC oven for 3 days before weighing.

We fitted a Generalized Linear Model (GLM) to analyze the effect of competition on *D. graveolens* biomass (car package; Fox and Weisberg 2019). The model used a Gamma distribution with a log link function; fixed effects were competition and soil. We initially used population and block as random effects, but both prevented model convergence and AIC scores showed that the random effects did not contribute importantly to the model, therefore they were not included. We evaluated the main and interaction effects using Type II likelihood ratio tests (car package; Fox and Weisberg 2019). We conducted post-hoc pairwise comparisons using estimated marginal means, comparing them using Welch t statistics with Satterthwaite degrees of freedom, and a Bonferroni adjustment for multiple comparisons (emmeans package; Lenth 2024).

Field experiment I: response to competition and disturbance

To assess the response of *D. graveolens* to competition in a field setting, we conducted an experiment at Blue Oak Ranch Reserve, part of the University of California Natural Reserve System. Blue Oak Ranch Reserve is located on the western slopes of Mount Hamilton in the Diablo Range, just east of San Jose, California, United States (37°22'54.89"N, 121°44'10.55"W). The reserve is a former rangeland, representing a key habitat type threatened by the invasion of *D. graveolens*. We established a 10 × 26 m fenced field site in a non-native grassland with a mixture of annual grasses and forbs.

We quantified *D. graveolens* response to four competition treatments: (1) control, the grassland including the year's plant growth as well as the previous year's thatch; (2) thatch removal, which involved raking and removing the previous year's thatch; (3) aboveground removal, where we used a string trimmer to trim grassland vegetation to 8 - 13 cm above the ground; (4) and above+below removal, where we tilled the soil completely to remove above- and belowground biomass.

In January 2021, we germinated *D. graveolens* seeds in the conditions described above and transplanted them into D16 Deepots (5 cm diameter × 18 cm height) with field topsoil collected from Blue Oak Ranch Reserve in December 2020. We grew the seedlings in the greenhouse for about eight weeks until the first true leaves had emerged and lengthened for all plants. We did not directly seed *D. graveolens* into the field site due to biosafety concerns about this noxious weed.

We used a randomized block design with 10 blocks of 1.5 m^2 plots (Figure S1). From 27 February - 24 March 2021, we planted 16 *D. graveolens* seedlings into each plot using dibblers (640 seedlings; 40 plots total). Seedlings were planted in a 4 × 4 grid centered on the plot. The distance between plants within plots was 33 cm, and plots were separated from each other by a 25 cm buffer. We surveyed plants weekly to assess *D. graveolens* survival and replaced any dead plants during the first month. We assessed bud development as a key phenology stage for terminating plants to ensure no seeds were released into the site. Weekly plant surveys continued until all plants had either produced buds or perished.

We measured height, biomass, and bud date as proxies for reproductive output. Aboveground biomass was harvested by cutting each plant at the root crown and drying the plant materials in a 60 ºC oven for three days before weighing. Height and biomass were correlated ($r = 0.58$, $N = 213$), and results for the response variables were similar. Therefore, we present only the results for final aboveground biomass.

We used a similar statistical approach for all response variables by fitting mixed effects models: survival (assessed both as total proportion surviving and time to death) and final biomass at budding. Competition treatment was the fixed effect and population and block were included as random effects. Here we describe the structures of the final models.

We analyzed survival in two ways. The probability of surviving to reproduction (budding) was compared across competition treatments with a GLMM using a binomial family with a logit link function (lme4 package; Bates et al. 2015). We evaluated the main effect of competition treatment using a Type II Wald Chi-Square test (car package; Fox and Weisberg 2019). Second, time to death was analyzed using a mixed-effects Cox proportional hazards model (coxme and survival packages; Therneau 2022a, 2023). We evaluated the main effect of competition treatment using likelihood ratio tests (car package; Fox and Weisberg 2019).

We analyzed biomass at reproduction using a linear mixed-effects model (lme4 package; Bates et al. 2015). The significance of the competition treatment was assessed using Type III Wald F test with Kenward-Roger degrees of freedom (car package; Fox and Weisberg 2019). We did post-hoc comparisons using the differences among estimated marginal means (emmeans package; Lenth 2024), using the Bonferroni method.

Field experiment II: separating response to above- and belowground competition

In January 2022, we germinated *D. graveolens* seeds in the conditions described above and transplanted them into 10.16 cm height × 8.89 cm width injection molded pots with potting media (ProMix® HP® BioFungicide™ + Mycorrhizae™) where they grew for about eight weeks until the first true leaves had emerged and lengthened for all plants. We conducted three experiments related to above and belowground competition using a subset of the same 1.5 m^2 plots as the previous year (Figure S2).

Aboveground and belowground competition

To replicate our test of the relative effect of above and belowground competition on the survival of *D. graveolens*, we conducted a second-year experiment that tested belowground competitor removal and background vegetation clipping. Here we randomly assigned three treatments to 54 planting locations: belowground competitor removal (holes dug to a depth of 45 cm), clipping (background grassland vegetation clipped to 1 - 3 cm high), and control (no manipulation of the soil or vegetation). We planted two *D. graveolens* seedlings in each planting location (N = 108) and maintained clipping treatments weekly, recording survival for nine weeks.

For the aboveground and belowground competition with clipping and belowground competitor removal data, we analyzed seedling survival using a mixed-effects Cox proportional hazards model (coxme and survival packages; Therneau 2022a, 2023), with treatment (control, clipping, belowground competitor removal) as a fixed effect, and a random effect of plot. We assessed the significance of the treatment using Type II likelihood ratio tests (car package; Fox and Weisberg 2019).

Aboveground shading

To separate effects of shading from other aboveground interactions, we used a shading experiment with 11 plots, which were each hoed to remove above and belowground competition. The plots were then divided into four quadrants and planted with two *D. graveolens* seedlings per quadrant (N = 88 plants total). Four bamboo stakes were placed in the corners of each quadrant and were randomly assigned a treatment of control (no shade cloth) or shade cloth (GCI Landscaper's Choice Premium 5-ounce Woven Landscape Fabric 500 Series). Shade cloth was clipped to the bamboo stakes and maintained for the duration of the experiment. We weeded the plots weekly and recorded D. graveolens survival for nine weeks.

For the aboveground shading data, we analyzed seedling survival using a mixed-effects Cox proportional hazards model (coxme and survival packages; Therneau 2022a, 2023), with treatment (no shade and shade) as a fixed effect, and a random effect of plot. We assessed the significance of the shading treatment using Type II likelihood ratio tests (car package; Fox and Weisberg 2019).

Belowground competition - trenching, water, and nutrients

To separate effects of belowground interactions, we used a trenching experiment with 28 grassland plots. Six 45-cm holes were trenched per plot with half lined with weed cloth fabric (GCI Landscaper's Choice Premium 5oz. Woven Landscape Fabric 500 Series) and the other half without weed cloth. We placed 15-diameter PVC collars in all holes, with a lip of 2 cm above the soil surface to prevent surface runoff and to a depth of 10 cm (PVC = 6 cm wide × 12 cm deep). The original soil was used to fill in the holes and we planted two *D. graveolens* seedlings in each treatment hole. Using a factorial design, treatments were assigned to each hole: control, the addition of 283.49 grams of water, and the addition of 283.49 grams of water plus 5 grams of fertilizer (Osmocote 14-14-14). The soil surface was scratched using a fork in all treatments to encourage infiltration. We maintained treatments weekly and recorded *D. graveolens* survival for nine weeks.

For the belowground competition data, we analyzed seedling survival using a mixed-effects Cox proportional hazards model (coxme and survival packages; Therneau 2022a, 2023), with treatment (control, water, water + nutrients) and competition (weed cloth, no weed cloth) as fixed effects, and a random effect of plot. We assessed the significance of the interaction and main effects by comparing models using likelihood ratio tests. This approach allowed us to sequentially test the interaction between competition and treatment, as well as the main effects of each variable.

Results

Seed behavior in roadside and field soil

The proportion of germinated seeds was high in both soil types (engineered fill = 81 %, field topsoil = 84 %), with seeds showing a slightly higher chance of germinating in field topsoil than in engineered fill (5 % higher; relative risk of 1.05 ± 0.06 SE; $X^2_1 = 105.57$, $P < 0.001$; Figure 2).

Plant growth response to disturbed soil

Plants growing in competition with non-native annual grasses were significantly smaller than those growing alone ($X^2_2 = 48.03$, $P < 0.001$), and plants grew much smaller in engineered fill ($X^2_1 = 431.87$, $P < 0.001$, Figure 3). In addition, we saw a significant interaction between competition and soil ($X^2_2 = 470.32$, $P < 0.001$). When grown with *B. hordeaceus*, competition reduced *D. graveolens* biomass by 14 fold in field topsoil ($\bar{X}_{\text{alone}} / \bar{X}_{\text{competitor}} = 14.3$, $t = 25.47$, $P < 0.001$) compared to only a 77 % reduction in biomass in engineered fill ($\bar{X}_{\text{alone}} / \bar{X}_{\text{competitor}} = 1.77$, $t = 5.45$, $P < 0.001$).

Similarly, competition with *F. perennis* caused a 55-fold reduction in *D. graveolens* biomass in field topsoil ($\bar{X}_{\text{alone}} / \bar{X}_{\text{competitor}} = 54.6$, $t = 38.16$, $P < 0.001$) and only a 91 % reduction in engineered fill ($\bar{X}_{\text{alone}} / \bar{X}_{\text{competitor}} = 1.91$, $t = 6.18$, $P < 0.001$).

Field experiment I: response to competition and disturbance

Overall survival to reproduction was strongly affected by treatment ($X^2_3 = 136.01$, $P < 0.001$), with about a 50 % increase in survival in the above+below removal treatment and, surprisingly, lower survival in the thatch removal treatment (Figure 4). Likewise, survival analysis showed variation in the timing of mortality, with an 84 % reduction in mortality risk in the above+below removal treatment ($X^2_3 = 200.1$, $P < 0.001$).

Aboveground biomass was also significantly different across the treatments ($F_{3,\,203.6} = 154.57$, $P < 0.001$; Figure 5). The post hoc pairwise comparison revealed that plants in the control treatment were significantly (almost 19-fold) smaller than in the above+below removal treatment ($\underline{X}_1$-$\underline{X}_2 = 0.18$, $P < 0.0001$), but did not differ from the aboveground removal ($\underline{X}_1$-$\underline{X}_2 = 0.14$, $P = 1.00$) or thatch removal ($\underline{X}_1$-$\underline{X}_2 = 2.9$, $P = 1.00$) treatments.

Field experiment II: separating response to above- and belowground competition

In the second year of experiments, above and belowground treatment (control, clipping, belowground competitor removal) had a significant effect on the survival of *D. graveolens* ($X^2_2 = 15.18$, $P < 0.001$; Figure 6a). Clipping marginally significantly increased survival by 32 % over the control ($Z = 1.64$, $P = 0.100$), and belowground competitor removal increased survival by 61 % ($Z = 3.85$, $P < 0.001$). Shading treatment reduced survival by almost 4-fold ($X^2_1 = 46.161$, $P < 0.001$; Figure 6b). In the trenching experiment, controlling belowground competition increased survival ($X^2_1 = 10.37$, $P = 0.0013$; Figure 6c). However, there was no significant effect of the watering and nutrient treatments ($X^2_2 = 2.94$, $P = 0.23$) or the interaction between trenching and water + nutrients ($X^2_2 = 1.11$, $P = 0.58$). We did not harvest biomass because intense heat and drought led to the death of all *D. graveolens* plants before they reached reproduction.

Discussion

Taken together, our results suggest that *D. graveolens* spread is limited by competition and that its association with roadsides reflects reduced competition than an affinity for the roadside soil conditions. Competition strongly reduced *D. graveolens* performance in two greenhouse and multiple field experiments conducted over two years with contrasting weather patterns, lending generality to the findings.

Other studies concur with our conclusion that *D. graveolens* is a poor competitor. Brownsey et al. (2014) found that *D. graveolens* develops shallow roots early, with significant growth starting in May, after most resident species are established. In contrast, *Bromus hordeaceus* initiates root growth by March and shoot growth by April, enabling it to outcompete *D. graveolens* by exploiting winter rains before senescing in summer. This delayed root development limits the ability of *D. graveolens* to compete with graminoids that capitalize on California's winter rains and senesce during the dry summer months. Similarly, Brinkmann (2020) observed that *D. graveolens* struggled to establish in straw mulching experiments when germinating alongside forbs. In our greenhouse experiment, *D. graveolens* aboveground biomass varied depending on competition from European annual grasses, with *F. perennis* producing more biomass than *B. hordeaceus*, potentially due to differences in root-shoot allocation or overall plant size. The rapid growth of annual grasses intensifies competition aboveground for light and belowground for nutrients and water (Coleman and Levine 2007), and their removal could facilitate *D. graveolens* spread into rangelands.

The primary mechanism limiting *D. graveolens* in field experiments appears to be belowground competition, although aboveground competition also plays a lesser role. In year one, removing aboveground biomass or thatch had no effect, while removing above + belowground competition resulted in both higher survival and higher biomass. In year two, removing belowground competition increased survival by 61 %, while removing only aboveground competition increased survival by 32 %. Our trenching and weed cloth treatments showed increased survival with reduced belowground competition, while adding water or nutrients had no effect, pointing to root competition as the primary driver. Finally, shading likely also negatively affected survival, consistent with findings by Brownsey et al. (2014) and Brinkmann (2020); however, disproportionate herbivory in shaded treatments may have contributed to this effect in our study. High mortality in year two, caused by unseasonal heat and intense drought, complicated efforts to fully disentable competition mechanisms.

Soil played an important role in *D. graveolens* performance. In our greenhouse experiment, *D. graveolens* performed worse in engineered fill (a roadside soil proxy) compared to nutrient-rich field topsoil. The reduced growth in engineered fill was likely due to its significantly lower nutrient content, with approximately 11 times less nitrogen, 13 times less carbon, and 22 times less phosphorus than field topsoil (Figure 1). These fertility differences likely drove the observed growth patterns. Seed germination was slightly lower in engineered fill than in field topsoil, contrasting with the species' frequent association with disturbed roadside soils. However, soil type had minimal overall impact on germination. Organic matter in field topsoil may have influenced soil moisture and microbial communities, subtly affecting germination outcomes.

Although *D. graveolens* is commonly found along transportation corridors (Melen et al. 2024), its ability to thrive in nutrient-rich soils suggests the potential to spread beyond these disturbed areas. This pattern mirrors other invasive species spreading from roadsides into adjacent habitats (Gelbard and Belnap 2003; e.g., Sărățeanu et al. 2010; McDougall et al. 2018). Across multiple experiments over two years, we consistently found evidence that *D. graveolens* is a poor competitor, primarily limited by belowground root competition. We also found that the effect of competition on *D. graveolens* in the greenhouse was much stronger in the nutrient-rich field soil than in the construction soil. Therefore, while *D. graveolens* can thrive in nutrient-rich soils, its distribution appears to be constrained to highly disturbed roadside soils by competition. Gioria et al. (2023) highlight that invasible ecosystems often include disturbance regimes, and both natural and anthropogenic disturbance creates space for colonization. Furthermore, proximity to roadways may increase NOx deposition from vehicle emissions (Bettez et al. 2013) or increase moisture from surface runoff (Trombulak and Frissell 2000), making these soils even more amenable to ruderal species.

Spread is opportunistic for *D. graveolens*, often exploiting disturbed or patchy areas. However, competition from resident plant communities restricts its range. Land managers should prioritize dense vegetation cover near spread vectors (e.g., transportation corridors, footpaths, riparian zones, construction areas) to protect sensitive habitats. Perennials and early-germinating annuals with dense canopies and root systems may provide strong early-season competition, limiting *D. graveolens* germination and growth before it bolts. Management practices that reduce graminoid competition in the spring may facilitate *D. graveolens* establishment by minimizing competition. Additionally, disturbances from cattle, mowing, and agricultural equipment create bare soil, likely increasing *D. graveolens* invasion risk.

Figures

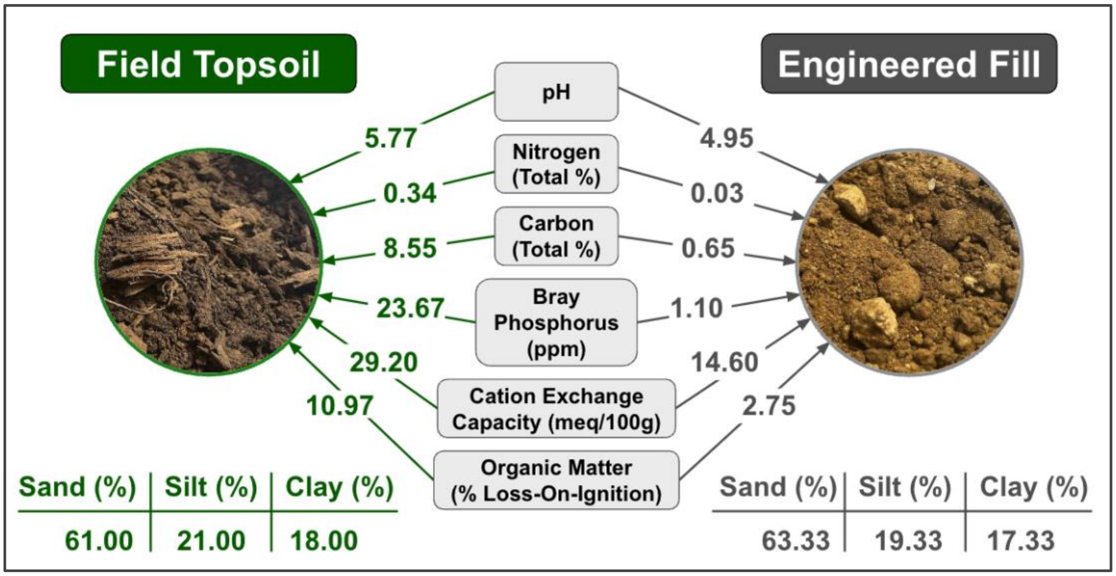


Figure 1. Soils used in germination and greenhouse experiments were collected from a central soil storage location at UC Santa Cruz. Field topsoil was collected from a woodland site on campus, and engineered fill was taken from a campus construction project. The soils were exposed to outdoor conditions, which allowed microbial communities to persist. We subsampled each soil type for our experiments. Samples were sent to UC Davis Analytical Laboratory, Davis, CA for analysis.

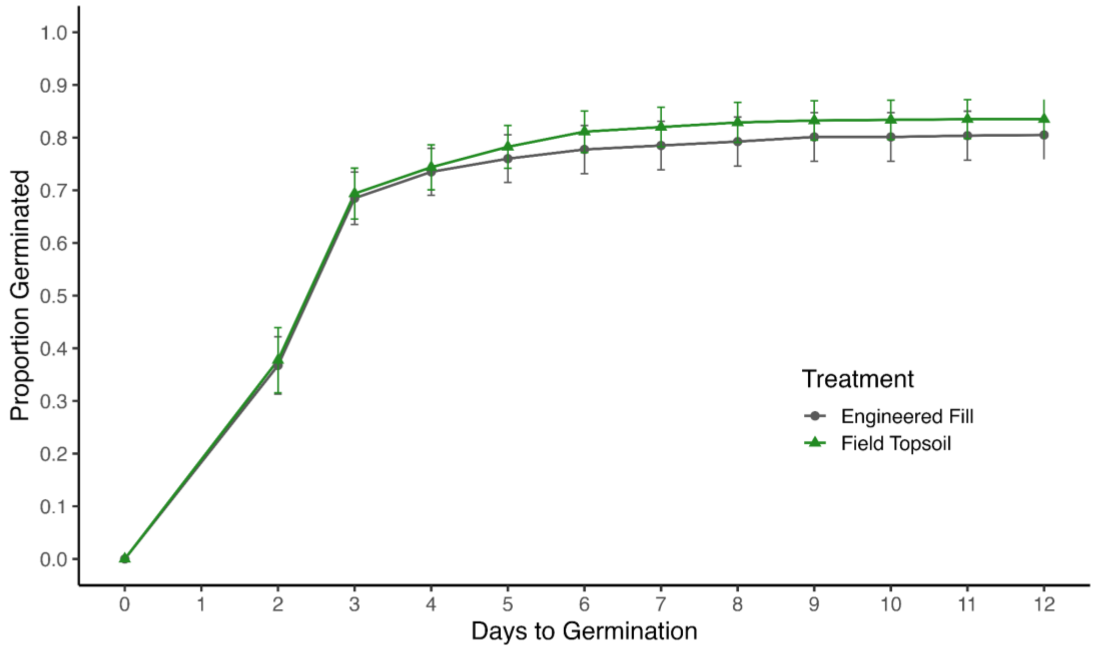


Figure 2: Cumulative proportion germinating per day of *D. graveolens* seeds germinated on engineered fill (circles) and field topsoil (triangles). Values shown are means ± 1 SE, showing variance across 16 seed sources (sites).

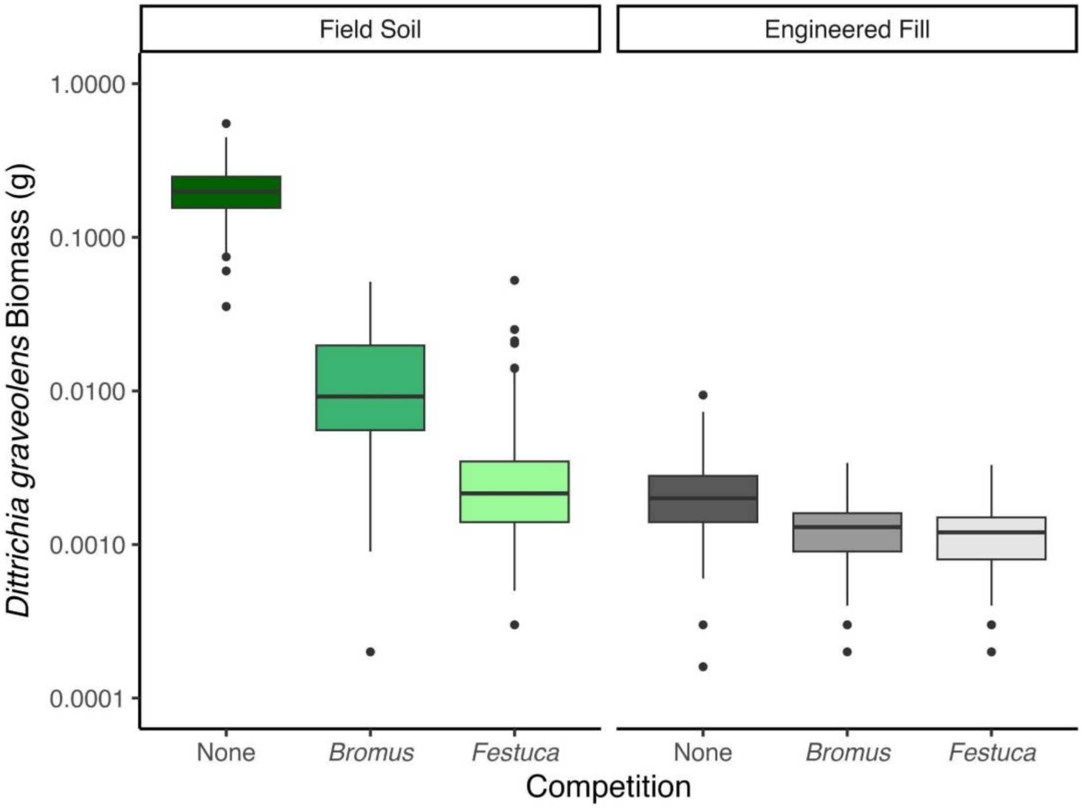


Figure 3. Biomass (g) of *D. graveolens* grown in a greenhouse experiment alone or with each of two grass competitors (*Bromus hordeaceus* and *Festuca perennis*), planted into field topsoil (a) or engineered fill (b). Boxes correspond to the median, first and third quartiles, and whiskers extend to the furthest value within 1.5 × the inter-quartile range. Note the log scale.

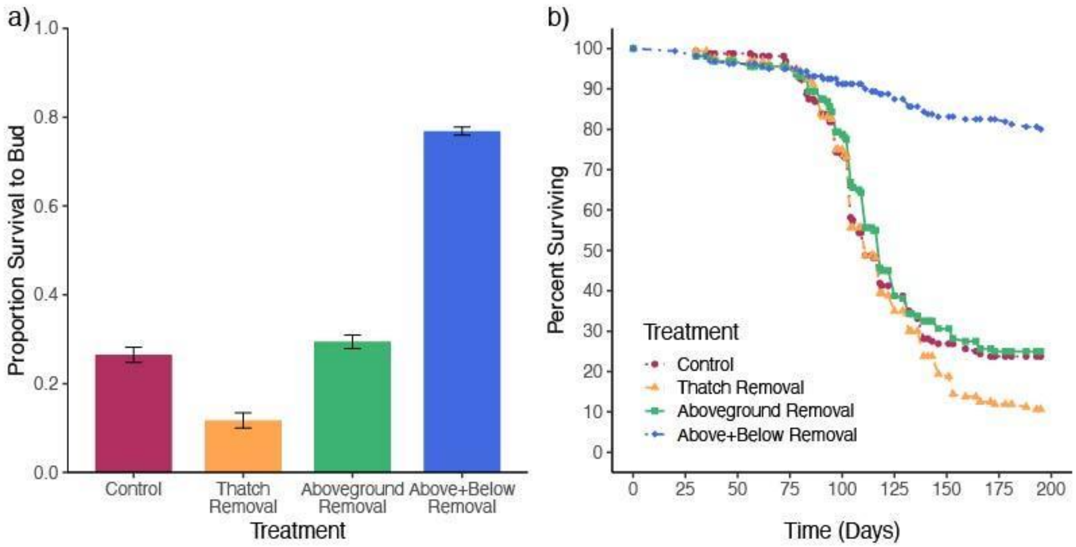


Figure 4. (a) Proportion of *D. graveolens* that survived to reproduction by treatment. Plants were transplanted as seedlings into control plots with undisturbed grassland, plots from which dry thatch was removed, plots where aboveground biomass was clipped, and plots where both above- and belowground biomass of all plant neighbors was removed. (b) Survival of *D. graveolens* over time for plants transplanted as seedlings into control plots with undisturbed grassland (circles), plots from which dry thatch was removed (triangles), plots where aboveground biomass was clipped (squares), and plots where both above- and belowground biomass of all plant neighbors was removed (diamonds).

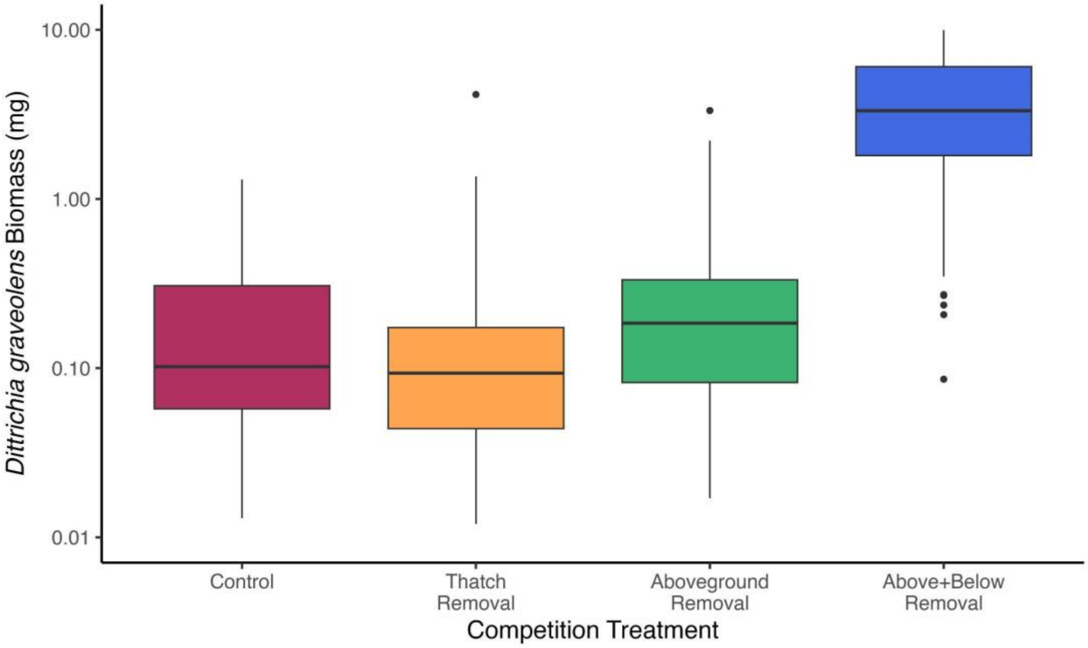


Figure 5. Differences in *D. graveolens* aboveground biomass for plants transplanted as seedlings into control plots with undisturbed grassland, plots from which dry thatch was removed, plots where aboveground biomass was clipped, and plots where both above- and belowground biomass of all plant neighbors was removed. Boxes correspond to the median, first and third quartiles, and whiskers extend to the furthest value within 1.5 × the interquartile range.

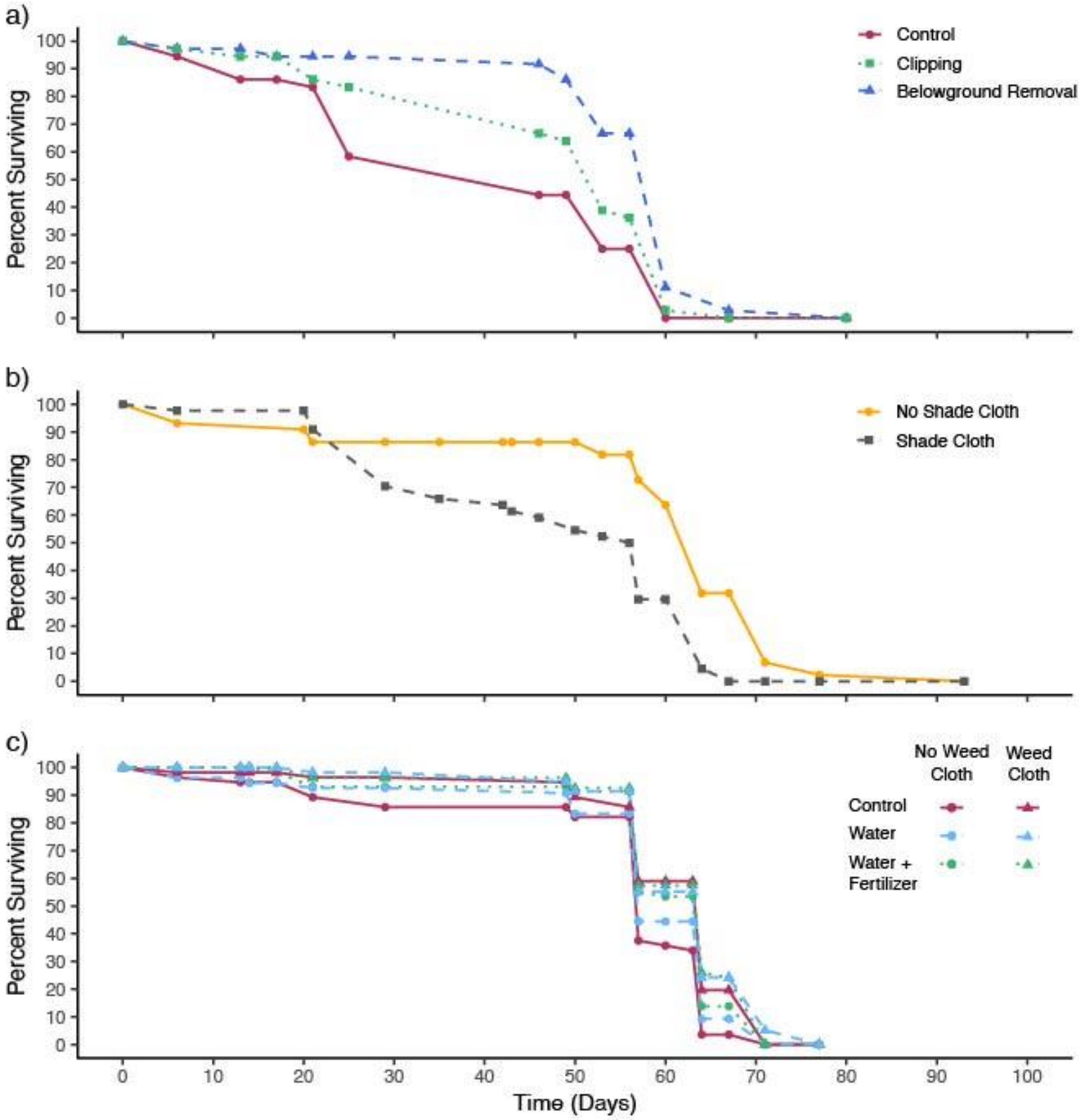


Figure 6. Survival of plants transplanted in 2nd-year experiment as seedlings into field plots. (a) Control plots with undisturbed grassland (circles), plots where belowground competitors were removed (triangles), and plots where aboveground biomass was clipped (squares). (b) Plots with no shade cloth (circles) and with shade cloth (squares). (c) Plots with three treatments (control, water, and water + fertilizer) and two competitive levels. Open triangles show plots where we trenched and then lined with weed cloth preventing root competition, while closed circles have no trenching or weed cloth.

Chapter 3

Seed bank behavior of an invasive annual Aster (*Dittrichia graveolens*)

Abstract

Seed dormancy and germination dynamics are critical for understanding the persistence and dynamics of invasive plant populations. We investigated ecological and evolutionary factors influencing seed dormancy and germination of a noxious plant in California, *Dittrichia graveolens* (stinkwort). To explore evolution with invasive spread, we compared four long-established core populations from the range of initial invasion with four recently established populations at the invasion front in the Sierra Nevada Mountain foothills. We used three experimental approaches: (1) a mesocosm study to assess germination of surface-sown seeds under outdoor conditions over three years, (2) a burial depth experiment to evaluate germination at six soil depths, and (3) a field dormancy experiment tracking seed survival in buried mesh bags over three years to assess seed dormancy. Overall, we found no significant difference in dormancy or germination between core and invading front populations over three years. Most seeds (83.9%) germinated in the first year, with only a small percentage germinating in later years. Burial depth significantly reduced germination, showing a sharp decline below 1cm. Finally, viability persisted through the first (65.9 %) and second (59.9 %) years in the field dormancy study, but dropped sharply by year three. These findings suggest that *D. graveolens* dormancy and germination behavior have not evolved on the spreading edge of the invasion front. They respond strongly to rainfall seasonality and burial, with most seeds germinating with the first moisture cues unless buried. Insights from this study highlight potential management strategies to limit *D. graveolens* spread in California, including control after the first rains and continued monitoring for more than three years.

Introduction

Seed banks are a critical phase of the plant life cycle and affect population dynamics and the maintenance of plant diversity (Gioria et al. 2012; Funk et al. 2020). Dormancy is a way for seeds to bet-hedge in variable environments (Baker and Stebbins 1965) to germinate under the right conditions (Fenner and Thompson 2005). Much of our understanding of seed viability and germination behavior comes from the agricultural sector (e.g., Dezfuli et al. 2008; Toscano et al. 2017; Hmissi et al. 2023). Despite its ecological and evolutionary importance, long-term seed bank behavior is often insufficiently studied because of the complexity of tracking seed fates over multiple years.

Long-lived seed banks are a key feature of many weedy species (Gioria et al. 2012), serving as a persistent source of invasive plants (Roberts and Boddrell 1984; Fletcher et al. 2015; Moravcová et al. 2018). For land managers, understanding seed bank dynamics is critical for determining how long dormant seeds may germinate and planning effective weed control efforts (Matzek et al. 2015; Funk et al. 2020). The dormancy rate of an invasive population directly influences how long managers must revisit a site to ensure successful removal, helping optimize time and funding allocation.

As populations spread across a landscape, they encounter and colonize new, often unoccupied habitats, a process that repeats across successive generations (Andow et al. 1990). This expansion creates opportunities for natural selection to favor traits that enhance dispersal and increase the population's ability to establish in these "empty" habitats (Phillips et al. 2010). Over time, selection pressures may optimize traits contributing to the density-independent population growth rate (r), enabling populations to spread more effectively across a fragmented dynamic landscape (Andow et al. 1990; Phillips et al. 2010; Perkins et al. 2013). For example, due to the selection for a shorter generation time, we predict dormancy will decline in rapidly spreading populations, resulting in higher rates of germination in the first year. Lower seed survival in invading front populations could also be caused by inbreeding depression, and loss of resistance to pathogens could occur through founder effects or genetic drift.

In California, *D. graveolens* was first observed in 1984 in Santa Clara County (Preston 1997), yet it is also actively spreading into new areas along the invading front in the northern and eastern edges of its invaded range (Figure 1). As a ruderal species, *D. graveolens* is most often found along roadsides (Melen et al. 2024) and is moving along transportation routes into the Sierra Nevada Mountain foothills. With core populations potentially experiencing different selective pressures than invading front populations, this system presents a unique opportunity to study the seed bank dynamics of an invasive plant species.

Previous research on *D. graveolens* seed behavior demonstrated that it has high germination (between 62 and 80 %) at a wide range of temperatures (12 to 34 °C) and germinates under various light conditions (9, 27, 50, and 100 % sunlight), indicating that primary dormancy is unlikely in this species (Brownsey et al. 2013b). In a seedling emergence study, Brownsey et al. (2013b) reported a 30 % germination rate over two years (2010 and 2011) for *D. graveolens* seed placed on sand in a field setting. Seed viability for this species is between 80 and 95 % (Brownsey et al. 2013b) at the time of dispersal, so this lower germination rate is uncommon for mature *D. graveolens* seeds and may not represent the seed bank capacity but does suggest a lack of primary dormancy. In addition, in the field, soil deposition occurs with time, accumulating on top of the seed, potentially changing the seed behavior. To adequately manage this invasive species, a more robust study is needed to assess how *D. graveolens* responds to field conditions where soil is allowed to naturally accrue over the top of the seed, in addition to testing germination for over two years.

Here, we assess *D. graveolens* seed germination, dormancy, and survival. We implemented experiments designed to assess complementary aspects of seed behavior, focusing on differences between core and invading front ranges. First, we placed seeds on the surface in an outdoor mesocosm to study germination over three years. Second, we studied the response of seed germination to sowing depth in a lab experiment. Finally, we buried seeds in the field in mesh bags to assess differences in seed dormancy and viability over three years. We predicted that invading front populations would show reduced dormancy and increased germination, and that seed germination patterns would be strongly affected by sowing depth.

Methods and Materials

Seed collection

We collected seeds from wild populations of *D. graveolens* in November and December 2018 from eight sites in California (Table S1; Figure 1): four from the core range of Santa Clara County, and four from the invasion front range, along four major transportation corridors leading into the Sierra Nevada Mountain foothills. We generated a list of potential locations in collaboration with local resource managers and using online sources (e.g., CalFlora and Google Maps), and drove and/or walked from those locations until we found the nearest roadside *D. graveolens* population. At each site, we collected seeds from 10 randomly selected plants (80 maternal families total).

To control for maternal effects, we grew an extra generation in a biosecurity greenhouse at the University of California Santa Cruz. In February 2019, we germinated seeds on filter paper in Petri dishes. We visually inspected seeds beforehand to ensure that only fully developed seeds were used for both experiments. Petri dishes were sealed with Parafilm M™ and placed in a randomized block design in an incubation chamber with a daytime temperature of 23 °C from 0900 to 0100 h and a nighttime temperature of 19 °C from 0100 to 0900 h. Once seedlings emerged, we transplanted them into potting media (ProMix® HP® Mycorrhizae™) in SC10 conetainer cells (3.81 cm diameter, 20.96 depth, 164 ml volume). Source populations were randomized with one offspring per maternal family represented in each of the two blocks.

To keep lineages pure, we bagged each plant with light-permeable mesh prior to budding; *D. graveolens* readily self-pollinates. In March 2020, the seeds produced by these plants were used to grow a second generation using the same methods and growing conditions. From December 2020 to January 2021, seeds were harvested from these plants for use in the experiments described below.

Mesocosm germination

To measure rates of germination over time, we sowed loose seeds onto the soil surface in ambient temperature and precipitation conditions on 15 October 2021. For biosafety reasons, this experiment was done in a mesocosm at the Coastal Science Campus at UCSC in an outdoor space adjacent to the greenhouses. Using two metal raised beds (0.9 × 1.2 m), we sunk 240 open-bottomed Anderson Band pots (6 × 9.5 cm) into 5 cm of filtered engineered fill soil which originated from the Kresge construction site at UCSC, leaving 9 cm of head space. We used engineered fill to mimic soil conditions in *D. graveolens*' typical roadside habitat. In each pot, we placed 200 seeds on the soil surface and then covered the raised beds with bird netting to prevent animal access but allow natural precipitation. The same 80 maternal families were used in this study, with three replicates in a randomized block design.

We checked the pots weekly for germination from October through March (typical California rainy season) and monitored occasionally throughout the rest of the year to attend to weeds and other maintenance needs. Immediately upon germination, seedlings were clipped at the soil level to remove them from the experiment and eliminate light competition from the pot. We used precipitation data from the Western Regional Climate Center (2024) weather station on-site to assess the start and end of each rainy season.

Burial depth

To understand how *D. graveolens* seeds respond to being buried, we conducted an experiment planting sterilized seeds at six different depths (0 mm, 1 mm, 5 mm, 10 mm, 15 mm, and 20 mm) in 50-mL Falcon centrifuge tubes (Corning, Tewksbury, Massachusetts, USA; 3.2 cm width × 11.7 cm height) filled 60 % with moist horticulture sand. We saturated the sand in each tube with deionized water and then used a paper towel to wick away water above the sand surface. In each of 10 replicate tubes per treatment, we placed 5 sterilized *D. graveolens* seeds from a population collected from Lexington Reservoir, California (10 replicates; 300 seeds total). We closed the lids on the Falcon tubes to maintain moisture and placed them in a randomized order in racks on a north-facing windowsill. We recorded germination weekly for eight weeks until no germination was recorded for seven days. After the experiment, the remaining seeds were exhumed for post-mortem analysis and treated with a Triphenyl tetrazolium chloride (TTC) solution. We bisected the seed along the longitudinal axis through the midsection of the embryo and then bathed it for 2.5 hours in a 0.25% Triphenyl tetrazolium chloride (TTC) solution. Afterward, seed halves were inspected for red or pink tissue using a microscope to assess whether any seeds were alive. None of the ungerminated seeds were viable.

Field dormancy

To measure dormancy rates and survival of dormant seeds in soil, we buried seeds in the field. A total of 30 seeds from each of 80 lineages were used. These lineages consisted of 10 maternal families from 8 field sites, grown for one generation in the greenhouse. Seeds were sown into light-permeable mesh bag pockets, arranged in a randomized block design. Each mesh sleeve contained 8 pockets, and 10 sleeves formed a block, with one maternal family per population represented in each sleeve. This ensured that all 80 lineages were included across the 10 sleeves. The three-year experiment was replicated at two sites, resulting in 14,400 seeds buried overall (30 seeds × 80 families × 3 years × 2 sites).

We installed the seed bags at two grassland sites at Blue Oak Ranch Reserve in California. Seed bags were staked into the ground and covered with about 2 cm of local soil. To prevent soil disturbance and herbivory, we placed a 120-cm high plastic plant protection fence around both sites. Each year in late spring, after the last winter rains and the soil starting to dry, we collected a block of seed bags from each site to test seeds for viability (Y1: June 18, 2022; Y2: May 10, 2023; Y3: May 25, 2024). In the lab, seed bags were opened, and seeds were sorted from soil and plant debris and placed in Petri dishes. We then counted the total number of seeds in the dish and sorted the seeds into categories based on a visual inspection and with a microscope. If seeds were firm, full, and had no visible damage, they were counted as presumed dormant (and alive), and they were transferred onto filter paper in a Petri dish. Seeds that were physically damaged (e.g., holes, broken in parts, etc.) were counted as dead, whereas seeds that were empty (e.g., seed coat split open but no seed within) were counted as germinated. In the first year only, we found two dried *D. graveolens* seedlings that were also counted as germinated.

Each Petri dish with presumed dormant seeds was moistened with 2.5 ml of a 250 mg/L gibberellic acid (GA3) solution and sealed with Parafilm M™. Dishes were placed in a randomized block design under shade cloth on outdoor tables with ambient average daily temperature highs of 16.5 °C and lows of 11.7 °C (Western Regional Climate Center weather station for Younger Lagoon). Each week seeds were checked for germination, and germinated seeds were removed. After seven days of no germination, the remaining seeds were tested for viability using a TTC solution (as above). Only one seed was alive after TTC addition, indicating that essentially all the live seeds were revealed by the GA3 treatment.

Data analysis

We used R version 4.3.1 (2023-06-16; R Core Team 2023) for all statistical analyses. We used Sankey diagrams to visualize the fates of seeds over time (Hernandez et al. 2020).

Mesocosm germination

We compared proportion of germinated seeds with a generalized linear mixed model fitted by a maximum likelihood (Laplace Approximation) using a binomial family with a logit link function (lme4 package; Bates et al. 2015). The fixed effect was range (core or invading front), and random effects were family nested within population. For each year, we evaluated the effect of range on germination using Type II Wald Chi-square tests (car package; Fox and Weisberg 2019).

Burial depth

We compared proportion of germinated seeds with a generalized linear mixed model using a binomial family with a logit link function; the fixed effect was burial depth, and the random effect was replicate (glmmTMB package; Brooks et al. 2017). We evaluated the effect of burial depth on germination using Type II likelihood ratio Chi-square tests (car package; Fox and Weisberg 2019). We conducted post-hoc pairwise comparisons using estimated marginal means, comparing them using z-ratios, and a Bonferroni adjustment for multiple comparisons (emmeans package; Lenth 2024).

Field dormancy

We compared proportion of alive, dormant seeds with a generalized linear mixed model fitted by maximum likelihood (Laplace Approximation) using a binomial family with a logit link function (lme4 package; Bates et al. 2015). The fixed effect was range (core or invading front), and random effects were replicate, family, and population. For each year, we evaluated the effect of range on dormancy using Type II Wald Chi-square tests (car package; Fox and Weisberg 2019).

Results

Mesocosm germination

We found no significant effect in germination between the core and invading front ranges for year one ($X^2 = 1.59$, DF = 1, P = 0.21; Figure 2), year two ($X^2 = 0.45$, DF = 1, P = 0.50), or year three ($X^2 = 1.12$, DF = 1, P = 0.29). Overall, 83.9 % of the seeds germinated in the first year time with rainfall, with an additional 1.3 % in year two and 0.04 % in year three (Figure 3).

Burial depth

Germination was greatest on the surface and declined with increasing burial to 10 mm, after which it leveled off ($X^2 = 53.95$, DF = 5, $P < 0.0001$; Figure 4). Seeds at 0 mm had the highest germination (80 % on average), while burial at 10 mm or deeper reduced germination to less than 10 %. Post hoc comparisons confirmed significantly higher germination at 0 mm compared to 10 mm, 15 mm, and 20 mm depths, with overlapping patterns at intermediate depths.

Field dormancy

Likewise, there was no significant difference between seeds originating from the core and invading front ranges for dormancy in any year (X^2 = 0.44-1.15, DF = 1, P = 0.28-0.51; Figure 5). Overall, 65.9 % of the seeds were dormant after one year, 59.9 % after two years, and only 29.6 % after three years (Figure 6).

Discussion

Most *D. graveolens* germination (83.9 % overall) for seeds on the soil surface in our mesocosm occurred in response to the first rains of the first year, with smaller germination events following rainfall events in years two and three. By the end of three years, only 0.04 % of the seeds overall were ungerminated. Brownsey et al. (2013b) also observed this pattern of high first-year germination in their two-year experiment, suggesting that this pattern is consistent across various *D. graveolens* populations, rainfall years, and locations in California. The dominance of a single germination cohort provides useful information for management, suggesting that land managers should be able to focus seedling control efforts after major rainfall events early in the season. In contrast to our findings, however, we have spoken to some land managers who have observed multiple seedling cohorts throughout the growing season all the way into June. Because this behavior was not seen in experiments in either coastal or central California, it may be particular to certain years, microsites, or management regimes. A broad survey of germination phenology would be useful to investigate these remaining questions.

Contrary to our expectations and prior research on other invaders, we found no significant difference in germination and dormancy between core and invading front *D. graveolens* populations. For example, Tabassum and Leishman (2018) found that *Gladiolus gueinzii* germination was slower in core populations than in the invading front range of invasion. However, Gong et al. (2022) found faster germination in core *Ageratina adenophora* populations compared to invading front populations, noting that selection pressures may have shifted energy investment to the core populations as a way to offset herbivory pressures. Several possible explanations may explain why we do not see a difference in germination for *D. graveolens*. First, selection may have acted on germination, but *D. graveolens* populations may have lacked sufficient genetic variation to respond to selection, possibly due to founder effects associated with the introduction (Dlugosch and Parker 2008b; Dlugosch et al. 2015) or relatively short time period since initial spread. Germination rates were high overall in the mesocosm (83.9 %), so there was generally low dormancy. On the other hand, the number of persistent seeds varied greatly among seed bags after three years, but that variability was not explained by the population type (core and invading front). Additionally, *D. graveolens* was identified in the early 1980s in the core range (County of Santa Clara) and has spread into the Sierra Nevada Mountain foothills only since the early 2000s (Table S1), which may not be enough time for dormancy traits to evolve. Finally, the benefits of short-term dormancy may remain even in the invading front populations, countering selection against long-term dormancy.

In our seed burial experiment, seeds at the soil surface germinated at around 80 %, as found in our mesocosm experiment and by Brownsey et al. (2013b); however, adding even a few millimeters of sand dramatically decreased germination. Light rarely penetrates deeper than a few millimeters below the soil surface (Bliss and Smith 1985); however, sand particles may extend that range by a few millimeters (Fenner and Thompson 2005, p. 116). For light-requiring species, such as those from the family Asteraceae, depth greatly affects germination, and small-seeded species often rely on light cues to assess their depth in the soil (Fenner and Thompson 2005, p. 117). In a greenhouse experiment using shade cloth, Brownsey et al. (2013b) found that *D. graveolens* had 50 to 75% germination success for all light environments under four different light levels (9, 27, 50, and 100 % sunlight), indicating a light requirement for germination. Presotto et al. (2014) found that light stimulation increased germination of the invasive wild-type *Helianthus annuus* and suggested that this adaptation may offer an advantage to species growing in disturbed soils, cueing germination to occur when soil is churned and seeds are brought to the surface. In fact, for species with long-lived seedbanks, tilling to bring seeds to the surface (followed by seedling control) is sometimes proposed as a management strategy. However, for a species with a shorter-lived seed bank, such as *D. graveolens*, burial to suppress germination may be a better strategy. Our findings suggest that covering the surface with at least 2 cm of soil post-dispersal and pre-rain events could nearly eliminate germination but would require further monitoring.

Our field dormancy experiment showed viability persisting through year one (65.9 %) and year two (59.9 %) but dropping sharply in year three (29.6 %). This classifies *D. graveolens* as a species with a short-term persistent seed bank (Fenner and Thompson 2005; Hu et al. 2017). Under agricultural field conditions, Brownsey et al. (2013b) observed germination patterns consistent with this trend, noting that the *D. graveolens* seedlings continued to emerge for at least two years in agricultural field conditions, although seed viability was not directly assessed.

Seed dormancy is a bet-hedging strategy that is one of Baker's (Baker and Stebbins 1965, p. 166) original 'ideal weed' traits. Dormancy allows a seed to germinate in the correct place and at the right time (Fenner and Thompson 2005, p. 97), but at the cost of not reproducing immediately. Other weedy invasive species show a similar pattern to *D. graveolens*, with short-term persistent seed banks (e.g., Roberts and Boddrell 1984; Presotto et al. 2014; Hu et al. 2017; Moravcová et al. 2018; Skálová et al.

2019). Although long seed dormancy is often associated with successful invaders (e.g., Bossard 1993; Passos et al. 2017), for a spreading population, such as *D. graveolens*, in theory, dormancy would be disfavored to promote traits to colonize empty space.

Since our three-year study shows an almost 30 % viability rate, the tail-end of the seed viability curve has not fully been assessed, and additional data would be valuable beyond this timeframe. Viability variability was observed in core and invading front populations over all three years, despite the average viability rate declining. Even with lower average viability, just a few plants reaching reproduction can maintain a persistent resident population and seed bank. Management implications suggest that more than a three-year control period might be necessary to manage *D. graveolens* populations, as some still retain high viability in year three. In year three, core populations had a maximum viability of 93.3 %, and invading front populations had a maximum viability of 73.3 %, suggesting that certain populations will require more targeted management in areas vulnerable to invasion and around previous stands of *D. graveolens* stands.

Conclusion

D. graveolens seed bank germination and dormancy showed little differentiation between populations in the core and invading front ranges of California. The high initial germination rates, followed by a dramatic decline after the first year suggest a life history strategy that favors the early establishment of the next generation over bet-hedging with a persistent seed bank. The steep decline in germination with increasing burial depth suggests that *D. graveolens* is photoblastic, which can influence its establishment in novel environments on the leading invading front. Our findings also suggest that shorter dormancy has not been selected for in invading front populations but is instead ubiquitous throughout the core and invading front populations sampled in this study. This strategy of favoring immediate colonization over longevity, a trait common in other ruderal species (Roberts and Boddrell 1984), likely contributes to its rapid spread in California. Based on our results, we recommend management strategies include monitoring and control after the first rains, as well as strategic burial to suppress germination.

Figures

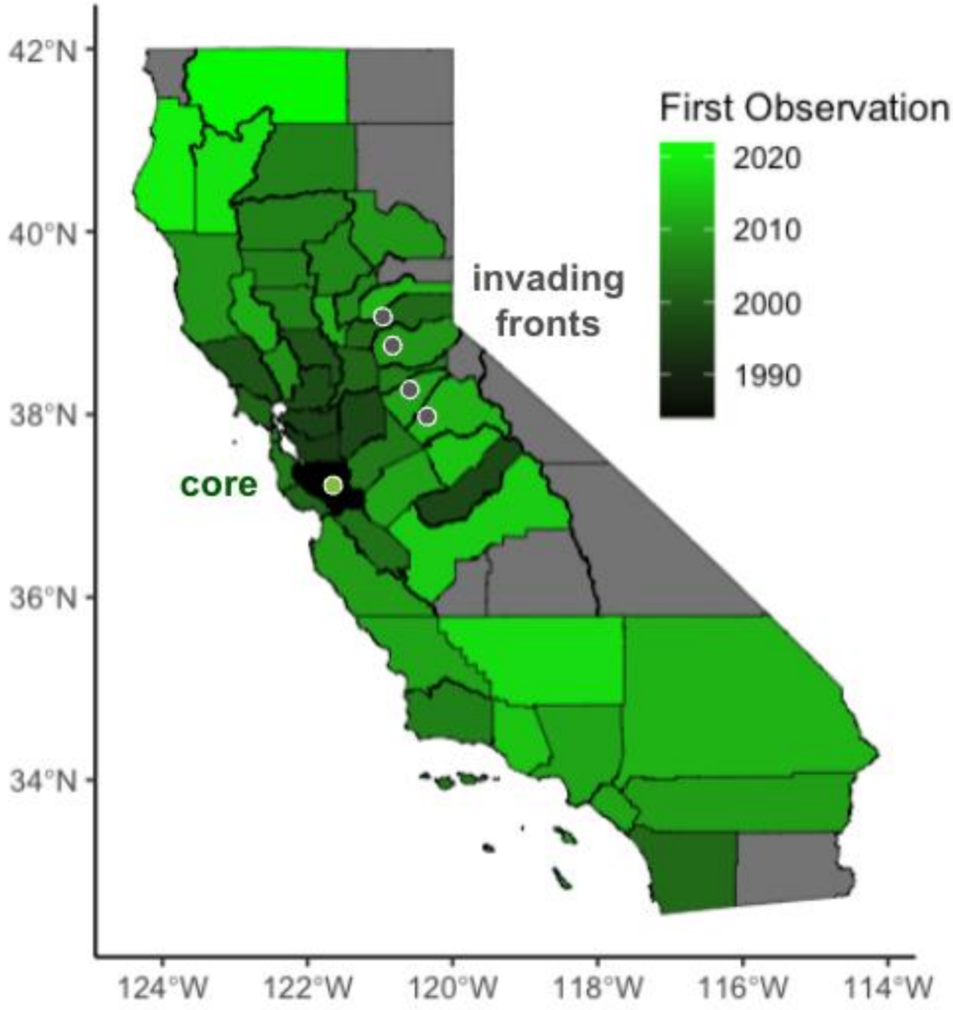


Figure 1. Map of California counties showing the year of first observation. Since its introduction in the County of Santa Clara (black polygon) in 1984, *D. graveolens* has been observed in 83 % of the counties. Counties in light gray have no data. The green dot shows core population county and the grey dots show invading front populations used in this study (see Table S1 for location details). Data retrieved from Calflora (2024), map created in R (Walker 2024).

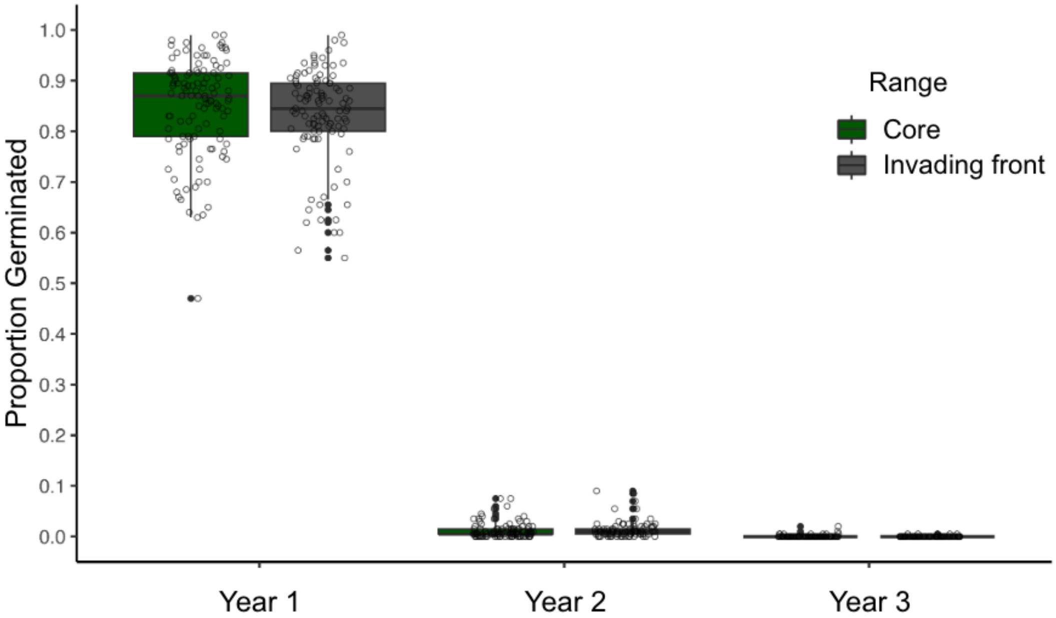


Figure 2. Proportion of germinated *D. graveolens* seeds from core and invading front ranges after each year of germination in an outdoor mesocosm at UC Santa Cruz greenhouses. Seeds were collected from the County of Santa Clara (core range) and along four major transportation corridors leading into the Sierra Nevada Mountain foothills (invading front). In the first year, 83.9 % of the seeds germinated, with greatly reduced germination in years two and three. Boxes correspond to the median, first and third quartiles, and whiskers extend to the furthest value within 1.5 × the inter-quartile range.

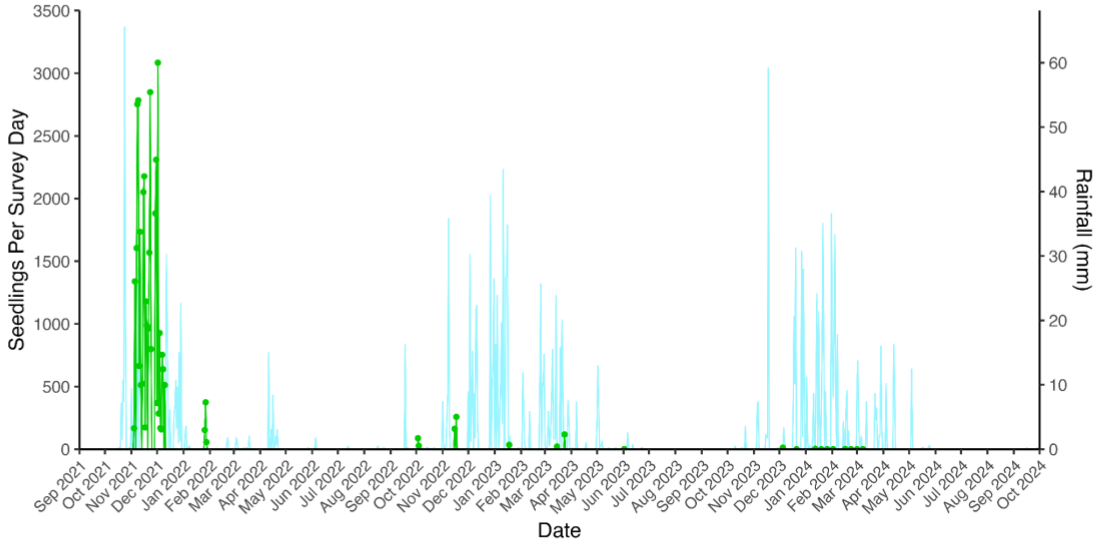


Figure 3. Number of *D. graveolens* seedlings per survey day (green circles) and total daily rainfall (mm) (blue lines). Seeds were placed on the surface on 15 October 2021. Overall, 83.9 % of the seeds germinated in the first year, with an additional 1.3 % in year two and 0.04 % in year three.

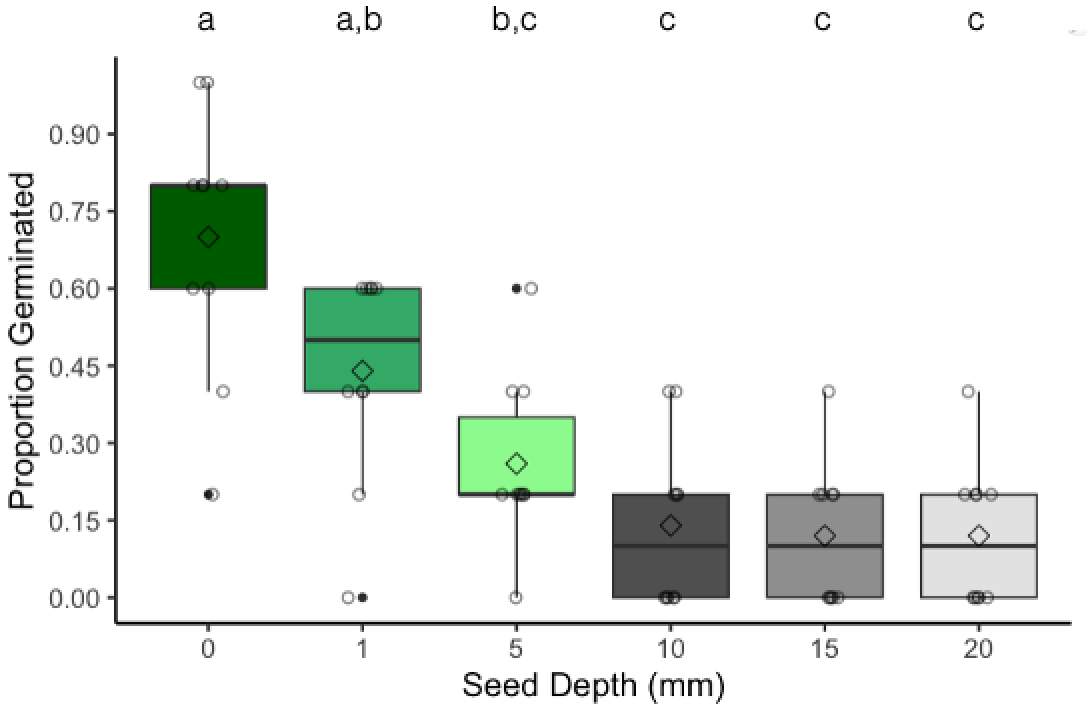


Figure 4. Proportion of germinated *D. graveolens* seeds at six different seed depths (0, 1, 5, 10, 15, and 20 mm below the surface). Boxes correspond to the median, first and third quartiles, and whiskers extend to the furthest value within 1.5 × the inter-quartile range. Outliers are represented as closed circles. Lowercase letters indicate significant differences in a pairwise post hoc test ($P < 0.05$), and open diamonds indicate means.

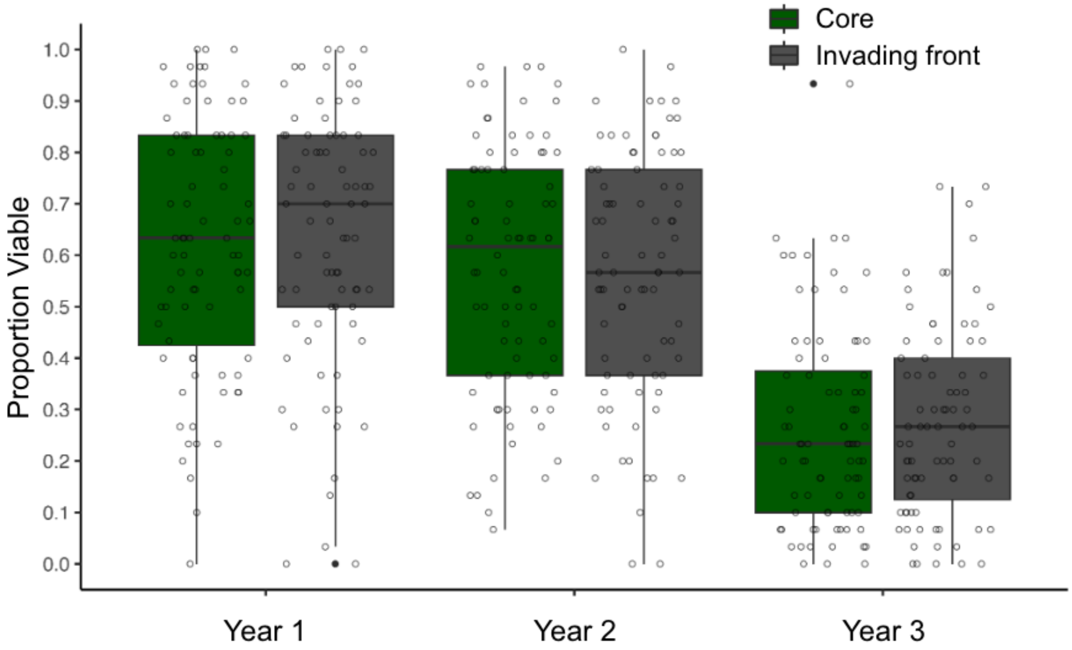
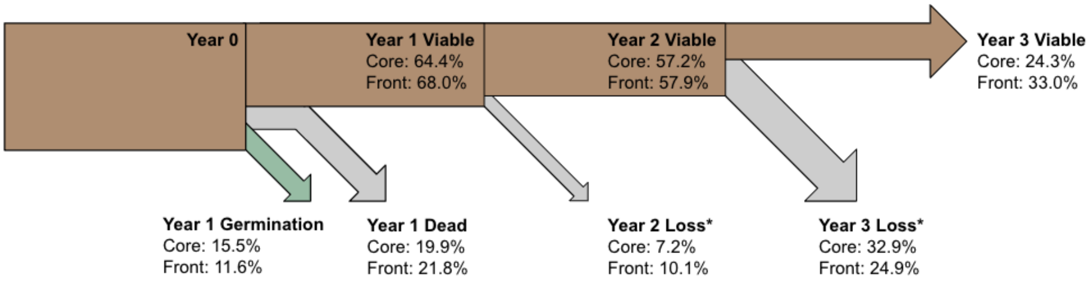


Figure 5. Proportion of viable *D. graveolens* seeds from core and invading front ranges after each year of burial in seed bags at Blue Oak Ranch Reserve. Seeds were collected from the County of Santa Clara (core range) and along four major transportation corridors leading into the Sierra Nevada Mountain foothills (invading front). Boxes correspond to the median, first and third quartiles, and whiskers extend to the furthest value within 1.5 × the inter-quartile range.



Core = populations from Santa Clara County
Front = invading front populations in Sierra Nevada Foothills

*Does not distinguish between possible germination and death

Figure 6. Sankey diagram for fates of buried seeds of *D. graveolens*. Estimates are averages for four populations in the core of the invasion (core) and four populations at the expanding invasion front (invading front). Each year, seed bags are removed and the remaining seeds are tested for viability. In year one, we could estimate germination (green arrow), death (gray arrow), and viable seed (brown arrow). In years 2 and 3, the gray arrows show seeds lost from the seed bank to seed death, disease, and predation, as well as germination. Seeds that remain viable in the seed bank are part of the brown arrow.

Conclusion

We have explored how ecological and evolutionary drivers influence the biological invasion of *D. graveolens* in California. With increasing observations of *D. graveolens* growing along roadsides and mounting evidence suggesting an ability to spread to nearby plant communities, we examined whether *D. graveolens* populations growing in habitats adjacent to roadsides have evolved competitive ability compared to roadside populations. We have also assessed whether *D. graveolens* has an affinity for abiotic roadside soil conditions or seeks it as a refuge from more competitive, nutrient-rich environments. Finally, we have asked critical questions about the seed bank of this invasive species and whether seed banks from populations on the leading front of the invasion behave differently from those from older populations near the core range of the invasion. We found (1) no evidence of adaptive differentiation for *D. graveolens* in non-roadside habitats, (2) competition with resident plants restricts distribution to roadsides rather than soil preference, and (3) dormancy and germination traits are similar across core and edge populations, and seed burial can be a beneficial management strategy. Overall, we have found a roadside invader that opportunistically invades nearby environments when competition is reduced, and has a seed bank with a short-term dormancy.

We combined lab, greenhouse, and field experiments to address our research questions from multiple perspectives, creating a deeper understanding of the invasion dynamics of *D. graveolens*. Our multi-faceted approach integrated the precision of controlled lab and greenhouse experiments with the contextual insights of field experiments, revealing aspects that may have been overlooked with a single method. For example, greenhouse experiments isolated competition effects on *D. graveolens* growth, while field studies placed these findings within the complexities of a natural system, such as intense heat causing high mortality, overriding other influences. Similarly, lab-based germination trials provided precise insights into seed dormancy, complementing the broader patterns observed in our seed bank greenhouse and field experiments. For studies of evolutionary divergence, lab experiments controlled for extraneous variation, making them ideal for detecting genetic differences. However, without the field context, it would be difficult to assess how natural selection might act on these genotypes. By combining approaches, we enhanced the validity of our findings and developed a more comprehensive understanding of the ecological and evolutionary factors driving invasion.

Finding a lack of adaptive differentiation across populations implies that in the 40+ years that *D. graveolens* has been present in the County of Santa Clara, this species has yet to adapt locally and evolve traits that make it a better invader. Our results suggest that even strong selection in less disturbed, more competitive environments may not result in the rapid evolution of invasive ability as plants spread away from roads. Opposing selection pressures on roads and away from roads, with gene flow linking close populations, may represent an insurmountable barrier to the evolution of increased competitive ability in invasive plants. To the extent that these barriers to adaptation persist over time, evolution will not represent an urgent threat to management activities or risk assessments. Another possible explanation is that expanding populations have limited genetic variation for these traits. The introduction of *D. graveolens* to California may have involved significantly reduced genetic diversity due to a strong founder effect or repeated bottlenecks. Such effects are common during invasions and often reduce variation in invasive species (reviewed in Dlugosch and Parker 2008a; Dlugosch et al. 2015). Additionally, McEvoy et al. (2023) suggest that *D. graveolens* has a history of selfing, which can further limit genetic variation and contribute to the lack of differentiation observed across California populations.

While *D. graveolens* grows well in different soil types, its spread is limited by competition from resident plants, suggesting that increasing competitive pressure and reducing disturbed, bare ground are two management strategies to curb its invasion. Managers can identify vectors of spread, such as nearby roadsides or soil disturbances, and focus resources to build a robust biotic barrier to inhibit new invasions. In conjunction, targeting areas with existing seed banks to practice early detection and rapid response (EDRR) methods for more than three years is a promising way to limit germination, and strategic burial may prevent new seedling cohorts from emerging without inducing secondary dormancy. Our seed bank experiment is continuing, and after 5 years we will have more complete information about the longer-term viability of *D. graveolens* seed. Using knowledge gained from these studies in combination with existing mechanical and chemical practices will strengthen our ability to mitigate new *D. graveolens* invasions and reduce the impact of current populations.

Similar to other researchers (Brownsey et al. 2013b), we found a strong association between large rainfall events and germination. Brownsey et al. (2013a) identified a germination window from November to March in the Central Valley of California, but vegetation management networks have reported repeated germination cohorts extending into June. In our study, germination did not occur after the rains stopped, though this result may reflect the limitations of our mesocosm experiment. Future studies should track germination year-round across different rain years, regions, substrates, and habitats. Late-season germination may occur in areas with groundwater movement, such as salt marshes, riparian zones, or drainage ditches. In these environments, soil moisture rather than precipitation might cue *D. graveolens* seeds to germinate, potentially enabling germination into late spring.

While we now better understand why *D. graveolens* thrives along roadsides and in disturbed soils, much remains to be learned about its potential response to climate change and other anthropogenic drivers. Climate change in California is expected to intensify droughts and flooding, likely increasing bare ground in plant communities – a known entry point for *D. graveolens* into more competitive ecosystems. As a

Mediterranean species, *D. graveolens* is adapted to mild winters and dry summers, suggesting potential drought tolerance that, combined with its ability to colonize bare ground, poses significant risks to vulnerable ecosystems. However, little is known about the drought tolerance of *D. graveolens* or how this late-season annual responds to hydrological fluctuations. While late-spring rains may increase competition with other species, vegetation management practices targeting grasses and other invasive species in spring could inadvertently create opportunities for late-spring cohorts of *D. graveolens* to establish. Further research on the response of *D. graveolens* to water availability is crucial to forecasting its behavior under California's changing climate.

Wildfire prevention methods and wildfire events themselves both contribute to the spread of *D. graveolens* by creating disturbed, bare ground that favors its establishment. Practices that reduce wildfire risk, such as removing vegetation to create defensible space around structures (FEMA 2020) and constructing firebreaks with large machinery like bulldozers, often result in soil disturbance and open patches – ideal conditions for *D. graveolens* to thrive. As an Asteraceae with small, lightweight seeds equipped with a pappus, *D. graveolens* is easily wind-dispersed, allowing its seeds to travel from nearby unburned areas into recently burned sites with minimal barriers. Similarly, wildfires themselves clear vegetation, reduce competition, and leave behind large areas of bare ground providing additional opportunities for *D. graveolens* to spread into new habitats. In addition to benefiting from wildfire-associated disturbance, *D. graveolens* may also influence fire regimes.

The chemical composition of its leaves and stems could contribute to flammability, potentially increasing wildfire risk in invaded areas. There is currently no evidence to suggest that *D. graveolens* can survive and resprout after wildfire (Kubiak 2009), however, this relationship between the plant and wildfire promotion remains poorly understood, and more empirical studies are needed to determine whether *D. graveolens* shows a positive feedback with wildfire. Understanding the dual relationship between wildfire and *D. graveolens* as both a potential benefitor and contributor to wildfire is critical to managing its spread and mitigating fire risks in California.

A deeper understanding of how *D. graveolens* responds to diverse edaphic conditions is urgently needed, particularly in sensitive habitats with soils rich in heavy metals (e.g., serpentine), elevated salinity (e.g., salt marshes, vernal pools), or frequent disturbance and bare ground (e.g., California native grasslands). Increasing reports from land managers suggest that *D. graveolens* is encroaching on serpentine soils – an uncommon soil type formed from the weathering of ultramafic rock. These soils are characterized by patchy, rocky terrain and high concentrations of heavy metals, which make it challenging for non-serpentine species to colonize (Whittaker 1954). Native plant communities on serpentine soils host rare and endemic species, but these can be outcompeted by invasive species, especially near roadsides with high nitrogen deposition (Weiss 1999). If *D. graveolens* can tolerate the particular edaphic conditions of serpentine plant communities, these unique ecosystems are at significant risk. Similarly, habitats with higher salinity could provide another pathway for *D. graveolens* invasion. The species has already been observed along the edges of salt marshes and salt pans in the southern San Francisco Bay, as well as encroaching on vernal pools in the East Bay. Within remnant California native grasslands, where bare ground naturally occurs around perennial bunchgrasses and shrubs, *D. graveolens* may exploit natural gaps and small mammal disturbances to establish itself. Further research is crucial to better assess the risks posed by *D. graveolens* and to inform management strategies for protecting these at-risk habitats.

What we lay out in this series of studies demonstrates that *D. graveolens*, although not a strong competitor, succeeds in bare soils, which often go hand in hand with anthropogenic habitats. Much of California and other parts of the globalized world are increasingly molded by human development, agriculture, and infrastructure. These disturbed conditions are where *D. graveolens* thrives, therefore human presence is ultimately the best indicator of *D. graveolens* risk. As a Mediterranean species, *D. graveolens* appears to be globally limited to similar climates (Lustenhouwer and Parker 2022), but a changing world and the plasticity of the species may propel it to move beyond these climate boundaries. Locally, early detection and rapid response are critical for eradicating new populations and slowing the spread. Using adaptive management to mitigate invasions is necessary to limit *D. graveolens* spread. Ultimately, plant invaders like *D. graveolens* are here to stay, but we can learn a lot from management efforts to curb the next invasion and mitigate current issues.

Appendix 1

Supplementary Tables and Figures for Chapter 1.

Table S1. Site characteristics for roadside and vegetated habitats at each of the 8 paired sites in the County of Santa Clara, California.

Site	Road Density (km/km2) within 1 km Radius		Elevation (m)		Road Width (m)	Distance Between Habitats (m)	Latitude/Longitude		Substrate Category	
	Roadside	Vegetated	Roadside	Vegetated			Roadside	Vegetated	Roadside	Vegetated
Baylands Park	7.659	6.702	3.05	3.05	34.6	539.72	37°24'50" N 122°0'5" W	37°24'50" N 121°59'43" W	soil	soil
Chesboro Reservoir	3.112	3.151	170.69	164.59	9.2	39.50	37°7'33" N 121°42'26" W	37°7'32" N 121°42'25" W	soil	soil
Guadalupe Reservoir	1.17	1.165	195.07	185.93	9.6	39.48	37°11'52" N 121°52'46" W	37°11'53" N 121°52'45" W	concrete	soil
Lexington Reservoir	4.744	4.495	207.26	210.31	10.1	92.66	37°12'2" N 121°59'9" W	37°11'59" N 121°59'9" W	concrete	rock (natural)
Oakridge Pond	13.03	12.029	57.91	57.91	25.7	252.91	37°15'22" N 121°51'58" W	37°15'16" N 121°52'5" W	soil	soil
Parkway Lakes RV	4.23	4.339	97.54	94.49	10.9	172.26	37°10'43" N 121°41'17" W	37°10'43" N 121°41'24" W	soil	rock (natural)
Penitencia Creek	12.785	12.925	70.10	79.25	9.4	122.70	37°23'38" N 121°50'23" W	37°23'38" N 121°50'18" W	soil	rock (natural)
South San Jose VTA	13.707	13.672	64.01	64.01	15.1	49.19	37°14'13" N 121°47'17" W	37°14'13" N 121°47'15" W	concrete	rock (natural)

Table S2. Resident species growing at each of the 8 paired sites in the County of Santa Clara, California. We surveyed the sites between July 1st and August 14th, 2020.

Populations	**Origin**	**Baylands Park**		**Chesboro Reservoir**		**Guadalupe Reservoir**		**Lexington Reservoir**		**Oakridge Pond**		**Parkway Lakes RV**		**Penitencia Creek**		**South San Jose VTA**	
Scientific Name		Roadside	Vegetated	Roadside	Vegetated	Roadside	Vegetated	Roadside	Vegetated	Roadside	Vegetated	Roadside	Vegetated	Roadside	Vegetated	Roadside	Vegetated
Anagallis arvensis	Non-native				X								X				
Baccharis pilularis	Native				X		X	X	X				X		X		
Brassica nigra	Non-native		X		X			X	X			X		X			
Centaurea solstitialis	Non-native											X	X				
Cyclospermum leptophyllum	Non-native													X			
Cynodon dactylon	Non-native						X										
Epilobium brachycarpum	Native				X		X						X			X	X
Erigeron bonariensis	Non-native	X												X			
Erigeron canadensis	Native	X															
Foeniculum vulgare	Non-native											X					
Genista monspessulana	Non-native							X	X								
Gnaphalium palustre	Native	X			X		X	X	X								
Heterotheca grandiflora	Native												X				
Medicago polymorpha/ arabica	Non-native			X			X				X		X	X			
Melilotus albus/ indicus	Non-native						X		X								
Plantago elongata/ erecta	Native							X				X					
Polycarpon tetraphyllum	Non-native													X			
Polypogon monspeliensis	Non-native				X		X		X								
Sonchus asper/ oleraceus	Non-native					X							X	X			
Spergularia rubra	Non-native			X													

Populations	**Origin**	**Baylands Park**		**Chesboro Reservoir**		**Guadalupe Reservoir**		**Lexington Reservoir**		**Oakridge Pond**		**Parkway Lakes RV**		**Penitencia Creek**		**South San Jose VTA**	
Scientific Name		Roadside	Vegetated	Roadside	Vegetated	Roadside	Vegetated	Roadside	Vegetated	Roadside	Vegetated	Roadside	Vegetated	Roadside	Vegetated	Roadside	Vegetated
Acmispon sp.	Native																X
Artemisia sp.	Native								X								
Avena sp.	Non-native		X	X									X				X
Bromus sp.	Non-native			X		X				X	X	X		X			X
Erodium sp.	Non-native			X	X						X						
Euphorbia sp.	Non-native							X									
Heliotropium sp.	Native				X												
Malva sp.	Non-native													X			
Polygonum sp.	Non-native						X		X								
Trifolium sp.	Unknown			X													
Verbena sp.	Unknown								X								
Unidentified annual Poaceae #1	Non-native		X														
Unidentified annual Poaceae #2	Non-native		X														
Unidentified annual Poaceae #3	Non-native	X															
Unidentified Brassicaceae	Unknown								X								

Table S3. Plant species growing within the fenced field plot at Blue Oak Ranch Reserve, California, surveyed on May 16, 2021.

Scientific Name	**Family**	**Abundance**	**Origin**
Amsinckia menziesii var. *intermedia*	Boraginaceae	Rare	Native
Avena barbata	Poaceae	Dominant	Non-native
Bromus diandrus	Poaceae	Common	Non-native
Bromus hordeaceus	Poaceae	Common	Non-native
Bromus madritensis ssp. *rubens*	Poaceae	Common	Non-native
Carduus pycnocephalus	Asteraceae	Uncommon	Non-native
Centaurea solstitialis	Asteraceae	Common	Non-native
Clarkia purpurea ssp. *quadrivulnera*	Onagraceae	Common	Native
Daucus pusillus	Apiaceae	Common	Native
Erodium cicutarium	Geraniaceae	Dominant	Non-native
Festuca myuros	Poaceae	Common	Non-native
Festuca perennis	Poaceae	Common	Non-native
Lupinus bicolor	Fabaceae	Common	Native
Lysimachia arvensis	Primulaceae	Uncommon	Non-native
Madia gracilis	Asteraceae	Rare	Native
Trifolium hirtum	Fabaceae	Dominant	Non-native
Vicia hirsuta	Fabaceae	Uncommon	Non-native

Table S4. We collected seeds from 8 paired populations in the County of Santa Clara, California, and took one homogenized sample of 30 seeds from each population and weighed them to the closest 0.001g. We calculated average seed mass for each source habitat using a Welch Two Sample t-test. Average seed mass varied from 0.243 to 0.333 and did not differ between source habitats (roadside = 2.26 mg, vegetated = 2.37 mg; $t_{12.11}$ = -1.18, P = 0.259).

Site Name	**Average Seed Weight (mg)**	
	Roadside	Vegetated
Baylands Park	0.273	0.277
Chesboro Reservoir	0.267	0.297
Guadalupe Reservoir	0.300	0.283
Lexington Reservoir	0.280	0.243
Oakridge Pond	0.267	0.333
Parkway Lakes RV	0.320	0.307
Penitencia Creek	0.273	0.320
South San Jose VTA	0.277	0.310

To visualize differences in plant community composition across our sites, we used the vegan package (Oksanen et al., 2022) in R (R Core Team, 2022) to perform an NMDS analysis on the plant community survey data collected from each paired site (Table S2). When we compared vegetated and roadside habitats, our NMDS analysis revealed overlapping ellipses and clustered points, indicating similarities in plant community composition. Specific plant species, including *Bromus* sp., *Epilobium brachycarpum*, and *Gnaphalium palustre*, correlated with values of the 2 NMDS factors as shown in Figure S1 ($P < 0.001$, $R^2 > 0.422$). We tested for differentiation between roadside and vegetated habitats with PERMANOVA and found no significant overall difference between the habitats using the ‘adonis2’ function from the vegan package ($F_{1,14} = 1.51$, $P = 0.227$).

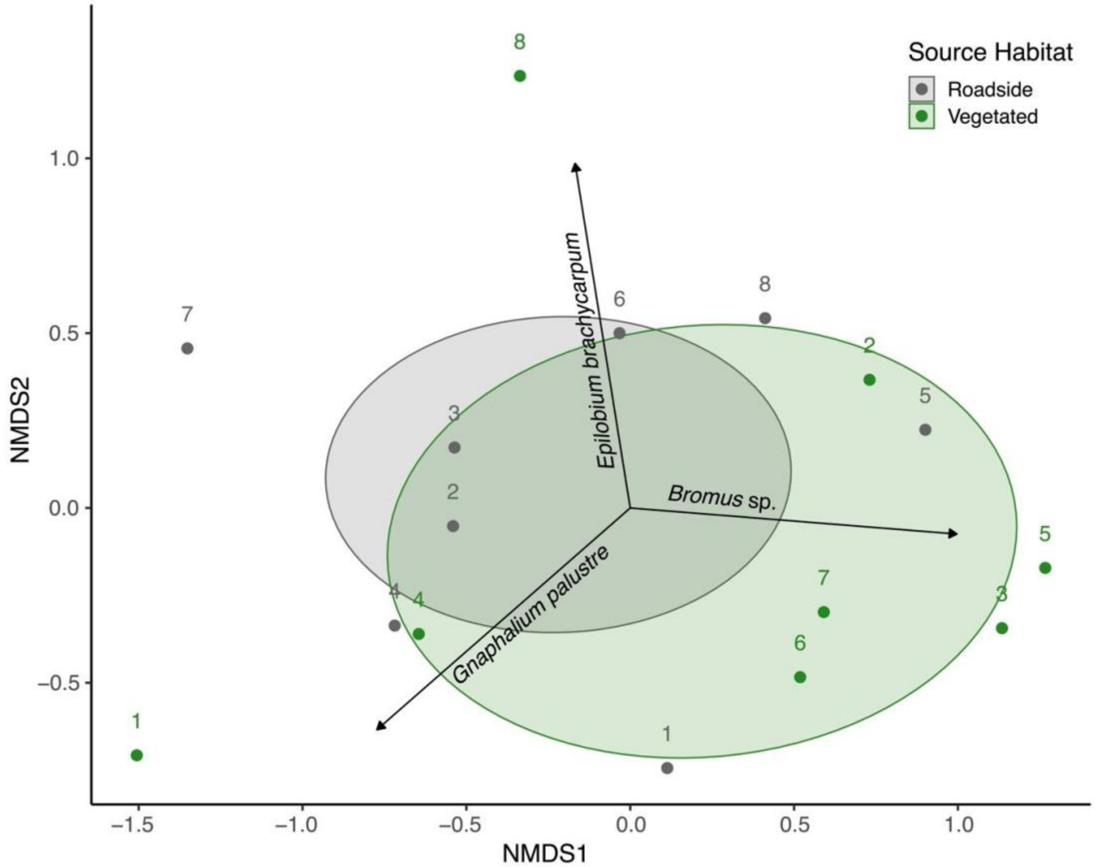


Figure S1. When we compared vegetated and roadside habitats, our NMDS analysis revealed overlapping ellipses and clustered points, indicating similarities in plant community composition. Specific plant species, including *Bromus* sp., *Epilobium brachycarpum*, and *Gnaphalium palustre*, correlated with values of the 2 NMDS factors. Numbers denote site pairs.

Appendix 2

Supplementary Tables and Figures for Chapter 2.

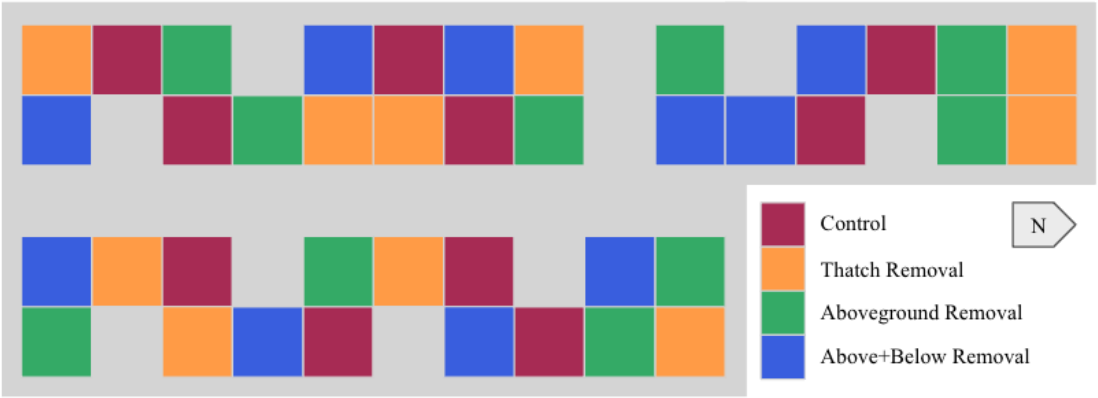


Figure S1. Experimental design of field experiment I: response to competition and disturbance. We used a randomized block design with 10 blocks of 1.5 m^2 plots. We planted 16 *D. graveolens* seedlings into each plot using dibblers (640 seedlings; 40 plots total). Seedlings were planted in a 4 × 4 grid centered on the plot. The distance between plants within plots was 33 cm, and plots were separated from each other by a 25 cm buffer.

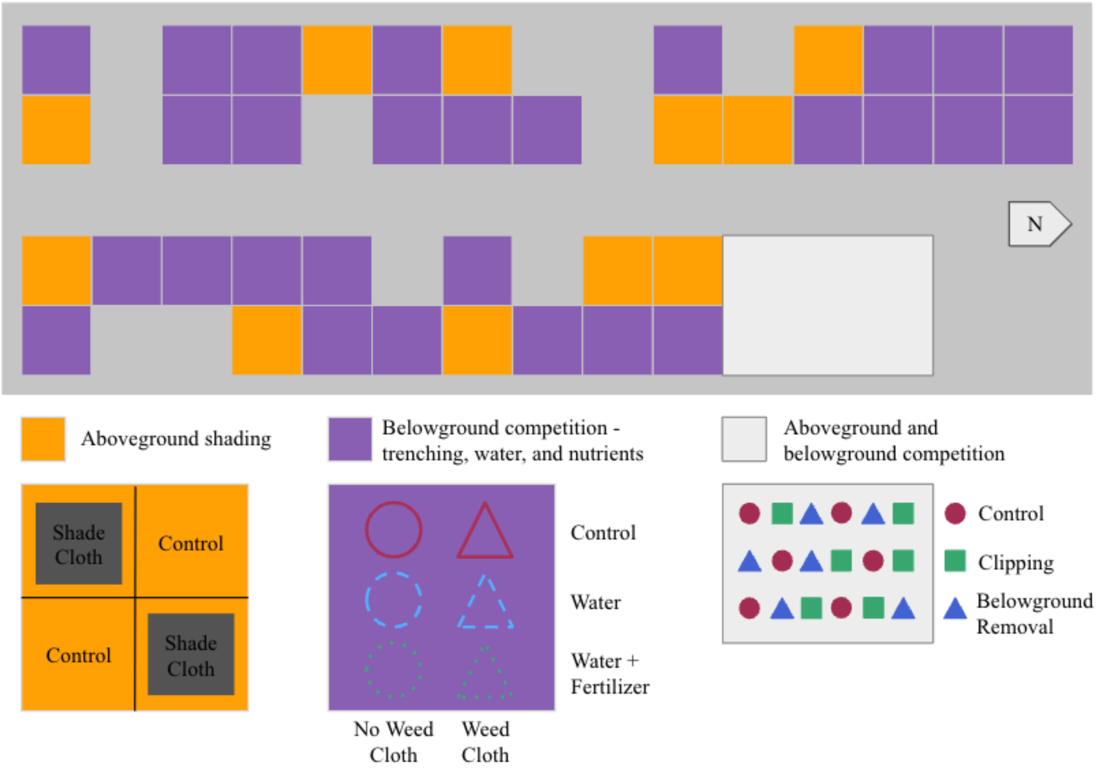


Figure S2. Experimental design of field experiment II: separating response to above- and belowground competition. We conducted three experiments related to above and belowground competition using a subset of the same 1.5 m^2 plots as the previous year: 1) Aboveground and belowground competition, 2) Aboveground shading, 3) Belowground competition - trenching, water, and nutrients. In the aboveground and belowground competition experiment, we randomly assigned three treatments to 54 planting locations: belowground competitor removal (holes dug to a depth of 45 cm), clipping (background grassland vegetation clipped to 1 - 3 cm high), and control (no manipulation of the soil or vegetation). We planted two *D. graveolens* seedlings in each planting location (N = 108). In the aboveground shading experiment we hoed 11 plots to remove above and belowground competition and then divided each plot into four quadrants and planted with two *D. graveolens* seedlings per quadrant (N = 88 plants total). Four bamboo stakes were placed in the corners of each quadrant and were randomly assigned a treatment of control (no shade cloth) or shade cloth). In the belowground competition - trenching, water, and nutrients experiment, we prepared 28 plots by trenching six 45-cm holes in each plot. We lined half of the holes with a weed cloth fabric and half without weed cloth. We placed 15-diameter PCV collars in all holes, with a lip of 2 cm above the soil surface to prevent surface runoff and to a depth of 10 cm (PVC = 6 cm wide × 12 cm deep). The original soil was used to fill in the holes and we planted two *D. graveolens* seedlings in each treatment hole. Using a factorial design, treatments were assigned to each hole: control, the addition of 283.49 grams of water, and the addition of 283.49 grams of water plus 5 grams of fertilizer (Osmocote 14-14-14). The soil surface was scratched using a fork in all treatments to encourage infiltration.

Appendix 3

Supplementary Tables and Figures for Chapter 3.

Table S1. Wild seed collection locations where we collected 10 maternal families from each site.

Range	Site	Elevation (m)	Latitude	Longitude	County	First County Observation
Core	Alviso	1	37.43305	-121.96277	Santa Clara	1984
	Page Mill Park & Ride	78	37.3875	-122.16333	Santa Clara	1984
	Almaden Quicksilver Park	88	37.2147	-121.88177	Santa Clara	1984
	Lexington Reservoir	191	37.19971	-121.98579	Santa Clara	1984
Invading Front	Triple L Ranch	393	37.91715	-120.45434	Tuolumne	2013
	Jesus Maria	428	38.3176	-120.65249	Calaveras	2011
	Sierra Rock	634	38.69128	-120.76408	El Dorado	2009
	Dutch Flats	1015	39.18778	-120.83264	Placer	2003

References

Aghel N, Mahmoudabadi AZ, Darvishi L (2011) Volatile constituents and anti candida activity of the aerial parts essential oil of *Dittrichia graveolens* (L.) Greuter grown in Iran. Afr J Pharm Pharmacol 5:772–775. https://doi.org/10.5897/AJPP10.145

Albaba I (2015) A list of important honeybee nectariferous and polleniferous plant species in the West Bank Governorates, Palestine. J Agric Sci Technol A 5:114–121. https://doi.org/10.17265/2161-6256/2015.02A.005

Alexander JM, Levine JM (2019) Earlier phenology of a nonnative plant increases impacts on native competitors. Proc Natl Acad Sci 116:6199–6204. https://doi.org/10.1073/pnas.1820569116

Amos W, Harwood J (1998) Factors affecting levels of genetic diversity in natural populations. Philos Trans R Soc Lond B Biol Sci 353:177–186. https://doi.org/10.1098/rstb.1998.0200

Anderson JT, Inouye DW, McKinney AM, et al (2012) Phenotypic plasticity and adaptive evolution contribute to advancing flowering phenology in response to climate change. Proc R Soc B Biol Sci 279:3843–3852. https://doi.org/10.1098/rspb.2012.1051

Anderson JT, Song B (2020) Plant adaptation to climate change - where are we? J Syst Evol 58:533–545. https://doi.org/10.1111/jse.12649

Andow DA, Kareiva PM, Levin SA, Okubo A (1990) Spread of invading organisms. Landsc Ecol 4:177–188. https://doi.org/10.1007/BF00132860

Aragón CF, Escudero A, Valladares F (2007) Stress-induced dynamic adjustments of reproduction differentially affect fitness components of a semi-arid plant. J Ecol 0:222–229. https://doi.org/10.1111/j.1365-2745.2007.01320.x

Bagavathiannan MV, Gulden RH, Van Acker RC (2011) Occurrence of alfalfa (Medicago sativa L.) populations along roadsides in southern Manitoba, Canada and their potential role in intraspecific gene flow. Transgenic Res 20:397–407. https://doi.org/10.1007/s11248-010-9425-2

Baker HG (1974) The evolution of weeds. Annu Rev Ecol Syst 5:1–24. https://doi.org/10.1146/annurev.es.05.110174.000245

Baker HG (1965) Characteristics and modes of origin of weeds. Charact Modes Orig Weeds 147–172

Baker HG, Stebbins GL (1965) Characteristics and modes of origin of weeds. In: The genetics of colonizing species. Academic Press, New York

Bakker J, Wilson S (2001) Competitive abilities of introduced and native grasses. Plant Ecol 157:117–125. https://doi.org/10.1023/A:1013972403293

Barrett SCH, Husband BC (1990) Genetics of plant migration and colonization. In: Brown AHD, Clegg MT, Kahler AL, Weir BS (eds) Plant population genetics, breeding and genetic resources. Sinauer Associates, Sunderland, MA, USA, pp 254–277

Bates D, Maechler M, Bolker BM, Walker S (2015) Fitting linear mixed-effects models using lme4. J Stat Softw 67:1–48. https://doi.org/10.18637/jss.v067.i01

Bazzaz FA (1986) Life history of colonizing plants: some demographic, genetic, and physiological features. In: Mooney HA, Drake JA (eds) Ecology of Biological Invasions of North America and Hawaii. Springer, New York, NY, pp 96–110

Bettez ND, Marino R, Howarth RW, Davidson EA (2013) Roads as nitrogen deposition hot spots. Biogeochemistry 114:149–163. https://doi.org/10.1007/s10533-013-9847-z

Blackburn TM, Pyšek P, Bacher S, et al (2011) A proposed unified framework for biological invasions. Trends Ecol Evol 26:333–339. https://doi.org/10.1016/j.tree.2011.03.023

Bliss D, Smith H (1985) Penetration of light into soil and its role in the control of seed germination. Plant Cell Environ 8:475–483. https://doi.org/10.1111/j.1365-3040.1985.tb01683.x

Blossey B, Nötzold R (1995) Evolution of increased competitive ability in invasive nonindigenous plants: a hypothesis. J Ecol 83:887–889. https://doi.org/10.2307/2261425

Blows MW, Hoffmann AA (2005) A reassessment of genetic limits to evolutionary change. Ecology 86:1371–1384. https://doi.org/10.1890/04-1209

Bossard CC (1993) Seed germination in the exotic shrub *Cytisus scoparius* (scotch broom) in California. Madroño 40:47–61

Bossdorf O, Auge H, Lafuma L, et al (2005) Phenotypic and genetic differentiation between native and introduced plant populations. Oecologia 144:1–11. https://doi.org/10.1007/s00442-005-0070-z

Brandes D (2009) Autobahnen als wuchsorte und ausbreitungswege von ruderal - und adventivpflanzen. Braunschweiger Naturkundliche Schriften 8:373–394

Brinkmann IJ (2020) *Dittrichia graveolens*, an invasive weed in exotic habitats: implications for management. Masters Thesis, California State University, Chico

Brooks ME, Kristensen K, van Betham KJ, et al (2017) glmmTMB balances speed and flexibility among packages for zero-inflated generalized linear mixed modeling. R J 9:378–400

Brownsey R, Kyser GB, DiTomaso JM (2013a) Stinkwort is rapidly expanding its range in California. Calif Agric 67:110–115. https://doi.org/10.3733/ca.v067n02p110

Brownsey R, Kyser GB, DiTomaso JM (2013b) Seed and germination biology of *Dittrichia graveolens* (stinkwort). Invasive Plant Sci Manag 6:371–380. https://doi.org/10.1614/IPSM-D-12-00072.1

Brownsey RN (2012) Biology of *Dittrichia graveolens* (stinkwort): implications for management. Ph.D. Dissertation, University of California, Davis

Brownsey RN, Kyser GB, DiTomaso JM (2014) Growth and phenology of *Dittrichia graveolens*, a rapidly spreading invasive plant in California. Biol Invasions 16:43–52. https://doi.org/10.1007/s10530-013-0501-4

Brullo S, de Marco G (2000) Taxonomical revision of the genus *Dittrichia* (Asteraceae). Port Acta Biol 19:341–354

Burton OJ, Phillips BL, Travis JMJ (2010) Trade-offs and the evolution of life-histories during range expansion. Ecol Lett 13:1210–1220. https://doi.org/10.1111/j.1461-0248.2010.01505.x

Buswell JM, Moles AT, Hartley S (2011) Is rapid evolution common in introduced plant species? J Ecol 99:214–224. https://doi.org/10.1111/j.1365-2745.2010.01759.x

Calflora (2024) Calflora: Information on California plants for education, research and conservation [web application]. Available at: https://www.calflora.org/. Accessed 1 Nov 2024

Calflora (2020) Calflora: Information on California plants for education, research and conservation [web application]. Available at: https://www.calflora.org/. Accessed 4 Apr 2021

California Rangeland Conservation Coalition (2014) California rangeland priority conservation areas - data basin. Available at: https://databasin.org/datasets/4e24786c97f04bdf8f91d0fc16013a88. Accessed 4 Apr 2021

Callaway RM, Lucero JE, Hierro JL, Lortie CJ (2022) The EICA is dead? Long live the EICA! Ecol Lett 25:2289–2302. https://doi.org/10.1111/ele.14088

Catford JA, Daehler CC, Murphy HT, et al (2012) The intermediate disturbance hypothesis and plant invasions: implications for species richness and management. Perspect Plant Ecol Evol Syst 14:231–241

Christen D, Matlack G (2006) The role of roadsides in plant invasions: a demographic approach. Conserv Biol 20:385–391. https://doi.org/10.1111/j.1523-1739.2006.00315.x

Clark CD (2018) Realised niche shifts, rapid evolution and phenotypic plasticity in introduced plants. Ph.D. Dissertation, Victoria University of Wellington

Clements DR, Ditommaso A (2011) Climate change and weed adaptation: can evolution of invasive plants lead to greater range expansion than forecasted? Weed Res 51:227–240. https://doi.org/10.1111/j.1365-3180.2011.00850.x

Cohen D (1966) Optimizing reproduction in a randomly varying environment. J Theor Biol 12:119–129. https://doi.org/10.1016/0022-5193(66)90188-3

Colautti RI, Barrett SCH (2013) Rapid adaptation to climate facilitates range expansion of an invasive plant. Science 342:364–366. https://doi.org/10.1126/science.1242121

Colautti RI, Lau JA (2015) Contemporary evolution during invasion: evidence for differentiation, natural selection, and local adaptation. Mol Ecol 24:1999–2017. https://doi.org/10.1111/mec.13162

Colautti RI, Maron JL, Barrett SCH (2009) Common garden comparisons of native and introduced plant populations: latitudinal clines can obscure evolutionary inferences: Evolution in invasive plants. Evol Appl 2:187–199. https://doi.org/10.1111/j.1752-4571.2008.00053.x

Coleman HM, Levine JM (2007) Mechanisms underlying the impacts of exotic annual grasses in a coastal California meadow. Biol Invasions 9:65–71. https://doi.org/10.1007/s10530-006-9008-6

Dawson K, Veblen KE, Young TP (2007) Experimental evidence for an alkali ecotype of *Lolium multiflorum*, an exotic invasive annual grass in the Central Valley, CA, USA. Biol Invasions 9:327–334. https://doi.org/10.1007/s10530-006-9036-2

Dezfuli PM, Sharif-zadeh F, Janmohammadi M (2008) Influence of priming techniques on seed germination behavior of maize inbred lines. ARPN J Agric Biol Sci 3:22–25

Diagne C, Leroy B, Vaissière A-C, et al (2021) High and rising economic costs of biological invasions worldwide. Nature 592:571–576. https://doi.org/10.1038/s41586-021-03405-6

Dietz H, Edwards PJ (2006) Recognition that casual processes change during plant invasion helps explain conflicts in evidence. Ecology 87:1359–1367. https://doi.org/10.1890/0012-9658(2006)87[1359:RTCPCD]2.0.CO;2

Diez JM, Hulme PE, Duncan RP (2012) Using prior information to build probabilistic invasive species risk assessments. Biol Invasions 14:681–691. https://doi.org/10.1007/s10530-011-0109-5

DiTomaso JM, Brownsey R (2013) Invasive stinkwort (*Dittrichia graveolens*) is quickly spreading in California. Calif Weed Sci Soc Res Update News 9:1–2

Dlugosch KM, Anderson SR, Braasch J, et al (2015) The devil is in the details: genetic variation in introduced populations and its contributions to invasion. Mol Ecol 24:2095–2111. https://doi.org/10.1111/mec.13183

Dlugosch KM, Parker IM (2008a) Founding events in species invasions: genetic variation, adaptive evolution, and the role of multiple introductions. Mol Ecol 17:431–449. https://doi.org/10.1111/j.1365-294X.2007.03538.x

Dlugosch KM, Parker IM (2008b) Invading populations of an ornamental shrub show rapid life history evolution despite genetic bottlenecks. Ecol Lett 11:701–709. https://doi.org/10.1111/j.1461-0248.2008.01181.x

El-Barougy RF, Dakhil MA, Halmy MW, et al (2021) Invasion risk assessment using trait-environment and species distribution modelling techniques in an arid protected area: towards conservation prioritization. Ecol Indic 129:107951. https://doi.org/10.1016/j.ecolind.2021.107951

Estoup A, Ravigné V, Hufbauer R, et al (2016) Is there a genetic paradox of biological invasion? Annu Rev Ecol Evol Syst 47:51–72. https://doi.org/10.1146/annurev-ecolsys-121415-032116

Ethridge SR, Chandra S, Everman WJ, et al (2023) Rapid evolution of competitive ability in giant foxtail (*Setaria faberi*) over 34 years. Weed Sci 71:59–68. https://doi.org/10.1017/wsc.2023.1

Felker-Quinn E, Schweitzer JA, Bailey JK (2013) Meta-analysis reveals evolution in invasive plant species but little support for Evolution of Increased Competitive Ability (EICA). Ecol Evol 3:739–751. https://doi.org/10.1002/ece3.488

FEMA (2020) Protect your property from wildfires

Fenner M, Thompson K (2005) The ecology of seeds. Cambridge University Press, United Kingdom

Fletcher CS, Westcott DA, Murphy HT, et al (2015) Managing breaches of containment and eradication of invasive plant populations. J Appl Ecol 52:59–68. https://doi.org/10.1111/1365-2664.12361

Fletcher RA, Atwater DZ, Haak DC, et al (2023) Adaptive constraints at the range edge of a widespread and expanding invasive plant. AoB PLANTS plad070. https://doi.org/10.1093/aobpla/plad070

Follak S, Eberius M, Essl F, et al (2018) Invasive alien plants along roadsides in Europe. EPPO Bull 48:256–265. https://doi.org/10.1111/epp.12465

Fox GA (1990) Drought and the evolution of flowering time in desert annuals. Am J Bot 77:1508–1518. https://doi.org/10.2307/2444763

Fox J, Weisberg S (2019) An R companion to applied regression, Third. Sage, Thousand Oaks CA

Frajman B, Kaligarič M (2009) *Dittrichia graveolens*, nova tujerodna vrsta slovenske flore. Hladnikia 24:35–43

Frenkel RE (1977) Ruderal vegetation along some California roadsides. University of California Press, United States

Funk JL, Parker IM, Matzek V, et al (2020) Keys to enhancing the value of invasion ecology research for management. Biol Invasions 22:2431–2445. https://doi.org/10.1007/s10530-020-02267-9

Gelbard JL, Belnap J (2003) Roads as conduits for exotic plant invasions in a semiarid landscape. Conserv Biol 17:420–432. https://doi.org/10.1046/j.1523-1739.2003.01408.x

Gioria M, Hulme PE, Richardson DM, Pyšek P (2023) Why are invasive plants successful? Annu Rev Plant Biol 74:635–670. https://doi.org/10.1146/annurev-arplant-070522-071021

Gioria M, Pyšek P, Moravcová L (2012) Soil seed banks in plant invasions: promoting species invasiveness and long-term impact on plant community dynamics. Preslia 84:327–350

Gong W, Wang Y, Chen C, et al (2022) The rapid evolution of an invasive plant due to increased selection pressures throughout its invasive history. Ecotoxicol Environ Saf 233:113322. https://doi.org/10.1016/j.ecoenv.2022.113322

Grace J (1990) On the relationship between plant traits and competitive ability. pp 51–65

Greenberg CH, Crownover SH, Gordon DR (1997) Roadside soils: a corridor for invasion of xeric scrub by nonindigenous plants. Nat Areas J 17:99–109

Greipsson S (2011) Restoration ecology. Jones & Bartlett Learning

Grime JP (1977) Evidence for the existence of three primary strategies in plants and its relevance to ecological and evolutionary theory. Am Nat 111:1169–1194. https://doi-org.oca.ucsc.edu/10.1086/283244

Grime JP (2006) Plant strategies, vegetation processes, and ecosystem properties. John Wiley & Sons

Grossinger RM, Striplen CJ, Askevold RA, et al (2007) Historical landscape ecology of an urbanized California valley: wetlands and woodlands in the Santa Clara Valley. Landsc Ecol 22:103–120. https://doi.org/10.1007/s10980-007-9122-6

Guo K, Pyšek P, Chytrý M, et al (2022) Ruderals naturalize, competitors invade: varying roles of plant adaptive strategies along the invasion continuum. Funct Ecol. https://doi.org/10.1111/1365-2435.14145

Guo W, Van Kleunen M, Winter M, et al (2018) The role of adaptive strategies in plant naturalization. Ecol Lett 21:1380–1389. https://doi.org/10.1111/ele.13104

Hairston NG, Ellner SP, Geber MA, et al (2005) Rapid evolution and the convergence of ecological and evolutionary time. Ecol Lett 8:1114–1127. https://doi.org/10.1111/j.1461-0248.2005.00812.x

Halekoh U, Højsgaard S (2014) A Kenward-Roger approximation and parametric bootstrap methods for tests in linear mixed models - the R package pbkrtest. J Stat Softw 59:1–30. https://doi.org/10.18637/jss.v059.i09

Hansen MJ, Clevenger AP (2005) The influence of disturbance and habitat on the presence of non-native plant species along transport corridors. Biol Conserv 125:249–259. https://doi.org/10.1016/j.biocon.2005.03.024

Hanson RT, Li Z, Faunt CC (2004) Documentation of the Santa Clara Valley Regional Ground-Water/Surface-Water Flow Model, Santa Clara County, California. Santa Clara Valley Water District

Harzallah-Skhiri F, Cheraif I, Jannet HB, Hammami M (2005) Chemical composition of essential oils from leaves-stems, flowers and roots of *Inula graveolens* from Tunisia. Pak J Biol Sci 8:249–254. https://doi.org/10.3923/pjbs.2005.249.254

Hernandez RR, Tanner KE, Haji S, et al (2020) Simulated photovoltaic solar panels alter the seed bank survival of two desert annual plant species. Plants 9:1125. https://doi.org/10.3390/plants9091125

HilleRisLambers J, Yelenik SG, Colman BP, Levine JM (2010) California annual grass invaders: the drivers or passengers of change? J Ecol 98:1147–1156. https://doi.org/10.1111/j.1365-2745.2010.01706.x

Hmissi M, Chaieb M, Krouma A (2023) Differences in the physiological indicators of seed germination and seedling establishment of durum wheat (*Triticum durum* Desf.) cultivars subjected to salinity stress. Agronomy 13:1718. https://doi.org/10.3390/agronomy13071718

Hobbs RJ, Huenneke LF (1992) Disturbance, diversity, and invasion: implications for conservation. Conserv Biol 6:324–337

Hogan KFE, Fogarty DT, Ellerman H, et al (2022) Rangelands in a fragmented grass-dominated landscape are vulnerable to tree invasion from roadsides. Biol Invasions 24:3341–3347. https://doi.org/10.1007/s10530-022-02869-5

Hu XW, Pan J, Min DD, et al (2017) Seed dormancy and soil seedbank of the invasive weed *Chenopodium hybridum* in north-western China. Weed Res 57:54–64. https://doi.org/10.1111/wre.12237

Hulme PE (2009) Trade, transport and trouble: managing invasive species pathways in an era of globalization. J Appl Ecol 46:10–18. https://doi.org/10.1111/j.1365-2664.2008.01600.x

Johnston FM, Johnston SW (2004) Impacts of road disturbance on soil properties and on exotic plant occurrence in subalpine areas of the Australian Alps. Arct Antarct Alp Res 36:201–207. https://doi.org/10.1657/1523-0430(2004)036[0201:IORDOS]2.0.CO;2

Kalwij JM, Milton SJ, McGeoch MA (2008) Road verges as invasion corridors? A spatial hierarchical test in an arid ecosystem. Landsc Ecol 23:439–451. https://doi.org/10.1007/s10980-008-9201-3

Kubiak PJ (2009) Fire responses of bushland plants after the January 1994 wildfires in northern Sydney

Kuznetsova A, Brockhoff P, Christensen R (2017) lmerTest package: tests in linear mixed effects models. J Stat Softw 82:1–26. https://doi.org/doi:10.18637/jss.v082.i13

Lande R (2015) Evolution of phenotypic plasticity in colonizing species. Mol Ecol 24:2038–2045. https://doi.org/10.1111/mec.13037

Lázaro-Lobo A, Ervin GN (2019) A global examination on the differential impacts of roadsides on native vs. exotic and weedy plant species. Glob Ecol Conserv 17:e00555. https://doi.org/10.1016/j.gecco.2019.e00555

Leblond M, Dussault C, Ouellet J –P. (2013) Avoidance of roads by large herbivores and its relation to disturbance intensity. J Zool 289:32–40. https://doi.org/10.1111/j.1469-7998.2012.00959.x

Leger EA, Rice KJ (2007) Assessing the speed and predictability of local adaptation in invasive California poppies (*Eschscholzia californica*). J Evol Biol 20:1090–1103. https://doi.org/10.1111/j.1420-9101.2006.01292.x

Lennon JT, Den Hollander F, Wilke-Berenguer M, Blath J (2021) Principles of seed banks and the emergence of complexity from dormancy. Nat Commun 12:4807. https://doi.org/10.1038/s41467-021-24733-1

Lenormand T (2002) Gene flow and the limits to natural selection. Trends Ecol Evol 17:183–189. https://doi.org/10.1016/S0169-5347(02)02497-7

Lenth R (2024) emmeans: Estimated Marginal Means, aka Least-Squares Means

Liu Z, Yang Y, Ji S, et al (2021) Effects of elevation and distance from highway on the abundance and community structure of bacteria in soil along Qinghai-Tibet Highway. Int J Environ Res Public Health 18:13137. https://doi.org/10.3390/ijerph182413137

Lustenhouwer N, Parker IM (2022) Beyond tracking climate: Niche shifts during native range expansion and their implications for novel invasions. J Biogeogr 49:1481–1493. https://doi.org/10.1111/jbi.14395

Lustenhouwer N, Wilschut RA, Williams JL, et al (2018) Rapid evolution of phenology during range expansion with recent climate change. Glob Change Biol 24:e534–e544. https://doi.org/10.1111/gcb.13947

Mack RN, Simberloff D, Mark Lonsdale W, et al (2000) Biotic invasions: causes, epidemiology, global consequences, and control. Ecol Appl 10:689–710. https://doi.org/10.1890/1051-0761(2000)010[0689:BICEGC]2.0.CO;2

Maron JL, Vilà M, Bommarco R, et al (2004) Rapid evolution of an invasive plant. Ecol Monogr 74:261–280. https://doi.org/10.1890/03-4027

Matzek V, Pujalet M, Cresci S (2015) What managers want from invasive species research versus what they get. Conserv Lett 8:33–40. https://doi.org/10.1111/conl.12119

May RM, Endler JA, McMurtrie RE (1975) Gene frequency clines in the presence of selection opposed by gene flow. Am Nat 109:659–676. https://doi.org/10.1086/283036

McDougall KL, Alexander JM, Haider S, et al (2011) Alien flora of mountains: global comparisons for the development of local preventive measures against plant invasions: Alien flora of mountains. Divers Distrib 17:103–111. https://doi.org/10.1111/j.1472-4642.2010.00713.x

McDougall KL, Lembrechts J, Rew LJ, et al (2018) Running off the road: roadside non-native plants invading mountain vegetation. Biol Invasions 20:3461–3473. https://doi.org/10.1007/s10530-018-1787-z

McEvoy SL, Lustenhouwer N, Melen MK, et al (2023) Chromosome-level reference genome of stinkwort, *Dittrichia graveolens* (L.) Greuter: a resource for studies on invasion, range expansion, and evolutionary adaptation under global change. J Hered esad033. https://doi.org/10.1093/jhered/esad033

McKee L, Leatherbarrow J, Pearce S, Davis J (2003) A review of urban runoff processes in the Bay Area: existing knowledge, conceptual models, and monitoring recommendations. San Francisco Estuary Institute, Oakland

Meadly GRW (1965) Stinkwort (*Inula graveolens* Desf.). J Dep Agric West Aust 6:7

Melen MK, Snyder ED, Fernandez M, et al (2024) Invasion away from roadsides was not driven by adaptation to grassland habitats in *Dittrichia graveolens* (stinkwort). Biol Invasions. https://doi.org/10.1007/s10530-024-03359-6

Mills SD, Mamo M, Schacht WH, et al (2020) Soil properties affected vegetation establishment and persistence on roadsides. Water Air Soil Pollut 231:568. https://doi.org/10.1007/s11270-020-04930-2

Minchinton TE, Bertness MD (2003) Disturbance-mediated competition and the spread of *Phragmites australis* in a coastal marsh. Ecol Appl 13:1400–1416. https://doi.org/10.1890/02-5136

Moran EV, Alexander JM (2014) Evolutionary responses to global change: lessons from invasive species. Ecol Lett 17:637–649. https://doi.org/10.1111/ele.12262

Moravcová L, Pyšek P, Krinke L, et al (2018) Long-term survival in soil of seed of the invasive herbaceous plant *Heracleum mantegazzianum*. Preslia 90:225–234. https://doi.org/10.23855/preslia.2018.225

Muñoz PT, Torres FP, Megías AG (2015) Effects of roads on insects: a review. Biodivers Conserv 24:659–682. https://doi.org/10.1007/s10531-014-0831-2

Nei M, Maruyama T, Chakraborty R (1975) The bottleneck effect and genetic variability in populations. Evolution 29:1–10. https://doi.org/10.2307/2407137

Oka H-I (1983) Life-history characteristics and colonizing success in plants. Am Zool 23:99–109. https://doi.org/10.1093/icb/23.1.99

Paini DR, Sheppard AW, Cook DC, et al (2016) Global threat to agriculture from invasive species. Proc Natl Acad Sci 113:7575–7579. https://doi.org/10.1073/pnas.1602205113

Parker IM, Rodriguez J, Loik ME (2003) An evolutionary approach to understanding the biology of invasions: local adaptation and general-purpose genotypes in the weed *Verbascum thapsus*. Conserv Biol 17:59–72. https://doi.org/10.1046/j.1523-1739.2003.02019.x

Parsons WT, Cuthbertson EG (2001) Stinkwort. In: Noxious Weeds of Australia, Second Edition. Csiro Publishing, Collingwood, Australia, pp 281–283

Passos I, Marchante H, Pinho R, Marchante E (2017) What we don't seed: the role of long-lived seed banks as hidden legacies of invasive plants. Plant Ecol 218:1313–1324. https://doi.org/10.1007/s11258-017-0770-6

Perkins AT, Phillips BL, Baskett ML, Hastings A (2013) Evolution of dispersal and life history interact to drive accelerating spread of an invasive species. Ecol Lett 16:1079–1087. https://doi.org/10.1111/ele.12136

Philbey A, Morton A (2000) Pyogranulomatous enteritis in sheep due to penetrating seed heads of *Dittrichia graveolens*. Aust Vet J 78:858–860. https://doi.org/10.1111/j.1751-0813.2000.tb10511.x

Philippi T, Seger J (1989) Hedging one's evolutionary bets, revisited. Trends Ecol Evol 4:41–44. https://doi.org/10.1016/0169-5347(89)90138-9

Phillips BL, Brown GP, Shine R (2010) Life-history evolution in range-shifting populations. Ecology 91:1617–1627. https://doi.org/10.1890/09-0910.1

Phillips BL, Shine R (2006) An invasive species induces rapid adaptive change in a native predator: cane toads and black snakes in Australia. Proc R Soc B Biol Sci 273:1545–1550. https://doi.org/10.1098/rspb.2006.3479

Pierce S, Negreiros D, Cerabolini BEL, et al (2017) A global method for calculating plant CSR ecological strategies applied across biomes world-wide. Funct Ecol 31:444–457. https://doi.org/10.1111/1365-2435.12722

Pimm S (1989) Theories of predicting success and impact of introduced species. Biol Invasions Glob Perspect 351–368

Poland TM, Patel-Weynand T, Finch DM, et al (eds) (2021) Invasive species in forests and rangelands of the United States: a comprehensive science synthesis for the United States forest sector. Springer International Publishing, Cham

Ponticelli M, Lela L, Russo D, et al (2022) *Dittrichia graveolens* (L.) Greuter, a rapidly spreading invasive plant: chemistry and bioactivity. Molecules 27:895. https://doi.org/10.3390/molecules27030895

Prentis PJ, Wilson JRU, Dormontt EE, et al (2008) Adaptive evolution in invasive species. Trends Plant Sci 13:288–294. https://doi.org/10.1016/j.tplants.2008.03.004

Presotto A, Poverene M, Cantamutto M (2014) Seed dormancy and hybridization effect of the invasive species, *Helianthus annuus*. Ann Appl Biol 164:373–383. https://doi.org/10.1111/aab.12104

Preston RB (1997) *Dittrichia graveolens* (Asteraceae), new to the California weed flora. Madroño 5

Pyšek P, Richardson DM (2010) Invasive species, environmental change and management, and health. Annu Rev Environ Resour 35:25–55. https://doi.org/10.1146/annurev-environ-033009-095548

R Core Team (2022) R: a language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria. https://www.R-project.org/

R Core Team (2023) R: a language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria. https://www.R-project.org/

Rameau J-C, Mansion D, Dumé G, Gauberville C (2008) Flore forestière française: guide écologique illustré. Région méditerranéenne. Institut pour le développement forestier, France

Reznick DN, Losos J, Travis J (2019) From low to high gear: there has been a paradigm shift in our understanding of evolution. Ecol Lett 22:233–244. https://doi.org/10.1111/ele.13189

Roberts HA, Boddrell JE (1984) Seed survival and seasonal emergence of seedlings of some ruderal plants. J Appl Ecol 21:617–628. https://doi.org/10.2307/2403433

Robinson AP, Walshe T, Burgman MA, Nunn M (2017) Invasive species: risk assessment and management. Cambridge University Press

Rollins LA, Richardson MF, Shine R (2015) A genetic perspective on rapid evolution in cane toads (*Rhinella marina*). Mol Ecol 24:2264–2276. https://doi.org/10.1111/mec.13184

Santilli L, Lavandero N, Montes O, Lustenhouwer N (2021) First record of *Dittrichia graveolens* (Asteraceae, Inuleae) in Chile. Darwiniana Nueva Ser 31–38. https://doi.org/10.14522/darwiniana.2021.91.938

Sărățeanu V, Moisuc A, Cotuna O (2010) *Ambrosia artemisiifolia* L. an invasive weed from ruderal areas to disturbed grasslands. Lucr Ştiinţ 53:235–238

Satterthwaite WH (2010) Competition for space can drive the evolution of dormancy in a temporally invariant environment. Plant Ecol 208:167–185. https://doi.org/10.1007/s11258-009-9696-y

Seabloom EW, Harpole WS, Reichman OJ, Tilman D (2003) Invasion, competitive dominance, and resource use by exotic and native California grassland species. Proc Natl Acad Sci 100:13384–13389. https://doi.org/10.1073/pnas.1835728100

Simons AM (2011) Modes of response to environmental change and the elusive empirical evidence for bet hedging. Proc R Soc B Biol Sci 278:1601–1609. https://doi.org/10.1098/rspb.2011.0176

Skálová H, Moravcová L, Čuda J, Pyšek P (2019) Seed-bank dynamics of native and invasive *Impatiens* species during a five-year field experiment under various environmental conditions. NeoBiota 50:75–95. https://doi.org/10.3897/neobiota.50.34827

Tabassum S, Leishman MR (2018) Have your cake and eat it too: greater dispersal ability and faster germination towards range edges of an invasive plant species in eastern Australia. Biol Invasions 20:1199–1210. https://doi.org/10.1007/s10530-017-1620-0

Takeno K (2016) Stress-induced flowering: the third category of flowering response. J Exp Bot 67:4925–4934. https://doi.org/10.1093/jxb/erw272

Therneau T (2022a) coxme: Mixed Effects Cox Models

Therneau T (2022b) A package for survival analysis in R

Therneau T (2023) A package for survival analysis in R

Thompson JN (1998) Rapid evolution as an ecological process. Trends Ecol Evol 13:329–332. https://doi.org/10.1016/S0169-5347(98)01378-0

Thong H-Y, Yokota M, Kardassakis D, Maibach HI (2007) Allergic contact dermatitis from *Dittrichia graveolens* (L.) Greuter (stinkwort). Contact Dermatitis 58:51–53. https://doi.org/10.1111/j.1600-0536.2007.01154.x

Toscano S, Romano D, Tribulato A, Patanè C (2017) Effects of drought stress on seed germination of ornamental sunflowers. Acta Physiol Plant 39:184. https://doi.org/10.1007/s11738-017-2484-8

Travis JMJ, Dytham C (2002) Dispersal evolution during invasions. Evol Ecol Res 4:1119–1129

Travis JMJ, Mustin K, Benton TG, Dytham C (2009) Accelerating invasion rates result from the evolution of density-dependent dispersal. J Theor Biol 259:151–158. https://doi.org/10.1016/j.jtbi.2009.03.008

Trombulak SC, Frissell CA (2000) Review of ecological effects of roads on terrestrial and aquatic communities. Conserv Biol 14:18–30. https://doi.org/10.1046/j.1523-1739.2000.99084.x

Turner KG, Hufbauer RA, Rieseberg LH (2014) Rapid evolution of an invasive weed. New Phytol 202:309–321. https://doi.org/10.1111/nph.12634

Tyser RW, Worley CA (1992) Alien flora in grasslands adjacent to road and trail corridors in Glacier National Park, Montana (U.S.A.). Conserv Biol 6:253–262. https://doi.org/10.1046/j.1523-1739.1992.620253.x

Ureta MS, Carrera AD, Cantamutto MA, Poverene MM (2008) Gene flow among wild and cultivated sunflower, *Helianthus annuus* in Argentina. Agric Ecosyst Environ 123:343–349. https://doi.org/10.1016/j.agee.2007.07.006

USDA (2013) Weed risk assessment for *Dittrichia graveolens* (L.) Greuter (Asteraceae). Animal and Plant Health Inspection Service, Plant Protection and Quarantine

van Boheemen LA, Atwater DZ, Hodgins KA (2019) Rapid and repeated local adaptation to climate in an invasive plant. New Phytol 222:614–627. https://doi.org/10.1111/nph.15564

van Kleunen M, Fischer M (2008) Adaptive rather than non-adaptive evolution of *Mimulus guttatus* in its invasive range. Basic Appl Ecol 9:213–223. https://doi.org/10.1016/j.baae.2007.03.006

van Kleunen M, Schmid B (2003) No evidence for an evolutionary increased competitive ability in an invasive plant. Ecology 84:2816–2823. https://doi.org/10.1890/02-0494

Venable DL, Brown JS (1988) The selective interactions of dispersal, dormancy, and seed size as adaptations for reducing risk in variable environments. Am Nat 131:360–384. https://doi.org/10.1086/284795

Vitousek PM, D’Antonio CM, Loope LL, et al (1997) Introduced species: a significant component of human-caused global change. N Z J Ecol 21:17

Von Der Lippe M, Kowarik I (2007) Long-distance dispersal of plants by vehicles as a driver of plant invasions. Conserv Biol 21:986–996. https://doi.org/10.1111/j.1523-1739.2007.00722.x

Walker K (2024) tigris: Load Census TIGER/Line Shapefiles

Wamelink GWW, van Dobben HF, Goedhart PW, Jones-Walters LM (2018) The role of abiotic soil parameters as a factor in the success of invasive plant species. Emerg Sci J 2:. http://dx.doi.org/10.28991/esj-2018-01155

Ward SF, Taylor BS, Dixon Hamil K-A, et al (2020) Effects of terrestrial transport corridors and associated landscape context on invasion by forest plants. Biol Invasions 22:3051–3066. https://doi.org/10.1007/s10530-020-02308-3

Weiss SB (1999) Cars, Cows, and Checkerspot Butterflies: Nitrogen Deposition and Management of Nutrient-Poor Grasslands for a Threatened Species. Conserv Biol 13:1476–1486. https://doi.org/10.1046/j.1523-1739.1999.98468.x

Western Regional Climate Center (2024) Younger Lagoon Reserve California. In: West. Reg. Clim. Cent. Accessed 29 Aug 2024

Whitney KD, Gabler CA (2008) Rapid evolution in introduced species, ‘invasive traits’ and recipient communities: challenges for predicting invasive potential. Divers Distrib 14:569–580. https://doi.org/10.1111/j.1472-4642.2008.00473.x

Whittaker RH (1954) The ecology of serpentine soils. Ecology 35:258–288. https://doi.org/10.2307/1931126

Williams JL, Kendall BE, Levine JM (2016) Rapid evolution accelerates plant population spread in fragmented experimental landscapes. Science 353:482–485. https://doi.org/10.1126/science.aaf6268

Williamson M, Fitter A (1996) The varying success of invaders. Ecology 77:1661–1666. https://doi.org/10.2307/2265769

Yuan Y, Wang B, Zhang S, et al (2013) Enhanced allelopathy and competitive ability of invasive plant Solidago canadensis in its introduced range. J Plant Ecol 6:253–263. https://doi.org/10.1093/jpe/rts033

Zenni RD, Bailey JK, Simberloff D (2014) Rapid evolution and range expansion of an invasive plant are driven by provenance–environment interactions. Ecol Lett 17:727–735. https://doi.org/10.1111/ele.12278