

UNIVERSIDAD DE CHILE

Facultad de Ciencias Forestales y de la Conservación de la Naturaleza
Magíster en Áreas Silvestres y Conservación de la Naturaleza

**GENERACIÓN DE UNA COMBINACIÓN DE PLANTAS PARA LA REHABILITACIÓN
ECOLÓGICA INICIAL DEL BOSQUE MEDITERRÁNEO DE CHILE**

Proyecto de grado presentado como parte de los requisitos para optar al grado de Magíster en Áreas Silvestres y Conservación de la Naturaleza.

ÁLVARO JAVIER PLAZA VALENCIA
Bioquímico y Licenciado en Bioquímica

SANTIAGO - CHILE

2019

Proyecto de Grado presentado como parte de los requisitos para optar al grado de Magíster en Áreas Silvestres y Conservación de la Naturaleza.

Profesora Guía

Nombre: Paulette Ivonne Naulin Gysling

Nota:

Firma:

Profesor Guía

Nombre: Miguel Eduardo Castillo Soto

Nota:

Firma:

Profesora Consejera

Nombre: Karen Angélica Peña Rojas

Nota:

Firma:

Profesor Consejero

Nombre: Álvaro Andrés Promis Baeza

Nota:

Firma:

DEDICATORIA

Dedico esta investigación a los cientos de jóvenes semillas y plántulas que vieron sus longevos y prometedores futuros truncados de la forma más inhumana en aras de la ciencia.

A ellas les pido perdón de todo corazón por el dolor y el sufrimiento que debieron experimentar a tan corta edad.

Les aseguro que fue por un bien mayor y que haré cuanto esté en mi mano para garantizar que estos resultados vayan en directo beneficio de tantas semillas, plántulas, hierbas, arbustos y árboles como sea posible.

Vuestro noble sacrificio nunca yacerá en el olvido.

AGRADECIMIENTOS

A mi familia, por apoyarme durante todo este tiempo.

A mis profesores guías, por ayudarme a ver el verdadero lado humano de la conservación.

A mi profesora de tiro con arco, por ayudarme a encontrar la serenidad para terminar esta investigación.

Y a mis amigos, por escucharme pacientemente hablar de mi investigación y fingir que entienden.

A todos ellos, GRACIAS.

ÍNDICE

RESUMEN	7
INTRODUCCIÓN	9
Ecología del fuego en climas mediterráneos	9
Cambios en los ecosistemas e invasiones biológicas	11
Las interacciones sostienen al ecosistema	12
Formulación de nuevas estrategias basadas en la interacción	13
Especies utilizadas	13
Objetivos	14
Bibliografía	15
GERMINATION RATES OF FOUR CHILEAN FOREST TREES SEEDS: <i>QUILLAJA SAPONARIA</i> , <i>PROSOPIS CHILENSIS</i> , <i>VACHELLIA CAVEN</i> , AND <i>CAESALPINIA SPINOSA</i>	18
Abstract	18
Keywords	18
Introduction	18
Methods	19
Data availability	21
Competing interests	21
Grant information	21
References	22
SEED AND SEEDLING INTERACTIONS IN FOUR TREE SPECIES FROM MEDITERRANEAN FORESTS	23
Abstract	23
Keywords	24
Highlights	24
Introduction	24
Materials and methods	25
Results	29
Discussion	34
Acknowledgements	37
References	37
CONCLUSIONES	42

Lista de Figuras

Figure 1. Time taken for 50% of seeds to germinate (TG50) for each species	21
Figure 1. RII values calculated from germination data of target species on day sixth	31
Figure 2. RII values calculated from seedling length of target species on day sixth	32
Figure 3. Survivorship of seedlings of target species growing in mono-specific pots or in mixtures with different species (day 27th)	33
Figure 4. Ideal representation of the interactions detected between the four species of this study ...	34

Lista de Tablas

Table 1. Percentage of germinated seeds of <i>Q. saponaria</i> and <i>P. chilensis</i> incubated for 19 days ..	20
Table 2. Percentage of germinated seeds of <i>V. caven</i> and <i>C. spinosa</i> incubated for 22 days ...	20
Table 1. Germinating fractions of each target species at day three and day sixth	30
Table 2. Average seedling length (cm) of each target species at day sixth	32

RESUMEN

En el mundo existen cinco regiones de clima templado mediterráneo, y una de ellas corresponde a la zona central de Chile. Como todas las regiones mediterráneas, esta zona se caracteriza por su alta biodiversidad y por su alta degradación antropogénica, principalmente producto de incendios forestales.

Con la finalidad de generar nuevas estrategias para la restauración de los biomas de esta zona, se buscó una combinación de especies arbóreas nativas que permitiera acelerar la regeneración del sustrato y facilitar el establecimiento de otras especies vegetales.

Para ello se seleccionaron cuatro especies arbóreas, basándose en dos criterios: 1) que estuviesen presentes en la zona mediterránea de Chile y 2) que fueran de germinación relativamente rápida. Las especies escogidas fueron *Acacia caven*, *Caesalpinia spinosa*, *Prosopis chilensis* y *Quillaja saponaria*.

En primer lugar, estas especies fueron ensayadas para determinar sus parámetros de germinación, encontrando que *A. caven*, *C. spinosa*, *P. chilensis* y *Q. saponaria* tenían tiempos de germinación medios (TG50) de 3,9 días, 25,8 días, 1,7 días y 4,9 días, respectivamente. A pesar de que *C. spinosa* tiene una germinación mucho más lenta que las otras especies, se optó por mantener la especie en la investigación.

Luego, se procedió a analizar cómo respondían las semillas de cada una de estas especies a la presencia de semillas de las otras especies, diferenciando el efecto de estas otras semillas cuando estaban latentes (mediante extractos de tegumento y testa), de cuando estaban germinadas (mediante extractos de cotiledones y embriones). Gracias a esto se encontraron una serie de alelopatías y facilitaciones, demostrando 1) que individuos no establecidos son capaces de interactuar activamente con otros organismos y 2) las afinidades u hostilidades entre unas y otras especies. Esto último es especialmente importante a la hora de proponer combinaciones de especies para reforestación o restauración de ecosistemas degradados.

Finalmente, se comparó la sobrevivencia y crecimiento en condiciones post-incendio de cada una de estas especies en dos situaciones, 1) cuando se encontraban creciendo en forma pura (control) o bien 2) en combinación con las otras especies (tratamientos). Se encontró que muchas

de las interacciones observadas entre las semillas se perpetuaban durante la etapa de plántula, aún en aquellos casos en que el individuo efector había muerto.

De los resultados de estos ensayos se pudo concluir que la combinación de *A. caven* y *P. chilensis* presenta las mejores características para la restauración, debido a que estas especies tienen menor TG50, presentan facilitaciones durante el estado de semilla, y producen un incremento mutuo de la sobrevivencia al crecer en combinación.

INTRODUCCIÓN

Ecología del fuego en climas mediterráneos

Para algunos ecosistemas, la falta de disturbios puede limitar la diversidad de especies; es necesario que los disturbios estén presentes a una escala tal que no sean estresantes pero tampoco demasiado infrecuentes (Odion y Sarr, 2007). Por ejemplo, en las regiones de clima templado mediterráneo la vegetación se ha adaptado para responder a regímenes de incendios y/o sequías prolongadas, y muchas plantas han evolucionado desarrollando relaciones específicas y muy estrechas con las condiciones dadas por el fuego y las altas temperaturas, como la floración y liberación de semillas estimulada por fuego, o la activación del banco de semillas del suelo por choque térmico, o por los compuestos químicos del humo o de la madera carbonizada (Montenegro et al., 2004; He et al., 2016).

Además, un fuego intenso expone nutrientes minerales que proveen una buena cama para las semillas (Moench, 2002; Reinhart et al., 2016), y abre espacios en el dosel permitiendo la aparición de plantas que normalmente no podrían crecer bajo los árboles, por ser intolerantes a la sombra.

Finalmente, existen variedades de insectos y hongos que infectan y se alimentan específicamente de plantas que murieron en un incendio, o de las que sobrevivieron al incendio pero resultaron dañadas (siendo este daño la “puerta de entrada” que las hace susceptibles) (Leatherman y Aguayo, 2007). Todos estos cambios en la abundancia y diversidad de especies regulados por el fuego, son parte de la ecología del fuego (Granados-Sánchez y López-Ríos, 1998).

El hombre ha alterado la ecología del fuego (Dennis, 2004 y 2007); ha cambiado la recurrencia, la intensidad y la extensión de los incendios, impidiendo que la vegetación nativa pueda recuperarse a tiempo; llevando a cambios en el patrón de la vegetación y volviendo al sistema vulnerable a las invasiones biológicas (Montenegro et al., 2004; Syphard et al., 2009; Bowman et al., 2011).

Esto se debe a que al aumentar el contacto humanidad-bosque aumenta el riesgo de incendios. Este problema es particularmente importante en las regiones de clima templado mediterráneo, que son las que sufren la mayor presión antrópica. Pero no sólo eso. También los patrones de sequías se ven alterados, ya que el hombre tiende a regular el flujo de agua mediante represas

y otros sistemas, haciéndolo más constante. En regiones como la zona central de Chile, que en su contexto natural era más propensa a las sequías que a los incendios, la mayoría de las especies vegetales desarrollaron mecanismos de regeneración por rebrote (Montenegro et al., 2004). En condición de sequía, el rebrote puede ser mucho más importante que la regeneración por semillas (Ferreira y Vieira, 2017); sin embargo, en condiciones post-incendio, esta estrategia podría ser menos eficiente que la regeneración por semillas de plantas introducidas, provenientes de otros ecosistemas mediterráneos, y que sí evolucionaron en adaptación a eventos más recurrentes de fuego. Por ejemplo, se ha visto que en las áreas quemadas, las semillas de *Pinus* afectadas por el fuego mejoraban su germinación y supervivencia (Sagra et al., 2018), mientras que las plantas chilenas en general carecen de adaptaciones específicas para la propagación en contexto de fuego, como floración estimulada por incendios o germinación estimulada por los compuestos del humo y las cenizas (Montenegro et al., 2004). Es por esto que al disminuir la ocurrencia de sequías y aumentar la incidencia de incendios, se favorece la colonización y propagación de especies exóticas como *Pinus* sp., cuya liberación de semillas se encuentra estimulada por el calor, además de que las condiciones generadas por el incendio cumplen un rol fundamental en la dispersión de sus semillas (Van Wilgen y Siegfried, 1986; Sagra et al., 2018).

Para enfrentar esta situación, se requiere de nuevas estrategias que permitan acelerar la regeneración de los bosques nativos luego de los incendios forestales, para evitar que las especies exóticas puedan ocupar los espacios que abre el fuego, y reparar el sustrato para que pueda sustentar a las especies originales. Esto se torna aún más urgente en el contexto del cambio climático, debido a que las regiones mediterráneas se tornan cada vez más secas, aumentando la disponibilidad de combustible y la intensidad de los incendios (Lee et al., 2015).

Particularmente en el contexto chileno, los megaincendios ocurridos en enero y febrero de 2017, precisamente en la región mediterránea del país, arrasaron con cientos de miles de hectáreas, de las cuales 187.906 Ha correspondían a matorrales nativos y 60.995 Ha a bosques nativos (de la Barrera et al., 2018). En respuesta al desastre ecológico, ambiental, social y económico que significaron estos incendios, la Corporación Nacional Forestal, que administra la mayoría de áreas silvestres protegidas del estado, recibió un aumento presupuestario (Gobierno de Chile), y se inició la tramitación del proyecto de ley que crea el Servicio Nacional Forestal y modifica la Ley General de Urbanismo y Construcciones. Esta ley tendrá por objeto, entre otros, la conservación, preservación y restauración de las formaciones vegetacionales del país, con especial atención a

la restauración de los bosques afectados por incendios (Boletín N° 11.175-01), lo cual conlleva la inherente necesidad de generar conocimiento en cuanto a mejores y más eficientes formas de llevar a cabo la restauración de los bosques mediterráneos en el contexto particular post-fuego.

Cambios en los ecosistemas e invasiones biológicas

En el pasado, han ocurrido eventos de fuego que han permitido a ecosistemas transitar desde un estado estable a otro, alcanzándose un sistema tal que la transición natural al estado anterior es imposible aún a escala geológica. Hablamos por ejemplo, de transiciones de bosque templado hacia humedales cubiertos de hierba (Fletcher et al., 2014).

Las condiciones que deben darse para este tipo de cambios, involucran (1) un disturbio lo suficientemente fuerte para mermar las poblaciones de aquellas especies que permiten que el ecosistema se autosustente, (2) la llegada de especies que inician el cambio de las condiciones bióticas y abióticas del sistema, hacia un estado estable alternativo, y (3) el establecimiento del nuevo ensamble de especies de manera tal que pueda autosustentarse a través del tiempo. En este contexto, si las respuestas de un ecosistema a los disturbios, por ejemplo de origen antrópico (condición 1), modifican la composición y funcionalidad (condición 2), estaríamos ante el colapso del estado original y el posible establecimiento de un nuevo estado, que podemos entender como degradado (condición 3) (Fletcher et al., 2014; Keith et al., 2015).

Después de los megaincendios registrados en Chile el 2017, la condición 1 podría estarse cumpliendo en las regiones más afectadas. Destaca en el planteamiento anterior, el rol que juega el establecimiento de nuevas especies, especies adventicias, funcionalmente distintas, en el cumplimiento de la segunda condición para que el cambio se vuelva irreversible.

Sobre la probabilidad de que una especie exótica se vuelva invasora, se suele hablar de la “regla de los 10”, de que sólo el 10% de las especies exóticas llegan a establecerse, y de las que se establecen, sólo el 10% se vuelven invasivas. Sin embargo, recientemente se ha puesto en entredicho esta “regla”; es muy probable que la cantidad de especies invasoras establecidas y su impacto se encuentren subestimados debido a nuestra falta de conocimiento (Jarić y Cvijanović, 2012).

Así, la competencia post-fuego podría cambiar la composición florística del paisaje (Moench, 2002). Debido a los efectos que pueden tener estas interacciones interespecíficas en que la

sucesión post-fuego lleve al sistema a un estado similar a lo que había antes del disturbio (regeneración) o bien, derive en un estado estable alternativo (cambio ecosistémico irreversible), es que cobra vital importancia conocer cómo las plantas interactúan durante las primeras etapas de la sucesión.

Las interacciones sostienen al ecosistema

Existe controversia acerca de si la diversidad de especies en un ecosistema es la que le otorga estabilidad ante los disturbios, o si acaso es la estabilidad del sistema (la baja frecuencia de disturbios) la que permite que la comunidad se vuelva diversa; sin embargo, algunos autores han propuesto, en base a modelos computacionales, que si bien diversidad y estabilidad tienden a darse juntas, no sería una la razón directa de la otra, sino que es la diversidad de relaciones interespecíficas (sólo posible, pero no *necesariamente* presente, en comunidades diversas) la que le otorga estabilidad al ecosistema (McCann, 2000). Por ejemplo, los sistemas en los que muchas de las relaciones sólo se dan entre dos especies particulares, son mucho más propensos a las extinciones secundarias (extinciones de una segunda especie debido a la extinción de una primera con la que mantenían una relación específica e irremplazable) que sistemas en que muchas especies pueden cumplir un rol determinado en una relación dada (Dunn et al., 2009).

Si la estabilidad de un sistema depende de las interacciones entre sus componentes, entonces es lógico que restaurar un ecosistema va a ser más fácil con especies que interactúen positivamente entre ellas y con las remanentes del sistema original, que con especies que no tengan interacciones entre ellas o con las especies locales, o que compitan entre ellas o con las especies locales.

Es por ello que muchas veces se opta por hacer uso de combinaciones en lugar de especies aisladas. Cuando se hace referencia a una combinación, ensamble, o consorcio, se está hablando de un conjunto de dos o más especies de organismos, generalmente de microorganismos o plantas, que se benefician mutuamente. Estas combinaciones pueden ser naturales o bien diseñadas por el hombre con un propósito específico; por ejemplo, hay numerosas combinaciones microbianas (Mukred et al., 2008) y vegetales (Kabra et al., 2011) que se ocupan en el campo de la biorremediación.

Formulación de nuevas estrategias basadas en la interacción

Como se revisó anteriormente, es necesario buscar nuevas estrategias para la restauración de los bosques mediterráneos del país, especialmente aquellos que han sido afectados por incendios forestales. Estas estrategias deben ser capaces de aprovechar y reforzar las interacciones biológicas de la comunidad afectada para potenciar la regeneración.

Por ello, planteamos determinar una combinación de plantas que permita acelerar la rehabilitación del sustrato y el establecimiento de plantas nativas en la zona mediterránea de Chile, en sectores que han tenido eventos recientes de incendios forestales, tanto para prevenir el establecimiento de especies exóticas dañinas (por ocupación del sitio), como para facilitar el re-establecimiento de las especies arbustivas y arbóreas.

Para esto se buscaron especies nativas que favorecieran la colonización de otras nativas. Nuestro foco de estudio fue el tipo forestal de Matorral y Bosque Esclerófilo, subtipos Matorral y Bosque Espinoso, y Bosque Esclerófilo, por lo que la búsqueda de especies se limitó a estas asociaciones.

Las características deseables en estas especies fueron que se dispersasen por semillas, ya que sembrar bulbos u otro tipo de propágulos conlleva una dificultad adicional, que fueran de rápida germinación, que se favorecieran unas a otras, y que fueran pioneras post-fuego.

Especies utilizadas

Se seleccionaron como base las siguientes especies:

- Espino (*Acacia caven*, Fabaceae)*
- Tara (*Caesalpinia spinosa*, Fabaceae)
- Algarrobo (*Prosopis chilensis*, Fabaceae)
- Quillay (*Quillaja saponaria*, Quillajaceae)

Estas especies corresponden a varias de las especies nativas arbustivas de bosque esclerófilo de germinación más rápida, según especialistas en semillas del CESAF de la Universidad de Chile (comunicación personal).

* En uno de los artículos, se nombra por el sinónimo *Vachellia caven*. Esto se debe a que la especie sufrió un cambio de nombre en el periodo en que dicho artículo fue publicado.

Al respecto de las especies mismas, se puede decir que *A. caven* y *P. chilensis* son las especies que delimitan el subtipo de Matorral y Bosque Espinoso (Gajardo, 1994).

Además, incluir en el estudio a *P. chilensis* tiene un valor de conservación adicional, puesto que esta especie se encuentra Casi Amenazada según la UICN, Vulnerable según la legislación chilena, y próxima a extinguirse en forma silvestre, según estudios recientes (Valdivia y Romero, 2013).

Si bien las poblaciones de *C. spinosa* se concentran más hacia el norte, el límite sur de su distribución se encuentra en la Región del Maule (GBIF 2019). Su presencia natural aunque ocasional en la región mediterránea, la convierten en una clase de *outgroup* para efectos comparativos: se encuentra naturalmente en la zona, pertenece a la misma familia que *A. caven* y *P. chilensis*, pero no es representativa de ninguna formación vegetal de la región y es poco probable que su evolución haya sido condicionada por sus interacciones con las demás especies estudiadas.

Por su parte, *Q. saponaria* ha sido descrita como una de las especies objetivo por Pérez-Quezada y Bown (2015) en la restauración del subtipo Bosque Esclerófilo, y se puede presentar en los mismos lugares que *A. caven*, aunque en distintas etapas sucesionales.

Objetivos

Objetivo General

Diseñar una combinación de especies arbóreas nativas que propicien la rehabilitación inicial de los bosques mediterráneos chilenos (de tipo Matorral y Bosque Esclerófilo) afectados por incendios forestales.

Objetivos Específicos

- a) Identificar los tipos de interacciones que se presentan entre las semillas de algunas de las especies de más rápida germinación.
- b) Identificar los tipos de interacciones que se presentan entre las plántulas de dichas especies cuando se desarrollan sobre sustrato quemado.
- c) Comparar la sobrevivencia de estas especies al desarrollarse sobre suelo quemado.

Bibliografía

- BOLETÍN N° 11.175-01. Proyecto De Ley, En Segundo Trámite Constitucional, Que Crea El Servicio Nacional Forestal Y Modifica La Ley General De Urbanismo Y Construcciones. CHILE.
- BOWMAN, D., BALCH, J., ARTAXO, P., BOND, W., COCHRANE, M., D'ANTONIO, C., DEFRIES, R., JOHNSTON, F., KEELEY, J., KRAWCHUK, M., KULL, C., MACK, M., MORITZ, M., PYNE, S., ROOS, C., SCOTT, A., SODHI, N. y SWETNAM, T. 2011. The human dimension of fire regimes on Earth. *Journal of Biogeography* 38:2223-2236.
- DE LA BARRERA, F., BARRAZA, F., FAVIER, P., RUIZ, V. y QUENSE, J. 2018. Megafires in Chile 2017: Monitoring multiscale environmental impacts of burned ecosystems. *Science of the Total Environment* 637-638(2018):1526-1536.
- DENNIS, F. 2004. Fire-Resistant Landscaping. USDA Natural Resources Conservation Service's Fact Sheet no. 6.303.
- DENNIS, F. 2007. Forest Home Fire Safety. USDA Natural Resources Conservation Service's Fact Sheet no. 6.304.
- DUNN, R., HARRIS, N., COLWELL, R., KOH, L. y SODHI, N. 2009. The sixth mass coextinction: are most endangered species parasites and mutualists? *Proceedings of the Royal Society B* 276:3037-3045.
- FERREIRA, M. y VIEIRA, D. 2017. Topsoil for restoration: Resprouting of root fragments and germination of pioneers trigger tropical dry forest regeneration. *Ecological Engineering* 103:1-12.
- FLETCHER, M., WOOD, S. y HABERLE, S. 2014. A fire-driven shift from forest to non-forest: evidence for alternative stable states? *Ecology* 95(9):2504-2513.
- GAJARDO, R. 1994. La vegetación natural de Chile. Clasificación y distribución geográfica. Editorial Universitaria, Santiago.
- GBIF.ORG (27 May 2019) GBIF Occurrence Download <https://doi.org/10.15468/dl.ilorcs>
- GOBIERNO DE CHILE. Estrategia contra los incendios forestales: un paso decisivo para proteger los bosques de Chile. 2017 [en línea] <<https://www.gob.cl/noticias/estrategia-contra-los-incendios-forestales-un-paso-decisivo-para-proteger-los-bosques-de-chile/>> [consulta: 30 mayo 2019]
- GRANADOS-SÁNCHEZ, D. y LÓPEZ-RÍOS, G. 1998. Ecología del Fuego. *Revista Chapingo Sene Ciencias Forestales y del Ambiente* 4(1):193-206.
- HE, T., LAMONT, B. y MANNING, J. 2016. A Cretaceous origin for fire adaptations in the Cape flora. *Scientific Reports* 6:34880.

- JARIĆ, I. y CVIJANOVIĆ, G. 2012. The Tens Rule in Invasion Biology: Measure of a True Impact or Our Lack of Knowledge and Understanding? *Environmental Management* 50:979-981.
- KABRA, A., KHANDARE, R., WAGHMODE, T. y GOVINDWAR, S. 2011. Differential fate of metabolism of a sulfonated azo dye Remazol Orange 3R by plants *Aster amellus* Linn., *Glandularia pulchella* (Sweet) Tronc. and their consortium. *Journal of Hazardous Materials* 190:424-431.
- KEITH, D., RODRÍGUEZ, J., BROOKS, T., BURGMAN, M., BARROW, E., BLAND, L., COMER, P., FRANKLIN, J., LINK, J., MCCARTHY, M., MILLER, R., MURRAY, N., NEL, J., NICHOLSON, E., OLIVEIRA-MIRANDA, M., REGAN, T., RODRÍGUEZ-CLARK, K., ROUGET, M. y SPALDING, M. 2015. The IUCN Red List of Ecosystems: Motivations, Challenges, and Applications. *Conservation Letters* 8(3):214-226.
- LEATHERMAN, D. y AGUAYO, I. 2007. Insects and Diseases Associated with Forest Fires. USDA Natural Resources Conservation Service's Fact Sheet no. 6.309.
- LEE, C., SCHLEMME, C., MURRAY, J. y UNSWORTH, R. 2015. The cost of climate change: Ecosystem services and wildland fires. *Ecological Economics* 116:261-269.
- MCCANN, K. 2000. The diversity–stability debate. *Nature* 405:228-233.
- MOENCH, R. 2002. Vegetative Recovery after Wildfire. USDA Natural Resources Conservation Service's Fact Sheet no. 6.307.
- MONTENEGRO, G., GINOCCHIO, R., SEGURA, A., KEELY, J. y GÓMEZ, M. 2004. Regímenes de incendios y respuestas de la vegetación en dos regiones de clima Mediterráneo. *Revista Chilena de Historia Natural* 77:455-464.
- MUKRED, A., HAMID, A., HAMZAH, A. y YUSOFF, W. 2008. Development of Three Bacteria Consortium for the Bioremediation of Crude Petroleum-oil in Contaminated Water. *OnLine Journal of Biological Sciences* 8(4):73-79.
- ODION, D. y SARR, D. 2007. Managing disturbance regimes to maintain biological diversity in forested ecosystems of the Pacific Northwest. *Forest Ecology and Management* 246:57-65.
- PÉREZ-QUEZADA, J. y BOWN, H. (Eds.). 2015. Guía para la restauración de los ecosistemas andinos de Santiago. Santiago, Universidad de Chile-CONAF. 115 p.
- REINHART, K., DANGI, S. y VERMEIRE, L. 2016. The effect of fire intensity, nutrients, soil microbes, and spatial distance on grassland productivity. *Plant and Soil* 409(1-2):203-216.
- SAGRA, J., FERRANDIS, P., PLAZA-ÁLVAREZ, P., LUCAS-BORJA, M., GONZÁLEZ-ROMERO, J., ALFARO-SÁNCHEZ, R., DE LAS HERAS, J. y MOYA, D. 2018. Regeneration of *Pinus*

- pinaster* Aiton after prescribed fires: Response to burn timing and biogeographical seed provenance across a climatic gradient. *Science of the Total Environment* 637-638:1550-1558.
- SYPHARD, A., RADELOFF, V., HAWBAKER, T. y STEWART, S. 2009. Conservation Threats Due to Human-Caused Increases in Fire Frequency in Mediterranean-Climate Ecosystems. *Conservation Biology* 23(3):758-769.
- VALDIVIA, C. y ROMERO, C. 2013. En la senda de la extinción: el caso del algarrobo *Prosopis chilensis* (Fabaceae) y el bosque espinoso en la Región Metropolitana de Chile central. *Gayana Botánica* 70(1):57-65.
- VAN WILGEN, B. y SIEGFRIED, W. 1986. Seed dispersal properties of three pine species as a determinant of invasive potential. *South African Journal of Botany* 52(6):546-548.

GERMINATION RATES OF FOUR CHILEAN FOREST TREES SEEDS: *QUILLAJA SAPONARIA*, *PROSOPIS CHILENSIS*, *VACHELLIA CAVEN*, AND *CAESALPINIA SPINOSA*

Alvaro Plaza¹, Miguel Castillo¹

¹Universidad de Chile.

Corresponding author

Alvaro Plaza (alvaro.plaza@ug.uchile.cl)

Published in F1000Research 2018, 7:1446,
(<https://doi.org/10.12688/f1000research.16091.1>).

Abstract

Data on the germination rates of four tree species, natively founded in the Chilean Mediterranean-climate zone, were determined by germination in crop chambers. The obtained data were used to interpolate or extrapolate the time taken for 50% of seeds to germinate in each case. These results are useful for regional native forest research and, in a broad sense, for its use in models to study germination dynamics in Mediterranean-climate zones.

Keywords

germination; native forest; Mediterranean-climate zone

Introduction

Knowledge of the germination rates of a species means that future determination of this rate is unnecessary, preventing the waste of time and seeds.

Quillaja saponaria and *Vachellia caven* are two of the most representative trees in the Chilean Mediterranean forest (Perez-Quezada & Bown, 2015), so information about these species will be useful for ecological investigation and restoration. *Prosopis chilensis* is vulnerable in the wild and is a key species of its community (Valdivia & Romero, 2013); data about its propagation is important for conservation biologists.

In this article, we present the germination rates of seeds of *Q. saponaria*, *P. chilensis*, *V. caven*, and *Caesalpinia spinosa*. Dataset 1 contains the raw data from which these germination rates are calculated (Plaza & Castillo, 2018).

Methods

Samples

All seeds were collected from adult trees. *Q. saponaria* seeds were collected in VIII Región, Chile; seeds from *V. caven*, *C. spinosa* and *P. chilensis* were from Región Metropolitana, Chile. The seeds were collected between February and April 2017. Information about collection was obtained from the seed provider, CESAF Antumapu, <http://cesaf.forestaluchile.cl/>.

Table 1 and Table 2 specify the initial number of seeds per plate and the percentage of germinated seeds in some days are shown. Figure 1 shows the obtained values of time taken for 50% of seeds to germinate (TG50).

Pretreatment

Pretreatment conditions were suggested by the provider. Briefly, seeds of *Q. saponaria* were hydrated in tap water overnight. Seeds of *P. chilensis* were scarified in 95-97%, analytical grade H₂SO₄ for 10 minutes and then hydrated in tap water overnight. Seeds of *V. caven* were scarified in 95-97%, analytical grade H₂SO₄ for 90 minutes and then hydrated in tap water overnight. Seeds of *C. spinosa* were scarified in 95-97%, analytical grade H₂SO₄ for 30 minutes and then hydrated in tap water overnight.

Germination

Activated seeds of *Q. saponaria*, *P. chilensis*, *V. caven*, and *C. spinosa* were placed in Petri plates over a filter paper bed (3 plates per species). Filter paper was then hydrated with distilled water. All plates were incubated in a crop chamber at 20°C, with light/dark cycles of 9 h/15 h. Germination is conditioned by temperature, so altering this factor could completely change the germination rates (Giuliani *et al.*, 2015).

Plates were monitored periodically to count the germinated seeds and refill distilled water. *Q. saponaria* and *P. chilensis* plates were monitored until day 19 (Table 1). After that, fungal development made it difficult to check the plates, and a tactile examination of seeds indicated that most of them were rotten.

Plates containing *V. caven* and *C. spinosa* were more resistant to contamination and could be monitored until day 22. After this point, germination was too slow, and it was decided to end the experiment. Results are shown in Table 2.

Table 1. Percentage of germinated seeds of *Q. saponaria* and *P. chilensis* incubated for 19 days.

	Initial seeds per plate, n	Seeds germinated, %				
		Day 0	Day 2	Day 5	Day 13	Day 19
<i>Q. saponaria</i> (n=3 plates)						
Average	100	0.0	0.0	52.0	68.3	68.6
Standard Error	10	0.0	0.0	4.3	5.4	5.6
<i>P. chilensis</i> (n=3 plates)						
Average	96	0.0	58.1	61.5	65.3	67.4
Standard Error	4	0.0	2.6	2.6	2.5	2.4

Table 2. Percentage of germinated seeds of *V. caven* and *C. spinosa* incubated for 22 days.

	Initial seeds per plate, n	Seeds germinated, %						
		Day 0	Day 2	Day 5	Day 7	Day 13	Day 19	Day 22
<i>V. caven</i> (n=3 plates)								
Average	56	0.0	20.0	66.6	69.9	71.7	74.2	74.2
Standard error	5	0.0	3.3	1.9	1.4	1.5	1.9	1.9
<i>C. spinosa</i> (n=3 plates)								
Average	61	0.0	0.0	17.1	32.5	44.8	48.0	48.5
Standard error	3	0.0	0.0	5.8	4.5	4.7	2.9	2.5

The sample size, provided in the tables, is considered important for the replicability of a germination assay (Ribeiro-Oliveira & Ranal, 2016).

TG50 calculation

For *Q. saponaria*, *P. chilensis* and *V. caven*, the TG50 was linearly interpolated from the two closest points (Figure 1A–C). *C. spinosa* didn't reach the 50% germination during the assay, so this was extrapolated using the last five points (Figure 1D). The TG50 of *Q. saponaria* was 4.9 days. *P. chilensis* had the fastest germination (TG50 = 1.7 days); *V. caven* had a TG50 of approximately 3.9 days, and the TG50 of *C. spinosa* was estimated to be 25.8 days.

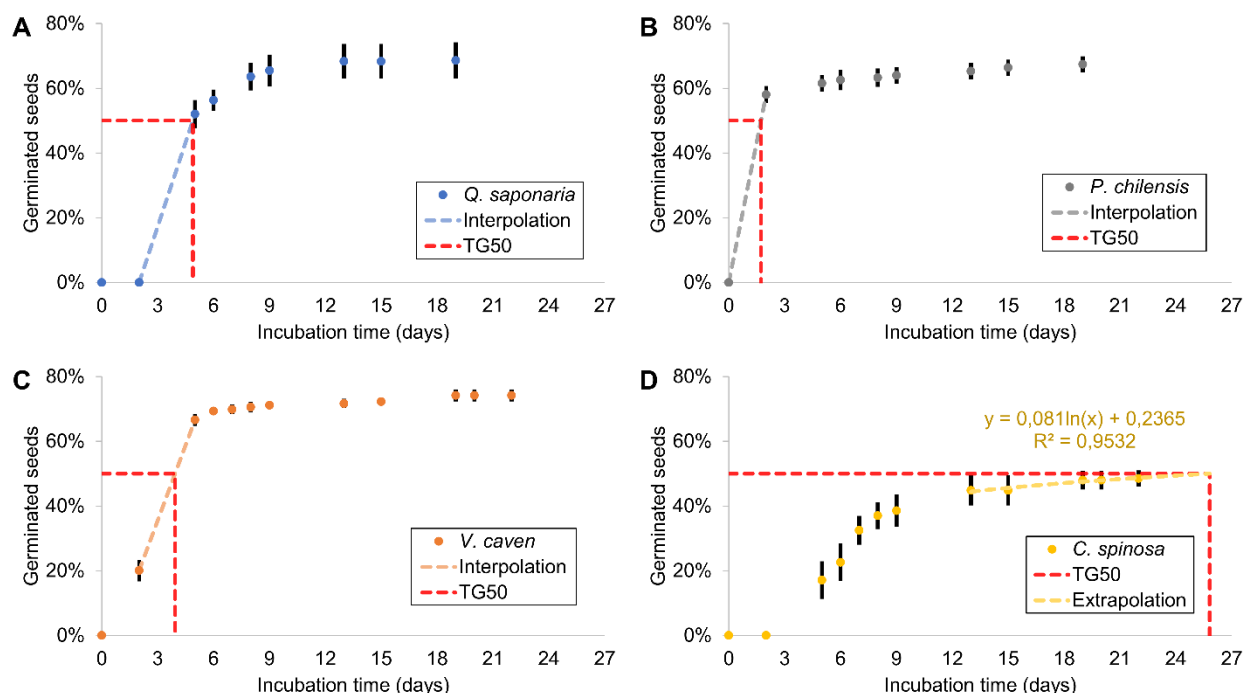


Figure 1. Time taken for 50% of seeds to germinate (TG50) for each species. Interpolation of *Q. saponaria* (A), *P. chilensis* (B) and *V. caven* TG50 (C), and extrapolation of *C. spinosa* TG50 (D).

Data availability

Dataset 1. Raw number of germinated seeds for each species, each repeat plate and each time point. Also included are cumulative number of germinated seeds, percentages of germinated seeds and calculation of the TG50 for each species. DOI: <https://doi.org/10.5256/f1000research.16091.d216429> (Plaza & Castillo, 2018).

Competing interests

No competing interests were disclosed.

Grant information

This work was supported by CONAF project 008/2016 "Pautas de terreno para la restauración de formaciones esclerófilas afectadas por incendios forestales. Regiones V, Metropolitana, VI y VII", and CONICYT-PCHA/MagísterNacional/2016 – 22161077.

The funders had no role in study design, data collection and analysis, decision to publish, or preparation of the manuscript.

References

Giuliani C, Lazzaro L, Mariotti Lippi M, *et al.*: Temperature-related effects on the germination capacity of black locust (*Robinia pseudoacacia* L., Fabaceae) seeds. *Folia Geobot.* 2015; 50(3): 275–282.

<http://doi.org/10.1007/s12224-015-9224-x>

Perez-Quezada J, Bown H: Guía para la restauración de los ecosistemas andinos de Santiago. Universidad de Chile-CONAF, Santiago, 2015.

<http://doi.org/10.13140/RG.2.1.3866.5441>

Plaza A, Castillo M: Dataset 1 in: Germination rates of four Chilean forest trees seeds: *Quillaja saponaria*, *Prosopis chilensis*, *Vachellia caven*, and *Caesalpinia spinosa*. F1000Research. 2018.

<https://doi.org/10.5256/f1000research.16091.d216429>

Ribeiro-Oliveira J, Ranal MA: Sample size in studies on the germination process. *Botany.* 2016; 94(2): 103–115.

<http://doi.org/10.1139/cjb-2015-0161>

Valdivia C, Romero C: En la senda de la extinción: el caso del algarrobo *Prosopis chilensis* (Fabaceae) y el bosque espinoso en la Región Metropolitana de Chile central. *Gayana Bot.* 2013; 70(1): 57–65.

<http://dx.doi.org/10.4067/S0717-66432013000100007>

SEED AND SEEDLING INTERACTIONS IN FOUR TREE SPECIES FROM MEDITERRANEAN FORESTS

Alvaro Plaza^a, Miguel Castillo^b & Paulette I. Naulin^a

^aLaboratorio Biología de Plantas, Universidad de Chile

^bLaboratorio de Incendios Forestales, Universidad de Chile

Corresponding author

Alvaro Plaza (alvaro.plaza@ug.uchile.cl)

Submitted to: Biodiversity and Conservation

Abstract

There is an increasing number of published studies on plant to plant interactions, with emphasis on adult individuals and mostly restricted to agricultural systems. The interactions between seeds and seedlings, as well as allelopathic interactions in natural communities, have both been largely neglected.

The objective of this study is to contribute to document interactions between seeds and seedlings from four forest species. We focused on the effects of seed extracts on germination and seed vigor and the interactions between seedlings growing together in monospecific and bispecific mixtures.

We exposed the seeds of these species to aqueous extracts of their seeds under lab conditions and measured germination rate and the growth of seedlings. In a second experiment we compared seedling survival and initial growth in monospecific vs bispecific mixtures.

Seed extracts promote germination in three cases and these effects are remarkably low. In five cases, the extracts inhibit germination of only one species, *Q. saponaria* with very high intensity. The effects of the extracts on seed vigor were more variable, including 12 significant cases. Only one case was positive. The negative effects included three cases of autotoxicity. Survival of seedlings growing in mixtures showed only three (out of 12) significant differences between mono-

and bispecific mixtures, all were positive, including a reciprocal effect. In only one case there was a significant difference in seedling initial growth, which was the inhibition of root growth.

The results disclosed a complex array of interactions between the four species. Type and intensity of the effects vary with the species, whether target or donor, or seed or seedling. The effects of seed extracts depend on tissue of origin and process affected. We interpret these interactions as allelopathic in nature and conclude that our results may help to open a window of innovation in this direction.

Keywords

plant–plant interactions; allelopathy; germination; seed vigor; growth; Mediterranean forest.

Highlights

- Seeds can alter other seed's germination through chemical interactions.
- Seedlings of several tree species are mutually facilitated in postfire conditions.
- Interactions inside seed bank or among seedlings could be a new tool for restoration.

Introduction

Interactions between plant species are extremely important for the structuring and functioning of plant communities. They also play an important role in community dynamics, both in succession after disturbance or in maintenance of stable communities (Inouye & Stinchcombe, 2001; Lortie et al., 2004). Interactions may be negative, as competition, or positive as facilitation, although in successional communities both types of interactions may act together (Brooker & Callaghan, 1998; Callaway & Walker, 1997). In recent times, positive interactions, specially facilitation, have received increased attention, although the emphasis has been on the effect of adult individuals on the growth of saplings and juveniles (Brooker et al., 2007). Positive interactions between seeds and seedlings have received less attention.

Increasingly, studies of interactions between seeds and seedlings are unveiling influences mediated by allelopathic compounds (Kruse et al., 2000; Masum et al., 2018). In recent decades, these studies are focused on control of weeds and pests, and invasive species in agricultural systems (Byun et al., 2015; Thorpe et al., 2009). However, allelopathic interactions in natural systems are receiving increased attention (Barbosa et al., 2008; de Souza Nascimento et al., 2014; Keeling et al., 2018). Allelopathy is considered important in forest management, in particular

interactions involving germination and growth (Pellissier & Souto, 1999; Reigosa & González, 2006). Furthermore, it is considered relevant for restoration programs following perturbations, especially those from wildfires (Caldararo, 2002; Syphard et al., 2009).

Several previous studies on Mediterranean tree species have focused on plant facilitation, particularly in relation to succession. *Acacia caven* has been found to facilitate establishment of four sclerophyllous tree species: *Quillaja saponaria*, *Maytenus boaria*, *Schinus latifolius*, and *Cryptocarya alba* (Root-Bernstein et al., 2017). Other studies have shown similar effects between some of these species as well as within one species (Fuentes et al., 1987; Peñaloza et al., 2001). However, although allelopathic effects on the germination and growth in plant species from Mediterranean forests have been the subject of various studies (Cavieres et al., 2007; Guerrero & Bustamante, 2007), interactions between seeds and seedlings of Mediterranean plant species have received little attention.

This study is focused on the detection of interactions between seeds and seedlings of four tree species from Mediterranean forests under controlled experimental conditions. We report the effects of seed extracts on germination and vigor and compare the growth of seedlings growing either with other seedlings of the same or other species. As far as we know, there are no previous reports of interactions between seeds and seedlings of Mediterranean communities. In addition, the novelty of these results is their contribution to document the existence of complex interactions between species of a natural ecosystem.

Materials and methods

Species

We selected four species which grow in the Chilean Mediterranean forest: "Espino", *Acacia caven* (Molina) Molina); "Tara", *Caesalpinia spinosa* (Molina) Kuntze; "Algarrobo", *Prosopis chilensis* (Molina) Stuntz, all three from the Fabaceae family; and "Quillay", *Quillaja saponaria* (Molina) (Quillajaceae). All four species are important from an ecological and forestry perspective. The seeds of these species were provided by CESAF Antumapu (<http://cesaf.forestaluchile.cl/>) and were collected between February and April 2017 in various regions of Central Chile.

Experiment 1

In this experiment we seek to detect the response of each species to extracts of seeds of each of the four species, in terms of rates of germination and vigor of seeds.

Extracts preparation

For the extraction of seed material, we used an aqueous solution of KNO_3 300 mOsm L^{-1} , as follows. Seed material was disaggregated in the aqueous medium to recover most seed compounds which are soluble in the aqueous phase of the soil. 65 seeds of *Q. saponaria* were incubated overnight in tap water (without previous treatments) and grounded in a mortar with 100mL of saline solution. This whole seed mixture was incubated overnight at 4° C.

Seeds of *A. caven*, *C. spinosa* and *P. chilensis* were scarified with sulfuric acid for 90, 30 and 10 minutes, respectively. Then, the seeds were placed on tap water overnight. After hydration, 65 seeds of each species were opened to separate the tegument from the embryo and cotyledons. Teguments were sliced into thin strips, mixed with 100mL of saline solution, and incubated overnight at 4° C. Embryos and cotyledons were grounded using a porcelain mortar, and then mixed with 100mL of saline solution and incubated overnight at 4° C.

Thereafter, these preparations were filtered using a poplin cloth (to remove major seed remnants) and were used for the different treatments in the experiment.

In order to refer to the different treatments we use the following abbreviations: *A. caven* teguments (AT), *A. caven* cotyledons and embryos (ACE); *C. spinosa* teguments (CT), *C. spinosa* cotyledons and embryos (CCE); *P. chilensis* teguments (PT), *P. chilensis* cotyledons and embryos (PCE); and *Q. saponaria* whole seeds (WS).

Germination experimental design

To promote germination, seeds of each of the four species were treated with sulfuric acid for 90, 30, 10 and 0 minutes, respectively, and then activated in tap water overnight. For each treatment we used five replicates of 15 seeds of each target species in culture plates with 3 g of each of the extracts. Five plates were used as control for each target species, using just 3g of saline solution. Plates were incubated at 20° C, with light/dark cycles of 9h-15h in a growth chamber. To check seeds germination time response to the extracts we counted the number of germinated seeds at day three and day sixth. To check initial vigor response to the extracts we measured seedling length (mm) from root apex to the base of the cotyledons at day sixth; non-germinated seeds were given a value of 0mm.

Experiment 2

In this experiment we seek to estimate survival and measure the growth of seedlings in monospecific and bispecific mixtures under controlled conditions.

Obtaining the seedlings

We obtained the seedlings by germinating the seeds of each species in the laboratory. Seeds of *A. caven*, *C. spinosa* and *P. chilensis* were previously scarified using analytic sulfuric acid (95-97%) for 90, 30 y 10 minutes respectively. Then we washed the seeds with a solution of sodium carbonate to neutralize the acid, followed by tap water to remove residues. Finally, the seeds were rinsed with distilled water.

Scarified seeds of *A. caven*, *C. spinosa* y *P. chilensis*, and non-scarified seeds of *Q. saponaria* were hydrated in distilled water at room temperature for 12 hours. Hydrated seeds were placed in culture dishes with wet filter paper and incubated in a growth chamber at 20° C and cycles of 9h light - 15 h darkness, simulating spring conditions.

The dishes were checked every two days, and seedlings were collected as seeds germinated. Before being used in the following stage, seedlings were washed in a suspension of cooper oxychloride 50 mgL⁻¹ to eliminate fungi and pathogenic bacteria.

Seedlings experimental design

We used plastic 200 ml-garden pots to transplant seedling combinations. In each pot we placed eight seedlings, 1-2 cm apart in a circle. In monospecific combinations the eight seedlings belonged to the same species. In bispecific combinations, we alternated four seedlings of one species with four seedlings of another species.

The substrate was a mixture of 70% commercial garden soil (mostly compost) and 30% of sandy mixture prepared in the lab, using 93% of filtered sand and 7% of plant ash, mostly from espino. These proportions were previously tested in the lab to obtain satisfactory growth. Furthermore, this mixture allowed us to emulate the outstanding features of the sclerophyllous forest after burning.

Pots with seedlings were placed in trays which were maintained with water (3 cm level) to make sure the substrate kept moist. The trays were placed in growth chambers at 20 °C and cycles of 9h light- 15h darkness.

To control pathogens, we applied 15 mL of a suspension of copper oxychloride 10 mgL⁻¹ to each pot, at the beginning of the experiment, every two-days during the first two weeks and once a week thereafter.

Replicas were limited by the availability of seedlings of the different species. Therefore, the number of replicas differed between treatments, varying within two and ten with a mean of six per group.

Survival and Growth

In each pot, we estimated survival at days 27th and 70th. At each time, we harvested all living seedlings, measured radicle length, stem length, neck diameter, presence of true-leaves and dry weight.

Treatment of Data

Results of Experiment # 1, responses to treatment with seed extracts in terms of germination rates and seed vigor, reflect interactions between seeds and between seeds and seedlings. To analyze these results, we used the Relative Interaction Index (RII) proposed by Armas et al. (2004). The equation for this index is:

$$RII = \frac{B_w - B_0}{B_w + B_0}$$

where B is any metric of plant growth or reproduction that could be altered by a possible interaction. In our case, B_w is germination percent (or seeds vigor) in the seed extract and B₀ is the germination or initial seedling growth in the control. This index has been frequently used to compare interactions (Howard et al., 2012; Le Bagousse-Pinguet et al., 2012). It varies from -1 to 1; if it is negative, the interaction could be the result of competitive or allelopathic interactions; if it is positive, it would be considered as facilitation; and if close to 0, it is a neutral interaction. The index has an asymptotic behavior, an approximately normal distribution, and can be used in parametric analysis. We calculated RII for each sample in each treatment (B_w) using the mean of the control group as B₀.

The mean RII obtained in each treatment in each species were compared using a two-tailed, Student-t test for homoscedastic samples, comparing each treatment group with the control group.

Results of Experiment #2, survival and growth of seedlings in monospecific and bispecific mixtures, were analyzed using two statistical approaches according to the type of results. For survival we estimated the percentage of living seedlings of each species in each pot, relative to the number initially planted. We then used Mann-Whitney test to compare the results of each bispecific combination to the results of the monospecific combination. N was not large enough to perform a reliable normal distribution test. For the number of true leaves, we compared individual plants. Those living seedlings with true leaves were assigned a number one, and those with only cotyledons were assigned a zero. We then compared the monospecific and bispecific groups using the Mann-Whitney test.

To compare the results of initial growth (radicle length, stem length, neck diameter and dry weight) we used the Student t-test corrected by Welch for disparate samples. For each variable, we compared the values obtained from the monospecific mixtures to those from the bispecific mixtures.

Graphics generation

All graphics were created using Microsoft Excel from Microsoft Office 365 ProPlus.

Results

Germination vs seed extracts

Germination was high and fast in *A. caven* and *P. chilensis*, with average percent germination higher than 80% at day three in all treatments (see Table 1). On the contrary, in the other two species germination was slower, with average germination below 6% in *Q. saponaria* and below 2% in *C. spinosa* at day three in all treatments. Average germination was still low at day sixth in *Q. saponaria* (50% or less), but in *C. spinosa* reached 70% or higher (see Table 1).

TABLE 1. Germinating fractions of each target species at day three and day sixth. Mean values of five replicates. Treatments are with aqueous extracts from seeds or seed parts from the four species as follows: *A. caven* teguments (AT), *A. caven* cotyledons and embryos (ACE), *C. spinosa* teguments (CT), *C. spinosa* cotyledons and embryos (CCE), *P. chilensis* teguments (PT), *P. chilensis* cotyledons and embryos (PCE), and *Q. saponaria* whole seeds (QWS).

TREATMENTS	DAY	TARGET SPP			
		<i>A. caven</i>	<i>C. spinosa</i>	<i>P. chilensis</i>	<i>Q. saponaria</i>
Control	day 3	0,88	0,01	0,88	0,07
	day 6	0,91	0,69	0,88	0,49
ACE	day 3	0,84	0,00	0,96	0,00
	day 6	0,87	0,77	0,97	0,00
AT	day 3	0,91	0,00	0,99	0,03
	day 6	0,96	0,80	0,99	0,37
CCE	day 3	0,81	0,00	0,91	0,00
	day 6	0,91	0,68	0,92	0,00
CT	day 3	0,91	0,00	1,00	0,00
	day 6	0,93	0,71	1,00	0,01
PCE	day 3	0,83	0,01	0,95	0,00
	day 6	0,92	0,75	0,95	0,21
PT	day 3	0,85	0,00	1,00	0,04
	day 6	0,89	0,85	1,00	0,32
QWS	day 3	0,88	0,00	0,91	0,04
	day 6	0,91	0,77	0,91	0,16

Interactions of Seed Extracts and Germination

The comparative analysis of Relative Interaction Index (RII) showed that with one exception, there are no differences between interactions at day three and at day sixth. The exception was the effects of the extracts of *Q. saponaria* on its own germination, which were no-significant at day three but were significant (and negative) at day sixth. Consequently, in Fig. 1 we present only the results of RII for day sixth, as follows.

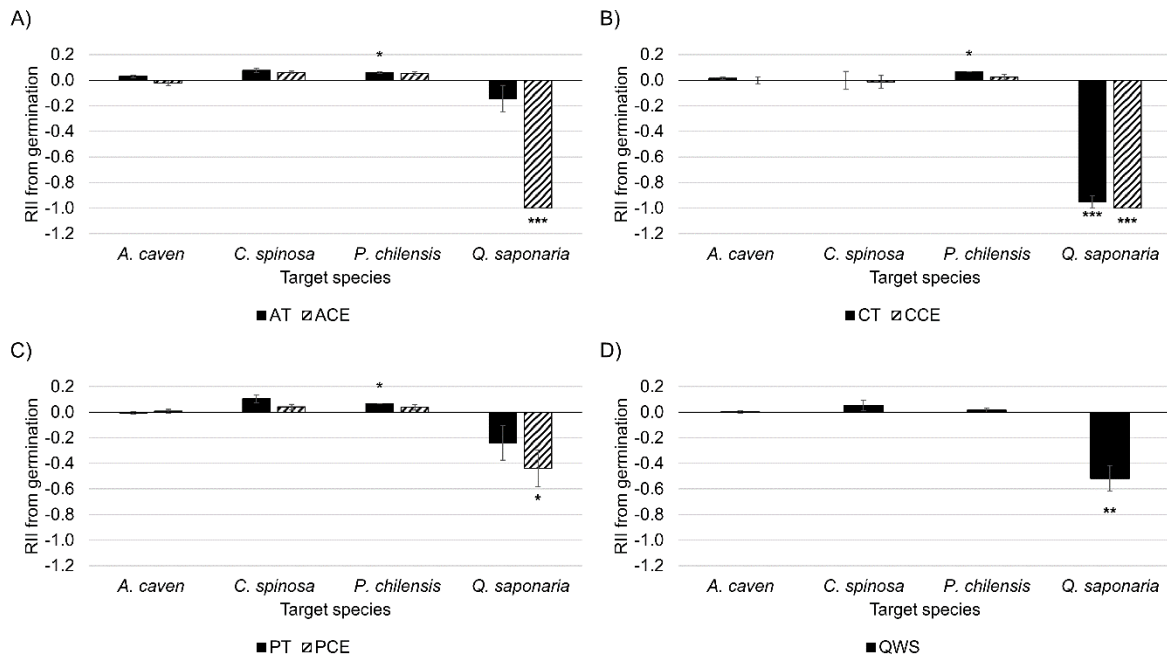


FIGURE 1. RII values calculated from germination data of target species on day sixth. A) with extracts of *A. caven*; B) with extracts of *C. spinosa*; C) with extracts of *P. chilensis*; D) with extracts of *Q. saponaria*. Asterisk indicates significant differences between the two modes (* p < 0.05; ** p < 0.01; *** p < 0.001).

Positive values of RII are remarkably low and significant only in three cases, all referred to the effects of tegument extracts on germination, as follows: *A. caven* on *P. chilensis* (1A); *C. spinosa* on *P. chilensis* (1B); and *P. chilensis* on its own germination (1C). Instead, negative values are much higher, with some values close to -1, and all restricted to *Q. saponaria* as a target species, in most cases significant.

Interactions of Seed Extracts and Seed Vigor

Seedling lengths measured at day sixth in all treatments are shown in Table 2.

TABLE 2. Average seedling length (cm) of each target species at day sixth. Treatments are with aqueous extracts from seeds or seed parts from the four species as follows: *A. caven* teguments (AT), *A. caven* cotyledons and embryos (ACE), *C. spinosa* teguments (CT), *C. spinosa* cotyledons and embryos (CCE), *P. chilensis* teguments (PT), *P. chilensis* cotyledons and embryos (PCE), and *Q. saponaria* whole seeds (QWS).

TREATMENTS	TARGET SPP (mm)			
	<i>A. caven</i>	<i>C. spinosa</i>	<i>P. chilensis</i>	<i>Q. saponaria</i>
Control	31,8	5,0	44,2	1,2
ACE	14,7	4,2	31,4	0,0
AT	38,2	9,3	53,5	0,6
CCE	15,3	2,2	29,5	0,0
CT	21,7	2,9	41,2	0,0
PCE	28,9	4,4	48,9	0,4
PT	37,4	5,9	60,6	0,7
QWS	13,2	4,9	31,7	0,2

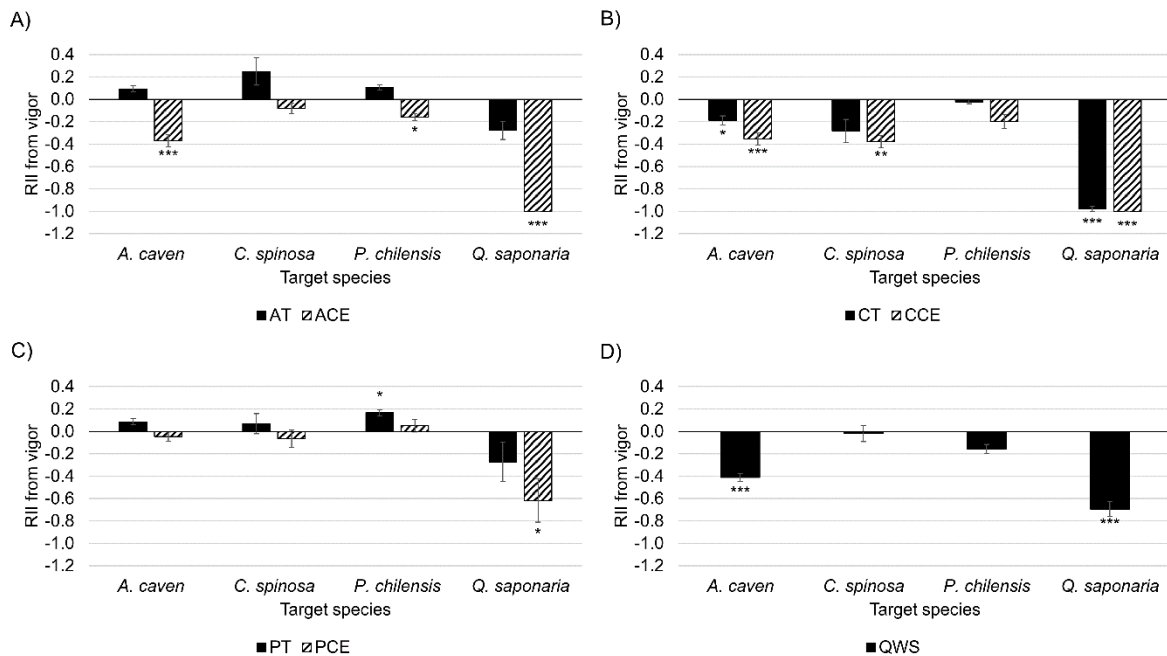


FIGURE 2. RII values calculated from seedling length of target species on day sixth. A) with extracts of *A. caven*; B) with extracts of *C. spinosa*; C) with extracts of *P. chilensis*; D) with extracts of *Q. saponaria*. Asterix indicates significant differences between the two modes (* $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$).

RII calculated for interactions of the seed extracts on initial seedling growth of target species (Fig. 2) showed more variable patterns than the effects on germination. There also were higher number

of effects (12) compared to eight in germination. In only one case, there was a positive effect, the effect of *P. chilensis* tegument extract on its own vigor (2C). All the other significant effects were negative. There were several autotoxic effects: *A. caven* CE extract (2A); *C. spinosa* CE extract (2B); and *Q. saponaria* seed extract (2D).

Effects of Seedlings on Survivorship

One of the results from Experiment #2 was about seedling survival in monospecific and bispecific mixtures. This allowed us to estimate the effects of companion species on seedling survival. Significant differences were restricted to day 27th monitoring, and these results are presented in Fig. 3.

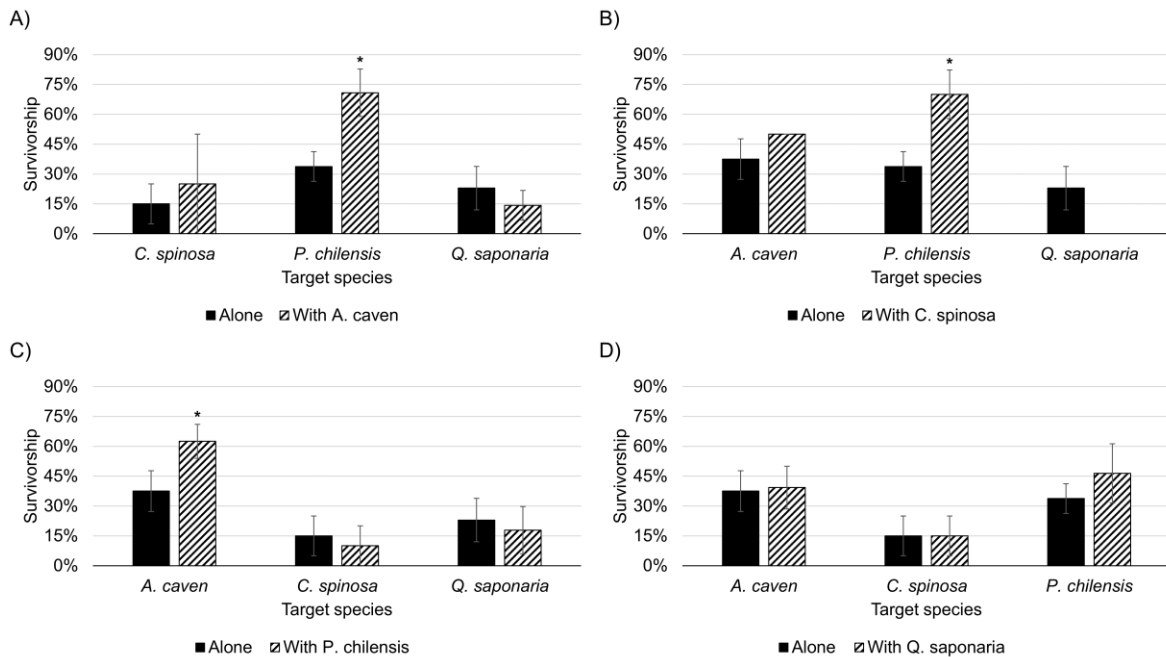


FIGURE 3. Survivorship of seedlings of target species growing in mono-specific pots or in mixtures with different species (day 27th): A) with *A. caven*; B) with *C. spinosa*; C) with *P. chilensis*; D) with *Q. saponaria*. Asterix indicates significant differences between the two modes ($p < 0.05$).

We registered only three significant effects, all positive: *P. chilensis* increased survival two times when growing with *A. caven* (3A) and with *C. spinosa* (3B); *A. caven* survival also increased when growing with *P. chilensis* (3C), showing a reciprocal effect between these two species. There were no significant effects of *Q. saponaria* on the survival of the other species (3D).

Effects of Seedlings on Seedling Growth

The one case of significant effects was the reduced growth of *A. caven* root in mixture with *Q. saponaria* when compared to monospecific mixture. By the date of measurement, all seedlings of *Q. saponaria* had died.

Discussion

Our results show a complex map of interactions between the seeds and the seedlings in these species. The type and intensity of the effects vary depending of the species, whether it is target or donor, or it is seed or seedling. Also, in the case of seed extracts, their effects differ substantially depending on the tissue of origin (tegument or cotyledons-embryo) and the process affected (germination or vigor). In Fig. 4 we try to resume this complex picture, distinguishing between target and donor species and the type (positive or negative) of interaction. As far as we know this is the first time such a set of complex interactions between seeds and seedlings is shown. However, complex interactions between plant species have been reported previously. Kushima et al. (1998) showed positive allelopathic effects of seed extracts of *Citrullus lanatus* on shoot growth of two Amaranthaceae species and inhibition of growth of seedlings of lettuce and tomato, whereas no effect was registered on the growth of barnyard grass.

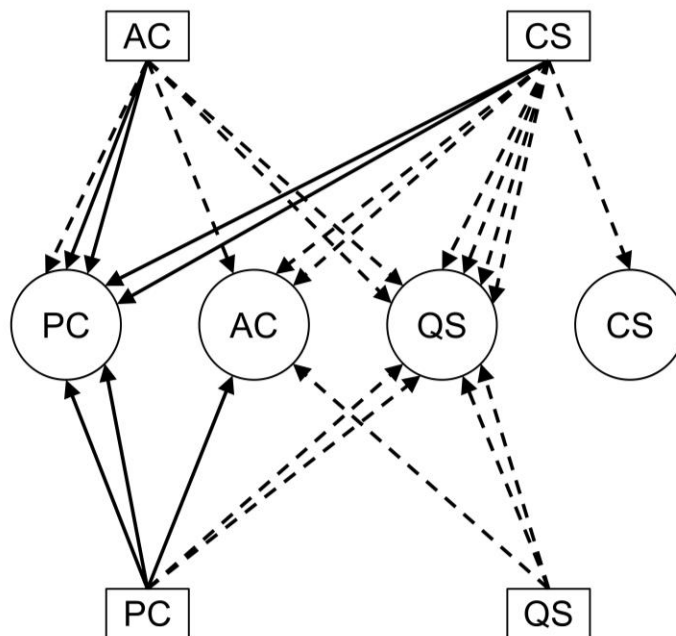


FIGURE 4. Ideal representation of the interactions detected between the four species of this study. Target species are within circles; donor species are within rectangles. Dash arrows are negative interactions; line arrows are positive interactions. AC: *Acacia caven*; CS: *Caesalpinia spinosa*; PC: *Prosopis chilensis*; QS: *Quillaja saponaria*.

Our results also showed that some species may exhibit opposite effects, even on the same target species. For instance, the tegument extract of *A. caven* favors germination of *P. chilensis* (Fig. 1A), whereas the extract of cotyledon-embryo of that donor reduces the vigor of this target (Fig. 2A).

The four species varied in their behavior either as donor or target. For instance, *C. spinosa* appears as target species only once, in response to its own seed extract (Fig. 2B). Instead, it appears as donor eight times. In contrast, *A. caven* was a target affected negatively for all species except *P. chilensis*; and *Q. saponaria* appeared as target of all species, including itself, and all interactions were negative. In *P. chilensis* as a target, it showed predominantly positive responses.

Germination appears as one the processes more affected by allelopathy (Huang et al., 2000; Kruse et al., 2000; Mallik, 1998; Masum et al., 2018; Zackrisson & Nilsson, 1992). In our study, only the germination of *Q. saponaria* was strongly inhibited by the extracts of all species, including itself, showing values of RII close to -1 (Fig. 1). In contrast, germination of *P. chilensis* was positively affected by tegument extracts in three of the four cases, although values of RII were very low (Fig. 1). Positive effects of seed extracts on germination have been previously reported. Yamada et al. (2010) showed that seed and grain exudates from *Helianthus annuus* and *Fagopyrum esculentum* stimulated seed germination of these and of other species. Positive allelopathic effects of extracts and exudates of seeds have also been reported for other species such as *Tamarindus indica* (Fabaceae; Parvez et al., 2004) and *Citrullus lanatus* (Cucurbitaceae; Kushima et al., 1998) among others.

We found important positive interactions between seedlings of different species growing in mixtures. *P. chilensis* and *A. caven* showed much higher survival when growing together than in monospecific mixtures (Fig. 3A, 3C). These two species grow together in the sclerophyllous forest (Gajardo, 1994), and their populations react in the same fashion to negative perturbations (Valdivia & Romero, 2013). Although there are abundant examples of negative allelopathic effects on seedling growth, (see for example, Kruse et al., 2000), there are previous reports of positive effects from adult plants (Schöb et al., 2013). It is likely that these effects are mediated by substances secreted to the substrate from growing plants (Yamada et al., 2010), altering and enriching the composition of the rhizosphere (Hartwig et al., 1991), and consequently facilitating growth for some species.

The four species included in our study are not commonly mentioned in the literature about allelopathic interactions. However, Muturi et al. (2017) reported the self-inhibitory effects of leaf litter of hybrids of *P. chilensis* x *P. juliflora* on their germination, in contrast to the positive effects of tegument extracts of *P. chilensis* reported in our study (Fig. 1C). Muturi et al., also reported that once leached, the litter loses the inhibitory effects and instead favor shoot growth of that species and also of *Acacia tortilis*. Aguilera et al. (2015) reported the negative effects of different tissues of *Acacia dealbata* on the growth of the radicle of *Q. saponaria*, which they explain as allelopathic effects on the radicle morphology. Those reports agree with our results of the loss of *Q. saponaria* seedling vigor when exposed to seeds extracts of the four species. On the other hand, Fuentes et al. (1987) reported that leaf extracts from adult plants of *Q. saponaria* increased germination rates of *Acacia caven* and *Q. saponaria* by 13 and 5% respectively. In addition, *A. caven* may act as nurse species for *Q. saponaria* (Root-Bernstein et al., 2017). Our results showed an opposite type of interaction between the seeds of these two species, suggesting that the ability for mutual facilitation develops later in their life cycle. Furthermore, we showed inhibition of radicle growth of *A. caven* seedlings growing in mixture with seedlings of *Q. saponaria*, even after the seedlings of this species have all died, indicating a negative allelopathic effect.

The interactions we detected in our study may be consequence of our particular experimental conditions, therefore they may differ from other results under different experimental designs. However, based on our experimental conditions, we can conclude that the interactions detected in this study should be intermediated by allelochemical substances liberated from the seed and seedling tissues (Aguilera et al., 2015; de Souza Nascimento et al., 2014).

If the interactions we report have any role at the ecological scale is a matter open to further investigation. Some authors have suggested the existence of allelopathic interactions between species resident in stable natural communities, although these would be weak as a consequence of the coevolution of those species (Callaway & Hierro, 2006). These ideas promote a third approach intermediary between the organismic and the Gleason individualistic theories of plant communities (Callaway, 1997). However, while there are numerous examples of allelopathic interactions between plant species in agricultural systems (Jabran et al., 2015; Masum et al., 2018), there are very few evidences for species resident in natural stable communities. Our results may help to open a window of innovation in this direction.

Acknowledgements

This work was supported by CONAF project 008/2016 "Pautas de terreno para la restauración de formaciones esclerófilas afectadas por incendios forestales. Regiones V, Metropolitana, VI y VII", and CONICYT-PCHA/MagísterNacional/2016 – 22161077.

We thank Juan F. Silva for help in editing and translating the manuscript.

Authors' contributions

Plaza, A.: Conceptualization, Methodology, Formal Analysis, Investigation, Writing – Original Draft, Writing – Review & Editing, Visualization ; Castillo, M.: Resources, Writing – Review & Editing, Supervision, Project Administration, Funding Acquisition; Paulette, P.I.: Conceptualization, Resources, Writing – Review & Editing, Supervision, Project Administration.

References

1. Aguilera, N., Guedes, L., Becerra, J., Baeza, C., & Hernández, V. (2015). Morphological effects at radicle level by direct contact of invasive *Acacia dealbata* Link. *Flora*, 215, 54–59. <https://doi.org/10.1016/j.flora.2015.07.007>
2. Armas, C., Ordiales, R., & Pugnaire, F. (2004). Measuring plant interactions: a new comparative index. *Ecology*, 85, 2682–2686. <https://doi.org/10.1890/03-0650>
3. Barbosa, E., Pivello, V., & Meirelles, S. (2008). Allelopathic evidence in *Brachiaria decumbens* and its potential to invade the Brazilian Cerrados. *Brazilian Archives of Biology and Technology*, 51(4), 825–831. <http://dx.doi.org/10.1590/S1516-89132008000400021>
4. Brooker, R., & Callaghan, T. (1998). The balance between positive and negative plant interactions and its relationship to environmental gradients: a model. *Oikos*, 81(1), 196–207. <https://doi.org/10.2307/3546481>
5. Brooker, R., Maestre, F., Callaway, R., Lortie, C., Cavieres, L., Kunstler, G., Liancourt, P., Tielbörger, K., Travis, J., Anthelme, F., Armas, C., Coll, L., Corcket, E., Delzon, S., Forey, E., Kikvidze, Z., Olofsson, J., Pugnaire, F., Quiroz, C., Saccone, P., Schifffers, K., Seifan, M., Touzard, B., & Michalet, R. (2007) Facilitation in Plant Communities: The Past, the Present, and the Future. *Journal of Ecology*, 96(1), 18–34. <https://doi.org/10.1111/j.1365-2745.2007.01295.x>
6. Byun, C., de Blois, S., & Brisson, J. (2015). Interactions between abiotic constraint, propagule pressure, and biotic resistance regulate plant invasion. *Oecologia*, 178(1), 285–296. <https://doi.org/10.1007/s00442-014-3188-z>

7. Caldararo, N. (2002). Human ecological intervention and the role of forest fires in human ecology. *Science of The Total Environment*, 292(3), 141–165. [https://doi.org/10.1016/S0048-9697\(01\)01067-1](https://doi.org/10.1016/S0048-9697(01)01067-1)
8. Callaway, R. (1997). Positive interactions in plant communities and the individualistic-continuum concept. *Oecologia*, 112(2), 143–149. <https://doi.org/10.1007/s004420050293>
9. Callaway, R., & Walker, L. (1997). Competition and facilitation: A synthetic approach to interactions in plant communities. *Ecology*, 78(7), 1958–1965. <https://doi.org/10.2307/2265936>
10. Callaway, R., & Hierro, J. (2006). Resistance and susceptibility of plant communities to invasion: revisiting Rabotnov's ideas about community homeostasis. In: Reigosa M., Pedrol N., González L. (eds) *Allelopathy*, pp 395–414. Springer, Dordrecht. https://doi.org/10.1007/1-4020-4280-9_17
11. Cavieres, L., Chacon, P., Peñaloza, A., Molina-Montenegro, M., & Arroyo, M. (2007). Leaf litter of *Kageneckia angustifolia* D. Don (Rosaceae) inhibits seed germination in sclerophyllous montane woodlands of central Chile. *Plant Ecology*, 190, 13–22. <https://doi.org/10.1007/s11258-006-9187-3>
12. de Souza Nascimento, C., Tabarelli, M., da Silva, C., Leal, I., de Souza Tavares, W., Serrão, J., & Zanuncio, J. (2014). The introduced tree *Prosopis juliflora* is a serious threat to native species of the Brazilian Caatinga vegetation. *Science of the Total Environment*, 481, 108–113. <https://doi.org/10.1016/j.scitotenv.2014.02.019>
13. Fuentes, E., Espinoza, G., & Gajardo, G. (1987). Allelopathic effects of the Chilean matorral shrub *Flourensia thurifera*. *Revista Chilena de Historia Natural*, 60, 57–62. http://rchn.biologiachile.cl/pdfs/1987/1/Fuentes_et_al_1987.pdf
14. Gajardo, R. (1994). *La vegetación natural de Chile: Clasificación y distribución geográfica*. Editorial Universitaria, Santiago. ISBN: 956-11-0825-8
15. Guerrero, P., & Bustamante, R. (2007). Can native tree species regenerate in *Pinus radiata* plantations in Chile?: Evidence from field and laboratory experiments. *Forest Ecology and Management*, 253(1-3), 97–102. <https://doi.org/10.1016/j.foreco.2007.07.006>
16. Hartwig, U., Joseph, C., & Phillips, D. (1991). Flavonoids released naturally from alfalfa seeds enhance growth rate of *Rhizobium meliloti*. *Plant Physiology*, 95, 797–803. <https://doi.org/10.1104/pp.95.3.797>
17. Howard, K., Eldridge, D., & Soliveres, S. (2012). Positive effects of shrubs on plant species diversity do not change along a gradient in grazing pressure in an arid shrubland. *Basic and Applied Ecology*, 13(2), 159–168. <https://doi.org/10.1016/j.baae.2012.02.008>

18. Huang, Z., Liao, L., Wang, S., & Cao, G. (2000). Allelopathy of phenolics from decomposing stump-roots in replant chinese fir woodland. *Journal of Chemical Ecology*, 26(9), 2211–2219. <https://doi.org/10.1023/A:1005580718765>
19. Inouye, B., & Stinchcombe, J. (2001). Relationships between ecological interaction modifications and diffuse coevolution: similarities, differences, and causal links. *Oikos*, 95, 353–360. <https://doi.org/10.1034/j.1600-0706.2001.950218.x>
20. Jabran, K., Mahajan, G., Sardana, V., & Chauhan, B. (2015). Allelopathy for weed control in agricultural systems. *Crop Protection*, 72, 57–65. <https://doi.org/10.1016/j.cropro.2015.03.004>
21. Keeling, C., Lewis, A., Kolotelo, D., Russell, J., & Kermode, A. (2018). Resin vesicles in conifer seeds: morphology and allelopathic effects. *Canadian Journal of Forest Research*, 48(12), 1515–1525. <https://doi.org/10.1139/cjfr-2018-0221>
22. Kruse, M., Strandberg, M., & Strandberg, B. (2000). Ecological Effects of Allelopathic Plants – a Review. National Environmental Research Institute, Technical Report No. 315, Silkeborg Bogtryk, Denmark. ISBN: 87-7772-540-9 https://www2.dmu.dk/1_viden/2_Publikationer/3_fagrapporter/rapporter/fr315.pdf
23. Kushima, M., Kakuta, H., Kosemura, S., Yamamura, S., Yamada, K., Yokotani-Tomita, K., & Hasegawa, K. (1998). An allelopathic substance exuded from germinating watermelon seeds. *Plant Growth Regulation*, 25(1), 1–4. <https://doi.org/10.1023/A:1005907101778>
24. Le Bagousse-Pinguet, Y., Forey, E., Touzard, B., & Michalet, R. (2012). Disentangling the effects of water and nutrients for studying the outcome of plant interactions in sand dune ecosystems. *Journal of Vegetation Science*, 24(2), 375–383. <https://doi.org/10.1111/j.1654-1103.2012.01462.x>
25. Lortie, C., Brooker, R., Choler, P., Kikvidze, Z., Michalet, R., Pugnaire, F., & Callaway, R. (2004). Rethinking plant community theory. *Oikos*, 107(2), 433–438. <https://doi.org/10.1111/j.0030-1299.2004.13250.x>
26. Mallik, A. (1998). Allelopathy and competition in coniferous forests. In: Sassa, K. (eds) *Environmental Forest Science*, pp 309–315. *Forestry Sciences*, vol 54. Springer, Dordrecht. https://doi.org/10.1007/978-94-011-5324-9_33
27. Masum, S., Hossain, M., Akamine, H., Sakagami, J., Ishii, T., Gima, S., Kensaku, T., & Bhowmik, P. (2018). Isolation and characterization of allelopathic compounds from the indigenous rice variety 'Boterswar' and their biological activity against *Echinochloa crus-galli* L. *Allelopathy Journal*, 43(1), 31–42. <https://doi.org/10.26651/allelo.j./2018-43-1-1127>

28. Muturi, G., Poorter, L., Bala, P., & Mohren, G. (2017). Unleached *Prosopis* litter inhibits germination but leached stimulates seedling growth of dry woodland species. *Journal of Arid Environments*, 138, 44–50. <https://doi.org/10.1016/j.jaridenv.2016.12.003>
29. Parvez, S., Parvez, M., Fujii, Y., & Gemma, H. (2004). Differential allelopathic expression of bark and seed of *Tamarindus indica* L. *Plant Growth Regulation*, 42(3), 245–252. <https://doi.org/10.1023/B:GROW.0000026493.95805.a5>
30. Pellissier, F., & Souto, X. (1999). Allelopathy in northern temperate and boreal semi-natural woodland. *Critical Reviews in Plant Sciences*, 18(5), 637–652. <https://doi.org/10.1080/07352689991309423>
31. Peñaloza, A., Cavieres, L., Arroyo, M., & Torres, C. (2001). Efecto nodriza intra-específico de *Kageneckia angustifolia* D. Don (Rosaceae) sobre la germinación de semillas y sobrevivencia de plántulas en el bosque esclerófilo montano de Chile central. *Revista Chilena de Historia Natural*, 74(3), 539–548. <http://dx.doi.org/10.4067/S0716-078X2001000300005>
32. Reigosa, M., & González, L. (2006). Forest Ecosystems and Allelopathy. In: Reigosa M., Pedrol N., González L. (eds) *Allelopathy*, pp 451–463. Springer, Dordrecht. https://doi.org/10.1007/1-4020-4280-9_20
33. Root-Bernstein, M., Valenzuela, R., Huerta, M., Armesto, J., & Jaksic, F. (2017). *Acacia caven* nurses endemic sclerophyllous trees along a successional pathway from silvopastoral savanna to forest. *Ecosphere*, 8(2), e01667. <https://doi.org/10.1002/ecs2.1667>
34. Schöb, C., Armas, C., & Pugnaire, F. (2013). Direct and indirect interactions co-determine species composition in nurse plant systems. *Oikos*, 122(9), 1371–1379. <http://doi.org/10.1111/j.1600-0706.2013.00390.x>
35. Syphard, A., Radeloff, V., Hawbaker, T., & Stewart, S. (2009). Conservation Threats Due to Human-Caused Increases in Fire Frequency in Mediterranean-Climate Ecosystems. *Conservation Biology*, 23(3), 758–769. <https://doi.org/10.1111/j.1523-1739.2009.01223.x>
36. Thorpe, A., Thelen, G., Diaconu, A., & Callaway, R. (2009). Root exudate is allelopathic in invaded community but not in native community: field evidence for the novel weapons hypothesis. *Journal of Ecology*, 97(4), 641–645. <https://doi.org/10.1111/j.1365-2745.2009.01520.x>
37. Valdivia, C., & Romero, C. (2013). En la senda de la extinción: el caso del algarrobo *Prosopis chilensis* (Fabaceae) y el bosque espinoso en la Región Metropolitana de Chile central. *Gayana Botánica*, 70(1), 57–65. <http://dx.doi.org/10.4067/S0717-66432013000100007>
38. Yamada, K., Hirose, K., Shigemori, H., & Hasegawa, K. (2010). Plant growth promotive allelochemicals. *The Society of Synthetic Organic Chemistry, Japan*, 68, 551–562.

http://www.naro.affrc.go.jp/archive/niaes/marco/marco2009/english/program/W3-10_Yamada_Kosumi.pdf

39. Zackrisson, O., & Nilsson, M. (1992). Allelopathic effects by *Empetrum hermaphroditum* on seed germination of two boreal tree species. *Canadian Journal of Forest Research* 22(9), 1310–1319. <https://doi.org/10.1139/x92-174>

CONCLUSIONES

De las especies analizadas, dos demostraron no ser buenas alternativas para la restauración post-fuego. Estas fueron:

- *Q. saponaria*, ya que experimentaba dificultades para crecer cerca de las otras plantas, incluso de su misma especie, y además posee baja viabilidad en suelo quemado.
- *C. spinosa*, ya que tiene el TG50 más largo de las especies analizadas, no genera interacciones con otras especies (y sus interacciones intraespecíficas son negativas), y posee, al igual que *Q. saponaria*, baja viabilidad en suelo quemado.

Por otra parte, *A. caven* y *P. chilensis* mostraron el mayor potencial como combinación de especies para la rehabilitación ecológica inicial del sustrato en bosques incendiados. Estas especies poseen desde estadios tempranos, fuertes interacciones que les permiten facilitarse mutuamente, y juntas mejoran significativamente su sobrevivencia sobre sustrato quemado.

Además, estas especies son representativas del Bosque Espinoso; ecológicamente, este subtipo forestal puede presentarse en algunos lugares como comunidad en estado estable, mientras que en otros lugares es sólo una de las etapas sucesionales tempranas de diferentes subtipos esclerófilos. Esto hace que las aplicaciones potenciales de esta combinación sean mucho más amplias, ya que no estarían limitadas al subtipo forestal del que son características.

Se demostró que entre las especies del Matorral y Bosque Esclerófilo, tanto semillas no germinadas, plántulas, o incluso semillas germinadas muertas, tienen el potencial de interactuar con otros organismos vegetales y alterar significativamente sus parámetros de desarrollo. Se trata de interacciones que no han sido descritas previamente para estas especies o para grupos de especies pertenecientes a comunidades naturales, y que deberán tenerse en cuenta tanto para estudios biológicos puros (por ejemplo, discriminar la acción de las semillas del efecto nodriza) como para tomar acciones concretas de restauración (por ejemplo, no sembrar o plantar juntas especies con alelopatías entre ellas).