

INSTITUTO POTOSINO
DE INVESTIGACIÓN
CIENTÍFICA Y TECNOLÓGICA, A.C.

IPICYT

POSGRADO EN CIENCIAS AMBIENTALES

**EFFECTOS INHIBITORIOS DE LA ESPECIE EXÓTICA *SCHINUS MOLLE* L.
(ANACARDIACEAE) SOBRE LA GERMINACIÓN DE
ESPECIES NATIVAS DE MÉXICO**

Tesis que presenta

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Para obtener el grado de

Maestra en Ciencias Ambientales

Director de tesis

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San Luis Potosí, S.L.P.

Agosto de 2014



Constancia de aprobación de la tesis

La tesis “**Efectos inhibitorios de la especie exótica *Schinus molle* L. (*Anacardiaceae*) sobre la germinación de especies nativas de México**” presentada para obtener el Grado de Maestra en Ciencias Ambientales fue elaborada por **Marcela Avendaño González** y aprobada el **ocho de agosto del dos mil catorce** por los suscritos, designados por el Colegio de Profesores de la División de Ciencias Ambientales del Instituto Potosino de Investigación Científica y Tecnológica, A.C.

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MAESTRA EN CIENCIAS AMBIENTALES

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sobre la Tesis intitulada:

*Efectos inhibitorios de la especie exótica *Schinus molle* L. (Anacardiaceae) sobre la germinación de especies nativas de México*

que se desarrolló bajo la dirección de

Dr. Ernesto Iván Badano

El Jurado, después de deliberar, determinó

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Acta de Examen

DEJAR ESTA PÁGINA EN BLANCO

Agradecimientos

Le doy gracias a mis padres, en especial a mi mamá porque sin su esfuerzo, dedicación y apoyo no sería la persona que soy. A mi hermana por siempre apoyarme y acompañarme. A mi familia.

De manera muy especial, quiero agradecer a mi director de tesis, el Dr. Ernesto Iván Badano por todas las enseñanzas, consejos y motivaciones. Por sacarme de mi zona de confort, pero principalmente por dedicar tiempo y esfuerzo en mi.

Al Dr Joel David Flores Rivas por los consejos, aporte y asesoría brindada para la presente tesis, así como por su disposición amable en todo momento. Al Dr Jorge Alberto Flores Cano por los aportes brindados a la presente tesis.

A la División de Ciencias Ambientales del Instituto Potosino de Investigación Científica y Tecnológica, así como a los profesores-investigadores por motivarme a aprender a lo largo del camino.

Al CONACYT por el apoyo económico brindado para la realización de la maestría.

A los técnicos de la División de Ciencias Ambientales, en especial al M. en C. Juan Pablo Rodas Ortíz por su ayuda, disposición y apoyo para la realización de los experimentos.

Finalmente, me gustaría agradecer a mis compañeros de maestría: Esmeralda, Tonatiuh, Alejandra y Daniela, fue un placer haber compartido estos dos años con ustedes. Gracias por sus enseñanzas y conocimientos compartidos, pero en especial por su amistad. Agradezco también a todos aquellos que de cierta forma me acompañaron en el camino, dentro y fuera del IPICYT, compartiendo momentos o apoyándome a distancia.

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Resumen

Algunas plantas exóticas liberan metabolitos secundarios al ambiente y estos inhiben la germinación y crecimiento de las especies nativas de sus alrededores. Este proceso de interferencia, generalmente conocido como alelopatía, es una interacción negativa planta-planta que ocurre sin involucrar una competencia directa por recursos. Especies alelopáticas invasoras pueden poner en riesgo la biodiversidad de plantas nativas si impiden su establecimiento. Este podría ser el caso del Pirul (*Schinus molle*; Anacardiaceae) en México. Esta especie arbórea dioica es nativa de América del Sur, pero fue introducida al centro de México a mediados del siglo 16 y estudios recientes indican que a lo largo de esta región se pueden encontrar poblaciones viables de este árbol exótico creciendo naturalmente. Se ha demostrado que el Pirul reduce la germinación y el crecimiento de especies agrícolas, pero su potencial alelopático sobre las plantas nativas de México aún no ha sido evaluada. Además, sus efectos alelopáticos sólo se han demostrado para árboles femeninos, los cuales producen una drupa de color rojo brillante a la cual se han atribuido la mayoría de los efectos alelopáticos, mientras que los árboles masculinos han sido ignorados. Este estudio se centró en evaluar (1) si los árboles de Pirul inhiben la germinación de especies nativas que colonizan terrenos agrícolas abandonados en el centro de México, y (2) si los árboles de Pirul femeninos y masculinos tienen efectos alelopáticos diferenciales sobre las especies nativas. Para ello, primero se realizaron los bioensayos dirigidos a comprobar si los extractos acuosos obtenidos a partir de flores estaminadas producidas por los árboles masculinos, frutos producidos por árboles femeninos, y las hojas de los dos géneros de árboles inhiben la germinación de seis especies mexicanas. Además de estos bioensayos, se realizaron

experimentos de invernadero para evaluar si el suelo debajo del dosel de los árboles de Pirul conserva la capacidad de reducir la germinación de las especies nativas. Los bioensayos realizados con extractos acuosos indicaron que los árboles de Pirul reducen la germinación de todas las especies nativas, en comparación con los controles en los que sólo se aplicó agua destilada. Los extractos acuosos obtenidos a partir de las flores estaminadas y los frutos tuvieron efectos alelopáticos más fuertes que los obtenidos a partir de hojas. No obstante se observaron efectos diferenciales de género en estos bioensayos, donde se obtuvo que los árboles masculinos de Pirul tienen efectos alelopáticos más fuertes que los árboles femeninos. En los experimentos de invernadero, todas las especies nativas mostraron tasas de emergencia más bajas en suelo colectado debajo de los árboles de Pirul que en el suelo control. Sin embargo, cuando se compararon los efectos del tipo de suelo entre Pirules masculinos y femeninos, las diferencias de los efectos entre género no fueron tan evidentes como en el primer experimento. Estos resultados sugieren que los Pirules que han invadido los campos agrícolas abandonados en el centro de México reducen la germinación y el crecimiento de las plantas nativas.

En esta tesis se presenta en idioma inglés, siguiendo el formato de un manuscrito que será enviado a una revista indexada.

Palabras clave: campos abandonados, invasiones biológicas, cactus, interferencia, ecosistemas semiáridos.

Abstract

Some exotic plants release secondary metabolites to the environment and this inhibits the germination and growth of native species in their surroundings. This interference process, usually referred to as allelopathy, is a negative plant-plant interaction that occurs without involving direct resource competition. Allelopathic invasive species can endanger native plant biodiversity if they impede the establishment of native plants. This could be the case of the Peruvian peppertree (*Schinus molle*; Anacardiaceae) in Mexico. This dioecious tree species is native to South America, but it was introduced in central Mexico by the middle of the 16th century and recent studies indicate that viable populations of this exotic tree are naturally occurring across this region. The peppertree has been shown to reduce germination and growth of several crops, but its allelopathic potential on Mexican native plants has not yet been assessed. Further, their allelopathic effects have been only proved for female trees, which produce red-brilliant drupes to which most allelopathic effects have been attributed, while male trees have been largely ignored. This study focused on assessing (1) whether pepper trees inhibit the germination of native plants that are colonizing abandoned agricultural fields in central Mexico, and (2) whether male and female pepper trees have differential allelopathic effects on native species. For this, we firstly conducted bioassays addressed to test whether aqueous extracts obtained from staminate flowers produced by male trees, fruits produced by female trees, and leaves of both tree genders inhibit the germination of six Mexican plant species. In addition to these bioassays, greenhouse experiments were conducted to assess whether the soil beneath the canopy of pepper trees retains the capability to affect germination of native species. Bioassays conducted with aqueous extracts indicated that peppertrees reduced germination of all native species, as compared to controls on which only distilled water was applied. Aqueous extracts obtained from male flowers and

fruits had stronger allelopathic effects than those obtained from leaves. Nevertheless, differential gender effects were observed in these bioassays, where male peppertrees were indicated to have stronger allelopathic effects than female trees. In the greenhouse experiments, all native species displayed lower rates of shoot emergence on soil collected beneath pepper trees than in the control soil. However, when the effects of the soil type were compared between male and female peppertrees, the different gender effects were not as evident as in the former experiment. These results suggest that peppertrees that have invaded abandoned agricultural fields in central Mexico reduce the germination and growth of native plants.

This thesis is presented in English by following the format of a manuscript that will be submitted to an indexed journal.

Keywords: abandoned fields, biological invasions, cacti, interference effects, semiarid ecosystems.

TENTATIVE TITLE OF THE ARTICLE: Allelopathy of a dioecious invasive plant on succulent species from central Mexico: does the gender of the invader differently affect the recovery of native vegetation?

INTRODUCTION

The spreading of plant biological invasions is directly linked to anthropic disturbances, where invaders behave as opportunistic species that rapidly colonize disturbed areas in response to the release of resources that occur after the original vegetation is partially or completely removed (Hobbs & Huenneke 1992; Lozon & Maclsaac 1997; Marvier et al. 2004; Alston & Richardson 2006). Once exotic plants establish viable populations, they may prevent the recovery of native plant communities by disrupting natural succession processes (Levine et al. 2002; Didham et al. 2005). Higher competitive ability for resources and space has been proposed as the major mechanism by which exotic plants prevent the establishment of native species in invaded sites (Eliason & Allen 1997; Bakker & Wilson 2001; Badano & Pugnaire 2004; Castro et al. 2010). Nevertheless, invasive plants may also interfere with the recovery of native vegetation in several other ways. Some exotic species, for instance, release secondary metabolites to the environment and inhibit the germination and growth of native species in their surroundings (del Moral & Muller 1970; Ridenour & Callaway 2001; Inderjit et al. 2008; Zhang & Fu 2009). This interference process, referred to as *allelopathy*, is a negative plant-plant interaction that occurs without involving direct resource competition. Nevertheless, allelopathic species take advantage from this process because they monopolize the resources within their area of influence by excluding other potential competitors (Muller 1969).

Some exotic plants have been shown to strongly inhibit the germination and growth of native species from invaded sites, even when these allelopathic effects are not evident in their native distribution ranges (Callaway & Aschehoug 2000; Ridenour & Callaway 2001; Bais et al. 2003; Vivanco et al. 2004). This differential impact of allelopathy has been proposed to occur because plants from invaded sites have never been exposed to the secondary metabolites released by exotic species and, thus, they lack of adaptations to face the biochemical arsenal of the invaders (Hierro & Callaway 2003; Callaway & Ridenour 2004). Therefore, if exotic plants that colonize human-disturbed sites release secondary metabolites that inhibit the establishment of other species, they may prevent the recovery of native biodiversity in these sites even after human activities were ceased.

To test this hypothesis we focused in the Peruvian peppertree (*Schinus molle* L.; Anacardiaceae). This dioecious species is native to South America, but was introduced to central Mexico by the middle of the 16th century as result of the commercial exchange between the Viceroyalties of Peru and New Spain (Kramer 1957). After that, this tree was widely propagated through the region by farmers and ranchers because of its rapid grow, and it was also quickly incorporated to the local culture because of its ethnobotanical uses (Bye & Linares 1983; Hurtado-Rico & Rodríguez-Jiménez 2006; Paredes-Flores et al. 2007; Blancas et al. 2010). Nevertheless, a recent study has indicated that this tree is an aggressive invader that has colonized abandoned agricultural and grazing fields from semiarid regions of central Mexico (Ramírez-Albores & Badano 2013). In addition to this widespread propagation of the Peruvian peppertree in México, this species has been reported to reduce germination of crops in America (Anaya & Gomez-Pompa 1971; Aceves-Ramírez 1987; Materechera & Hae 2008; Zahed et al. 2010; Borella, et al. 2011). Moreover, a recent study conducted in Brazil by Trindade & Coelho 2012) indicated that plant communities naturally established in the

understory of human-induced plantations of Peruvian peppertree are less diverse than those communities established beneath the canopy of other tree species used for silvicultural practices. These authors then suggested that the reduced plant diversity observed within peppertree plantations could be at least partially due to allelopathy (Trindade & Coelho 2012). Allelopathic effects of Peruvian peppertrees on crops have been attributed to several biochemical compounds that are accumulated in their fruits, including flavonoids, oleoresins, tannins, terpenes and saponins (Zahed et al. 2010; Barroso et al. 2011). However, as far as we are aware, no previous studies have evaluated whether this tree species also inhibits the establishment of wild plants in the new sites it has invaded, including Mexico.

In central Mexico, several succulent plant species are currently colonizing abandoned agricultural and grazing fields, together with peppertrees (Calderon & Rzedowsky 2005). Because several of these native species seem to depend on the presence of nurse plants that provide suitable microhabitats for their establishment (Flores & Jurado 2003), it could be firstly hypothesized that peppertrees might act as facilitators for the recovery of native vegetation in human-disturbed sites from central Mexico. However, field observations indicate that native species are rarely found in the surroundings of pepper trees. Thus, instead of acting as nurse plants, peppertrees may be interfering with the recovery of plant communities because of their allelopathic effects.

Besides these potential negative effects of peppertrees, there is an additional issue that should be taken into account before testing the previous hypothesis. Although the peppertree is a dioecious species, the bioassays addressed to prove its allelopathy on crops species have been conducted with the female trees that produce the red-brilliant drupes to which these effects have been attributed (Anaya & Gomez-Pompa 1971; Aceves-Ramírez 1987; Materechera & Hae 2008; Zahed et al. 2010). Therefore, the allelopathic effects of male

peppertrees have been largely ignored. As far as we are aware, no previous studies have assessed whether the allelopathic effects of dioecious plants may differ between genders.

In this study we conducted a series of experiments addressed to determine (1) whether peppertrees has allelopathic effects on those native plants that are colonizing abandoned agricultural fields in semiarid zones of central Mexico, and (2) whether male and female peppertrees have differential allelopathic effects on these native species. In a first instance, we tested whether aqueous extracts obtained from staminate flowers, fruits, and leaves of both genders inhibit the germination of Mexican plants. After that, given that the inhibitory activity of allelopathic compounds is usually reduced once they reach the soil (Kobayashi 2004), we conducted greenhouse experiments for assessing whether the soil beneath the canopy of male and female trees retains the capability for affecting the germination and growth of native species.

MATERIALS AND METHODS

Native species and seed collection

Six species endemic to Mexico were used in the experiments addressed to determine the allelopathic effects of male and female peppertrees. These species involved four cacti, including *Echinocactus platyacanthus* Link et Otto, *Mammillaria longimamma* DC, *Ferocactus latispinus* (Haw.) Britton et Rose, and *Myrtillocactus geometrizans* (Mart. ex Pfeiff.) Console. The other two species were *Agave salmiana* Otto ex Salm-Dyck and *Yucca filifera* Chabaud, which are monocots of the family Asparagaceae widely distributed across semiarid ecosystems of Mexico. We focused on these species because they were found with mature fruits in an abandoned agricultural field invaded by peppertrees, which were required to obtain seeds of these species for the experiments described below. This abandoned field is located near the city of San Luis Potosí, central Mexico (22° 5' N; 100° 45' O; 1976-2015 masl). Mean annual temperature is 16.9 °C and precipitation averages 352 mm per year (Medina-García et al. 2005). Up to 90% of rainfalls occur between June and October, while a markedly dry season occurs between and November and May (Medina-García et al. 2005). Although other cacti were also found in this abandoned field, these species were not included in this study because they lacked of mature fruits at the moment of seed collection. A preliminary sampling conducted in this site indicated that the density of adult peppertrees is 28.4 individuals per hectare and the proportion of sexes is almost 1:1 between male and female trees. On the other hand, the density of recruits (i.e., non-reproductive individuals of undetermined sex) was 5.3 individuals per hectare.

Seeds of the species mentioned above were obtained by collecting mature fruits from parental plants between October 2012 and January 2013. All these species are well known to produce fruits with large amounts of viable seeds (between 50 and 300 seeds per fruit,

depending on the species; Arredondo-Gómez & Sotomayor 2009). However, to capture any potential variability in the reproductive features of species (e.g., maternal effects), we collected fruits from several different parental plants. For this, 15 different parental plants of each species were randomly selected in the target site and 2-5 fruits were collected on each plant. Collected fruits were pooled across plants of the same species to obtain large representative seed samples of each species. These fruits were carried to the laboratory and cleaned to recover the seeds. These seeds were disinfected by immersing them in 1% sodium hypochlorite solution during 3 min. After disinfection, seeds were air-dried during 24 h under the laboratory condition (18-20 °C) and stored in ventilated plastic flasks until their use.

Allelopathic effects of peppertrees

In a first instance, we were interested in assessing whether male and female peppertrees reduce the germination of native plants. For this, we performed *in vitro* germination bioassays where seeds of native species were watered with aqueous extracts obtained from male flowers, fruits, and mature leaves of both genders. All these plant organs were collected in April 2013, when adult male and female peppertrees from the site described above were found with staminate flowers and mature fruits, respectively. In the field site we randomly selected ten individuals of each gender. On each male tree we collected 100 g of mature leaves and 100 g staminate flowers, while on each female tree we collected 100 gr of leaves and 100 g of fruits. Plant organs were pooled across the ten trees to obtain a composite sample of 1 kg per plant organ. These samples were carried to the laboratory and rinsed with distilled water to remove dust and any other solid particles. Plant organs were later carefully dried with paper towels and stored in hermetic plastic bags at 5 °C.

Aqueous extracts were prepared the next day after the collection of plant organs. For this, 400 g of staminate flowers, fruits, and leaves of male and female peppertrees were separately liquefied with 4 liters of distilled water. The obtained blends were collected in amber flasks that that hermetically sealed and incubated at 20 °C during 48 h. Later, blends were later filtered and the resulting aqueous extracts were used in the germination bioassays described below. This procedure concurs with those used by the authors that have assessed allelopathic effects of peppertree fruits on crops (Aceves 1987; Materechera & Hae 2008). Nevertheless, because the inhibitory effects of allelopathic compounds can decrease as their concentrations are reduced (Rietveld 1983; Batish et al. 2002; Bogatek 2006; Materechera & Hae 2008), we diluted part of our aqueous extracts to the half of their original concentrations. In this way, for each plant organ (staminate flowers, fruits, and leaves of male and female peppertrees), we obtained two aqueous extracts which were equivalent to 100 g of plant organs per liter of distilled water (concentrated extract) and 50 g of plant organs per liter of distilled water (diluted extract).

To test the allelopathic effects of these aqueous extracts were sowed seeds of native species on plastic Petri dishes using sterile cotton pads as germination substrate. For each native species, 45 Petri dishes containing 20 seeds each were placed in a grow chamber (25 °C, 60% relative humidity, photoperiod 12 h dark/light). After that, we randomly selected a dish of each species and applied 5 ml of a given aqueous extract. We repeated this procedure until the eight aqueous extracts (male flowers, fruits, and leaves of male and female trees; all extracts at two concentrations) were applied on five dishes of all native species (i.e., a total of 100 seeds of each species received each aqueous extract). Further, five dishes of each species (100 seeds in total) were used as germination control and only received 5 ml of distilled water. Each dish was sealed with Parafilm® to avoid the loss of

humidity and labeled indicating the aqueous extract that was applied and the native species it contained. Aqueous extracts, and distilled water in the case of controls, were only applied at the beginning of the experiment; no additional watering was provided to seeds. Germination within each dish was monitored every two days during 30 days by recording the date on which each seed germinated; in all cases, a seed was considered as successfully germinated after the radicle emergence was observed.

Allelopathic effects of soils beneath peppertrees

To assess whether the soil beneath male and female peppertrees retains the allelopathic compounds that inhibit the germination of native species we collected samples from the first ten centimeters of the soil profile. These samples were taken beneath the canopy of five adult peppertrees of each gender randomly selected in the field site on May 2013; approximately ten liters of soil were collected beneath the canopy of each selected individual. Soil samples from male and female trees were stored separately. Soil samples of the same volume were also taken in five sites without vegetation cover located at least 20 m away from the canopy any peppertree (control soil). The soil samples that belonged to the same habitat type (male trees, female trees and control soil) were pooled and homogenized to obtain a composite soil sample. Composite soil samples were meshed to remove leaf litter and other coarse materials (e.g., fruits, flowers and stones) and stored in cloth bags.

These soil samples were used to perform a greenhouse experiment in which seeds of the native species were sowed on the different soil types. For this, 10 plastic pots (100 ml) were filled to $\frac{3}{4}$ of their capacity with each soil type and 15 seeds of each native species were sowed on each pot (i.e., a total of 150 seeds of each species were sowed on each soil type). These pots were labeled indicating the soil type and the species it contained. Pots were later

arranged within the greenhouse (temperature 25-28 °C; relative humidity 40%) by following a complete random design. All pots were watered by applying 30 ml of distilled water every three days. Most seeds were superficially buried after the first watering because of their small size, which made hard to assess the date in which the radicle emerged. Therefore, in this experiment we assumed that the germination had successfully occurred after the aerial shoot was observed. Seedling emergence was monitored every three days during 60 days.

Besides testing whether the soil beneath peppertrees affects germination, this experiment was also used to assess whether male and female peppertrees affect the growth of native plants. For this, at the end of the experiment, we carefully removed all seedlings from each experimental pot to avoid damage of aerial shoots and radicular systems. Each seedling was placed in a paper bag labeled with the name of the species and the pot to which it belonged to (one bag per seedling). Seedlings were dried at 60 °C in an air-forced stove until they were completely desiccated. Each seedling was later weighed in an analytical balance (accuracy 0.00001 g) to assess its dry biomass. Dry biomasses were later averaged across seedlings belonging to the same experimental pot to avoid pseudoreplication in the statistical analyses described below (Hurlbert 1984).

Statistical analyses

Failure-time-analyses were used to compare germination rates among the watering treatments we applied on the seeds of native species in the *in vitro* germination experiment (i.e., aqueous extracts at different concentrations of male and female peppertrees organs, as well as distilled water). In these analyses, the germination of a seed in a given monitoring date is taken into account as a 'failure event' (Aalen et al. 2008). Thus, these analyses considered each seed as a replicate of the respective treatment (n = 100 per treatment).

Failure-time-analyses were conducted separately for each native species and the Kaplan-Meier's method (Kaplan & Meier 1958) was used to estimate the germination rates of seeds in each treatment. The Gehan's generalized Wilcoxon chi-square test (Lee 1980) was used to assess whether germination rates differed among aqueous extracts. When differences were detected, pairwise comparisons between these treatments were conducted by using the Gehan-Wilcoxon's two-sample test (Lee et al. 1975). Besides comparing germination rates, we also compared the total number of germinated seeds among the treatments applied on each native species. For these comparisons, we taken into account the accumulated number of seeds that germinated throughout the experiment within the Petri dishes belonging to each treatment (n = 5 per treatment). These data was compared among treatments with one-way ANOVAs and the *post-hoc* Tukey test was used to assess differences between treatments.

Failure-time-analyses similar to those described above were conducted to compare germination rates of native species among the different soil types used greenhouse germination experiment (soil beneath male peppertrees, soil beneath female peppertrees, and control soil from the surrounding open spaces). The accumulated number of emerged seedlings within each plot was also recorded in this experiment and, for each native species, we compared this variable among soil types (n = 10 per soil type) by using one-way ANOVAs and Tukey tests. The same analyses (ANOVAs and Tukey tests) were used to compare the average dry biomass of seedlings among soil types.

RESULTS

Allelopathic effects of peppertrees

The *in vitro* experiments indicated that all native species displayed higher germination rates and higher accumulated numbers of germinated seeds when only distilled water was added (Figure 1 and 2). Indeed, for all native species, these two germination variables significantly differed among the different peppertree aqueous extracts applied on seeds (Table 1). These results indicate that the aqueous extracts obtained from the different organs of both peppertree genders (leaves of male and female individuals, staminate flowers and seeds) can inhibit the germination of native species at some degree. Nevertheless, the different native species used in this experiment showed a wide variety of responses to these aqueous extracts.

The aqueous extracts of staminate flowers significantly reduced the germination rates of *Echinocactus platyacanthus*, as compared with those seeds that received distilled water or the extracts obtained from other peppertree organs (Figure 1A). These inhibitory effects occurred irrespectively of the concentration of staminate flowers (10% or 5%) in the extracts (Figure 1A). For this cactus, no differences in germination rates were observed among seeds that received the other aqueous extracts of peppertrees and the control seeds that only received distilled water (Figure 1A). The accumulated number of germinated seeds of *E. platyacanthus* showed a similar pattern, but in this case the strongest inhibitory effect on germination was indicated for the concentrated aqueous extract of staminate flowers (Figure 2A).

The germination rates of *Ferocactus latispinus* were reduced by all aqueous extracts of peppertrees, as compared to control seeds (Figure 1B). In this case, the diluted extract of staminate flowers (5%) had the strongest inhibitory effect on germination rates (Figure 1B). It

was followed by the concentrated extracts (10%) of staminate flowers, and leaves of male and female peppertrees (Figure 1B). The concentrated extract of peppertree fruits (10%) had similar effects than the diluted extracts (5%) of male leaves and fruits on the germination rates of this cactus (Figure 1B). The accumulated number of germinated seeds at the end of the experiment showed a slightly different pattern than that observed in germination rates. In for this variable, staminate flowers strongly reduced the final germination of seeds of *F. latispinus* as compared to control seeds, and these negative effects occurred irrespectively of the concentration of flowers in the extracts (Figure 2B). Similar negative effects were observed for the concentrated extracts of leaves of male and female peppertrees when compared to the control plots (Figure 2B). This germination variable did not differ between all the other aqueous extracts and the control plots (Figure 2B).

All aqueous extracts of peppertrees reduced the germination rates of *Mammillaria longimamma* as compared to the control seeds (Figure 1C). Nevertheless, the concentrated extracts (10%) of all peppertree organs had higher inhibitory effects than the diluted extracts (5%) on the germination rates of this cactus (Figure 1C). For *M. longimamma*, the slower germination rate was observed on seeds that received the concentrated extract of peppertree fruits, followed by the seeds that received the concentrated extracts of leaves of both tree genders (Figure 1C). The lower effects on the germination rates were obtained in the seeds that received the diluted extracts of fruits and leaves, as well as the concentrated extract of staminate flowers (Figure 1C). On the other hand, pairwise comparisons of the accumulated number of germinated seeds at the end of the experiment indicated that the experimental plots that received the concentrated extracts of peppertree fruits, male leaves and female leaves had fewer germinated seeds of *M. longimamma* than all the other treatments; no differences were observed in the inhibitory capacity of these three concentrated aqueous

extracts (Figure 2C). With the exception of these three treatments, the accumulated number of germinated seeds did not differ between all the other aqueous extracts of peppertrees and the control that only received distilled water (Figure 2C).

The seeds of *Myrtillocactus geometrizans* also showed slower germination rates in the experimental plots that were watered with aqueous extracts of peppertrees than in those plots in which only distilled water was used (Figure 1D). The stronger inhibitory effects on germination rates were observed for the concentrated aqueous extracts (10%) of staminate flowers and fruits of peppertrees (Figure 1D). The diluted extract (5%) of staminate flowers had a similar effect than the concentrated extract of fruits, while all the other extracts had smaller negative effects on the germination rates of this cactus (Figure 1D). The final amount of germinated seeds of *M. geometrizans* was significantly reduced by most aqueous extracts of peppertrees, as compared to the values of this variable obtained for the control seeds (Figure 2D). These negative effects were stronger for the extracts at both concentration of staminate flowers, as well as for the concentrated extract of peppertree fruits (Figure 2D). Only the seeds treated with diluted extracts of fruits and female leaves showed similar amounts of germinated seeds when compared to control seeds (Figure 2D).

The seeds of the two succulent monocots in this experiment, *Agave salmiana* and *Yucca filifera*, displayed lower germination rates in the plots watered with concentrated extracts of staminate flowers, as compared to the control plots and those plots that received any other aqueous extract (Figure 1E and 1F). Nevertheless, while germination rates of *A. salmiana* were reduced by all peppertree extracts as compared to control seeds (Figure 1E), in the case of *Y. filifera* there were four treatments (concentrated extract of female leaves, and diluted extracts of staminate flowers, male leaves and female leaves) in which germination rates did not differ from that observed for the control (Figure 1F). The other three aqueous

extracts applied on seeds of *Y. filifera* (diluted extract of staminate flowers and concentrated extracts of fruits and male leaves) were found to reduce germinations rates (Figure 1F).

The cumulative number of germinated seeds of *A. salmiana* showed a different patten from that observed for germination rates. In this species, the experimental plots watered with the concentrated extract of staminate flowers showed the lowest amount of germinated seeds, followed by the diluted extract of staminate flowers and the extracts obtained from fruits at both concentrations (Figure 2E). The final amount of germinated seeds of *A. salmiana* in plots watered with the concentrated extract of female leaves, as well as in the plots watered with extracts of leaves of both peppertree genders, did not differ from that observed in the control plots (Figure 2E). On the other hand, the values of this variable for *Y. filifera* concurred with that observed for the germination rates (Figure 2F).

Allelopathic effects of soils beneath peppertrees

The greenhouse experiment indicated that soils beneath male and female peppertrees can also affect native species. All native species displayed slower rates of seedling emergence in soils collected beneath both peppertree genders than in the control soil collected in the surrounding open areas (Table 2; Figure 3). This experiment also indicated that both, the total number of emerged seedlings and their dry biomasses at the end of the experiment were smaller in soil collected beneath peppertrees (Table 2; Figure 4). However, as occurred in the *in vitro* germination bioassays, the magnitude with which male and female peppertree soils affected these variables varied among native species.

Seedling emergence rates of *Echinocactus platyacanthus* were slower in the soils collected beneath both peppertrees genders than in the control soil from the surrounding open areas, but soil beneath female trees had the strongest inhibitory effects on the germination of this

cactus species (Figure 3A). The final number of emerged seedlings was also lower in soils collected beneath peppertrees but, in this case, no differences between peppertree genders were found (Figure 4A). The dry biomass of the *E. platyacanthus* seedlings that grew on both peppertree soils was lower than those harvested on the control soil, but seedlings that grew on soils from male peppertrees were even smaller than those that grew on soils from female trees (Figure 4A).

All these response variables showed opposite patterns in *Ferocactus latispinus*. This cactus displayed slower seedling emergence rates on soils collected beneath peppertrees than in the control soil, but slower rates of shoot emergence were found on soil from male peppertrees (Figure 3B). The final number of emerged seedlings was lower in soils collected beneath peppertrees than in the control soil; the values of this variable did not differ between soils collected beneath male and female peppertrees (Figure 4B). The dry biomass of the seedlings was also lower in soils from peppertrees than in the control soil, but the lowest values of this variable were obtained on soils from female peppertrees (Figure 4B).

The cacti *Mammillaria longimamma* (Figure 3C) and *Myrtillocactus geometrizans* (Figure 3D), as well as the succulent monocot *Agave salmiana* (Figure 3E), displayed significantly slower seedling emergence rates on soils collected beneath both peppertree genders, as compared to the control soil. Nevertheless, no differential effects of soils from male and female peppertrees were observed on the seedling emergence rates of any of these native species (Figure 3). The final number of emerged seedlings of all these native species, as well as their dry biomasses, showed similar negative responses to peppertree soils (Figure 4).

Although *Yucca filifera* displayed the slowest rate of seedling emergence on the soil collected beneath male peppertrees, no differences were indicated between the control soil and the soil collected beneath female peppertrees (Figure 3F). When the cumulative number

of emerged seedlings was compared among soils types at the end of the experiment, only the soil from male peppertrees was indicated to have significant effects on this variable; no differences were found between the soil collected beneath female peppertrees and the control soil (Figure 4F). Conversely, both peppertree genders were indicated to have significant inhibitory effects on the growth of *Y. filifera* when the dry biomasses of their seedlings were compared across soil types (Figure 4F).

DISCUSSION

The results of the *in vitro* germination experiments conducted in this study suggest that the Peruvian peppertree can reduce the germination of native species that colonize abandoned agricultural fields in central Mexico. These negative effects could be due to a number of allelochemical compounds that have been previously detected in Peruvian peppertrees (Zahed et al. 2010; Barroso et al. 2011). Nevertheless, these results also indicated that the magnitude of the allelopathic effects of Peruvian peppertrees might vary depending on the plant organ from which these compounds are obtained (reproductive or vegetative organs). On this issue, Yang et al. (2012) have previously shown that the accumulation of secondary metabolites in living tissues of plants depends on the organ in which they are biosynthesized. In our case, the stronger allelopathic effects observed for staminate flowers and fruits of peppertrees could be linked to higher concentrations of secondary metabolites in these reproductive organs than in their leaves. This suggestion concurs with the results of the biochemical analyses conducted by Barbosa et al. (2007) for a species closely related to the Peruvian peppertree, namely *Schinus terebinthifolius* (Brazilian peppertree). These authors indicated that the concentration of secondary metabolites in the Brazilian peppertrees is higher in the reproductive structures than in leaves, but these differences in the concentration of secondary metabolites increase during the reproductive season (Barbosa et al. 2007). Nevertheless, biochemical analyses are still being required to determine the concentration of secondary metabolites in the different plant organs of Peruvian peppertrees inhabiting Mexico.

Besides these differential effects of reproductive and vegetative plant organs, the *in vitro* germination experiments also indicated that the magnitude of the allelopathic effects of Peruvian peppertrees can differ between tree genders. With the exception of the cactus

Mammillaria longimamma, these experiments suggested that staminate flowers produced by male peppertrees have stronger allelopathic effects than fruits produced by female peppertrees. Indeed, in most native species used in this study, the inhibitory effects of staminate flowers occurred irrespectively of the biomass of flowers used to prepare the aqueous extracts. These results allow proposing that male peppertrees are more aggressive than female peppertrees in inhibiting the germination of Mexican native species. As far as we are aware, no previous studies have reported such differential gender effects for allelopathic invasive species with dioecious reproductive system. These differential allelopathic effects of male and female peppertrees could be explained by differences in the rates of biosynthesis and accumulation of secondary metabolites between tree genders. Moreover, it could also be proposed that male and female peppertrees produce different bioactive substances with the potential to inhibit the germination of Mexican species, where secondary metabolites produced by male peppertrees would have stronger allelopathic effects than those produced by female individuals. However, besides determining the concentration of secondary metabolites in both tree genders, testing this hypothesis would also require conducting detailed biochemical analyses addressed to identify the nature and activity of the secondary metabolites produced by male and female peppertrees.

Although these *in vitro* bioassays clearly indicated that secondary metabolites contained in living tissues of peppertrees can interfere with the germination of Mexican native species, determining if this invasive species interferes with the recovery of native vegetation in the field requires assessing whether these bioactive compounds retain their allelopathic capacity after they are released from peppertrees. This was the aim of our greenhouse germination experiments, which indicated that the soil beneath peppertrees also inhibits the emergence of seedlings of native species. Moreover, those seedlings that grew on soils collected beneath

the canopies of peppertrees were smaller, in terms of their dry biomass, than the seedlings grew on soil collected in the open areas that surround peppertrees. These results then suggest that the allelopathic compounds produced by this invasive species are released to the environment and accumulated in the soil beneath their canopies, where reduce germination and growth of native species.

The differential allelopathic effects of the male and female peppertrees in the greenhouse experiments, however, were not as evident as occurred in the *in vitro* germination experiments. In the greenhouse experiment, seedling emergence of all native species was inhibited in soils collected beneath peppertrees. Nevertheless, only two native species (*Ferocactus latispinus* and *Yucca filifera*) showed a markedly negative response on soils collected beneath male peppertrees, while only the cactus *Echinocactus platyacanthus* displayed slower seedling emergence rates on soils collected beneath female peppertrees. Seedling emergence of the other three native species (*Mammillaria longimamma*, *Myrtillocactus geometrizans* and *Agave salmiana*) was similarly inhibited on soils collected beneath both tree genders. Moreover, when dry biomasses of seedlings were compared across soil types, only *Echinocactus platyacanthus* was indicated to have smaller seedlings on soils collected beneath male peppertrees, while only *Ferocactus latispinus* had smaller seedlings on soils collected beneath female peppertrees. The dry biomass of seedlings of the other four native species was smaller on soils collected beneath peppertrees than in the control soil, but no differences were observed between soils collected beneath the different tree genders. Thus, although the *in vitro* experiments showed that staminate flowers produced by male peppertrees had strongest inhibitory effect on most native species than fruits produced by female trees, the greenhouse experiments suggested that the allelopathic effects of the different peppertree genders are similar in the soil beneath their canopies.

This homogenization of inhibitory effects of male and female peppertrees in the soil beneath their canopies may be due to a number of factors. In first instance, it can be proposed that male peppertrees, which seem having stronger inhibitory effect on the germination of native species, release to the environment lower concentrations of allelopathic compounds than female peppertrees. This could lead to similar concentrations of secondary metabolites beneath the canopy of the different tree genders and, thus, cause similar allelopathic effects of male and female peppertrees on other species. On the other hand, it has been shown that the phytotoxic activity of secondary metabolites released by allelopathic plants depend upon their residence time in the soil, which can be reduced because of their degradation by microorganisms (Inderjit & Duke 2003; Inderjit & Nilsen 2003). Thus, although male and female peppertrees may differ in the amount of secondary metabolites they release to the environment, the degradation rates of these compounds might be higher beneath that tree gender that release more allelopathic compounds because this could promote microbial activity in the soil. As a consequence of this degradation process, the concentration of allelochemical substances in the soil could be similar between peppertree genders. Nevertheless, these suggestions would remain as hypotheses until conducting experiments specifically addressed to measure the amount of allelopathic metabolites released by male and female peppertrees, as well as to assess the microbial activity in the soil beneath their canopies.

Regardless of the nature, concentration and inhibitory capacity of secondary metabolites produced by male and female peppertrees, the results of this study allow proposing that this invasive species may threaten the recovery of native vegetation in abandoned agricultural fields of central Mexico, and also suggests that these negative effects would occur irrespectively of the gender to which peppertrees belong to. However, an important caveat about these

detrimental effects of peppertrees is related to the moment of the year in which they would effectively affect to native plants. Because the reproductive structures of peppertrees (staminate flowers and fruits) had the stronger allelopathic effects on native species, it could be argued that this invasive species precludes the recovery of native vegetation during its reproductive season only. Nevertheless, this tree has not the typical reproductive cycle of plants of temperate-semi arid regions. In its native distribution range (South America), the Peruvian peppertree produces flowers and fruits continuously between spring and fall, but reproduction abruptly stops at the beginning of winter (Copeland 1959). Conversely, in Mexico, this invasive species produces leaves, flowers and fruits during all year round (Martínez 1989; Goldstein & Coleman 2004; Ramírez-Albores & Badano 2013). Thus, if their reproductive structures are the main source of allelopathic compounds, invasive peppertrees would threaten the recovery of native vegetation in central Mexico irrespectively of the season of year.

Within the theoretical framework of biological invasions, the results of this study may fit to the *novel weapons hypothesis* (Callaway & Ridenour 2004). This hypothesis attempts to explain why some plants, which have no inhibitory effects on other species in their native distribution ranges, are strongly allelopathic in the new sites they invade. It proposes that these allelopathic effects would occur because plants from invaded sites have never been exposed to the secondary metabolites released by the new species and, thus, native plants lack of adaptations to face the biochemical arsenal of the invader (Hierro & Callaway 2003; Callaway & Ridenour 2004). As far as we are aware, the allelopathic effects of Peruvian peppertrees on other native species from South America have not yet been evaluated (but see Trindade & Coelho 2012). Nevertheless, our results indicate that succulent plant species from semiarid regions of Mexico would be extremely sensitive to the allelochemical

compounds released by this invasive species. Indeed, these results allow proposing that Peruvian peppertrees may be playing a critical role in determining the structure of plant communities that are colonizing abandoned agricultural fields in these regions.

ACKNOWLEDGMENTS

This study was supported by Secretaría de Educación Pública-Consejo Nacional de Ciencia y Tecnología, project CB-2010-156205 to JFR.

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Table 1

Statistical results from the in vitro experiments addressed to test the allelopathic effects of aqueous extracts obtained from different plant organs of male and female peppertrees (staminate flowers, fruits and leaves) on Mexican native species. All these aqueous extracts were used at two concentrations (10% and 5%) and distilled water was used as control treatment. Germination rates were compared among treatments by using the Gehan's generalized Wilcoxon chi-square test, while the accumulated numbers of germinated seeds were compared among treatments by using one-way ANOVAs. The table shows the results of both statistical analyses for each native species (critical $\alpha = 0.05$ in all cases).

Target species	Germination rates	Accumulated numbers of germinated seeds
<i>Echinocactus platyacanthus</i>	Chi ² = 44.629; d.f. = 8; p < 0.001	F _(8,36) = 405.823; p < 0.001
<i>Mammillaria longimamma</i>	Chi ² = 188.694; d.f. = 8; p < 0.001	F _(8,36) = 663.002; p < 0.001
<i>Ferocactus latispinus</i>	Chi ² = 108.896; d.f. = 8; p < 0.001	F _(8,36) = 219.133; p = 0.016
<i>Myrtillocactus geometrizans</i>	Chi ² = 175.039; d.f. = 8; p < 0.001	F _(8,36) = 628.811; p < 0.001
<i>Agave salmiana</i>	Chi ² = 117.895; d.f. = 8; p < 0.001	F _(8,36) = 304.944; p < 0.001
<i>Yucca filifera</i>	Chi ² = 202.539; d.f. = 8; p < 0.001	F _(8,36) = 1260.570; p < 0.001

Table 2

Statistical results from the greenhouse experiments addressed to test the allelopathic effects of soils collected beneath male and female peppertrees on Mexican native species. Soil from the open areas that surround peppertrees was used as control substrate in all cases. Seedling emergence rates were compared among soil by using the Gehan's generalized Wilcoxon chi-square test, while one-way ANOVAs were used to compare the accumulated numbers of emerged seedlings and their dry biomass. The table shows the results of both statistical analyses for each native species (critical $\alpha = 0.05$ in all cases).

Target species	Seedling emergence rates	Accumulated numbers of emerged seedlings	Dry biomass of seedlings
<i>Echinocactus platyacanthus</i>	Chi ² = 28.634; d.f. = 2; p < 0.001	F _(2,27) = 1039.436; p < 0.001	F _(2,27) = 783.667; p < 0.001
<i>Mammillaria longimamma</i>	Chi ² = 58.535; d.f. = 2; p < 0.001	F _(2,27) = 2029.167; p < 0.001	F _(2,27) = 1694.118; p < 0.001
<i>Ferocactus latispinus</i>	Chi ² = 27.895; d.f. = 2; p < 0.001	F _(2,27) = 1872.320; p < 0.001	F _(2,27) = 1384.189; p < 0.001
<i>Myrtillocactus geometrizans</i>	Chi ² = 25.355; d.f. = 2; p < 0.001	F _(2,27) = 866.422; p < 0.001	F _(2,27) = 552.7855; p < 0.001
<i>Agave salmiana</i>	Chi ² = 38.930; d.f. = 2; p < 0.001	F _(2,27) = 881.803; p < 0.001	F _(2,27) = 403.324; p < 0.001
<i>Yucca filifera</i>	Chi ² = 9.216; d.f. = 2; p = 0.009	F _(2,27) = 4893.136; p < 0.001	F _(2,27) = 205.734; p < 0.001

FIGURE CAPTIONS

Figure 1. Germination curves estimated with the Kaplan-Meier's method for the different native species used in the *in vitro* experiments addressed to test the allelopathic effects of aqueous extracts obtained from different plant organs of male and female peppertrees (staminate flowers, fruits, and leaves of both tree genders). All these aqueous extracts were used at two concentrations (10% and 5%) and distilled water was used as control treatment. The legend on the side of each figure indicates the treatment to which each curve belongs. Different letters accompanying the symbols of the curves indicate statistical differences in germination rates between treatments (Gehan-Wilcoxon's two-sample test critical $\alpha = 0.05$ in all cases).

Figure 2. Accumulated number of germinated seeds (averages ± 2 S.E.) obtained for each native species at the end of the *in vitro* experiments addressed to test the allelopathic effects of aqueous extracts obtained from different plant organs of male and female peppertrees (staminate flowers, fruits, and leaves of both tree genders). All these aqueous extracts were used at two concentrations (10% and 5%) and distilled water was used as control treatment. Different letters above the bars of treatments indicate statistical differences between treatments (Tukey test critical $\alpha = 0.05$ in all cases).

Figure 3. Seedling emergence curves estimated with the Kaplan-Meier's method for the different native species used in greenhouse experiments addressed to test the allelopathic effects of soils collected beneath male and female peppertrees. Soil collected in the open areas that surround peppertrees was used as control substrate. The legend on the side of each figure indicates the treatment to which each curve belongs. Different letters

accompanying the symbols of the curves indicate statistical differences in seedling emergence rates between treatments (Gehan-Wilcoxon's two-sample test critical $\alpha = 0.05$ in all cases).

Figure 4. Accumulated number of emerged seedlings (white bars; left scaling) and their dry biomass (grey bars; right scaling) obtained for each native species at the end of the greenhouse experiments addressed to test the allelopathic effects of soils collected beneath male and female peppertrees. Soil from the open areas surrounding peppertrees was used as control substrate. Bars are average values for these two variables (± 2 S.E.) at each soil type and different letters above the bars indicate statistical differences between treatments (Tukey test critical $\alpha = 0.05$ in all cases).

Figure 1

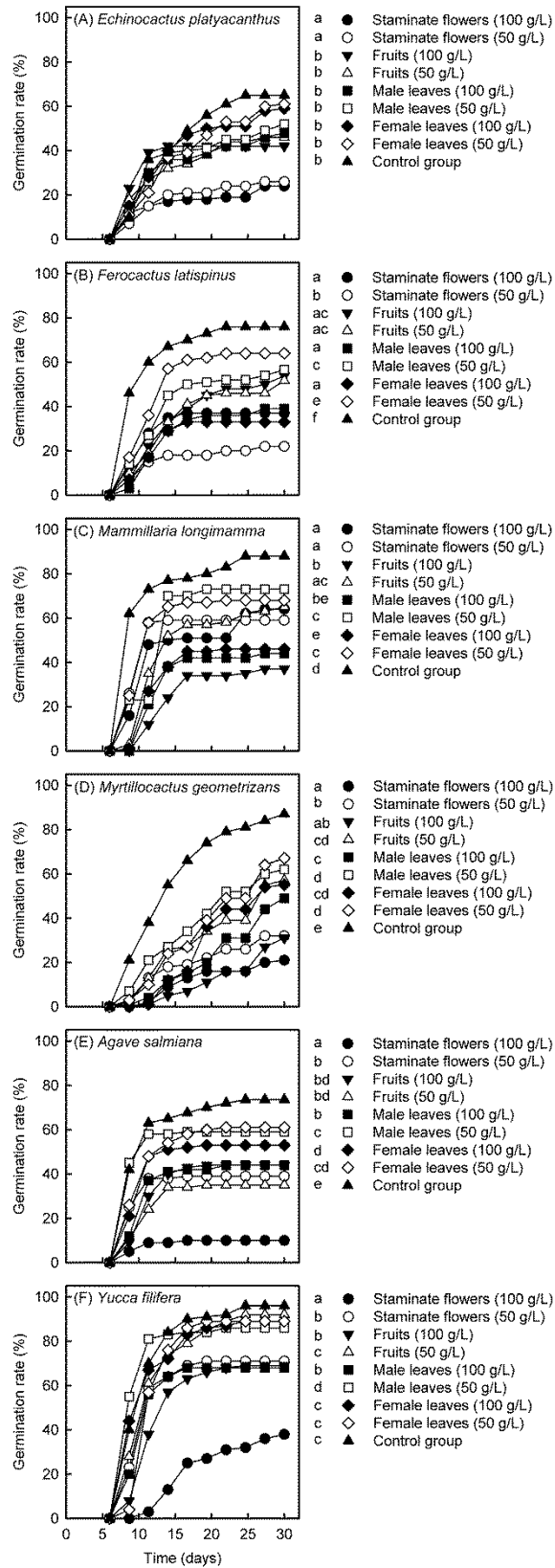


Figure 2

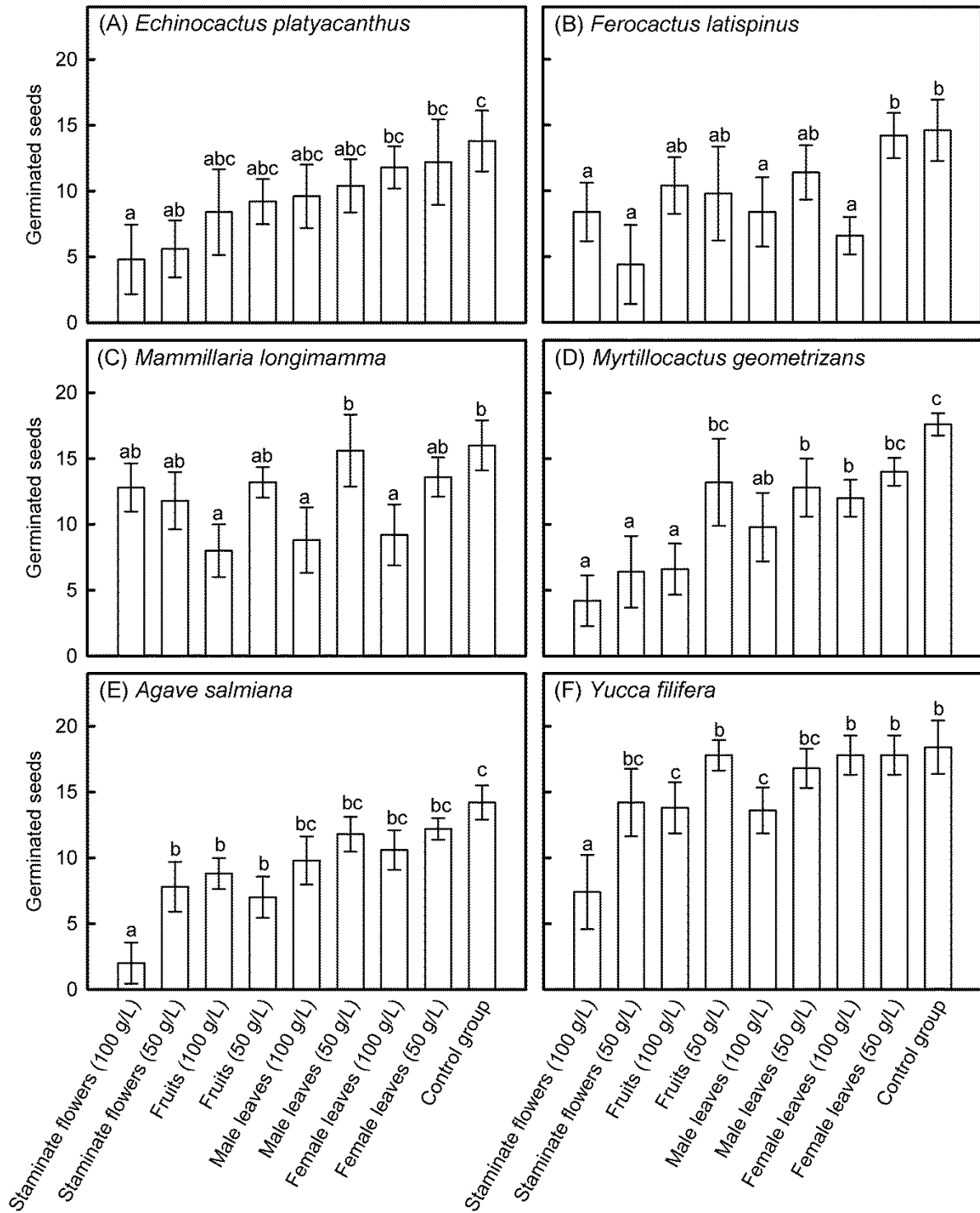


Figure 3

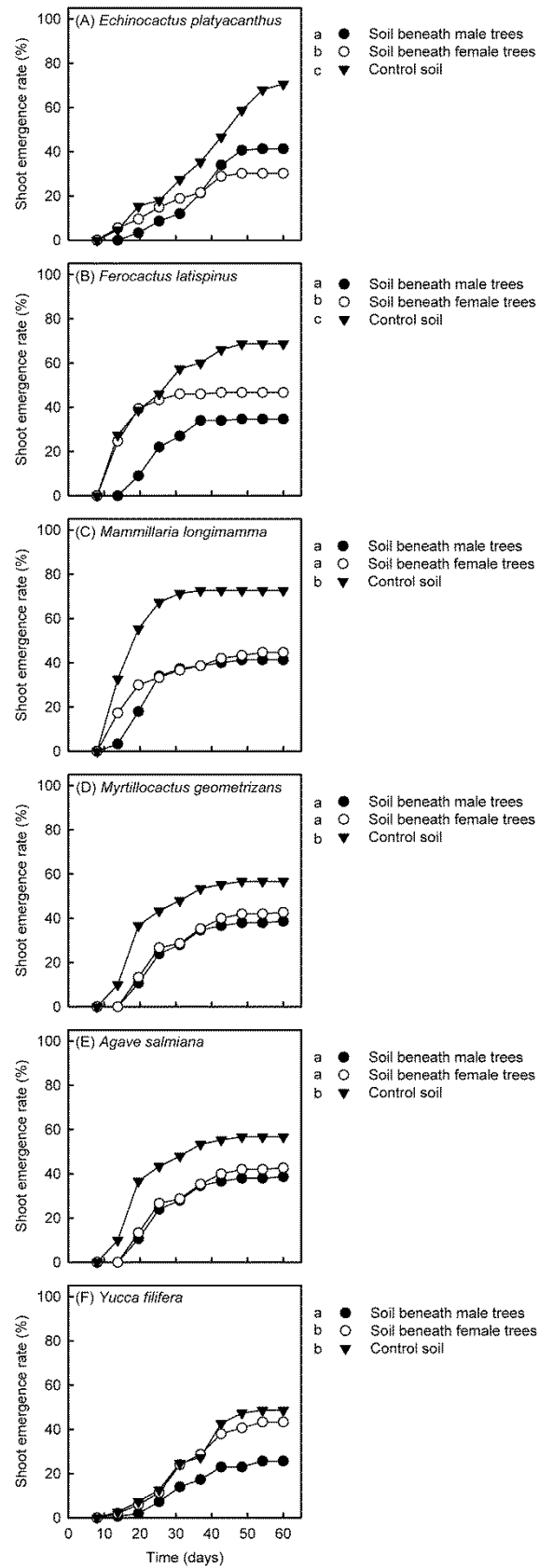


Figure 4

