ROLE OF FIRE AND PLANT-DERIVED SMOKE IN SEED GERMINATION AND SEEDLING EMERGENCE IN FESCUE PRAIRIE

A Thesis Submitted to the College of Graduate Studies and Research

In Partial Fulfillment of the Requirements

For the Degree of Doctor of Philosophy

In the Department of Plant Sciences

University of Saskatchewan

Saskatoon

By

Lei Ren

PERMISSION TO USE

In presenting this thesis/dissertation in partial fulfillment of the requirements for a Postgraduate degree from the University of Saskatchewan, I agree that the Libraries of this University may make it freely available for inspection. I further agree that permission for copying of this thesis/dissertation in any manner, in whole or in part, for scholarly purposes may be granted by the professor or professors who supervised my thesis/dissertation work or, in their absence, by the Head of the Department or the Dean of the College in which my thesis work was done. It is understood that any copying or publication or use of this thesis/dissertation or parts thereof for financial gain shall not be allowed without my written permission. It is also understood that due recognition shall be given to me and to the University of Saskatchewan in any scholarly use which may be made of any material in my thesis/dissertation.

Requests for permission to copy or to make other use of material in this thesis in whole or part should be addressed to:

Head of the Department of Plant Sciences
College of Agriculture and Bioresources
51 Campus Drive
University of Saskatchewan
Saskatoon, Saskatchewan S7N 5A8, Canada

ABSTRACT

Fire, a natural disturbance, regulates species composition in Fescue Prairie. However, little is known about the contribution of different regeneration strategies in altering species composition after burning in Fescue Prairie. The present study was conducted to determine if and how fire and associated fire cues regulate species composition in Fescue Prairie through their effects on seedling recruitment. The effects of fire and various fire cues, including smoke, ash, and smoke plus ash on seedlings emerging in the field and/or from litter, 0-1 cm, and 1-5 cm layers of the soil seed banks were therefore examined. These studies were complimented by a study of testing effects of smoke originated from different plant materials on seed germination and seedling growth of species from Fescue Prairie. Chemical analyses were also conducted to determine whether different active compounds existed in smoke made from different materials, which in turn affect germination and seedling growth differently. Burning increased densities, richness, and diversity of seedlings emerging in the field. This was possibly attributed to direct fire cues of burning. Seedling densities of native forbs and non-native graminoids emerging from the soil seed bank were increased and decreased by burning, respectively. Ash and smoke plus ash increased density of forbs emerging from the soil seed bank. Species composition of seedlings emerging in the field and from the soil seed bank was altered by burning. Complex responses were observed for the effects of smoke on seedling establishment, which depended on the type and dilution of smoke solutions, as well as germination conditions. Smoke solutions partly substituted light requirement for germination of *Artemisia ludoviciana*. Germination of *Cirsium arvense* and *Conyza canadensis* only responded to smoke solutions at 25/15°C, but not at 10/0 °C. Diluted smoke solutions increased radical length of *Artemisia ludoviciana*. Karrikinolide (KAR_1) was in the smoke made from prairie hay and wheat straw, but not in that made from alfalfa. This is the first report that different active compounds existed in smoke made from different materials. Highly concentrated smoke solutions made from alfalfa increased germination and radical length of *Conyza canadensis*, while the same concentrated smoke solutions made from prairie hay and wheat straw reduced germination of *Conyza canadensis* at 25/15 °C in darkness. Priming in KAR_1 solutions and active fractions obtained from prairie hay and/or wheat straw increased germination of *Artemisia frigida*, *Artemisia ludoviciana*, and *Conyza canadensis* at certain germination conditions. In summary, fire and direct fire cues,

smoke and ash specifically, stimulated recruitment of some species, especially early seral species and native forbs, contributing to potential changes in species composition of the Fescue Prairie. Different compounds existed in smoke solutions made from alfalfa as compared with those from prairie hay and wheat straw, showing different effects on seed germination and seedling growth. KAR_1, the most important active compound discovered in smoke, was present in the smoke made from prairie hay and wheat straw, but was not in that made from alfalfa.

ACKNOWLEDGEMENTS

I would like to express my sincerest appreciation to my supervisor Dr. J. T. Romo and Dr. Y. Bai for their mentoring, support, and motivation through my graduate study and research. Their extensive knowledge, serious attitude, and commitment to research made this study a success. My sincere thanks extend to all my committee members, Dr. K. Tanino, Dr. E. Lamb, Dr. B. Coulman and Dr. P. Yu. Their questions, suggestions, and comments guided me to conquer various difficulties throughout the project. My special thanks go out to P. A. Ryan for helping me collecting data in the field and greenhouse. Special thanks also go to Dr. M. Reaney and staff in his lab, especially P. Burnett and P. Jadhav, for helping me with chemical analysis. I am also grateful to my external examiner.

My thanks also go to staff at the Kernen Crop Research Farm for allowing me to make smoke solutions there. Special thanks are extended to E. Siemens at the Agriculture Greenhouse for assisting me manage the experiment conducted there. I would like also thank Dr. Y. Wei who gave me tremendous help in setting up experiment in the lab. My thanks are also extended to H. Wang, Y. Abu, R. Bibi, F. Jia, and P. Nick for their assistance and resourcefulness towards the success of this study. I also want to express my thanks to fellow graduate students and the outstanding faculty and staff of the Department of Plant Sciences.

Financial support provided through the China Scholarship Council, University of Saskatchewan, and W. J. White Professorship to J.T. Romo has made my research and life much easier over the last few years. I am grateful to have received the F.V. MacHardy Graduate Fellowship twice in Plant Sciences. I am also grateful for the travel awards received through the University Postgraduate Scholarships for my conference in US.

Last but not least, I would like to thank my family members and friends, especially my mother Qingling Liu and my wife Jun Liu, who supported and encouraged me through it all.

TABLE OF CONTENTS

Page

LIST OF TABLES

LIST OF FIGURES

1.0 INTRODUCTION

As a natural disturbance, fire plays a key role in maintaining ecosystem stability, functioning, and biodiversity of the Fescue Prairie (Romo, 2003; Gross and Romo, 2010). Burning can exert positive (Anderson and Bailey, 1980) or neutral effects (Bailey and Anderson, 1978) on species richness and species diversity in Fescue Prairie. Plant species respond differently to burning (Dejong and Macdonald, 1975; Van De Venter and Esterhuizen, 1988; Van Staden *et al.*, 2000; Nelson *et al.*, 2012), with species adapted to arid conditions being favoured (Dejong and Macdonald, 1975), due to the reduced soil water content after burning (Redmann *et al.*, 1993). Different functional groups in Fescue Prairie have various responses to burning, with perennial forbs being favored (Bailey and Anderson, 1978; Wright and Bailey, 1982). Regeneration after burning in grasslands occurs through vegetative reproduction and seedling emergence from seeds (Everson and Tainton, 1984; Fenner and Thompson, 2005). Fire may initiate seedling establishment by opening gaps or by triggering germination of seeds buried in the soil seed bank, which plays an important role in influencing the dynamics of plant communities (Thompson and Grime, 1983; Leck *et al.*, 1989). Fire is a selective pressure in the evolution of seedling traits expressed through seed germination and seedling establishment (Keeley *et al.*, 2012). Although it has been well known that different fire regimes, including fire severity (Mithcell, 1958; Wright, 1971), frequency (Anderson and Bailey, 1980), and seasonality (Bailey and Anderson, 1978) regulate species composition in Fescue Prairie, it is not clear about the contribution of regeneration from seeds to the altered species composition after burning.

Burning effects can be broadly divided into direct and indirect (Santana *et al.*, 2013). Direct effects include heat shock, ash, and smoke produced by burning. Changes in the micro-environmental conditions after burning, including soil temperatures, soil water content, light quality and quantity, as well as nutrient availability are indirect effects (Nelson *et al.*, 2012). Independent or combined effects of direct fire cues regulate seedling emergence in mesic grasslands in South Africa (Ghebrehiwot *et al.*, 2012), woodlands in Australia (Enright and Kintrup, 2001), chaparrals in southern California (Keeley and Fotheringham, 2000), but not in shrublands in the Mediterranean Basin in Spain (Santana *et al.*, 2013).

Seed germination and seedling establishment cannot occur if seeds are dormant (Finch-Savage and Leubner-Metzger, 2006). Seed dormancy is an adaptive trait in temperate species

and can be broken by periodic disturbances including burning (Schwilk and Zavala, 2011). Heat shock, one of the fire-related cues, is regarded as beneficial in breaking physical dormancy in legumes (Auld and O'Connell, 1991). In addition, chemicals, including ethylene, ammonia, and nitrogenous compounds generated by burning can break seed dormancy and stimulate seed germination (Van De Venter and Esterhuizen, 1988; Keeley and Fotheringham, 1997). Ash inhibits seed germination of ten species from a gorse shrubland (Gonzalez-Rabanal and Casal, 1995). Henig-sever *et al.* (1996) suggested that the high pH of soil caused by ash influences regeneration of forest species.

Plant-derived smoke can break seed dormancy and stimulate germination (De Lange and Boucher, 1990; Van staden *et al.*, 2000). Smoke stimulates seed germination of plants from the Australian kwongan (Roche *et al.*, 1997), Californian chaparrals (Keeley and Fotheringham, 1998), Western Cape fynbos (Brown *et al.*, 2003) and the Mediterranean basin (Crosti *et al.*, 2006). Species that are responsive to smoke have been found in all continents except Antarctica, representing a wide diversity of ecosystems that are either prone or not prone to burning (Baxter and Staden, 1994; Baxter *et al.*, 1995; Tieu *et al.*, 1999; Chiwocha *et al.*, 2009). Seed germination for over 1,200 species responds positively to plant derived smoke (Dixon *et al.*, 2009). Stimulation of seed germination by smoke is independent of seed size, shape, and life form (Dixon *et al.*, 1995). Plant species have different sensitivities to the active compounds in smoke (Adkins and Peters, 2001), with high concentrations of smoke inhibiting seed germination (Drewe *et al.*, 1995 and Kulkarni *et al.*, 2007). Smoke can also increase hypocotyl development, root elongation, and flowering (Taylor and Van Staden, 1996).

Different theories have been proposed to explain the mechanisms of smoke in breaking seed dormancy and stimulating germination. Smoke acted as chemical scarification and increased germination of *Emmenanthe penduliflora* Benth. by increasing seed coat permeability to water and oxygen (Egerton-Warburton, 1998). Smoke may also induce germination by altering the sensitivity of seeds to phytohormones (Schwachtje and Baldwin, 2004; Nelson *et al.*, 2009). Acid compounds generated by smoke stimulated germination of annuals after burning in chaparral (Keeley and Fotheringham, 1998). The mechanism by which smoke promotes seed germination has not been determined, likely because it varies among species (Baxter *et al.*, 1995; Keeley and Fotheringham, 1997).

More than one active compound is present in smoke created by burning plant materials. Twelve compounds were identified in smoke obtained from burning *Themeda triandra* Forssk.; seven of these compounds were also present in extracts of smoke from *Passerina vulgaris* Meisn. (Jager *et al.*,1996). At least three compounds in smoke promote germination of *Nicotiana attenuata* Steyd. (Baldwin *et al.*, 1994). Among these possible active components, butenolide, 3-methyl-2*H*-furo [2,3-c]-pyran-2-one, renamed as karrikinolide (KAR_1), was first isolated and shown to be the active compound in smoke solution from burned plant materials (Van Staden *et al.*, 2004; Flematti *et al.*, 2004). Five other KAR_1 analogs (KAR_2-KAR_6) and KAR_1, collectively known as karrikins, were confirmed in smoke solution (Flematti *et al.*, 2009). Synthetic KAR_1 can be active at a range of concentrations from 10^{-9}M to 10^{-7}M, depending on species (Flematti *et al.*, 2004; Baldwin *et al.*, 1994). While high concentrations of smoke solution may inhibit seed germination, KAR_1 does not (Van Staden *et al.*, 2004). KAR_1 stimulates seed germination in many species, including plants from fire-prone environments (Merritt *et al.*, 2006), arable weeds (Daws *et al.*, 2007; Stevens *et al.*, 2007), and crops (Kulkarni *et al.*, 2006; van Staden *et al.*, 2006; Jain *et al.*, 2008). KAR_1 also widens the environmental conditions under which seeds can germinate. KAR_1 favors germination of tomato (*Lycopersicon esculentum* Mill.) seeds at sub- and supra-optimal temperatures (Jain *et al.*, 2006). In addition to stimulating seed germination, KAR_1 increases seedling growth (Daws *et al.*, 2007; Kulkarni *et al.*, 2007). Another active compound, cyanohydrin, is also present in smoke of burned plant material (Flematti *et al.*, 2011). Although KAR_1 is the major stimulant to germination, some species, such as *Anigozanthos manglesii* D. respond to cyanohydrin but not KAR_1 (Flematte *et al.*, 2011).

The stimulating effects of smoke and KAR_1 on seed germination are dependent on temperature and light (González-Rabanal and Casal, 1995; Van Staden *et al.*, 1995; Ghebrehiwot *et al.*, 2009). Ghebehiwot *et al.* (2009) reported that the positive effects of smoke solutions on seed germination of *Aristida junciformis* Trin. & Rupr., *Hyparrhenia hirta* (L.) Staph., and *Panicum maximum* Jacq. were more notable with increasing temperature. Smoke solutions or KAR_1 can, however, eliminate light requirements for seed germination of *Lactuca sativa* L., a photoblastic species (Drewes *et al.*, 1995). Smoke concentrations also influence seed germination. Aqueous smoke solutions promoted seed germination of *L. sativa* over a range of concentrations 1:100 v/v and 1:10000 v/v, but germination was reduced at concentrations of 1:1 v/v to 1:10 v/v (Drewes *et al.*, 1995).

Effects of burning on species density and species composition have been previously studied in the Fescue Prairie (Bailey and Anderson, 1978; Anderson and Bailey, 1980; Grilz and Romo, 1995; Bork et al., 2002). However, none of these studies disentangles the effects of burning on seed reproduction from vegetative reproduction. Different fire cues, including direct and indirect, regulate regeneration from seeds after burning (Keeley and Fotheringham, 2000). Which fire cues are more important in affecting seedling emergence in Fescue Prairie are still unknown. Although the importance of smoke on seed germination has been investigated in many species (Dixon *et al.*, 2009), further research is needed to determine the ecological significance of this environmental factor (Reyes and Trabaud, 2009). Furthermore, most previous studies focusing on the effects of smoke on seed germination and seedling establishment were completed in Africa, Australia, America and Europe. No such studies have been conducted in the Canadian Prairies, specifically the Fescue Prairie. In conclusion, disentangling effects of fire-related cues, smoke specifically, on germination and regeneration ecology of species in Fescue Prairie can help us better understand the mechanisms of fire in shaping species composition in Fescue Prairie.

The objectives of these studies were to: 1) determine seedling emergence in the field and from the soil seed bank after burning Fescue Prairie; 2) determine the effect of fire cues of smoke, ash, and smoke plus ash on seedling emergence from the soil seed bank; 3) determine the interacting effects of different types of smoke and smoke solution concentrations on seed germination and seedling growth of species from the Fescue Prairie within different germination conditions, and; 4) evaluate the effects of known active components in smoke and identify potentially new, active compounds. It was hypothesized that: 1) seedling density, seedling emergence rates, and species composition are affected by burning in the field and in the seed bank under controlled environmental conditions; 2) seedling density, seedling emergence rates, and species composition are affected by the direct effects of burning, which includes smoke, ash, and smoke plus ash; 3) stimulating effects of smoke made by burning plant materials are dependent on temperatures and light; 4) germination and seedling growth of different species respond differently to smoke made by burning plant materials, and; 5) smoke solutions produced from different plant materials contain different active compounds, which in turn affect germination and seedling growth differently.

2.0 LITERATURE REVIEW

2.1 Fire and Fescue Prairie

Although fire is one of the key factors shaping ecosystem composition and distribution (Bond *et al.*, 2005), its importance in ecosystem processes is underestimated (Pausas and Keelkey, 2009), due to the dated notion that soils and climate are the major components determining community characteristics (Pausas and Keelkey, 2009). Recently, the significance of fire was revealed during attempts to model vegetation change worldwide (Bond and Keeley, 2005). Different adaptive traits of plants to burning are acquired by experiencing different fire regimes. This phenomenon is apparent in different ecosystems (Biswell, 1974; Naveh, 1974; Gill, 1981; Frost, 1984; Tucker and Cadotte, 2013).

Wright and Bailey (1982) separated Fescue Prairie into two sections. The mountain and foothill section ranges from the eastern foothills of the Rocky Mountains in southwestern Alberta to the boundary of United States along the foothills. The plain or aspen parkland section occupies central Alberta and Saskatchewan (Moss and Campbell, 1947; Coupland and Brayshaw, 1953). The annual mean precipitation is between 38-61 cm and 36-46 cm in the foothill and plain sections, respectively (Wright and Bailey, 1982), with half accumulating between April and July (Coupland, 1961). The annual mean temperature in fescue prairie is 0-6 ℃. The number of frost-free days ranges from about 100 to 125 days (Coupland, 1961). Looman and Best (1979) subdivided rough fescue (*Festuca scabrella* Torr.), the dominating species in fescue prairie, into the foothills rough fescue (*Festuca campestris* Rydb.) and the plains rough fescue (*Festuca hallii* (Vassey) Piper), based on the different morphology of these two species (Moss and Campbell, 1947; Wright and Bailey, 1982). *Agropyron* spp. and *Carex* spp. are frequently codominant with rough fescue in the foothills (Coupland, 1961). The drier condition in the plain region favors western porcupine (*Stipa curtiseta* Hitchc.) as the codominant species with plains rough fescue.

Fescue Prairie is well adapted to burning (Wright and Bailey, 1982). Fire suppression and the reduced probability of large-scale fires due to settlement and landscape fragmentation, have increased fire return intervals, leading to the encroachment of trees and shrubs (Coupland and Brayshaw, 1953; Anderson and Bailey, 1980). Reintroducing fire may play a crucial role in conserving biodiversity in remnant Fescue Prairies (Romo, 2003), and in enlarging the area of the remnant Fescue Prairies (Bailey and Wroe, 1974). Without fire, Fescue Prairie is subject to

brush invasion (Moss and Campbell, 1947; Bailey and Wroe, 1974). Brush area doubled from 1907 to 1966 in the parkland region of southcentral Alberta (Bailey and Wroe, 1974). Coverage of aspen forest increased 13-fold between 1940 and 1975 in the aspen parkland of central Alberta (Wright and Bailey, 1982). Brush invasion significantly reduces the yield of forage. Johnston and Smoliak (1968) claimed forage yield under a closed canopy was only one third of that in open Fescue Prairie. Fire is the key factor preventing invasion of shrubs and trees and maintaining biodiversity of herbaceous species in Fescue Prairie (Anderson and Bailey, 1980; Wright and Bailey, 1982). Long term annual early spring burning reduced the canopy cover of *Symphoricarpos occidentalis* Hook., the most frequent shrub, from 31% to 2%, and maintained the forest cover at low levels in aspen parkland in central Alberta (Anderson and Bailey, 1980). The responses to fire vary among species. Species adapted to burning increased growth and reproduction (Nelson, 2012). Burning in Fescue Prairie favors perennial forbs and grasses but tends to discriminate against annuals (Bailey and Anderson, 1978).

The timing (Bailey and Anderson, 1978; Romo *et al.*, 1993) and frequency (Anderson and Bailey, 1980; Gross and Romo, 2010) of burning regulate plant community composition in Fescue Prairie. *Festuca hallii* can tolerate a single burn, with an early spring burn increasing its tillering and standing crop (Gerling *et al.*, 1995). However, mid-spring burning has detrimental effects on the coverage and biomass of *Festuca hallii* in the first growing season (Sinton, 1980; Wright and Bailey, 1982). Early spring or late autumn burning did not affect herbaceous biomass, but altered species composition in the first growing season in Fescue Prairie. Spring burning reduces sedge and Hooker's oatgrass (*Helictotrichon hookeri*) and has detrimental effects on the seed production of rough fescue (Wright and Bailey, 1982). Production of inflorescences by porcupine grass (*Stipa curtiseta*) was favored by spring burning and negatively affected by fall burning (Bailey and Anderson, 1978). Species composition shifted in favor of perennial forbs, regardless of the time of burning in Fescue Prairie (Bailey and Anderson, 1978; Wright and Bailey, 1982). Repeated annual, early spring burning of grassland in east-central Alberta for 24 years eliminated woody species and expanded the coverage of herbaceous species, with a notable increase in the number of forbs (Anderson and Bailey, 1980). The structural, spatial, and temporal heterogeneity in plant species composition of Fescue Prairie was not affected by the time of burning and burning frequency (Gross and Romo, 2010).

2.2 Fire and plant reproduction

Mechanisms allowing species to persist in habitats that are prone to burning include asexual reproduction (resprouters) and seedling recruitment from seeds in the soil seed bank and canopy (seeders) (Keeley and Zedler, 1978; Paula and Pausas, 2004, 2008). A key difference between these two strategies lies in the different allocation of resources to reproductive organs (Fenner and Thompson, 2005). Pausas and Verdú (2005) proposed a negative correlation between the traits of seeders and resprouters in the Mediterranean Basin, suggesting that there may be a trade-off relationship between these two reproductive strategies (Paula and Pausas, 2008). Regeneration of plants after burning can occur by resprouting (obligate resprouters), seeding (obligate seeders), or both (facultative seeders) (Keeley, 1986; Pausas and Keeley, 2014). It is common for perennials to reproduce from seeds and vegetative propagation (Enright and Goldblum, 1999; Fenner and Thompson, 2005). Resprouting is common in perennial plants (Wells, 1969). After evaluating species in 139 families in Mediterranean ecosystems in Europe and North America, Pause and Keeley (2014) claimed that 57% of species studied have the ability to resprout.

Environmental alterations play crucial roles in modifying plant traits (Lathi *et al.*, 2009). Resprouting is prevalent for plants living in moist and fertile habitats (Grime, 2005). However, decreased moisture after burning in shrublands favors obligate seeders over obligate resprouters (Pause and Keeley, 2014). Compared with resprouters, germination of seeders is more likely to be promoted by heat (Paula and Pausas, 2008). Higher temperatures during more intense fires may break seed dormancy (Moreira and Pausas, 2012). Seedling survivorship can also be enhanced after burning because of reduced competition (Grime, 2005). In addition, Steven *et al.* (2014) claimed seedling emergence could be enhanced by the higher soil temperatures after burning, even with limited water availability. The need for fire to break seed dormancy and stimulate germination of obligate seeders after burning favors accumulation of a seed bank during period of no burning (Pause and Keeley, 2014). Early emergence and quick growth enhance the possibility of survival of obligate seeders after burning (de Luis *et al.*, 2008). The seeding strategy after burning is also positively correlated with plant flammability (Schwilk and Ackerly, 2001; He *et al.*, 2012).

Coexistence status, distribution, and density of the seeders and resprouters are determined by the frequency of fires (Crandall and Platt, 2012). A short interval between burning may

reduce the density of seeds in the soil seed bank for seeders and eliminate those species (Keeley and Zedler, 1978; Wooller *et al.*, 2002; Watson *et al.*, 2009). On the other hand, frequent fires favor reprouters (Bell, 2001; Bond and Midgley, 2001; Drewa *et al.*, 2002). In contrast, resprouters are more vulnerable if the fire return interval is longer than the life cycle of established plants (Enright *et al.*, 2011).

2.3 Effects of fire on seedling emergence from the seed bank

2.3.1 Soil seed banks

A soil seed bank is a reserve of viable, ungerminated seeds buried in the soil (Harper, 1977), left on the soil surface (Roberts, 1981), or in the litter (Komarova, 1985). A soil seed bank is dynamic, with the composition and seed density changing seasonally and annually (Thompson and Grime, 1979). Seeds can remain viable for variable lengths of times depending on their dormancy status and suitable environmental conditions for germination. Thompson and Grime (1979) classified soil seed banks as transient and persistent. The ability of seeds to retain their viability until the second germination season separates transient and persistent soil seed bank (Baskin and Baskin, 1998). These categories are based on the amount of time (1 year) that seeds remain viable in the soil. They further divided transient seed banks into two types, based on the season of seed germination. Persistent seed bank are also classed into two types, based on the ratio of germinated to non-germinating seeds. Rather than the age *per se*, it is suggested that the season of germination should be used to define these two groups (Walck *et al.*, 1996). The time of sample collection should correspond to the type of seed bank studied (Champness and Morris, 1948; Archibold, 1981; Pratt *et al.*, 1984). For example, samples should be collected in early spring to include transient plus persistent seed banks when plant communities are dominated by summer annuals or perennials. The transient seed bank can be eliminated, however, if samples are collected in summer, when germination of seeds from the last season is complete and the maturation and germination of the new seeds have not started (Warr *et al.*, 1994).

High spatial heterogeneity of seed distribution leads to aggregation of many species in the soil seed bank (Thompson, 1986; Chauvel *et al.*, 1989; Dessaint *et al.*, 1991). It is common for soil seed bank studies to have high variability in seed densities and composition (Matlack and Good, 1990). Increasing the sample size is an effective approach to reduce this high variation (Thompson, 1986). To quantify species density in the soil seed bank, at least 15 locations with

20 soil cores should be taken (Gross, 1990). Benoit *et al.* (1989) suggested the sample size should be increased to 60 to determine the number of *Chenopodium* spp. in the soil seed bank in cultivated fields.

Seed densities in the persistent soil seed bank vary considerably among habitats, ranging from 0-9/m^2 in subarctic forests to 1,900-24,000/m^2 in pastures (Baskin and Baskin, 2004). Densities of buried seed were negatively correlated with altitude, latitude, and successional stage (Thompson, 1978). Generally, plant communities in grasslands have more persistent seeds compared with forests (Baskin and Baskin, 1998), and even more buried seeds can occur in disturbed soils such as arable land (Thompson, 1978).

2.3.2 Effects of fire on seedling emergence from soil seed banks

Seedling recruitment from soil seed bank is fundamental in predicting plant species composition after disturbances (Harper, 1977). Rather than the burning *per se*, seedling recruitment in different plant communities is affected by fire regimes (Keeley *et al.*, 2011). Five distinct components of fire regimes include fire severity, frequency, seasonality, spread pattern, and fire distribution (Keeley, 2009). Relatively high fire intensity favors seedling establishment of fugitive species from the soil seed bank compared with low fire intensity in the South American steppe (Ghermandi *et al.*, 2013). Increased fire frequency increases density of seedlings emerging from the soil seed bank in tropical savannas (Anderson *et al.*, 2012). A mild increase of fire frequency favors seedling establishment of forbs and perennial graminoids and increased species richness in the Mediterranean Basin (Santana *et al.*, 2014). However, by favoring seedling recruitment of short life span and fast growing species, frequent fire increases the density of alien species and reduces diversity of native species in shrubland ecosystem (Keeley and Brennan, 2012). Species diversity of emerged seedlings from the soil seed bank was reduced by summer or autumn burning compared with early spring burning in Fescue Prairie (Romo and Gross, 2011). Densities of seedlings emerging from the soil seed bank in semi-arid grassland respond more positively to head fire as compared with the back fire (Snyman, 2005). Burning increases annuals and biennials, but reduces perennials emerging from the soil seed bank in Patagonia grasslands (Gonzalez and Ghermandi, 2008). Density and richness of seedlings emerging from the soil seed bank in deciduous savanna are negatively affected by burning (Mamede and Araujo, 2008). Density and richness of seedlings emerging from the transient soil seed bank in a Mediterranean pasture are significantly reduced by burning but well

recovered one year after burning (Ferrandis *et al.*, 2001). Hence, soil seed banks respond to fire in complex ways, depending on the effects of different fire regimes, dormancy mechanisms, and the depth of dormancy in seeds (Enright *et al.*, 1997).

2.3.3 Effects of heat shock on seedling emergence from soil seed banks

Heat produced during burning exerts dual effects on seeds in the soil seed bank. On one hand, the number of viable seeds in the litter may be reduced because of lethal temperatures (Bailey and Anderson, 1980; Archibold *et al.*, 1998). On the other hand, heat can break seed dormancy, especially physical dormancy (Floyd, 1976; Shea *et al.*, 1979; Warcup, 1980; Baskin and Baskin, 1998), and stimulate germination of seeds buried in the soil (Morrison *et al.*, 1998). By fracturing the seed coat of hard-seeded species, heat allows seeds to absorb water, the prerequisite for germination (Bewley, 1997; Morrison *et al.*, 1998). To break dormancy for species with physical and physiological dormancy, heat shock must often be coupled with other stimuli, such as cold (Keeley, 1991) or light (Keeley, 1987; Bell, 1994). The effects of heating on stimulating seed germination are related to intensity and duration (Keeley and Fotheringham, 1997). While small-seeded species survive a long duration at lower temperatures, they are killed by a short duration exposure to higher temperature; large-seeded species exhibit an opposite pattern (Keeley *et al.*, 1985).

Species density and richness of seedlings emerging from the soil seed bank can be increased by heat treatment (Read *et al.*, 2000; Izhaki *et al.*, 2000; Wills and Read, 2007). Heat produced during burning alters the composition of plant communities, because germination, seedling emergence, and establishment of some species from soil can be triggered by heat (Bossuyt and Honnay, 2008). The effect of heat on seedling establishment from the soil seed bank in Fescue Prairie is restricted to seeds located in the upper few millimeters of the soil profile (Bailey and Anderson, 1980; Archibold *et al.*, 1998) because insignificant temperature change occurs deeper than 1 cm in the soil profile (Archibold *et al.*, 1998, 2003).

2.3.4 Effects of smoke on seedling emergence from soil seed banks

The discovery of the effect of plant-derived smoke and aqueous smoke on breaking seed dormancy and stimulating seed germination (De Lange and Boucher, 1990; Dixon *et al.*, 1995; Enright *et al.*, 1997; Keeley and Fotheringham, 1997) has major implications for understanding community ecology (Roche *et al.*, 1997). Aerosol smoke at ambient temperature stimulates

densities of natives, perennial forbs and graminoids, as well as *Digitaria breviglumis* Domin. and *Heteropogon triticeus* R. Brown, two common grasses in tropical savannas (Williams *et al.*, 2005); as well as stimulates density of seedlings emerging from the soil seed bank in a cultivated field (Cochrane *et al*., 2007). Active compounds in smoke are heat stable, water soluble, and long lasting in aqueous solutions (Van Staden *et al.*, 2000). Exposing seeds to smoke solutions increases seedling density and species richness of seedlings emerging from the soil seed bank in the grasslands in Australia (Lloyd *et al.*, 2000; Enright and Kintrup, 2001); promotes seedling emergence in mesic grasslands in South Africa (Ghebrehiwot *et al.*, 2012) and Mediterranean grasslands (Tormo *et al.*, 2014). The most important active component in smoke derived from plant is 3-methyl-2H-furo[2,3-c]pyran-2-one (KAR_1) (Van Staden *et al.*, 2004; Flematti *et al.*, 2004). KAR_1 has been extracted from the top 8 cm soil profiles after burning (Ghebrehiwot *et al.*, 2011), providing further evidence that smoke stimulates seedling emergence from soil seed banks. Smoke effects on the soil seed banks is commonly studied with heat effects (Wills and Read, 2002; Thomas *et al.*, 2003; Ghebrehiwot *et al.*, 2012). Some studies indicate heat and smoke are exclusive triggers, with annuals responding to smoke and legumes responding to heat (Grant and Koch, 1997). Germination of seeds with physical dormancy can be stimulated by heat, but not by smoke (Moreira *et al.*, 2010). However, other studies indicate the interactive effect between these two fire cues. Germination of 14 populations responded to the interaction of smoke and heat; negative heat effects were partially reversed when combined with smoke (Thomas *et al.*, 2007). Smoke or smoke combined with heat significantly increase densities and biomass production of seedlings emerging from the soil seed bank in mesic grassland in South Africa (Ghebreiwot *et al.*, 2012).

2.3.5 Effects of ash on seedling emergence from soil seed banks

Except for nitrogen (Humphreys and Craig, 1981), ash from burned plant material increases the soil pH and releases macro-nutrients into the soil (Siddiqui *et al.*, 1976). Change in soil chemistry may play a crucial role in seedling emergence from the soil seed bank (Enright *et al.*, 1997). The inhibitory effect of ash on seed germination and seedling establishment was shown in *Calluna vulgaris* L. Hull and *Erica ciliaris* L. (Gonzalez-Rabanal and Casal, 1995), *Pinus banksiana* Lamb. (Thomas and Wein 1990, 1994) and annual species (Ne'eman *et al.*, 1993). Several hypotheses were proposed to explain the inhibitory effect of ash. High pH is believed to lower the osmotic potential of soil which in turn suppresses the water uptake by seeds

(Thomas and Wein, 1990; Henig-Sever *et al.*, 1996). Mayer and Poljakoff-Mayber (1989) claimed high pH inhibits the activity of proteolytic enzymes in seed storage compounds, which in turn affected germination. The toxic ions involved in ash may also inhibit germination (Ne'eman *et al.*, 1993). The effect of ash on seed germination and seedling establishment varies among species (Gonzalez-Rabanal and Casal, 1995), with species adapted to arid conditions being favoured by fire (Dejong and Macdonald, 1975).

2.3.6 Micro environmental conditions after burnings

Micro-environmental conditions are modified by burning, including soil temperatures, soil water content, light intensity at ground level, nutrient availability and soil biota (Nelson *et al.*, 2012). Altering all of these factors in turn influences the persistence of seeds in seed bank, dormancy, and germination (Walck *et al.*, 2011). More specifically, soils are warmer in the summer and colder in the winter after fire (Archibold *et al.*, 2003). Though soil water content is reduced (Redmann *et al.*, 1993; Romo *et al.*,1993; Grilz and Romo, 1994), gap formation after fire reduces competition for water for new seedlings (Keeley and Fotheringham, 2000). Light intensity is markedly increased after burning (Nelson *et al.*, 2012). Available nutrients can increase because of accelerated rates of mineralization after a moderately intense fire (Dunn and DeBano, 1977; Wells *et al.*, 1979) or decrease because of volatilization after an intense fire (Hones and Richards, 1977). Microbial populations are also altered and pathogens are reduced after burning (Fletcher, 1910; Wicklow, 1988).

Archibold *et al*. (2003) recorded effects of spring, summer, and autumn burning on the microenvironment in Fescue Prairie. Fire temperatures exceeded 300 ^{o}C at 5-10 cm above the canopy. Summer burn increased soil temperature at a depth of 1 cm to 40 ^{o}C. Soil surface albedos reduced to 15% of the level in the pre-burn plots after burning. Spring, summer, and autumn burning reduced snowpack by 34%, 52% and 66%, respectively, as compared with the control.

2.4 Effects of smoke in breaking seed dormancy and stimulating seed germination

Seed germination starts with the absorption of water and ends with extrusion of the radical through the surrounding embryo (Bweley, 1997). Seed dormancy, *per se*, is intrinsically associated with the seed preventing germination, even under appropriate germination conditions

that favor non-dormant seeds (Baskin and Baskin, 1998). Hence, seed germination will not occur unless seed dormancy is broken and a suitable physical environment is provided.

Smoke breaks seed dormancy and stimulates germination of *Audouinia capitata* (L.) Brongn. (De Lange and Boucher, 1990). The significance of smoke as a crucial germination cue was proven in comprehensive germination of taxa from Asteraceae, Ericaceae, Proteaceae (Brown, 1993; Brown *et al.*, 1993), Restionaceae (Brown *et al.*, 1994), Poaceae (Baxter *et al.*, 1994) and Mesembryanthemaceae (Pierce *et al.*, 1995). Smoke stimulates germination of species from fire-prone and fire-free habitats (Van Staden *et al.*, 2000). Promotive effects of smoke are independent of seed size, shape, and the life form of plants (Dixon *et al.*, 1995).

Smoke also broadens the environmental conditions for seed germination and enhances seedling growth. Germination of tomato seeds (*Lycopersicon esculentum*) occurs between 10 ℃ and 40 ℃ after treating with KAR_1 (Jain *et al.*, 2006). Seeds of *Kunzea ambigua* (Sm.) Druce. and *Kunzea capitata* (Sm.) Heynh. can germinate at lower water potentials in smoke solutions (Thomas *et al.*, 2010). Smoke solutions increased seedling growth of some species from South Africa and the Mediterranean Basin (Sparg *et al.*, 2006; Moreira *et al.*, 2010). Exposing seeds to smoke solutions increased the length of roots in tomato seedlings by 10-fold (Van Standen and Jain, 2006).

2.4.1 Priming seeds with aqueous smoke solutions

Although the discovery that seed germination is promoted after exposure to aerosol smoke from burned plant material has major implications in restoration ecology and rangeland management (Baxter *et al.*, 1995; Roche *et al.*, 1997), finding the active components in smoke from plants that are heat stable, water soluble, and remain active for a long period has ecological significance (Van Staden *et al.*, 2000). How long the active component(s) may remain in the aqueous smoke solution is dependent on the temperatures and rate of combustion (Brown and Van Staden, 1997). Jager *et al.* (1996) claimed the active compound(s) was trapped into water more efficiently when *Themeda triandra* Forssk. leaves were heated between 160 ℃ and 200 ℃ at a slow rate as compared with those heated at 220 ℃ and 240 ℃.

Priming is a pre-sowing treatment in which seeds are soaked in an osmotic solution that partially hydrates seeds, followed by drying, allowing seeds to start germination, but not permit radical emergence (Heydecker, 1973). Priming is a common technique to increase the rate and uniformity of seedling emergence (Parera and Cantliffe, 1994). Priming can break seed

dormancy and improve seed germination for some species, especially under unoptimal conditions (Tavili *et al.*, 2010; Rouhi *et al.*, 2011). Priming effects are determined by various factors, including temperature and duration of priming, seed vigour, and water potential of priming agents (Parera and Cantliffe, 1994). The promotive effect of priming with smoke solutions on seed germination can be retained for varying lengths of time (Baxer and Van Staden, 1994). In addition, this effect depends on seed dormancy, with fresh seeds being affected more than seeds that have after-ripened (Baxer and Van Staden, 1994).

Promotive effects of smoke solutions or active compounds in smoke on germination depend on the dormancy state of tested seeds (Nelson *et al.*, 2012). Germination of *Brassica tournefortii* Gouan. improved from 4% in the control to over 90% after treating in butenolide, the active compounds in smoke (Stevens *et al.*, 2007). However, Long *et al.* (2011) reported responses of germination of *Brassica tournefortii* to smoke varied among seed lots and years. Germination of seeds collected in 2005 and 2006 were less than 10% and above 90% after applying same smoke treatment, respectively. After-ripening seeds collected in 2005 for 6 month increased their germination sensitivity to smoke (Long *et al.*, 2011), illustrating germination responses to smoke depend on dormancy status. Germination of *Stylidium affine* Sonder. and *Stylidium crossocephalum* F. Muell. responded more positively to smoke solutions after soil burial (Tieu *et al.*, 2001).

2.4.2 Factors influencing the effects of smoke on breaking seed dormancy

Seed dormancy and germination are affected by environmental conditions (Benech-Arnold *et al.*, 2000). Germination of non-dormant seeds occurs in a wide range of environmental conditions, but a relatively narrow range of environmental conditions break seed dormancy (Knapp, 2000). Temperature and light are the most important factors changing seed dormancy (Basking and Baskin, 1987). High temperatures break seed dormancy and broaden the range of germination conditions (Standifer and Wilson, 1988), whereas low temperatures are needed to break dormancy in other species (Baskin and Baskin, 1998). Fluctuating soil temperatures break dormancy in seeds of some species (Brits, 1986; Pierce and Moll, 1994), and light promotes germination and seedling growth of many species (Maloof *et al.*, 2000). Light inhibits germination of negatively photoblastic seeds, such as *Bromus sterilis* L. and *Anemone coronaria* L. (Bullowa *et al.*, 1975; Hilton, 1982), while it promotes germination of positively photoblastic seeds (Chen *et al.*, 2013).

Various factors influence the effect of smoke on seed germination, including temperature (Brown *et al.*, 1994; Ghebehiwot *et al.*, 2009), light (Brown and Van Staden, 1997), and the concentration of smoke solution (Drewes *et al.*, 1995). Stimulation of seed germination of *Aristida junciformis*, *Hyparrhenia hirta*, and *Panicum maximum* by smoke solutions increases with temperature (Ghebehiwot *et al.*, 2009). Negatively photoblastic seeds can germinate in light (Brown, 1993; Brown and Van Staden, 1997). Whereas, photoblastic seeds germinate in darkness (Drewes *et al.*, 1995; Thomas and Van Staden, 1995). Highly concentrated smoke solutions may inhibit seed germination while diluted solutions have the opposite effect (Brown and Van Staden, 1997). This effect suggests inhibitors are present in smoke (Drewes *et al.*, 1995). Different species, however, have varying sensitivities to aqueous smoke concentrations (Adkins and Peters, 2001). By testing germination responses of 13 species to smoke solutions, only *Aristolochia debilis* Sieb. et Zucc. showed positive response to 1/10 v/v smoke solution (Zhou *et al.*, 2014).

2.5 Stimulants and mechanisms of the stimulating effects of smoke

2.5.1 Active stimulants in smoke solutions

After eliminating nitrates, carbon dioxide, ethylene, and methane as the germination cues in smoke, Keeley and Fotheringham (1997) reported that NO_2 or NO were possibly responsible for increased seed germination of *Emmenanthe penduliflora*. However, the proposed role of NO_x as a cue in breaking seed dormancy in smoke is not clear. NO_x generated from sources other than smoke does not stimulate seed germination of smoke-responsive species (Preston *et al.*, 2004). Similarly, smoke solutions prepared by burning pure cellulose have no NO_x compounds, but can break dormancy in seeds (Baldwin *et al.*, 1994; Preston *et al.*, 2004).

The structure of the first compound identified in smoke was 3-methyl-2*H*-furo [2,3-c]-pyran-2-one (Van Staden *et al.*, 2004; Flematti *et al.*, 2004). This compound was initially called butenolide, but it was later renamed as karrikinolide (KAR_1) (Commander *et al.*, 2008). The stimulation of germination by low concentrations KAR_1 was confirmed in many species (Flematti *et al.*, 2004). KAR_1 and five KAR_1 analogs (KAR_2-KAR_6), also known as karrikins, were discovered and later confirmed by chemical synthesis of smoke (Flematti *et al.*, 2009). Although KAR_1 is the most important stimulant for most species, other analogs can stimulate

germination in other species. For example, KAR_2 is the most active stimulant for germination of *Arabidopsis* seeds (Nelson *et al.*, 2009).

Seeds of *Tersonia cyathiflora* (Fenzl) J.W.Green and *Anigozanthos manglesii* responded to smoke solutions but not to karrikins, suggesting that components other than karrikins are present in smoke (Downes *et al.*, 2010). Cyanohydrin glyceronitrile is another component in smoke that increases seed germination (Flematti *et al.*, 2011). This is because cyanide is released after contacting with water from glyceronitrile, which is a stimulant to promote seed germination (Dziewanowska *et al.*, 1979; Hendricks and Taylorson, 1972; Flematti *et al.*, 2011).

2.5.2 Mechanisms of active compounds in smoke that stimulate seed germination

Components of smoke promote seed germination by changing the morphology and the permeability of the seed coat (Egerton-Warburton, 1998) or by altering the sensitivity of seed to phytohormones (Schwachtje and Baldwin, 2004; Nelson *et al.*, 2009). Seeds of *Emmenanthe penduliflora*, an obligate fire species (Wicklow, 1977; Christensen an Muller 1975), have physical dormancy (Egerton-Warburton, 1998; Baskin and Baskin, 1998). Smoke acted as chemical scarification to the seed coat of *Emeenanth. penduliflora*, altered the permeability of the sub-testa cuticle and broke seed dormancy (Egerton-Warburton, 1998).

Gebberellin acid (GA) and Abscisic acid (ABA) are key phytohormones with antagonistic effects on seed dormancy and germination. While GA releases seed dormancy and promotes germination, ABA induces and prolongs dormancy (Bewley, 1997; Kucera *et al.*, 2005; Finkelstein *et al.*, 2008). Exposing seeds to smoke or the active components of smoke increases endogenous GA and decreases endogenous ABA in *Lactuca sativa* and *Nicotiana attenuate* (Gardner *et al.*, 2001; Schwachtje and Baldwin, 2004). Rather than altering the endogenous levels of ABA, GA_1, GA_3 and GA_4 before germination, KAR_1 increased sensitivity to the exogenous GA_3 and GA_4 in *Stylidium maritium* Lowrie. (Chiwocha *et al.*, 2009). KAR_1 induces expression of two GA biosynthesis genes *GA3ox1* and *GA3ox2* in the dormant *Arabidopsis* seeds (Nelson *et al.*, 2009). It thus appears that the mechanism in which smoke and its active components break dormancy in seeds varies among species, either by acting as chemical scarification or by changing GA and ABA metabolism in seeds.

3.0 BURNING MODIFIES COMPOSITION OF SEEDLING EMERGENCE IN FESCUE PRAIRIE

Abstract

Fire may regulate species composition in Fescue Prairie through its effects on seedling recruitment. Seedlings emerging in the field and from soil seed banks incubated in a greenhouse were examined after burning and mowing in the spring of 2012 and 2013. Soil seed bank samples were taken from the top 5 cm of the soil profile, separated into litter, 0-1 cm, and 1-5 cm layers. In the 2-year field study, 11 plant families with 1 graminoid and 22 non-graminoids were identified among emerged seedlings. Burning significantly increased the number of *Artemisia frigida*, *Artemisia ludoviciana*, *Conyza canadensis*, and *Cirsium arvense* seedlings emerging as well as total seedlings emerging in both years ($P<0.05$). Species richness and diversity were increased by burning. Species composition of emerged seedlings was significantly altered by burning in 2012 ($P=0.03$) and 2013 ($P=0.002$). In the 2-year soil seed bank study, 19 plant families with 10 graminoids and 56 non-graminoids emerged. Burning had more prominent effects on seedling density and richness of native species and forbs, rather than non-native species and graminoids. Species composition was altered by burning in all studied soil layers ($P<0.05$). Fire appears to stimulate recruitment of some species, especially early seral species, contributing to potential changes in species composition of the Fescue Prairie.

3.1 Introduction

Fescue Prairie is located mainly in central and southwestern Alberta and west central Saskatchewan (Moss and Campbell, 1947; Coupland and Brayshaw, 1953). As a natural disturbance, fire plays a crucial role in regulating community composition in Fescue Prairie (Romo, 2003; Gross and Romo, 2010). Effects of burning vary among different functional groups. For example, burning favors the establishment of perennial forbs in Fescue Prairie (Bailey and Anderson, 1978; Wright and Bailey, 1982). Density and diversity of native species in savannah grasslands of North America were increased by burning (DiTomaso *et al.*, 1999). Regeneration after burning in grasslands occurs with vegetative reproduction (resprouters) and seedling emergence from seeds (seeders) (Everson and Tainton, 1984). Fire is a selective force in grasslands, leading to high germination and seedling establishment immediately after burning (Keeley *et al.*, 2012). Although it has been well known that different fire regimes, including fire

severity (Mithcell, 1958; Wright, 1971), frequency (Anderson and Bailey, 1980) and seasonality (Bailey and Anderson, 1978) regulate species composition in Fescue Prairie, it is not clear what the relative contribution of seed reproduction is in altering species composition after burning.

Broadly, burning effects on seed germination and seedling emergence can be divided into direct and indirect effects (Santana *et al.*, 2013). Direct effects include heat shock, ash, and smoke produced by burning. While burning kills many viable seeds in the litter layer due to the high temperature and flame on the soil surface (Bailey and Anderson, 1980; Archibold *et al.*, 1998), heat shock may break seed dormancy, especially physical dormancy, and stimulate germination of seeds buried in the soil (Shea *et al.*,1979; Warcup, 1980; Baskin and Baskin, 1998). It has been well established that plant-derived smoke can break seed dormancy and stimulate germination (Dixon *et al.*, 1995; Ghebrehiwot *et al.*, 2012; Nelson *et al.*, 2012). Germination of different species can be increased, decreased, or unaffected by ash (Sweeney, 1956; Enright *et al.*, 1997; Zuloaga-Aguilar *et al.*, 2011). For indirect effects, burning increases temperature fluctuation, light, and nutrient availability, but reduces soil water content, which in turn influences germination and seedling emergence (Walck *et al.*, 2011). Archibold *et al.* (2003) tested effects of spring, summer, and autumn burns on modifying micro-environmental conditions in Fescue Prairie. Temperature at the 1 cm soil depth exceeded 40 ℃ during the summer burn, but was not affected during spring and autumn burns. Surface albedos dropped to 0.03 from 0.27 immediately after burning. Spring, summer, and autumn burns reduced winter snowpack by 36%, 53%, and 67% as compared with the control, respectively.

As an immediate source of recruitment after disturbance, soil seed banks play a significant role in determining the plant communities (Leck *et al.*, 1989). Various fire-related factors have been shown to exert effects on seed germination and seedling emergence of soil-stored seeds from a range of vegetation types worldwide (Roche *et al.*, 1997). Some researchers focused on the effects of specific fire-related factors on seedling emergence from the soil seed banks, such as heat and smoke (Izhaki *et al.*, 2000; Ghebrehiwot *et al.*, 2012), others studied the overall impact of burning (Cespedes *et al.*, 2012). Responses of seedling emergence to different fire cues are species-dependent, either positive, negative, or no response (Van De Venter and Esterhuizen, 1988). Burning history and seasons of burning modified species richness, diversity, and composition of seedlings emerging from the soil seed bank in Fescue Prairie (Romo and Gross, 2011).

Most previous studies focused on responses of plant community to burning without disentangling the effects of burning on seedling emergence in Fescue Prairie (Anderson and Bailey, 1980; Pylypec and Romo, 2003; Gross and Romo, 2010). While seeds buried in the soil seed bank exhibit the potential capability of new seedling recruitment in the habitat (Ghebrehiwot *et al.*, 2012), seedling emergence in the field is regulated by multiple factors, such as temperature, light, moisture, nutrition, and species interactions (Baskin and Baskin, 1998). The objective of this study was to determine the effects of burning on modifying seedling emergence in Fescue Prairie. The following hypotheses were tested: 1) burning modifies the density, composition, and emergence rate of seedlings emerging from the field and soil seed bank in Fescue Prairie; 2) species density and richness of different functional groups emerging from the soil seed banks response differently to burning, and; 3) effects of burning on density and composition of seedlings emerging from the soil seed bank vary among different depths of the soil profile.

3.2 Material and Methods

3.2.1 Study site

Field studies were conducted at the University of Saskatchewan's Kernen Prairie in 2012 and 2013. Kernen Prairie is about 130 ha in size and near Saskatoon, SK (52°10′N, 106°33′W, elevation 510 m). The long-term annual temperature averages 2.2 ℃ (Environment Canada, 2013), while the long-term average temperatures in May, June, July, and August are 11.5 ℃, 16.0 ℃, 18.2 ℃, and 17.3 ℃, respectively (Environment Canada, 2013). The long-term annual precipitation averages 350 mm, with over half of it received during May to August (Environment Canada, 2013). In 2012, average temperatures were 10.1 ℃ in May, 15.8 ℃ in June, 19.7 ℃ in July, and 17.3 ℃ in August. Precipitation totalled 108 mm in May, 121.1 mm in June, 80.9 mm in July, and 48.5 mm in August 2012 (Environment Canada, 2012). In 2013, temperatures averaged 13.0 ℃ in May, 15.5 ℃ in June, 17.4 ℃ in July, and 18.9 ℃ in August. Total precipitation was 15.2 mm in May, 115.9 mm in June, 35.2 mm in July, and 14.7 mm in August (Environment Canada, 2013). Kernen Prairie is dominated by *Fescuta hallii* Vasey. and other C3 plants (Coupland and Brayshaw, 1953). Soils in Kernen Prairie are Bradwell and Sutherland Orthic Dark Brown Chernozems (Acton and Ellis, 1978). The growing season has approximately 110 frost-free days (Gross and Romo, 2010).

3.2.2 Experimental design

An area about 45 × 30 m in size was chosen for field studies in 2012 and 2013. In total, 150, 3 × 3 m experimental plots were established on 17 April 2012 (Figure 3.1). Among the 75 plots established for study in each of 2012 and 2013, 25 were untreated controls; 25 were mowed to a 2-cm stubble height with plant material removed by raking; and 25 were burned with headfires. Mowing is to create a similar micro-environmental condition as burning does, representing the indirect effects caused by burning. The experimental design was a randomized-complete-block design (RCBD) with 25 replicates. Plots were burned once on 17 April 2012 or 4 May 2013. Seedling emergence was determined in a 1 x 1m subplot in the center of each plot one, which was set up immediately after burning and mowing. After species identification, new seedlings were pulled out gently from the soil to confirm they were not coming from the remnants.

3.2.3 Data collection

Fire temperatures during burning were recorded using pyrometers, as described by Wally *et al.* (2006). Twenty four lacquers manufactured to melt at specific temperatures (Tempil Division, Big Three Industries Inc., NJ, US), ranging from 66 ℃ to 704 ℃, were painted on oval copper tags. After drying, another copper tag was used to sandwich the painted surface and these two tags were linked by a gauge wire. The longer edge of each pyrometer was placed in contact with the soil surface when recording fire temperatures. Fire temperatures were estimated by determining the highest temperature at which a lacquer melted. Three sub-samples were used for recording fire temperatures in each burned plot. Immediately after burning and mowing, soil temperatures at the 2 cm depth were recorded hourly using a Campbell Scientific 21X data logger (21X micrologger, Campbell Scientific, USA). Temperature probes were installed in 10 plots for each of the three treatments during each year study. Volumetric soil water content at the 12 cm soil depth was determined with a CD620 hydrosense system (Campbell Scientific, USA) at weekly intervals with three sub samples in each plot. In 2013, light intensity was determined with an AccuPAR LP-80 system (Decagon Devices, USA) measured weekly with three sub-samples in each plot. Emerged seedlings were marked at weekly intervals from the first week after burning and mowing with coloured paper clips for 14 and 12 weeks in 2012 and 2013, respectively. Marked seedlings were allowed to grow to the stage when they could be identified

to species. Numbers of seedlings that could not be identified before dying were recorded. At the end of the growing season, seedlings that could not be identified in the field were transplanted to a greenhouse and grown until they could be identified.

3.2.4 Soil seed bank study

Twenty five, 25 cm^2 soil core samples were collected from the top 5 cm of the soil profile in burned and mowed plots outside of the 1 x 1 sub-plot used determining seedling emergence. Soil samples from the burned plots were collected before and immediately after burning, which were regarded as control and burning treatments, respectively. Soil samples collected from the mowed plots were regarded as mowing treatment. Soil samples collected from the same block in the field were placed as one block for the soil seed bank study. Each soil sample was divided into the litter layer on the soil surface, as well as the 0-1cm, and 1-5cm depths. Same layers for the 25 soil cores from each plot were combined, mixed, and placed in plastic trays (52×26×7cm) to keep the depth of soil < 1cm in each tray (Cespedes *et al.*, 2012). Root fragments, rhizomes, and plant materials were removed. The litter layer was put on a 1 cm layer of sterile sand placed on the bottom of a plastic tray measuring 26×26×6 cm. Trays within blocks were randomly placed on benches in a greenhouse. The air temperature during the growing season in the greenhouse averaged 27 ± 3°C during the day and 21 ± 4°C at night. Natural light was supplemented with two 400 W high-pressure sodium lights (18 h photoperiod, average of 600 $\mu mol \cdot m^{-2} \cdot s^{-1}$) for one bench. All trays were watered daily. Seedling emergence was recorded weekly for 14 weeks and new seedlings were transplanted to pots until they were identified. In total, seedling emergence data from five layers (litter layer, 0-1 cm depth, 1-5 cm depth, 0-5 cm depth, and all layers combined (litter layer plus 0-5 cm depth soil layer) were analyzed.

3.2.5 Data analysis

Data of fire temperature, soil water content, and light intensity within each year were subjected to Analysis of Variance (ANOVA) in a randomized completed block design (RCBD) with 25 replicates. Data of soil temperature within each year was subjected to ANOVA in a RCBD with 10 replicates. Fire temperature, soil water content, light intensity, or soil temperature were taken as dependent variable, and in each case, different treatments were used as independent variables. Block was factored into the model as random effects.

Seedling densities of individual species in five blocks, which were in the same column of the experimental layout in each year (Figure 3.1), were combined for data analysis because of high variability in the density of seedlings emerging among replicates in the field and the soil seed bank study. Data normality was tested using the Shapiro-Wilk test before each analysis. Total seedling densities and emergence rate for individual species were square root, log, or log (x+1) transformed before being subjected to ANOVA for a split-plot in a Completely Randomized Design (CRD) design with 5 replicates, if they did not meet normality requirement. Total seedling densities, species richness, densities and richness of functional groups of forb, graminoid, native, and non-native species, species diversity, and seedling emergence rate in the field and from the soil seed bank in the greenhouse were square root, log, or log (x+1) transformed before subjected to ANOVA for a split-plot in a CRD design with 25 replicates, if they did not meet normality requirement. Year was the whole plot factor, while mowing and burning treatments were subplot factors. When the interaction of year x treatment was significant ($P \leq 0.05$), data were analyzed within each year. Treatment means were separated using Tukey test at $P \leq 0.05$. The mixed model procedure in SAS version 9.3 was used for data analysis (SAS Institute Inc., USA), taking main effects and possible interactions of year and treatments as fixed factors. Block (the whole-plot unit) was factored into the model as random effects.

Differences in species composition among treatments within years were tested using a PerMANOVA test, a permutation-based multivariate analysis of variance in PC-ORD version 6.0 (McCune and Grace, 2002). Permanova controls for variation contributed by a blocking factor. Densities of seedlings emerging for individual species in each plot were relativized by being divided by the total density of seedlings emerging in each plot. Sorenson distances were used to express similarity of species composition among treatments (McCune and Grace, 2002). Data were tested using 999 random permutations. Blocked indicator species analysis was used to determine the frequency and abundance of species from the soil seed bank among treatments (Dufrene and Legendre, 1997). Densities of seedlings emerging for individual species in each plot were relativized by being divided by the total density of seedlings emerging in each block (McCune and Grace, 2002). A Monte Carlo simulation of 10,000 runs was used to evaluate the statistical significance of indicator values (McCune and Grace, 2002).

M1	C1	B1	C2	B2	M2	M3	B3	C3	M4	B4	C4	C5	M5	B5
C6	B6	M6	C7	M7	B7	C8	M8	B8	B9	C9	M9	C10	B10	M10
M11	B11	C11	M12	B12	C12	B13	M13	C13	C14	M14	B14	M15	C15	B15
M16	B16	C16	B17	M17	C17	C18	M18	B18	C19	B19	M19	B20	C20	M20
M21	B21	C21	M22	B22	C22	C23	M23	B23	B24	M24	C24	C25	M25	B25
C26	M26	B26	B27	C27	M27	C28	M28	B28	M29	C29	B29	C30	M30	B30
C31	M31	B31	C32	B32	M32	C33	B33	M33	C34	B34	M34	C35	B35	M35
B36	M36	C36	M37	C37	B37	B38	C38	M38	M39	B39	C39	M40	C40	B40
C41	B41	M41	C42	M42	B42	M43	B43	C43	M44	B44	C44	B45	C45	M45
B46	M46	C46	M47	B47	C47	B48	M48	C48	B49	M49	C49	M50	C50	B50

Figure 3.1 Layout of burning (B), mowing (M), and control (C) plots for the Kernen Prairie seed bank and seedling emergence study in 2012 and 2013. Twenty-five replicate plots were treated in 2012 (Gray shading) and 25 replicate plots were treated in 2013 (white shading). Plots were 3 m X 3 m; a 1 m X 1 m subplot for measurements will be centered in the 3 m X 3 m plots. The study site is 45 m long and 30 m wide.

3.3 Results

3.3.1 Microenvironment conditions as affected by mowing and burning

Fire temperatures averaged 548 ± 24 °C (mean ± SE) in 2012 and 349 ± 26 °C in 2013. Average soil temperatures at the 2-cm depth were greater in burning and mowing treatments than that in the control ($P = 0.02$ and $P < 0.01$ in 2012 and 2013, respectively) (Figures 3.2a, b). Soil water content in control plots was significantly greater than that in mowing and burning plots ($P = 0.01$ and $P = 0.01$ in 2012 and 2013, respectively) (Figures 3.2c, d). Light intensity at the soil surface in mowing and burning plots was significantly higher than in control plots ($P = 0.01$ in 2013) (Figure 3.2e). Soil temperatures, soil water content, and light intensity did not vary significantly between mowing and burning in both years.

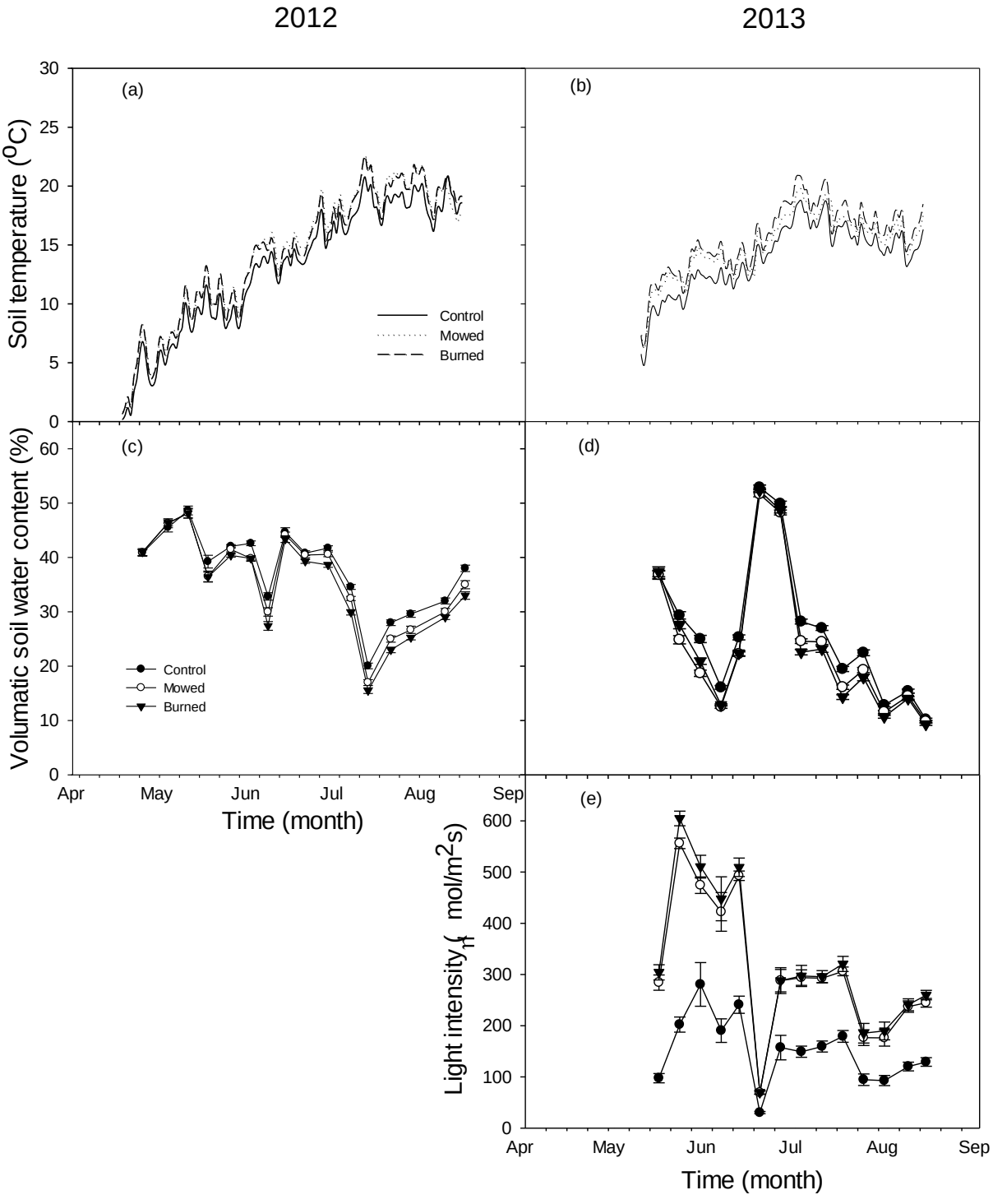

Figure 3.2 Soil temperatures (at 2 cm depth), soil water content at the 0-12 cm depth, and light intensity at the soil surface after mowing and burning in 2012 and 2013. Values are means ± SE of 25 replicates.

3.3.2 Effects of burning and mowing on seedling emergence in the field

Seven species, including *Androsace septentrionalis*, *Artemisia frigida*, *Artemisia ludoviciana*, *Campanula rotundifolia*, *Cirsium arvense*, *Sonchus arvensis*, and *Taraxacum officinale* occurred in more than half of the burned plots in either 2012 or 2013 (Table 3.1). Among these species, *Cirsium arvense*, *Sonchus arvensis*, and *Taraxacum officinale* are non-native.

Table 3.1 Frequency (%) of emerged species from control, mowed, and burned plots in 2012 and 2013.

	2012			2013		
Species	Control	Mowed	Burned	Control	Mowed	Burned
Achillea lanulosa	8	12	8	4	8	4
Androsace septentrionalis	44	48	68	44	36	48
Artemisia frigida	28	28	76	24	32	68
Artemisia ludoviciana	16	12	68	0	20	64
Aster ericoides	4	8	4	0	0	4
Astragalus agrestis	0	12	16	0	0	0
Campanula rotundifolia	40	40	52	20	20	28
Cerastium arvense	0	0	0	4	0	4
Chenopodium album	0	0	0	0	0	4
Cirsium arvense	24	16	60	16	8	44
Conyza canadensis	12	4	28	12	0	20
Epilobium ciliatum	0	4	12	0	0	0
Galium boreale	44	36	48	12	12	24
Heterotheca villosa	0	0	12	0	0	0
Monolepis nuttalliana	0	0	0	8	0	0
Oxytropis sericea	8	8	20	0	0	8
Poa pretensis	0	0	0	0	4	4
Populus balsamifera	0	0	4	0	0	0
Potentilla gracilis	0	0	4	0	0	0
Rosa sp.	4	0	0	4	8	4
Salix sp.	0	0	0	0	4	4
Sonchus arvensis	32	44	52	24	40	52
Taraxacum officinale	72	84	68	76	80	96

Compared with the control, burning significantly increased seedling densities of *Artemisia frigida* ($P < 0.01$), *Artemisia ludoviciana* ($P < 0.01$), *Conyza canadensis* ($P < 0.01$), *Cirsium arvense* ($P < 0.01$) and total emerged seedlings ($P < 0.01$) in both years (Figures 3.3, 3.4). Seedling densities of *Taraxacum officinale* were significantly increased by burning in 2013 ($P < 0.01$), but not in 2012 ($P = 0.42$) as compared with the control. Mowing had no effects on seedling densities of individual species and total emerged seedlings in 2012 and 2013 as compared with the control.

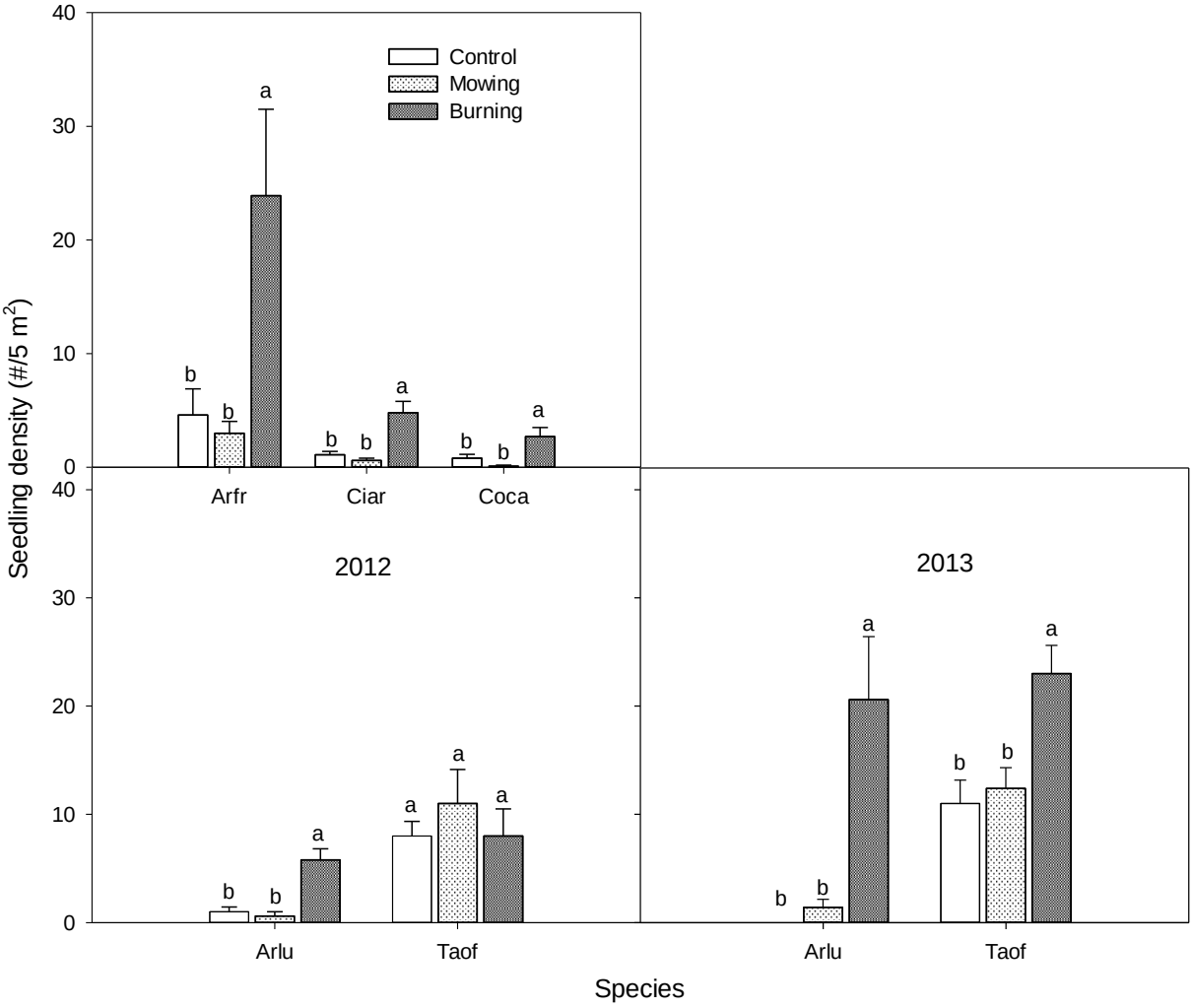

Figure 3.3 Densities of species in which total seedling densities were significantly affected by burning and/or mowing in the field in 2012 and 2013. Bars represent means ± SE. Means with different letters within each species of seedlings emerging were significantly different ($P \leq 0.05$). Arfr: *Artemisia frigida*, Arlu: *Artemisia ludoviciana*, Ciar: *Cirsium arvense*, Coca: *Conyza canadensis*, and Taof: *Taraxacum officinale*.

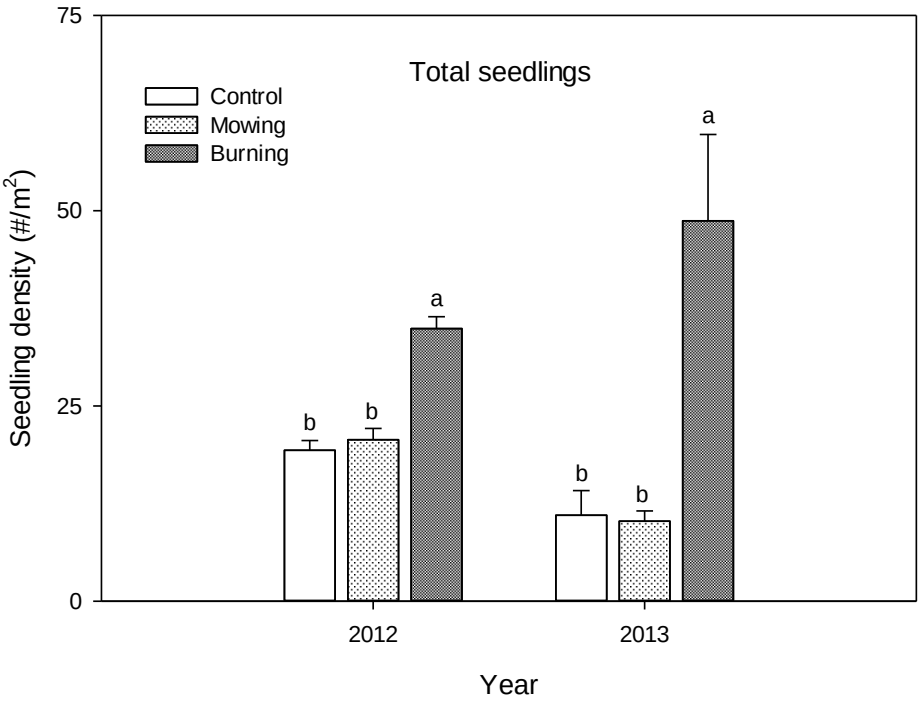

Figure 3.4 Densities of total seedlings emerging as affected by burning and mowing in the field in 2012 and 2013 combined. Bars represent means ± SE. Means with different letters within years were significantly different ($P \leq 0.05$).

3.3.3 Effects of burning and mowing on species composition of seedlings emerging in the field

The Permanova tests indicated species composition of emerged seedlings in the field was significantly different between burning and the control as well as between burning and mowing in both years (Table 3.2). Species composition in the control and the mowing treatment was not significantly different.

Table 3.2 Pairwise comparison on species composition of emerged seedlings among the control, mowing, and burning treatments in the field in 2012 and 2013 based on PERMANOVA.

	2012		2013	
Comparison	t	P	t	P
Control vs Mowing	1.30	0.11	0.76	0.81
Control vs Burning	1.47	0.03	1.88	<0.01
Mowing vs Burning	2.44	<0.01	1.69	0.01

Burned plots were characterized by greater frequency and abundance of native species, including *Androsace septentrionalis*, *Artemisia frigida*, *Artemisia ludoviciana*, and *Campanula rotundifolia* and the non-native species *Cirsium arvense*, *Sonchus arvensis*, and *Taraxacum officinale* in at least one year (Table 3.3). Control plots had high frequency and abundance of *Androsace septentrionalis*, *Galium boreale*, *Monolepis nuttalliana*, *and Rosa sp.* in at least one year, although they were not significantly different from the mowing or burning treatment. In 2012, the frequency and abundance of *Achillea lanulosa*, *Aster ericoides*, and *Taraxacum officinale* were higher in mowed plots compared with the control and the burn treatment in 2012. However, no significant differences existed among these 3 treatments.

Table 3.3 Blocked indicator species analysis for seedlings emerging in the field in the control, mowing, and burning treatment in 2012 and 2013.

Species	2012			2013		
	Max Group	Indicator value	P	Max Group	Indicator value	P
Achillea lanulosa	Mowing	4.0	0.95	Burning	3.0	1.00
Androsace septentrionalis	Burning	37.7	**0.01**	Control	20.1	0.53
Artemisia frigida	Burning	55.1	**<0.01**	Burning	49.2	**<0.01**
Artemisia ludoviciana	Burning	53.4	**<0.01**	Burning	57.9	**<0.01**
Aster ericoides	Mowing	4.8	0.37	Burning	4.0	1.00
Astragalus agrestis	Burning	9.4	0.27	--[a]	--	--
Campanula rotundifolia	Burning	31.3	**0.05**	Burning	12.5	0.55
Cerastium arvense	--	--	--	Burning	2.5	1.00
Chenopodium album	--	--	--	Burning	2.0	1.00
Cirsium arvense	Burning	41.9	**<0.01**	Burning	33.5	**<0.01**
Conzya Canadensis	Burning	21.4	**0.02**	Burning	16.0	0.06
Epilobium ciliatum	Burning	7.2	0.32	--	--	--
Galium boreale	Burning	20.2	0.51	Control	7.3	0.86
Heterotheca villosa	Burning	12.0	0.12	--	--	--
Monolepis nuttalliana	--	--	--	Control	8.0	0.33
Oxytropis sericea	Burning	10.9	0.25	Burning	8.0	0.32
Poa pretensis	Burning	9.1	0.17	Burning	3.3	1.00
Populus balsamifera	Burning	4.0	1.00	--	--	--
Potentilla gracilis	Burning	4.0	1.00	--	--	--
Rosa sp.	Control	4.0	1.00	Control	3.0	0.97
Salix sp.	--	--	--	Burning	2.5	1.00
Sonchus arvensis	Burning	20.2	0.49	Burning	29.8	**0.04**
Taraxacum officinale	Mowing	37.8	0.06	Burning	48.1	**<0.01**

[a] No seedlings emerged

During the 2 years of study, 21 forbs, 1 graminoid, and 1 shrub species emerged in the field. Species richness was 87% greater in the burning treatment than that in the control ($P < 0.01$) (Figure 3.5a). Species diversity index (H') of emerged seedlings was 1.6-fold greater in the burning treatment compared with the control ($P < 0.01$) (Figure 3.5b). Species richness and species diversity were not significantly different between the control and mowing treatment.

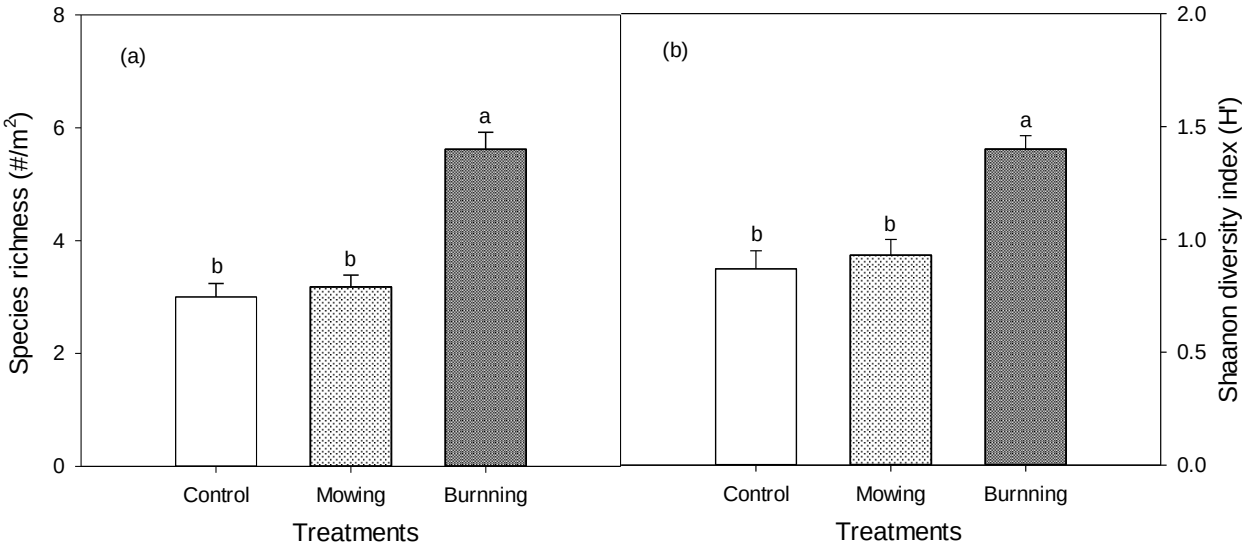

Figure 3.5 Species richness (a) and shannon diversity index (H') (b) for seedlings emerging in the field as affected by burning and mowing in 2012 and 2013 combined. Bars represent means ± SE of 25 replicates. Means with different letters within treatments were significantly different ($P \leq 0.05$).

3.3.4 Effects of burning and mowing on species densities of seedlings emerging from the soil seed bank

A total of 5,394 and 17,116 seedlings were identified from the soil seed bank samples in a 4.69 m^2 sampling area in 2012 and 2013, respectively. The percentage of unidentified emerged seedlings was <1%. *Androsace sepentrionalis* was the most abundant forb emerging (61% and 80% in 2012 and 2013, respectively), while *Poa pratensis* was the most abundant graminoid emerging (85% and 83% in 2012 and 2013, respectively). Burning significantly reduced densities of *Agrostis scabra* ($P < 0.01$), *Artemisia frigida* ($P < 0.01$), *Poa pratensis* ($P < 0.01$), native species ($P < 0.01$), non-native species ($P < 0.01$), forbs ($P < 0.01$), graminoids ($P < 0.01$), and total seedlings ($P < 0.01$) emerging from the litter layer (Figures 3.6, 3.7).

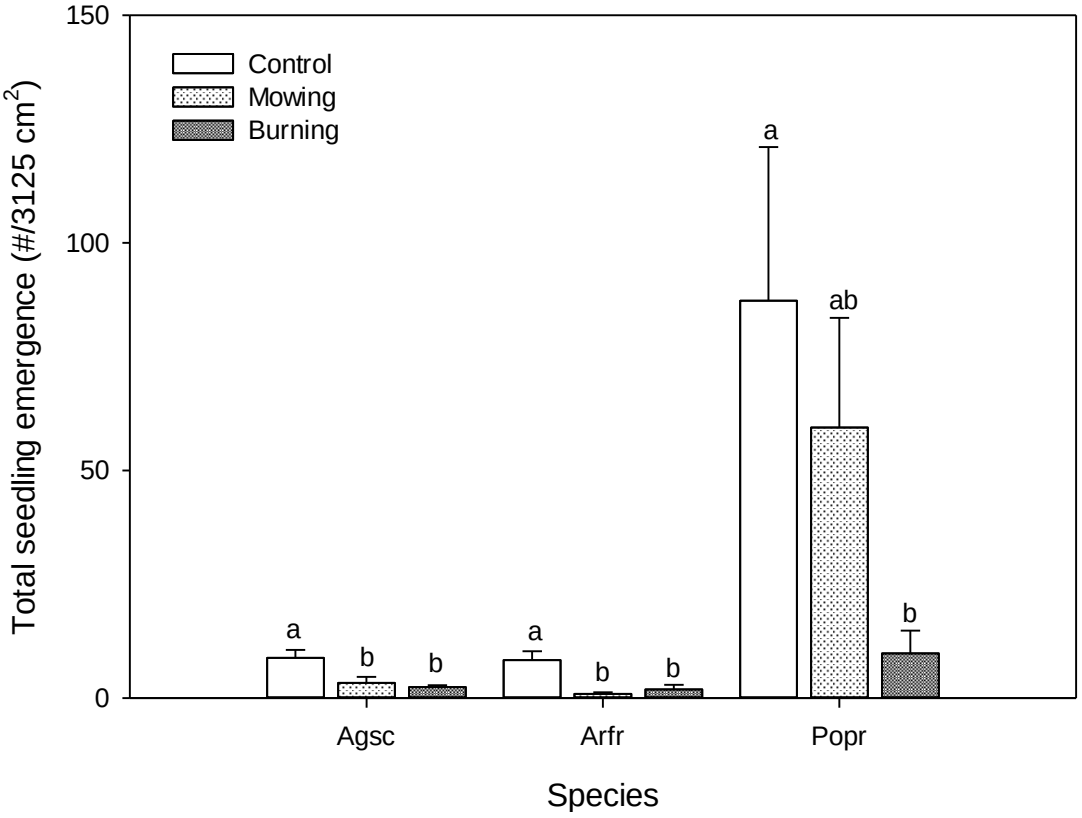

Figure 3.6 Densities of species in which total seedling densities in the litter layer were significantly affected by burning and mowing in 2012 and 2013 combined. Values represent means ± SE. Means with different letters within species were significantly different ($P \leq 0.05$). Agsc: *Agrostis scabra*, Arfr: *Artemisia frigida*, and Popr: *Poa pratensis*.

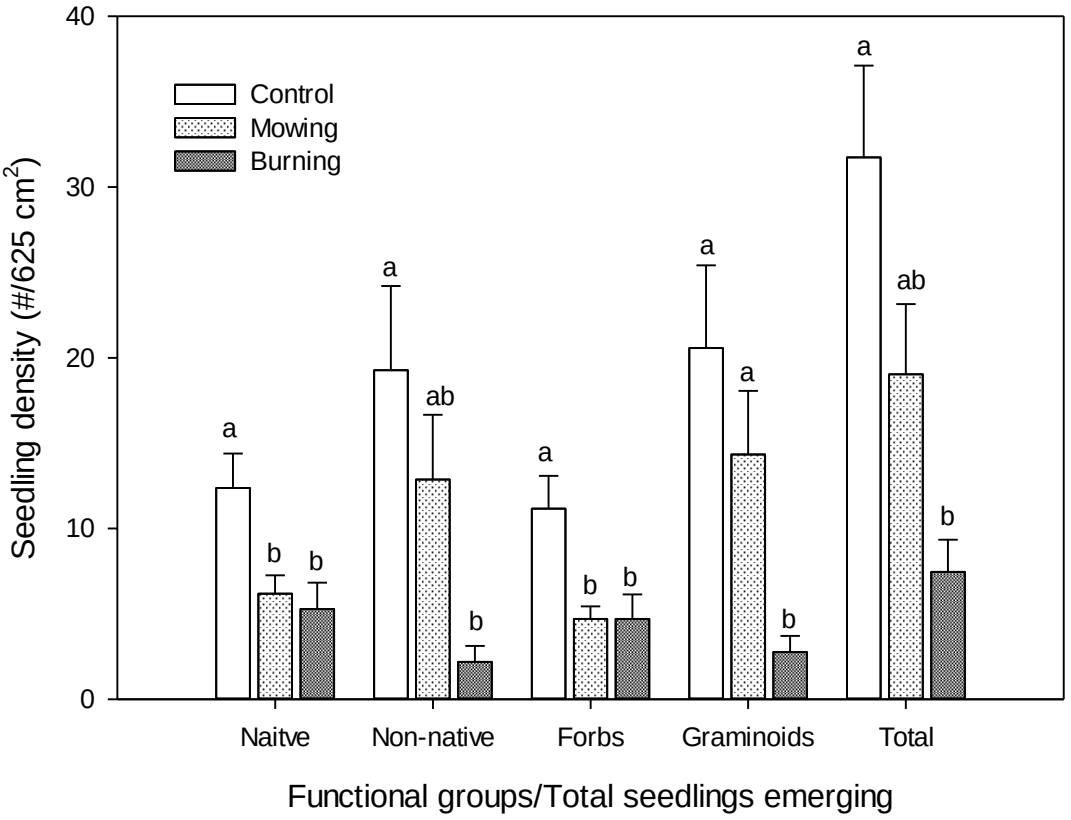

Figure 3.7 Densities of functional groups and total seedlings emerging in the litter layer as affected by burning and mowing in 2012 and 2013 combined. Values represent means ± SE. Means with different letters within each functional groups or total seedlings emerging were significantly different ($P \leq 0.05$).

Burning and mowing effects on seedling density varied between native and non-native species in different soil layers (Figure 3.8). Seedling densities of native species responded positively to burning in the 0-1 cm soil layer; 26% and 53% more seedlings emerged after burning compared with the control in 2012 ($P = 0.74$) and 2013 ($P = 0.02$), respectively. In the control, 71% and 67% of the seedlings of native species emerging from the 0-5 cm soil layer emerged from the 0-1 cm soil layer in 2012 and 2013, respectively. Densities of non-native species were negatively affected by burning in all layers combined; 45% fewer seedlings emerged after burning compared with the control ($P < 0.01$). Mowing significantly increased densities of native species emerging from the 0-1 cm soil layer ($P = 0.02$) as compared with the control in 2013, but not in 2012 ($P=0.74$).

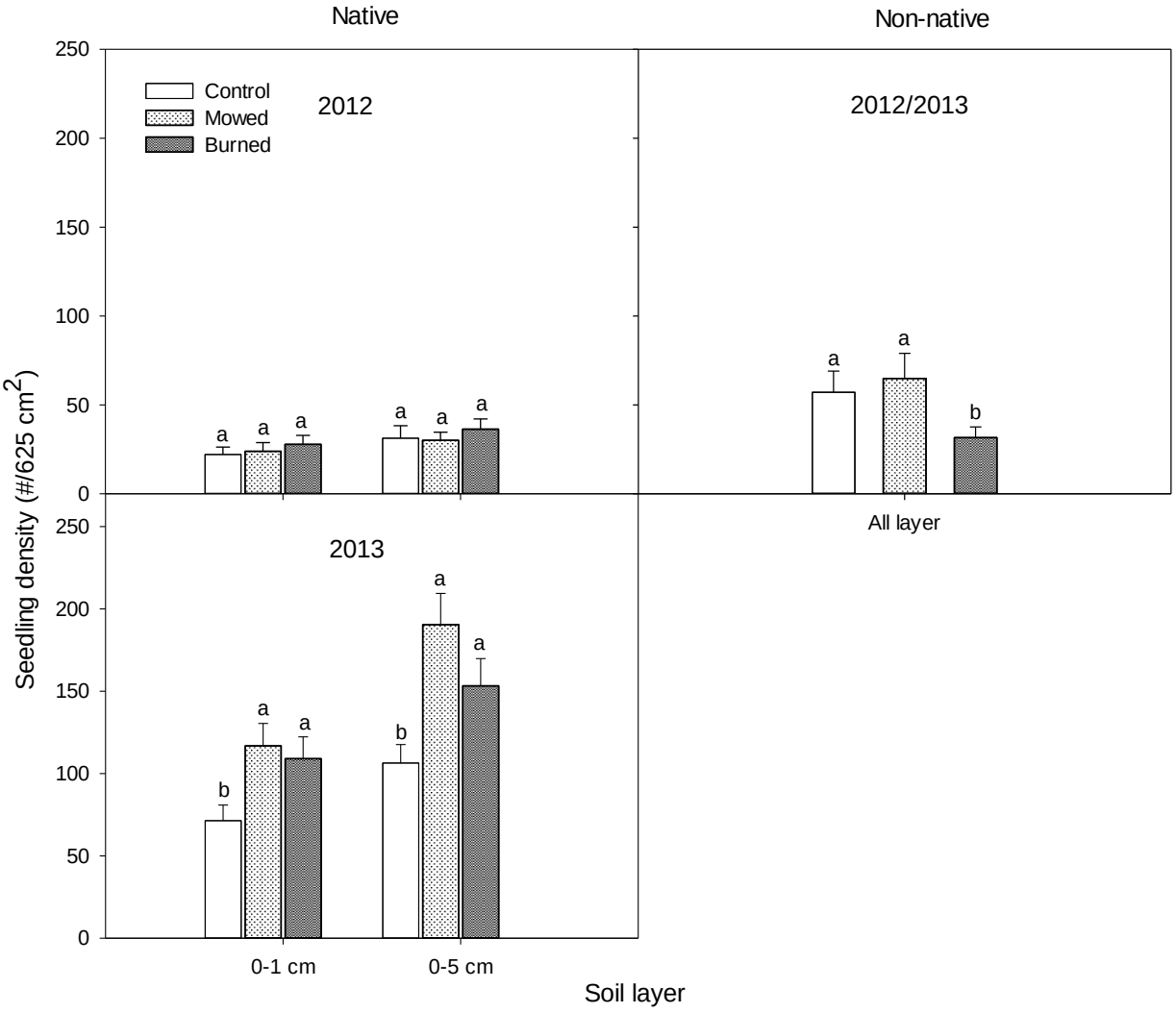

Figure 3.8 Densities of natives and non-native seedlings emerging from the soil seed bank (excluding the litter layer) as affected by burning and mowing in 2012 and 2013. Bars represent means ± SE of 25 replicates. Means with different letters for native or non-native species within soil layers were significantly different ($P \leq 0.05$).

Densities of forbs and graminoids responded differently to burning and mowing in different soil layers (Figure 3.9). Burning and mowing significantly increased densities of forbs emerging from the 0-1 cm soil layer by 60% and 68% in 2013 ($P = 0.01$), respectively, as compared with the control. In the control, 52% and 65% of forb seedlings emerged from the 0-1 cm soil layer in 2012 and 2013, respectively. Seedling densities of graminoids were reduced by burning in all layers combined; 44% fewer seedlings emerged after burning compared with the control ($P < 0.01$).

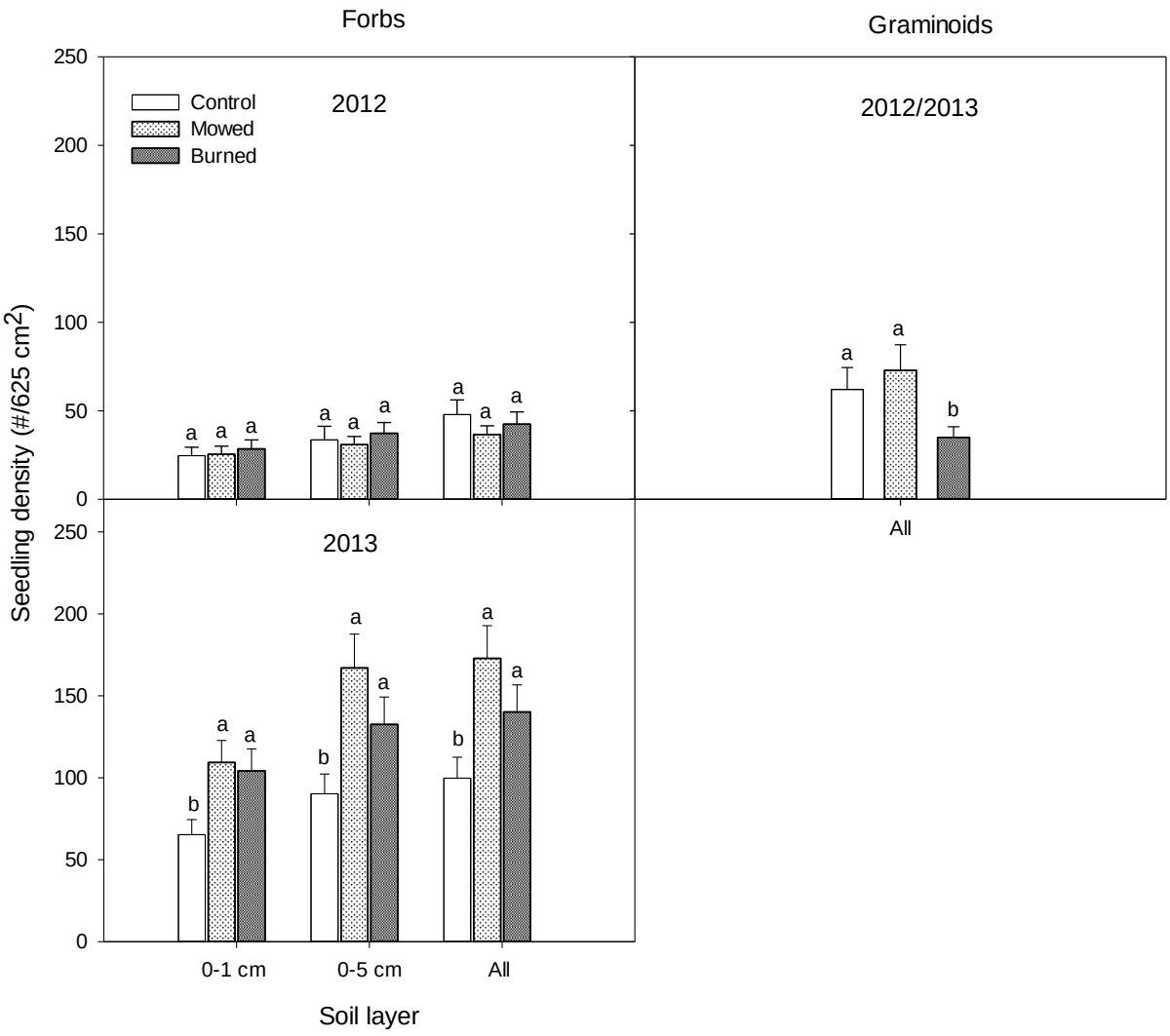

Figure 3.9 Densities of forb and graminoid seedlings emerging from the soil seed bank (excluding the litter layer) as affected by burning and mowing in 2012 and 2013. Bars represent means ± SE of 25 replicates. Means with different letters for forbs or graminoids within soil layers were significantly different ($P \leq 0.05$).

3.3.5 Effects of burning and mowing on species composition of seedlings emerging from the soil seed bank

Burning significantly altered species composition as compared with the control for species in all but the 1-5 cm soil layer in 2012 and 0-5 cm and all layers combined in 2013 (Table 3.4). In 2012, species composition in the mowing treatment was also significantly different from that in the burning treatment in the litter, 0-5 cm depth, and all layers combined. There was no significant difference in species composition in all soil layers between the control and the mowing treatment in both years.

Table 3.4 Pairwise comparison from multivariate permutational analysis (PERMANOVA) of differences in species composition among the control, mowing, and burning treatments in different soil layers in soil seed bank in 2012 and 2013.

		2012		2013	
Comparison	Soil layer	t	P	t	P
Control vs Burned	Litter	2.27	<0.01	1.79	0.03
Mowed vs Burned	Litter	2.33	<0.01	1.29	0.14
Control vs Burned	0-1 cm	2.04	<0.01	2.08	<0.01
Control vs Burned	1-5 cm	1.42	0.06	1.92	0.01
Control vs Burned	0-5 cm	1.56	0.02	1.00	0.40
Mowed vs Burned	0-5 cm	1.67	0.01	0.73	0.70
Control vs Burned	All	2.55	<0.01	1.29	0.17
Mowed vs Burned	All	2.30	<0.01	0.91	0.48

Species richness of native species ($P < 0.01$), non-native species ($P < 0.01$), forbs ($P < 0.01$), graminoids ($P < 0.01$), and total seedlings emerging ($P < 0.01$) from the soil seed bank was significantly reduced by burning compared with the control in the litter layer (Figure 3.10).

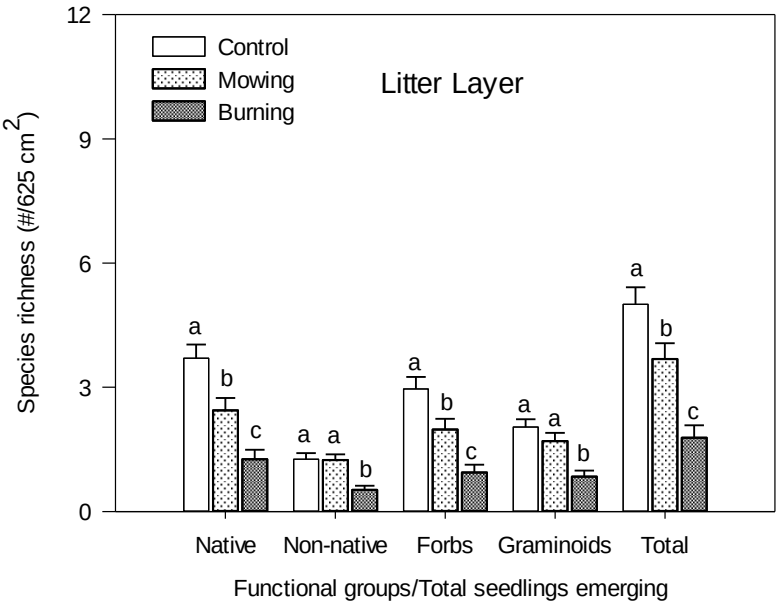

Figure 3.10 Species richness for seedlings within functional groups as well as total seedlings emerging from litter layer in the soil seed bank as affected by burning and mowing in 2012 and 2013 combined. Bars represent means ± SE of 25 replicates. Means with different letters within functional groups or total seedlings emerging were significantly different ($P \leq 0.05$).

3.3.6 Effects of burning and mowing on the rate of seedling emergence from the soil seed bank

For total seedlings, seedlings emerged faster after burning and mowing in the 0-1 cm and 0-5 cm soil layers (Figure 3.11). Compared with non-native species, burning and mowing had more prominent effects on the rate of seedling emergence of native species in both years. Seedlings of native species emerged faster after burning and mowing in the 0-1 cm ($P < 0.01$), 1-5 cm ($P < 0.01$), and all layers combined ($P < 0.01$). For non-native species, seedlings emerged faster after burning in the 0-5 cm soil layer ($P = 0.01$). Forb ($P < 0.01$) and graminoid ($P < 0.01$) seedlings emerged faster after burning and mowing in the 0-1 cm soil layer. Graminoid seedlings emerged significantly faster in all layers combined after burning and mowing compared with the control ($P < 0.01$).

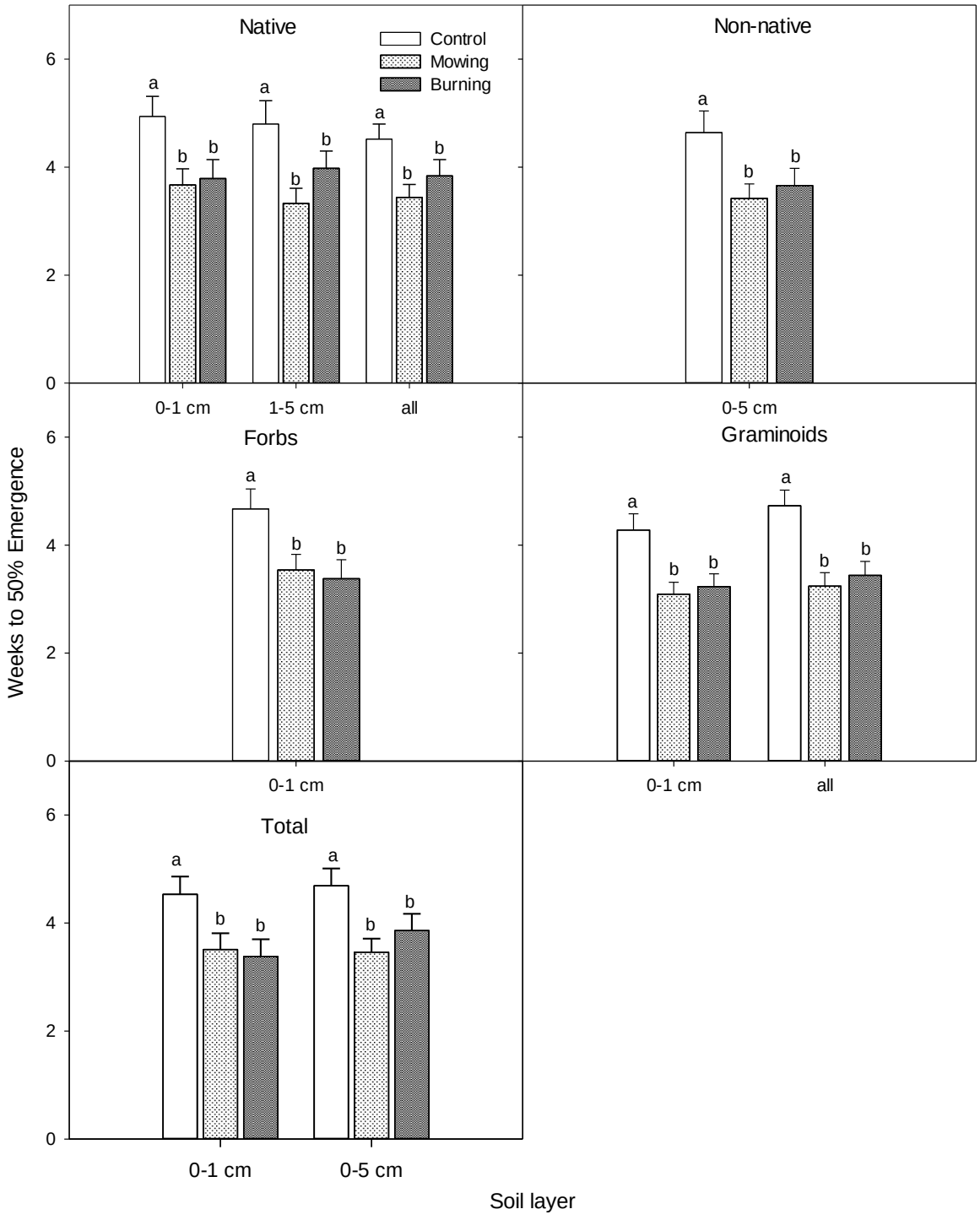

Figure 3.11 Seedling emergence rate (weeks to 50% seedling emergence) of functional groups and total seedlings emerging from different soil layers in the soil seed bank after burning and mowing in 2012 and 2013 combined. Data represent means ± SE. Means with different letters for each species within soil layers were significantly different ($P \leq 0.05$).

3.4 Discussion

3.4.1 Burning had positive effects on seedlings emerging in the field

Previous studies reported spring burn increased canopy cover of *Artemisia frigida* (Anderson and Bailey, 1980), *Artemisia ludoviciana* (Bailey and Anderson, 1978), and *Cirsium arvense* (Romo and Gross, 2011) in Fescue Prairie and that of *Conyza canadensis* in tallgrass prairie (Collins, 1987). Our results indicated this increase could be partially attributed to the increased seedling densities after burning for all these four species. The interaction between fire behaviour, seed biology, and ecology of different species determine the success of seedling establishment of individual species after burning (Keeley and Fotheringham, 2000). The positive responses of seedling emergence of *Artemisia frigida* to burning can be attributed to the fact that seed production of *Artemisia frigida* is prolific with a large amount of viable seeds buried in the soil (Bai and Romo, 1997). In addition, the relatively arid condition created by fire (Dejong and Macdonald, 1975) favors seedlings emergence of *Artemisia frigida*, which can withstand a short-term drought and exhibit relative high competitiveness and vigor (McWilliams, 2003). Several characteristics shared by *Artemisia ludoviciana*, *Cirsium arvense*, and *Conyza canadensis* including high fecundity (Stranathan *et al.*, 1986; Heimann and Cussans, 1996; Weaver, 2001), good dispersal capability (Romme *et al.*, 1995; Wood and del Moral, 2000; Weaver, 2001) and quick germinating ability (Moore, 1975; Welsh *et al.*, 1987; Weaver, 2001), benefit the occupancy and seedling establishment of these species after burning. In addition, seedlings of *Cirsium arvense* (Donald, 1994) and *Conyza canadensis* (Leroux *et al.*, 1996) are sensitive to competition. The less competitive conditions after burning also contribute to the increased seedling emergence of these species. During the two-year study, only one graminoid (*Poa pratensis*) emerged in the field, which is in line with previous studies that seeds of resprouters were more vulnerable to heat, leaving few viable seeds for seedling emergence after burning (Paula and Pausas, 2008).

Compared with the control, densities of total seedlings emerging in the field increased after burning, agreeing with the notion that high seed germination and seedling establishment occur after fire (Keeley *et al.*, 2012). Burning increased species richness and diversity of emerged seedlings, which in turn might alter species compostion of emerged seedlings. Seedlings of *Astragalus agrestis*, *Aster ericoides*, *Epilobium ciliatum*, *Heterotheca villosa*, and *Potentilla gracilis* emerged only after burning, because their seedlings were not found in the

control plots. It has been reported that spring and fall burns increased the canopy of *Astragalus agrestis* in Fescue Prairie (Bailey and Anderson, 1978). Repeated burning favors *Aster ericoides* in tallgrass prairie of Kansas (Towne and Owensby, 1984). Burning increases canopies of *Epilobium ciliatum* in a mesic hardwood forest (Murray *et al.*, 2012) and *Potentilla gracilis* in the Gallatin National Forest in the US (Nimir and Payne, 1978).

3.4.2 Factors associated with burning in affecting seedling emergence

In the present study, mowing created an open habitat just like burning did. Soil temperatures, soil water content, and light intensity did not vary significantly between mowing and burning. Mowing did not affect densities of individual species and total seedlings emerging from the filed, indicating that the altered environmental conditions after burning play minor role in affecting seedling emergence in Fescue Prairie. In contrast to our findings, Santana *et al.* (2013) states that increased alternating temperatures experienced after burning rather than burning *per se* had a greater influence on seedling emergence in Mediterranean Basin. Because species in different habitats have different sensitivities to various germination cues (Santana *et al.*, 2013), this may explain the different responses of seedling emergence to altered environmental conditions after burning. Smoke or ash stimulated seed germination and seedling emergence of *Artemisia frigida, Artemisia ludoviciana* and *Conyza canadensis* (results in chapter 4 and 5). This is in line with previous findings that single or interactive direct fire cues increased seedling emergence of species in South Africa mesic grasslands and naive Australian grasslands (Dixon *et al.*, 1995; Clarke and French, 2005; Ghebrehiwot *et al.*, 2009). It is also reported that concentrated smoke solutions increased germination of *Artemisia ludoviciana* (Jefferson *et al.*, 2014). Karrikinolide, the active compound in smoke, can broaden the environmental conditions for seed germination (Jain *et al.*, 2006) and alter the sensitivity of seeds to phytohormones (Schwachtje and Baldwin, 2004; Nelson *et al.*, 2009), explaining the positive effects of smoke on seed germination and seedling emergence. Ash can also affect seed germination and seedling emergence by altering soil pH (Henig-Server *et al.*, 1996). In addition, soil nutrients and microbial activites can be influenced by burning (Nelson *et al.*, 2012), which in turn affect below-ground competition. Further studies may be needed to determine whether changed below-ground conditions partly account for the different responses of seedling emergence after burning, as compared with mowing and control.

3.4.3 Effects of burning on seedling emergence from the soil seed bank

Differences in seedling numbers from the soil seed bank between years was not uncommon and can be explained by the temporal influence of variable environmental conditions on seed production, seed dormancy, and seed viability (Baskin and Baskin, 1998). In addition, seed bank in this study included transient and persistent seed bank because samples were collected in early spring, which was after seed dispersal but before germination (Baskin and Baskin, 1998), and seedling densities from the transient seed bank is more susceptible to temporal variation (Thompson and Grime, 1979).

Effects of fire on seedling emergence varied in different soil layers. Seedling densities of *Agrostis scabra*, *Artemisia frigida*, and *Poa pratensis*, together with species density, richness and diversity of total seedlings or different functional groups emerging from the litter layer were all reduced by burning. This is likely due to the damage to the seeds by burning, which reduces viable seeds above the soil surface (Bailey and Anderson, 1980; Archibold *et al.*, 1998). Burning reduced densities of non-native species and graminoids emerging from all layers in both years, but increased densities of native species and forbs emerging from the 0-1 cm soil layer in 2013. The positive effects of burning on species densities of forbs emerging from the soil seed bank agree with former findings that species composition shifted in favor of perennial forbs after a burn, regardless of the time of burning in Fescue Prairie (Daubenmire, 1968; Bailey and Anderson, 1978; Anderson and Bailey, 1980). More specifically, increased densities of native forbs accounted for the increased densities of native species and forbs. The positive responses of densities of native forbs to burning support the notion of using fire to restore native species and control non-native species in various grasslands (DiTomaso *et al.*, 1999; Prober *et al.*, 2005; MacDonald *et al.*, 2007). The existence of karrikinolide residues is partly responsible for increased seedling densities of native forbs after burning (Ghebrehiwot *et al.*, 2013). As discussed above, karrikinolide can stimulate seed germination and seedling emergence for certain species.

Total seedlings emerged faster in the 0-1 cm soil layer after burning compared with the control. Emergence rate for seedlings from the soil seed bank was accelerated by smoke and heat in a mesic grassland in South Africa (Ghebrehiwot *et al.*, 2012). Increased emergence rate of non-native seedlings emerging from the 0-5 cm soil layer by burning was because of the responses of emergence rate of non-native graminoids rather than that of non-native forbs. More

specifically, emergence rate of *Poa pratensis* rather than other non-native graminoids leads to the observed responses of emergence rate of non-native species to burning. Increased emergence rate of forbs emerging from the 0-1 cm soil layer by burning was caused by the responses of emergence rate of native forbs rather than that of non-native forbs, further proving the importance of fire on regeneration of native forbs from seeds.

Unexpectedly, mowing significantly increased densities of forbs and native species from the soil seed bank in 2013 and accelerated the rate of seedling emergence of total seedlings and functional groups. Soil samples for the control and burning treatments were collected in the same plot before and after burning, reducing spatial variability. Further studies may be needed to determine the mechanism in the effect of mowing on seedling emergence from the soil seed bank in Fescue Prairie.

3.4.4 Factors accounting for different responses of seedlings emerging in the field and from soil seed bank to burning

Burning had more positive effects on seedling emergence in the field compared with those from the soil seed bank. For example, burning increased species densities of five species in the field, but had no positive effects on seedling densities of any individual species emerging from the soil seed bank. As discussed above, the characteristics of individual species, direct fire cues, and the less competitive environment conditions after burning, contributed to the positive effects of burning on seedlings emerging in the field. Optimal conditions in the greenhouse may dampen the effects of direct fire cues. For example, the effects of smoke on increasing seed germination are compensated by most suitable germinating conditions for many species (Drewes *et al.*, 1995; Gonzalez-Rabanal and Casal, 1995). Ash changes the post-fire seedling emergence partly through its effect on changing pH and osmotic potential of soil (Gonzalez-Rabanal and Casal, 1995; Enright *et al.*, 1997). However, watering soil daily diminishes ash effect considerably. In addition, established seedlings were pulled out of the trays once they were identified for all treatments in the greenhouse. Destructing established vegetation greatly released species competition, which diminishs the effectiveness of burning on creating a less competitive environment in the field.

3.5 Conclusion

In conclusion, burning acts as a selective pressure by favoring seedling emergence in species and community levels and by altering species composition in Fescue Prairie. Burning altered species composition of emerged seedlings, increased seedling densities of *Artemisia frigida*, *Artemisia ludovicana*, *Conyza canadensis*, *Cirsium arvense*, and *Taraxacum officinale* and density, richness, and diversity of total seedlings emerging in Fescue Prairie. Density, richness, diversity, and species composition of total seedlings had no responses to mowing treatment, indicating observed positive effects of burning were mainly attributed to the direct fire cues and the lower competitive status, rather than the changed micro-environmental conditions created by burning, suggesting a relatively high resilience of species in Fescue Prairie to climate change. Consistent with our hypothesis, densities, richness, and emergence rate of different functional groups responded differently to burning. Reduced non-native graminoids accounted for the decreased densities of non-native species and graminoids emerging from all layers in the soil seed bank. Burning increased emergence rate of functional groups and total seedlings emerging from the soil layer in the soil seed bank. Emergence rate of *Poa pratensis* determined responses of emergence rate of non-native species to burning. Burning increased densities and emergence rate of native forbs emerging from the soil seed bank, indicating the importance of regeneration from seeds on shaping species composition in favor of forbs after burning and the possibility of using fire to restore Fescue Prairie.

4.0 EFFECTS OF SMOKE AND ASH APPLICATION ON SEEDLING EMERGENCE FROM THE SOIL SEED BANK IN FESCUE PRAIRIE

Abstract

Direct fire cues may regulate species composition in Fescue Prairie through their effects on seedling recruitment. Seedlings emerging from the soil seed banks incubated in the greenhouse were examined after applying smoke, ash, and smoke plus ash in 2013 and 2014. Soil seed bank samples were taken from the top 5 cm of the soil profile, and separated into litter, 0-1 cm, and 1-5 cm layers. Smoke plus ash significantly increased the number of *Artemisia ludoviciana* seedlings emerging from 0-1 cm soil layer and *Conyza canadensis* seedlings emerging from the litter layer ($P<0.05$), while ash significantly increased the number of *Artemisia frigida* seedlings emerging from 0-1 cm soil layer ($P<0.05$). Densities of total seedlings emerging from the 0-1 cm soil layer were increased by smoke plus ash in 2013 and by ash in 2014 ($P<0.05$). Smoke plus ash and ash had more prominent effects on seedling density and richness of native species and forbs, rather than non-native species and graminoids. Species composition was altered by ash in the 0-1 cm, 0-5 cm, and all layers combined in 2013 ($P<0.05$). Direct fire cues appear to stimulate recruitment of some species, especially native forbs, contributing to potential changes in species composition of the Fescue Prairie.

4.1 Introduction

Fescue Prairie is well adapted to burning (Wright and Bailey, 1982; Romo, 2003). Annual burns may increase, decrease, or have no effects on species frequency and canopy cover in Fescue Prairie (Anderson and Bailey, 1980). Responses of species composition in Fescue Prairie vary with burning season (Bailey and Anderson, 1978; Redmann *et al.*, 1993) and frequency (Anderson and Bailey, 1980). Different functional groups respond differently to burning in Fescue Prairie, with species composition shifts in favor of perennial forbs, regardless of the time of burning (Bailey and Anderson, 1978; Wright and Bailey, 1982). Burning increases density and diversity of native species in savannah grasslands in North America (DiTomaso *et al.*, 1999). Regeneration after burning can occur with vegetative reproduction (resprouters), seedling recruitment from seeds in the soil seed bank (seeders), or both (Keeley and Zedler, 1978; Paula and Pausas, 2008), and trade-off exists between the two (Paula and Pausas, 2008). The role

played by resprouters or seeders in altering species composition after burning in Fescue Prairie is not well known.

The soil seed bank is regarded as a biodiversity reservoir and plays a crucial role in restoring degraded or invaded ecosystems (Thompson and Grime, 1979). High germination and seedling establishment occur immediately after burning (Keeley *et al.*, 2012). Although seedling recruitment from the soil seed bank is fundamental in predicting plant species composition in response to disturbances (Harper, 1977), limited attention has been given to the effects of burning on seedling emergence from the soil seed bank in Fescue Prairie. Romo and Gross (2011) claimed species composition of seedlings emerging from the soil seed bank in Fescue Prairie were affected by burning history and season of burning.

Burning alters soil nutrient, light availability, and fluctuation of temperatures on the soil surface (Auld and Bradstock, 1996; Nelson *et al.*, 2012), but reduces competition (Keeley *et al.*, 1985). Altered environmental conditions partly account for the massive regeneration from soil or canopy stored seed banks after burning. In addition, single or combination of direct fire cues, including heat shock (Keeley *et al.*, 1985; Auld and O'Connell, 1991), plant-derived smoke (Brown, 1993; Dixon *et al.*, 1995; Roche *et al.*, 1997), and ash (Gonzalez-Rabanal and Casal 1995; Izhaki *et al.*, 2000) have been reported to exert promotive, inhibitive, or neutral effects on seedling emergence from the soil seed banks.

Smoke can break seed dormancy and stimulate seed germination (De Lange and Boucher, 1990; Dixon *et al.*, 1995; Enright *et al.*, 1997; Keeley and Fotheringham, 1997). Active components in smoke are heat stable, water soluble, and have long lasting effectiveness (Van Staden *et al.*, 2000). Smoke increases seedling densities and species richness of seedlings emerging from the soil seed bank in mesic grasslands in South Africa (Ghebrehiwot *et al.*, 2012). With increased soil depth, the extracted content of 3-methyl-2H-furo[2,3-c]pyran-2-one (KAR_1), the major active compound in smoke (Van Staden *et al.*, 2004; Flematti *et al.*, 2004) decreased (Ghebrehiwot *et al.*, 2011). Smoke increases germination of species native to semi-arid grasslands in North America (Schwilk and Zavala, 2012). Inhibiting effects of ash on seed germination and seedling establishment have been reported in herbaceous (Sweeney, 1956), shrub (Gonzalez-Rabanal and Casal, 1995), and tree species (Izhaki *et al.*, 2000). However, germination of four montane species in Mexico was positively affected by ash (Zuloaga-Aguilar *et al.*, 2011). Plants from different families react differently to ash (Gonzalez-Rabanal and Casal,

1995), but the mechanism is unclear. The combination of different fire cues, specifically heat and smoke, influences germination independently (Keith 1997; Kenny 2000) or synergistically (Gilmour *et al.*, 2000; Kenny 2000). However, the interactive effect of smoke and ash on seedling emergence from soil seed bank is still little known.

Despite the importance of seed bank in short and long term vegetation dynamics, the roles of different fire cues in general, smoke and ash specifically, on seedling emergence from the soil seed bank in Fescue Prairie are poorly understood. The objective of this study is to determine the effects of smoke, ash, and smoke plus ash on the density and composition of seedlings emerging from the soil seed bank in Fescue Prairie. The following hypothesis were tested: 1) smoke, ash, and smoke plus ash affect species composition and seedling emergence from soil seed bank in the Fescue Prairie; 2) effects of smoke, ash, and smoke plus ash on density and composition of seedlings emerging from soil seed bank vary among soil layers, and; 3) different functional groups emerging from the soil seed bank respond differently to smoke, ash, and smoke plus ash.

4.2 Material and Methods

4.2.1 Study site

This study was conducted at the Kernen Prairie in 2013 and 2014. Descriptions of the environmental conditions of Kernen Prairie can be found in section 3.2.1. In 2014, temperatures averaged 10.1 ℃ in May, 14.1 ℃ in June, 18.3 ℃ in July, and 17.9 ℃ in August. Total precipitation was 61.1 mm in May, 94.8 mm in June, 44.5 mm in July, and 18.5 mm in August (Environment Canada, 2014).

4.2.2 Experimental design

In total, 50, 3 × 3 m experimental plots were established on 17 April 2012 for this study. Among the 25 plots for study in 2013 and 2014, four subplots with the same size (25×25 cm) were randomly selected within each plot. Twenty five, 25 cm^2 soil core samples were collected from the top 5 cm of the soil profile in each subplot, which were subjected to a specific treatment, including control, smoke, ash, and smoke plus ash. Soil samples were collected on 4 May 2013 and 10 May 2014. Except for the control, soil cores were collected after clipping the phytomass to the ground in each subplot. Clipped phytomass was collected and dried in an 80 ℃ oven for two days with dry weight being recorded. Phytomass from each subplot was then combusted to

generate smoke solution or ash. The apparatus in producing smoke solutions and ash included a wooden board with an electric ring heater firmly fixed on, enough room was left for the power cord of the ring heater to exit from under the board; a 75L metal garbage can with two opposite holes attached with pressure gauges, connecting an air supplying hose on one side and a silicon tube on the other side; a pot for hodling plant material; and two weights (Figure 4.1). Each smoke, ash, or smoke plus ash sample was produced by smouldering the corresponding phytomass, which was put into container and placed on the electric ring heater. The 75L metal garbage can was inverted placed on the wooden board, enclosing the ring heater and the metal container containing plant materials. Two weights were placed on the top of the garbage can to eliminate the leakage of smoke. Air was forced into the combustion chamber at a pressure of 70-100 kPa. Smoke produced from the samples was continuously passed through the silicon tube and bubbled into 500 mL of distilled water in a water bottle to make smoke solutions. Ash was left in the container after combustion. In total, 25 smoke solution samples, 25 ash samples, and 25 smoke plus ash samples were collected.

Figure 4.1 Apparatus used for producing smoke solutions and ash. After drying, phytomass from each subplot was smouldered in a 10 L metal container placed on an electric heater fitted to a wooded board. A 75 L metal garbage can is inverted placed on the wooden board, enclosing the ring heater and the 10L metal container containing plant materials. Air at 70-100 kPa was forced into the combustion chamber. Smoke produced was continuously passed through the silicon pipe and bubbled into 500 mL of distilled water. Ash was left in the metal container after combustion.

Each soil sample was divided into the litter layer on the soil surface, 0-1cm, and 1-5cm depths. Same layers for the 25 soil cores from each subplot were combined, mixed, and spread in plastic trays (52×26×7cm) to < 1cm in depth (Cespedes *et al.*, 2012). Root fragments, rhizomes, and plant materials were removed. The litter layer was put on a 1 cm layer of sterile sand placed

at the bottom of plastic trays measuring 26×26×6 cm. Trays containing soil cores from the same plot were randomly placed as blocks on benches in a greenhouse. The air temperature during the growing season in the greenhouse averaged 27 ± 3°C during the day and 21 ± 4°C at night. Natural light was supplemented with two 400 W high-pressure sodium lights (18 h photoperiod, average of 600 $\mu mol \cdot m^{-2} \cdot s^{-1}$) for one bench. Each of the 25 blocks had four treatments including soil cores without any treatment, soil cores being applied with smoke, soil cores being applied with ash, and soil cores being applied with smoke plus ash. The amount of smoke, ash, or smoke plus ash applied into litter layer and two soil layers were based on the volumetric ratio. More specifically, for the soil cores collected within each subplot, litter volumes were measured with a volumetric cylinder and averaged at 220 and 270 ml in 2013 and 2014, respectively. Volumes in 0-1 cm and 1-5 cm soil layers were calculated as 625 cm^3 and 2500 cm^3, respectively, in both years. Hence, the ratios for applying smoke, ash, or smoke plus ash obtained from the phytomass collected from a specific subplot to the litter, 0-1 cm and 1-5 cm soil layers collected from the corresponding subplot were 220: 625: 2500 and 270: 625: 2500 in 2013 and 2014, respectively. All trays were watered daily. Seedling emergence was recorded weekly for 14 weeks and new seedlings transplanted to pots until they were identified. In total, data of five layers (litter layer, 0-1 cm depth, 1-5 cm depth, 0-5 cm depth and all layers combined (litter layer plus 0-5 cm depth soil layer) were analyzed.

4.2.3 Data analysis

Seedling densities of individual species in five blocks were combined because of high variability. Data for total seedling densities and emergence rate for individual species were square root, log, or log (x+1) transformed before subjected to ANOVA for a split-plot in a Completely Randomized Design (CRD) with 5 replicates. Data normality was tested using the Shapiro-Wilk test before each analysis. Total seedling densities, species richness, seedling densities and species richness of functional groups of forbs, graminoids, native, and non-native plants, species diversity, and seedling emergence rate from the soil seed bank in the greenhouse were square root, log, or log (x+1) transformed and subjected to ANOVA for a split-plot in a CRD design with 25 replicates, if they did not meet normality requirement. Year was the whole plot, while smoke, ash, and smoke plus ash treatments were subplots. When the interaction of year x treatment was significant ($P \leq 0.05$), data were analyzed within each year. Treatment means were separated using Tukey test at $P \leq 0.05$. The mixed model procedure in SAS version 9.3 was

used for data analysis (SAS Institute Inc., USA), taking main effects and possible interactions of year and treatments as fixed factors. Block (the whole-plot unit) was factored into the model as random effects.

Differences in species composition among treatments were tested using a PerMANOVA test, a permutation-based multivariate analysis of variance in PC-ORD version 6.0 (McCune and Grace, 2002). Permanova controls for variation contributed by a blocking factor. Densities of seedlings emerging for individual species in each plot were relativized by being dividing by the total seedlings emerging each plot. Sorenson distances were used to express similarity of species composition among treatments (McCune and Grace, 2002). Data were tested using 999 random permutations. Blocked indicator species analysis was used to determine the frequency and abundance of species emerging from the soil seed bank for specific treatments (Dufrene and Legendre, 1997). Densities of seedlings emerging for individual species in each plot were relativized by being dividing by the total seedlings emerging from each corresponding block (McCune and Grace, 2002). A Monte Carlo simulation of 10,000 runs was used to evaluate the statistical significance of indicator values (McCune and Grace, 2002).

4.3 Results

4.3.1 Effects of smoke, ash, and smoke plus ash on seedling density

A total of 21,434 and 32,895 seedlings were identified from the soil seed bank samples in a 4.69 m^2 sampling area in 2013 and 2014, respectively. Seedling numbers of native species were 1.5-fold and 1.7-fold greater than those of non-native species in 2013 and 2014, respectively. Seedling numbers of forbs were 1.3-fold greater than those of graminoids in both years. The percentage of unidentified seedlings was <1%. *Androsace septentrionalis* was the most abundant forb emerging (73% and 69% of total forbs in 2013 and 2014, respectively). *Poa pratensis* was the most abundant graminoid emerging (84% and 81% of total graminoids in 2013 and 2014, respectively).

In the control plots, 81% of *Artemisia frigida* seedlings and 75% of *Artemisia ludoviciana* seedlings from the 0-5 cm soil layer emerged from the 0-1 cm soil layer in both years. Ash increased densities of *Artemisia frigida* emerging from the 0-1 cm soil layer by 110% compared with the control (Figure 4.2). Densities of *Artemisia ludoviciana* emerging from the 0-1 cm soil layer were significantly increased by smoke plus ash by 253% compared with the

control. Densities of *Conyza canadensis* emerging from the litter layer and all layers combined were 2.6-fold and 1.7-fold greater in smoke plus ash than those in the control, respectively.

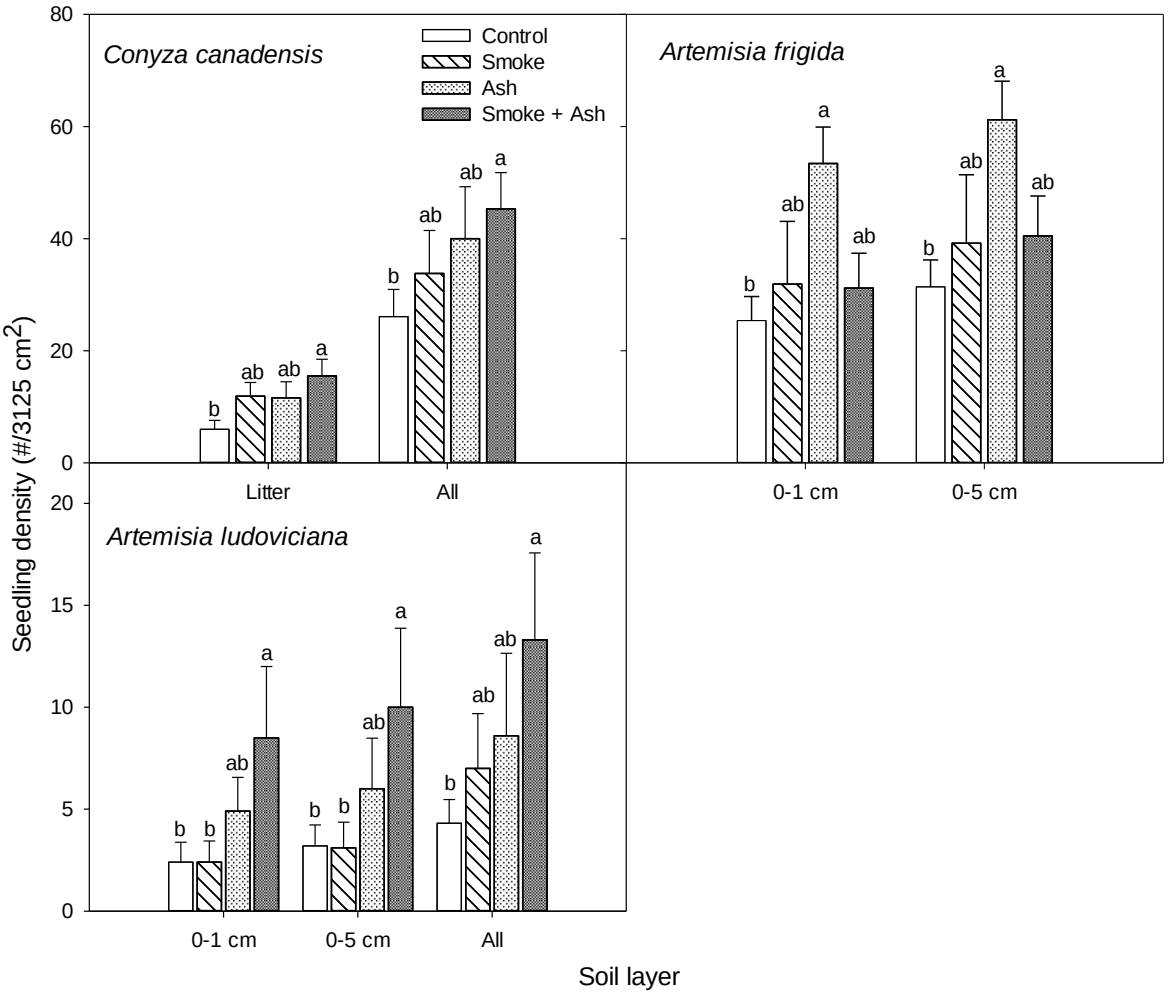

Figure 4.2 Densities of species in which total seedling densities emerging from the soil seed bank were significantly affected by smoke, ash, or smoke plus ash in 2013 and 2014 combined. Values represent means ± SE of 5 replicates. Means with different letters within soil layers were significantly different ($P \leq 0.05$).

Densities of total seedlings emerging from the litter layer were increased by ash in both years (Figure 4.3). Densities of total seedlings emerging from the 0-1 cm soil layer and all layers combined were affected by the interaction of treatment and year, which were significantly increased by smoke plus ash in 2013 and by ash in 2014 as compared with the control. Smoke reduced densities of total seedlings emerging from the 0-1 cm soil layer in 2013.

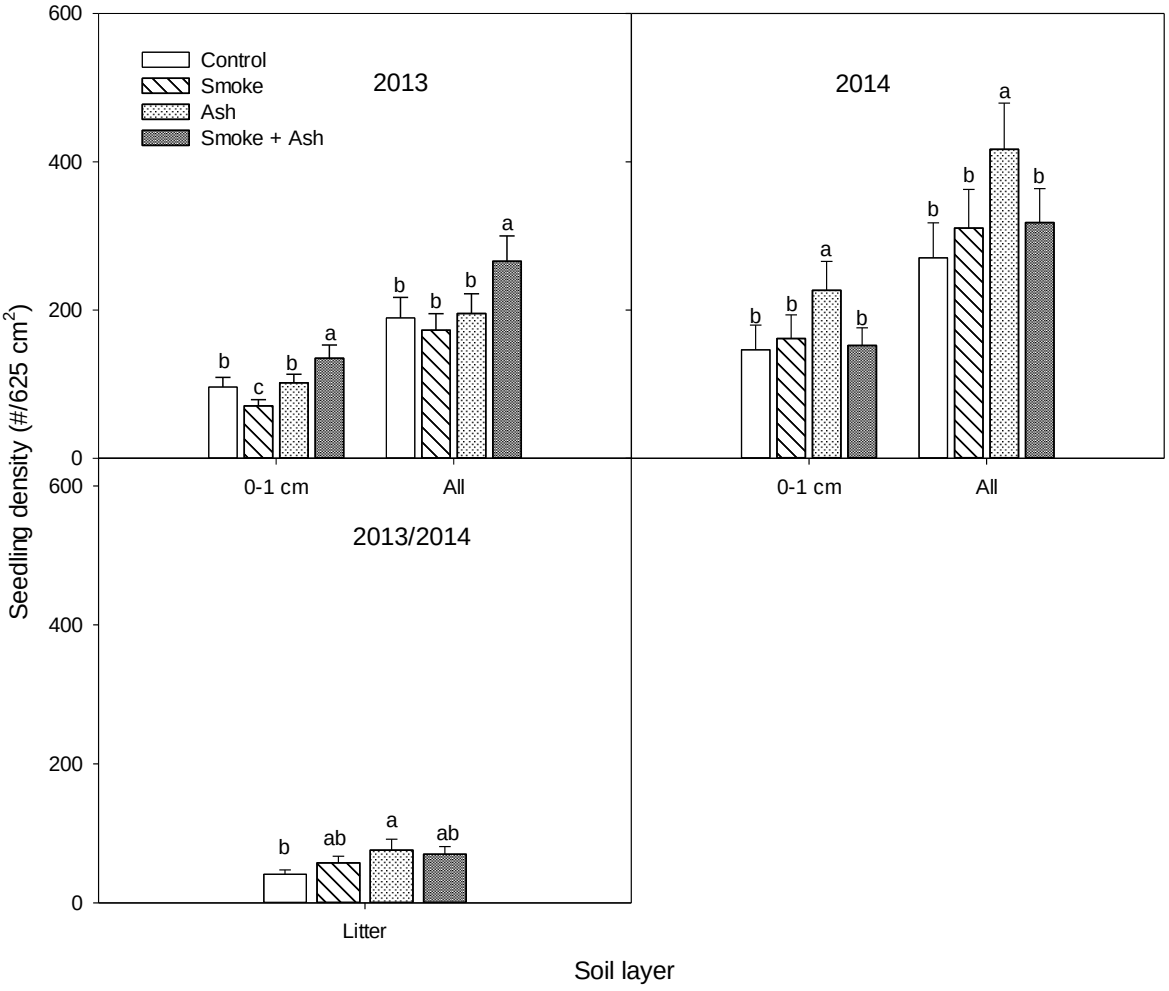

Figure 4.3 Total seedling densities emerging from the soil seed bank as affected by smoke, ash, and smoke plus ash in 2013 and 2014. Values represent means ± SE. Means with different letters within soil layers were significantly different (P≤0.05).

Seedling densities of functional groups emerging from different soil layers in the soil seed bank responded differently to smoke, ash, and smoke plus ash (Figure 4.4). In the control, 60% of native species or forbs emerging from the soil seed bank emerged from the 0-1 cm soil layer in both years. Densities of native species in the 0-1, 0-5 cm, and all layers combined were affected by the interaction of treatment and year. Smoke plus ash increased densities of native species emerging from the 0-5 cm soil layer in 2013. In 2014, densities of native species responded positively to ash in the 0-1 cm soil layer, with 67% increase in their abundance. Ash increased densities of forbs in the 0-1 cm and all layers combined by 44% and 34%, respectively, as compared with the control. Smoke, ash, or smoke plus ash did not significantly affect densities of non-native species and graminoid seedlings emerging from the soil seed bank.

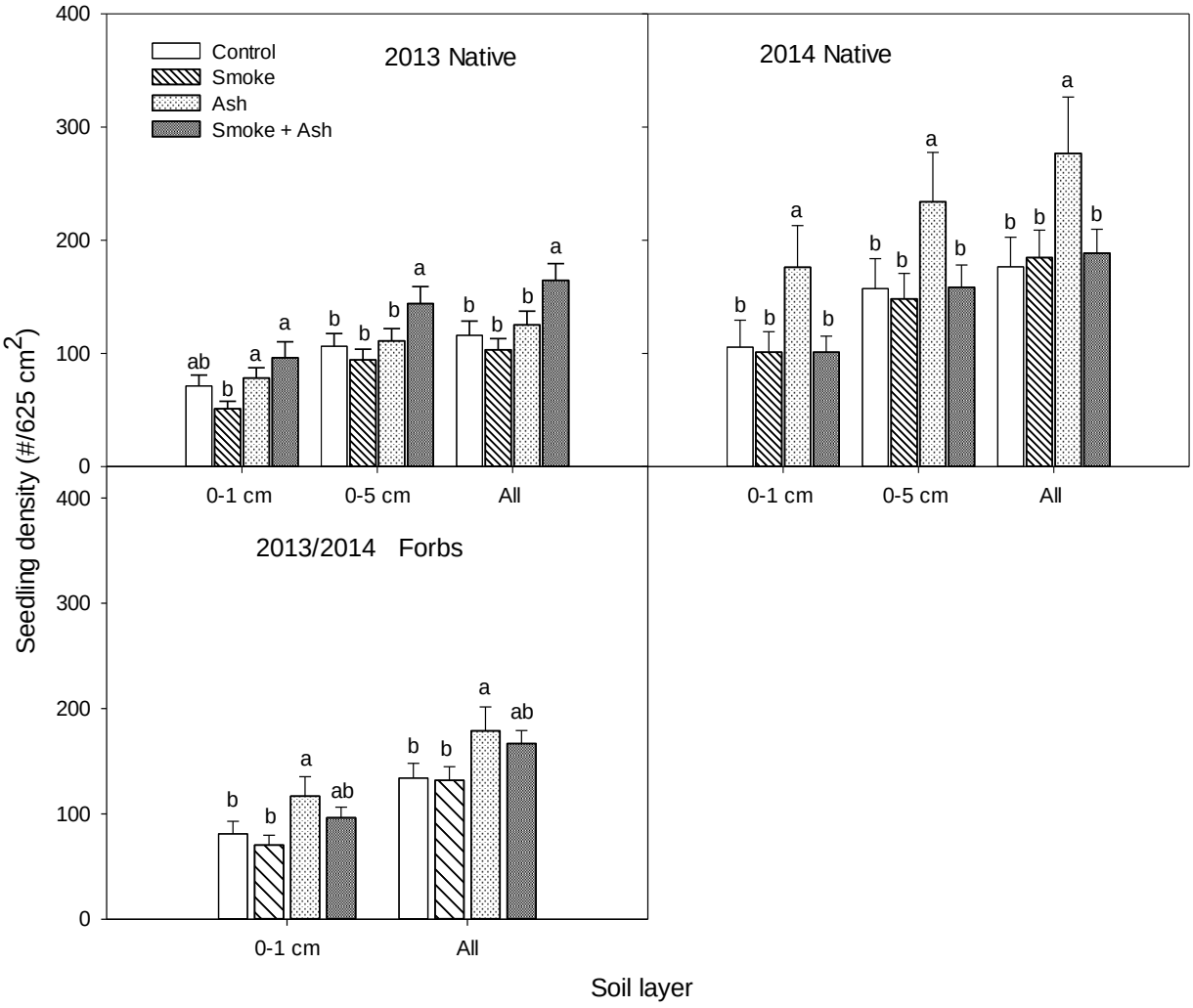

Figure 4.4 Densities of native species and forbs seedlings emerging from different soil layers in the soil seed bank as affected by smoke, ash, and smoke plus ash in 2013 and 2014. Bars represent means ± SE of 25 replicates. Means with different letters for native species or forbs within soil layers were significantly different ($P \leq 0.05$).

4.3.2 Effects of smoke, ash, and smoke plus ash on species composition of seedlings emerging from the soil seed bank

Smoke significantly altered species composition as compared with the control in the litter layer in 2013 (Table 4.1). Species composition in ash was significantly different from that in the control in 0-1 cm and 0-5 cm soil layers in 2013. As compared with the control, none of the three treatments significantly altered species composition in any soil layers in 2014.

Table 4.1 Pairwise comparison from multivariate permutational analysis (PERMANOVA) of differences in species composition among the control, smoke, ash, and smoke plus ash treatments in different soil layers in soil seed bank in 2013 and 2014.

		2013		2014	
Comparison	Soil layer	T	P	t	P
Control vs Smoke	Litter	1.57	0.01	0.75	0.84
Control vs Ash	0-1 cm	1.63	0.04	0.81	0.64
Smoke vs Ash	0-1 cm	1.63	0.05	1.20	0.28
Control vs Ash	0-5 cm	1.57	0.04	0.84	0.57
Smoke vs Ash	0-5 cm	1.65	0.04	0.91	0.40
Smoke vs Ash	All layer	1.63	0.04	1.00	0.43

Species richness of total seedlings emerging from the soil seed bank responded consistently to treatment effects in both years (Figure 4.5). Species richness of total seedlings emerging from all soil layers in the soil seed bank was significantly increased by smoke plus ash as compared with the control. Except for the 1-5 cm and 0-5 cm soil layers, species richness of total seedlings from all soil layers in the soil seed bank was significantly increased by ash as compared with control. Smoke increased species richness of total seedlings emerging from the litter layer.

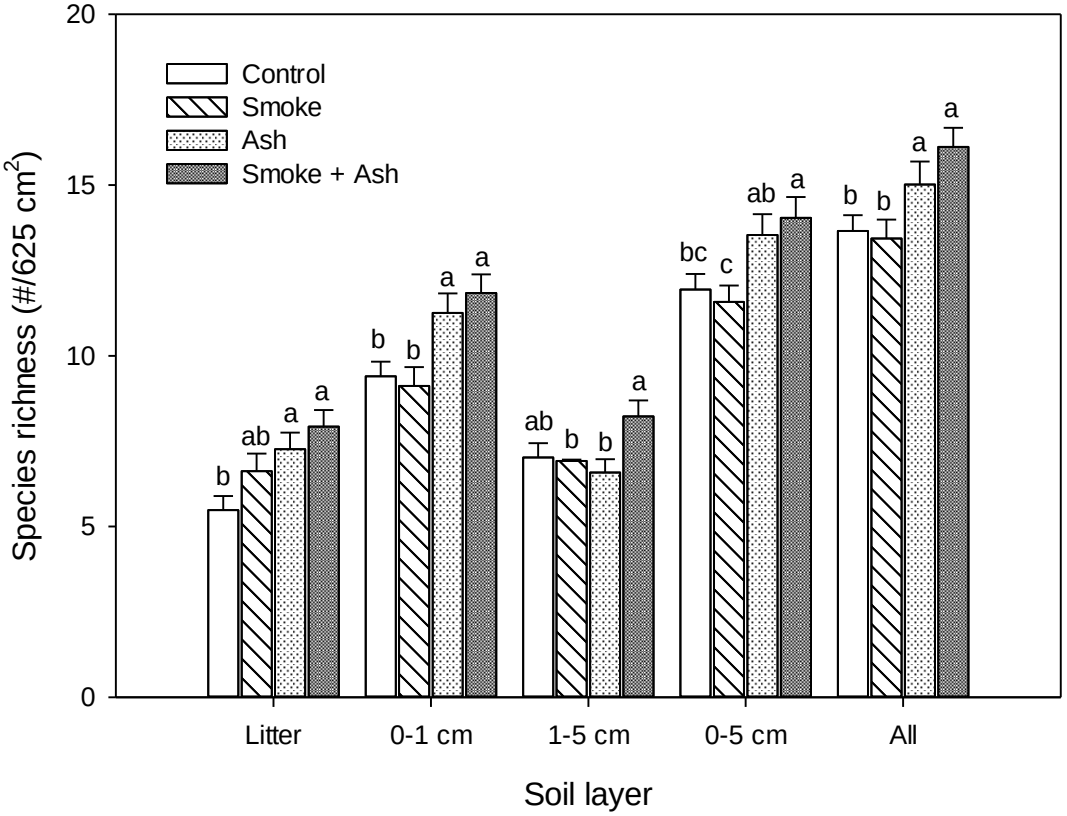

Figure 4.5 Species richness for total seedlings emerging from different soil layers in the soil seed bank as affected by smoke, ash, and smoke plus ash in 2013 and 2014 combined. Bars represent means ± SE of 25 replicates. Means with different letters within soil layers were significantly different at $P \leq 0.05$.

Effects of smoke, ash, and smoke plus ash on species richness of native species varied in different soil layers (Figure 4.6). Species richness of native species was enhanced by smoke plus ash in all soil layers as compared with the control. Ash increased species richness of native species emerging from the litter and 0-1 cm soil layer. Species richness of native species in the litter layer was increased by smoke.

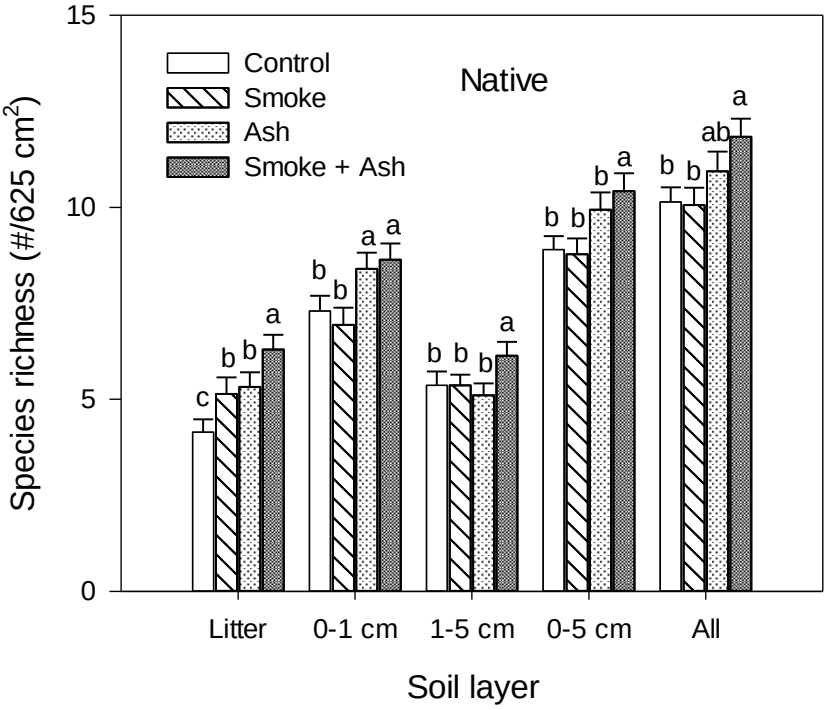

Figure 4.6 Species richness of native species emerging from different soil layers in the soil seed bank as affected by smoke, ash, and smoke plus ash in 2013 and 2014 combined. Bars represent means ± SE of 25 replicates. Means with different letters for either native or non-native species within soil layers were significantly different ($P \leq 0.05$).

Species richness of forbs and graminoids responded differently to smoke, ash, and smoke plus ash in different soil layers, with forbs being more positively affected (Figure 4.7). Species richness of forbs was significantly increased in all soil layers by smoke plus ash. Ash increased species richness of forbs emerging from the litter and 0-1 cm soil layers. Species richness of graminoids emerging from all soil layers did not vary significantly among treatments. Smoke, ash, and smoke plus ash did not affect species diversity and evenness of seedlings emerging from different soil layers in the soil seed bank (data not shown).

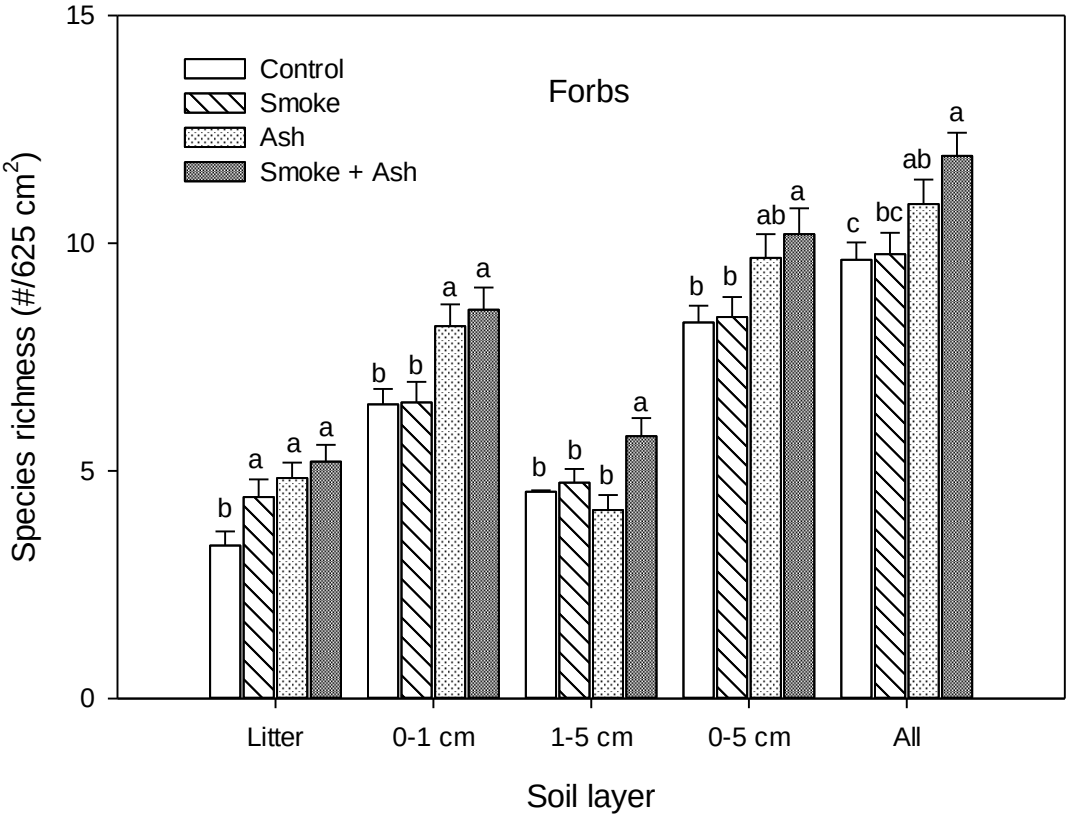

Figure 4.7 Species richness of forbs emerging from different soil layers in the soil seed bank as affected by smoke, ash, or smoke plus ash in 2013 and 2014 combined. Bars represent means ± SE of 25 replicates. Means with different letters within soil layers were significantly different ($P \leq 0.05$).

4.3.3 Effects of smoke, ash, and smoke plus ash on the rate of seedling emergence from the soil seed bank

The rate of seedling emergence of total seedlings and functional groups emerging from different soil layers in the soil seed bank only responded to smoke, ash, or smoke plus ash in 2013 (Figure 4.8). Total seedlings emerged faster in ash and smoke + ash in the litter and all layers combined. Total seedlings emerged faster in the litter layer but slower in the 0-1 cm and 0-5 cm soil layers in smoke as compared with the control. Seedlings of forbs and native species emerged faster in smoke plus ash in the litter, 0-5 cm, and all layers combined. Smoke plus ash increased the rate of emergence of fobs and native species in the litter layer. Seedlings of forbs in the 0-1 cm and 0-5 cm soil layers and seedlings of native species in the 0-1 cm soil layer emerged slower in smoke. Seedlings of graminoids emerged faster in smoke, ash, and smoke plus ash in the litter layer. Seedlings of graminoids emerged slower in smoke in the 0-1 cm soil layer, but faster in smoke plus ash in all layers combined. Seedlings of non-native species emerged faster in ash and smoke plus ash in the litter layer, but slower in smoke in the 0-1 cm soil layer.

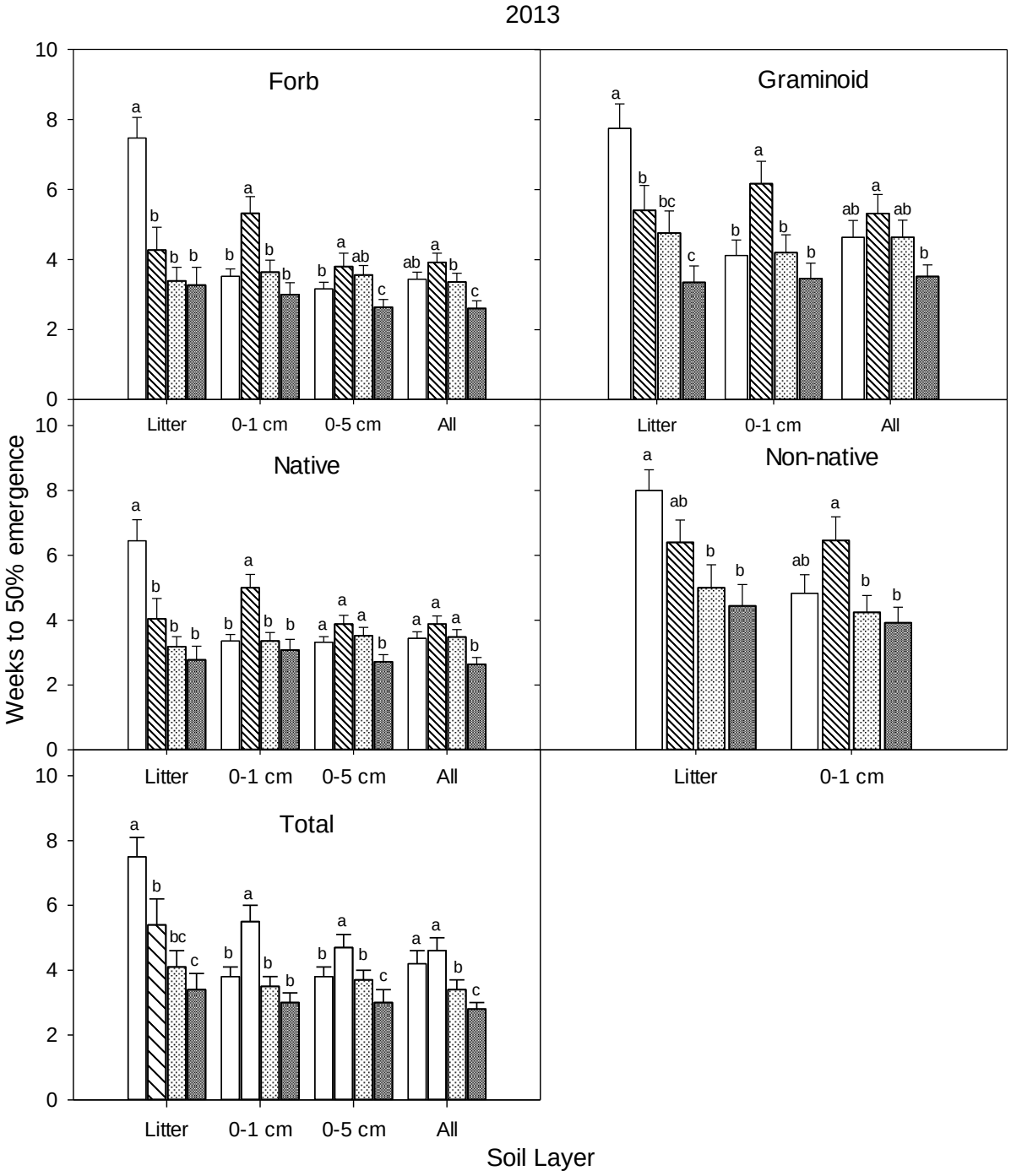

Figure 4.8 Weeks to 50% emergence of functional groups and total seedlings emerging from different soil layers in the soil seed bank as affected by smoke, ash, or smoke plus ash in 2013. Bars represent means ± SE of 25 replicates. Means with different letters within soil layers were significantly different ($P \leq 0.05$).

4.4 Discussion

Smoke, ash, and smoke plus ash had various effects on densities, richness, diversity and composition of seedlings emerging from the soil seed bank in Fescue Prairie. These effects also varied among plant functional groups and sampling depth, suggesting that regeneration from seeds after burning plays an important role in shaping species composition in Fescue Prairie in a complicated manner.

4.4.1 Seedling emergence from the soil seed bank in Fescue Prairie

The dominant species in the mature Fescue Prairie, including *Festuca hallii*, *Stipa curtiseta* and *Agropyron dasystachyum* (Coupland, 1961), were poorly represented in the soil seed bank. *Androsace septentrionalis* and *Poa pratensis* were the most abundant forb and graminoid in the soil seed bank, respectively. The lack of resemblance between the soil seed bank and above-ground in species composition is common in grasslands (Major and Pyott, 1966; Rabinowitz, 1981; Baskin and Baskin, 1998), which can be attributed to seed longevity, seed dispersal, seed predation, and the temperate and spatial variation of seed rain (Romo and Bai, 2004). *Androsace septentrionalis* is described as an annual (Weber and Wittmann, 2001) or short-lived perennial (Inouye *et al.*, 2003), which accumulates in the soil seed bank during a period of no burning (Pausas and Keeley, 2014). *Poa pratensis* is known to produce abundant seeds, accounting for its high proportion in the soil seed bank (Uchytil, 1993). The little contribution of *Stipa curtiseta* and *Agropyron dasystachyum* to the soil seed bank was reported before (Romo and Bai, 2004). *Festuca hallii* mainly reproduces from rhizomes with an infrequent seed production (Palit, 2013), which explains its small fraction in the soil seed bank (Romo *et al.*, 1991).

The number of seedlings emerging from the soil seed bank varied between 2013 and 2014. Densities of seedlings emerging from the soil seed bank are subjected to the variation of seed production, seed viability, and seed dormancy status in each year (Baskin and Baskin, 1998). In addition, soil cores were collected between seed dispersal and germination in this study, which include transient and persistent seed banks (Baskin and Baskin, 1998). Seedlings emerging from transient soil seed banks are more subjected to temporal variability (Thompson and Grime, 1979).

4.4.2 Effects of smoke and ash on seedling emergence from the soil seed bank

Contrary to the well-reported promotive effects of smoke on seedling emergence from soil seed banks in various ecosystems including mesic grasslands in South Africa (Ghebrehiwot *et al.*, 2012), South African fynbos (Brown, 1993), and California chaparrals (Keeley and Fotheringham, 1997; 1998), smoke had neutral or negative effects on densities and emergence rate of total seedlings and functional groups emerging from the soil seed bank in the present study. Species in different habitats may respond differently to smoke, for example, species in the Mediterranean Basin have been claimed to be insensitive to the promotive effects of smoke (Rivas *et al.*, 2006; Reyes and Casal, 2006). However, the highly concentrated smoke solutions applied in the present study are more likely the reason for the observed responses. Diluted smoke solutions made from *Festuca hallii* have proved to stimulate germination of native species in Fescue Prairie, including *Artemisia frigida*, *Artemisia ludoviciana*, and *Conyza canadensis* (Chapter 5). High concentration of aqueous smoke solutions reduces germination of *Acacia robusta* (Kulkarni *et al.*, 2007). This reduction may be due to the presence of inhibiting or toxic compounds within concentrated smoke solutions (Dixon *et al.*, 1995). Diluted smoke solutions were used in most studies in which seedling emergence from the soil seed bank responded positively to smoke (Lloyd *et al.*, 2000; Wills and Read, 2002; Abella, 2009; Ghebrehiwot *et al.*, 2012). In addition, different methodologies used to apply smoke solutions may also contribute to the different responses. For example, Ghebrehiwot *et al.* (2012) applied diluted smoke solutions to the soil seed bank weekly during the whole experimental period. Enright *et al.* (1997) used aqueous smoke solutions to water soil samples every two days for the first four weeks. In our case, in order to mimic the field scenario, smoke solutions were applied to the soil seed bank only once, and then tap water was used for watering to mimic the precipitation after burning. It is worth noting that the amount of smoke being trapped using the combustion chamber was much greater than that occurred in the field during burning.

Ash had positive effects on densities of *Artemisia frigida* seedlings emerging from the soil seed bank. Izhaki *et al.* (2000) attributed the effects of ash on germination and seedling establishment to blocked light and alternated soil chemistry. In the present study, approximately 27 g and 21 g of ash were divided and applied to the litter, 0-1 cm and 1-5 cm soil layer based on volumetric ratio among these 3 layers in each replicate for ash treatment in 2013 and 2014, respectively. The amount of ash could barely cover surface of trays used in the tests, which

eliminated the light blocking effect of ash. The high pH of ash, together with soil property and germination characteristics of specific species can also affect seed germination (Henig-Sever *et al.*, 1996) and seedling establishment (Ne'eman & Izhaki, 1998). For example, it was reported that *Pinus halepensis*, *Cistus salviifolius* and *Cistus creticus*, the three dominant plant species in native forests in Israel, were all sensitive to high pH (Henig-Sever *et al.*, 1996; Izhaki *et al.*, 2000). Ash decreased seedling emergence of these three species because it increased soil pH from 7.0 to 8.5 (Henig-Sever *et al.*, 1996). In the present study, soils are Bradwell and Sutherland Orthic Dark Brown Chernozems (Acton and Ellis, 1978) with a pH of 5.4 (Curtin and Ukrainetz, 1997). The high pH of the ash can neutralize the acidic soil, creating a more suitable germination conditions for certain species. The optimum pH for germination of *Artemisia frigida* is between 5.8 and 7.0 (Wilson, 1982).

Smoke plus ash, but not smoke or ash alone increased seedling densities of *Artemisia ludoviciana* and *Conyza canadensis* from the soil seed bank, which is supported by the notion that combinations of fire cues can positively or negatively affect seed germination than a single cue (Keeley and Fotheringham, 1998; 2000). For example, germination of *Aphanes arvensis* did not respond to heat or smoke, but was significantly reduced by smoke plus heat (Figueroa and Cavieres, 2012). Similarly, germination of three common shrub species in south-eastern Australia, including *Epacris microphylla* var. *rhombifolia*, *Baeckea linifolia*, and *Epacris muelleri* was only increased by the combination of added smoke and heat (Thomas *et al.*, 2007). Germination of *Artemisia ludoviciana* was favored by neutral to alkaline conditions and can be stimulated by highly concentrated smoke solutions (Lara Vanessa *et al.*, 2014), which favors the notion that smoke and ash work synergistically on *Artemisia ludoviciana*. *Conyza canadensis* germinates better in a neutral-to-alkaline soil than an acidic one (Nandula *et al.*, 2006). Although the possible interactive effect of smoke and ash could be attributed to the fact that the stimulant effects of smoke on germination varied in different pH conditions (Keeley and Fotheringham, 1998), the underlying mechanisms are still vague and further studies are needed. The positive responses of densities of *Artemisia frigida*, *Artemisia ludoviciana*, and *Conyza canadensis* seedlings from the soil seed bank to ash and smoke plus ash indicate that direct fire cues encourage regeneration from seeds for these 3 species, which partly account for the increased abundance and canopy cover of these 3 species in the Fescue Prairie and tallgrass prairie after

burning (Coupland and Brayshaw, 1953; Bailey and Anderson, 1978; Anderson and Bailey, 1980; Collins, 1987).

4.4.3 Responses of functional groups to smoke and ash

Seedling densities for different functional groups responded differently to smoke, ash, and smoke plus ash in the two-year study. Smoke plus ash and ash increased seedling densities of native species from the soil seed bank in 2013 and 2014, respectively. Increased seedling densities of native species were due to the positive responses of seedling densities of native forbs to treatments. Native and non-native graminoids emerging from the soil seed bank had no responses to treatments in both years, agreeing with the observation that seedling emergence of Poaceae was insensitive to ash (Gonzalez-Rabanal and Casal, 1995). Ash increased seedling densities of forbs in the soil seed bank in both years. Seedling densities of native forbs and non-native forbs were positively affected. Species composition shifted in favor of perennial forbs, regardless of the time of burning in Fescue Prairie (Bailey and Anderson, 1978; Wright and Bailey, 1982). Frequent burning also increases the number of forbs in Fescue Prairie (Anderson and Bailey, 1980). The positive responses of seedling densities of native and non-native forbs to ash indicate that improved soil pH conditions after burning is perhaps one of the reasons for the shift in species composition in favor of forbs in the Fescue Prairie.

Species richness of different functional groups responded consistently to different treatments in both years. Increased species number of native forbs after smoke, ash, and smoke plus ash accounted for the increased species richness of native species from different soil layers in the soil seed bank. Smoke plus ash was the most effective treatment in increasing species richness for functional groups as compared with the control in both years. Native forbs, including *Astragalus flexuosus*, *Draba nemorosa*, *Hackelia deflexa*, *Stellaria longipes* and non-native forbs, including *Chenopodium album*, *Gnaphalium uliginosum*, *Rorippa islandica* emerged from the soil seed bank after applying smoke plus ash but not in the control in the two-year study. Spring and fall burns increase canopy cover of *Astragalus flexuosus* in Fescue Prairie (Bailey and Anderson, 1978). Increased burning frequency favors seedling emergence of *Chenopodium album* from the soil seed bank in Fescue Prairie (Romo and Gross, 2011). All three non-native forbs are annuals, indicating fire-related cues may favor seedling emergence of species with short life cycles for non-native forbs. Forbs but not graminoids were positively affected by

smoke, ash, or smoke plus ash, further supporting the notion that direct fire-related cues might be partly attribute to increased forbs in the field after burning.

Emergence rate of functional groups and total emerged seedlings responded to the treatments in 2013 but not 2014. In 2013, emergence rate of forbs, graminoids, native species, and non-native species emerging from the soil seed bank were all affected by treatments. Seedling number of *Poa pratensis*, a non-native graminoids, accounted for 84% and 94% of the total seedlings in graminoids and non-native species, respectively. Thus, observed responses of emergence rate of graminoids and non-native species to treatments are mainly due to the presence of *Poa pratensis*. Observed responses of emergence rate of forbs and native species to treatments were most likely due to the presence of native forbs, but not non-native forbs. Smoke plus ash increased seedling emergence rate for native forbs emerging from the soil seed bank, further proving the importance of direct fire cues in shaping species composition in favor of forbs after burning, because early recruitments were being favored in a low competition habitat (Fenner and Thompson 2005).

4.4.4 Seedlings emerging from different soil layers responded differently to smoke and ash

Densities of seedlings from the litter layer and 0-1 cm soil layer responded more to the independent and interactive effect of smoke and ash, as compared with those from the 1-5 cm soil layer. This may be due to the fact that seeds distribute unevenly in different depth of soil profiles (Everson, 1994) with more viable seeds occupying the top of the soil profile (Izhaki *et al.*, 2000; Ghebrehiwot *et al.*, 2012). For example, most seedlings emerged from the litter layer and 0-1 cm soil layer, only 11% and 18% of total seedlings of *Artemisia frigida* and *Artemisia ludoviciana* emerged from the 1-5 cm soil layer in 2013 and 2014, respectively, in the control. Lack of responses of seedlings of *Artemisia frigida* and *Artemisia ludoviciana* emerging 1-5 cm soil layer to treatments may be due to their low seedling counts, which may be under the detectable threshold. This notion is supported by Bargmann *et al.* (2014), who claimed that low seedling counts may be the reason accounting for the lack of responses of herbs to smoke and heat treatments in heathlands in Norway.

Total seedling densities in the 1-5 cm soil layer were less than that in the 0-1 cm soil layer but were 3 times greater than those in the litter layer in both years in the control, indicating seedling numbers of total seedlings in the 1-5 cm soil layer were big enough to detect effects of treatment. Seed dormancy status of buried seeds varies among different soil depth. Seeds of

Aeschynomene indica remain dormant in buried conditions but release dormancy on the soil surface (Fukumi and Nakata, 2008). Dormancy release speed decreased in *Lespedeza potaninii* and *Peganum nigellastrum* as soil burial depth increased (Wang *et al.*, 2012). Individual species has its own optimal depth for seedling emergence and survival (Kozlowski, 1972). Below the optimum depth secondary dormancy can be induced (Cumming and Hay, 1958; Banting, 1966). However, in the present study, different dormancy status for seeds buried in different soil layers were not tested, which need further study to determine whether and how it can be used to explain the lack of response in total seedling emergence from 1-5 cm soil layer to treatments.

It should be noted in the present study, smoke solutions and ash were applied to different soil layers based on the volumetric ratio among different soil layers. However, in reality, smoke and ash distribute unevenly among soil profile, with majority accumulated in top soil profiles (Ghebrehiwot *et al.*, 2013), which leads to a pattern of deceasing effectiveness of treatments on seedling emergence from soil seed bank with increasing soil depth.

4.5 Conclusions

Direct fire cues, smoke and ash specifically, played a crucial role in modifying seedlings emerging from the soil seed bank in Fescue Prairie. Although effects of smoke, ash, and smoke plus ash may interact with year due to the variation of seeds in the soil seed bank and amount of phytomass collected for producing different treatments, ash and smoke plus ash had positive effects on the density, richness and seedling emergence rate of seedlings emerging from the soil seed bank. Densities of *Artemisia frigida*, *Artemisia ludovicana*, *Conyza canadensis,* and total seedlings emerging from certain soil layers in the soil seed bank responded positively to ash or smoke plus ash. Contrary to our hypothesis, smoke solutions had few positive effects on density, richness and emergence rate of seedlings emerging from the soil seed bank, which may be due to the relative high concentration applied. Further studies are required to determine effects of smoke on seedlings emerging from soil seed bank in Fescue Prairie with different dilutions. Consistent to our hypothesis, density, richness and emergence rate of different functional groups responded differently to the treatments, with forbs and native species being affected more. More specifically, native forbs rather than native graminoids or non-native forbs responded to the treatments. The responses of densities and emergence rate of graminods and non-native emerging from the soil seed bank to treatments were mainly determined by *Poa pratensis*, a non-native graminoids whose seedling numbers accounted for 84% and 94% of the total seedlings in

graminoids and non-native species in the two years, respectively. Seedlings emerging from the 1-5 cm soil layer were mostly insensitive to treatments as compared with those emerging from the litter and 0-1 cm soil layers. This study highlights the importance of direct fire cues in shaping species composition through seed regeneration in Fescue Prairie and implies the possibility to use these fire cues as effective approach to restore Fescue Prairie.

5.0 SMOKE AFFECTS SEED GERMINATION AND SEEDLING GROWTH OF SPECIES IN FESCUE PRAIRIE

Abstract

Smoke has the potential to regulate species composition in Fescue Prairie through its various effects on seed germination and seedling growth of different species. Seeds of four forbs in Fescue Prairie, whose seedling emergence was significantly increased after burning, were primed for 24 h in darkness using serial dilutions (1/1000v/v, 1/100v/v, 1/10v/v and 1/1v/v) of the aqueous smoke solutions produced from alfalfa ((*Medicago sativa*), prairie hay (*Festuca hallii*), and wheat straw (*Triticum aestivum*). After priming, seeds were dried at 20°C in darkness for 7d before distilled water was applied. Seeds were incubated at 10/0°C or 25/15°C in 12h light /12h darkness or 24 h darkness for 49 d. Non-primed seeds and those primed in distilled water (0/1v/v) were used as controls. Within each germination condition, germination varied significantly ($P<0.05$) among smoke dilution, smoke type, and their interactions. Priming in highly concentrated smoke solutions made from prairie hay and wheat straw had more negative effects on germination and seedling growth for *Artemisia frigida* and *Conyza canadensis*, compared with those made from alfalfa. Smoke substituted light requirement for germination of *Artemisia ludoviciana* and *Conyza canadensis*. Germination of *Cirsium arvense* and *Coynza canadensis* only responded to smoke solutions at 25/15°C. Low concentrated smoke solutions increased radical length of *Artemisia ludoviciana*. At 25/15°C in 24 h darkness, concentrated smoke solutions made from alfalfa increased seed germination of *Coynza canadensis*, while same concentrated smoke solutions made from prairie hay and wheat straw decreased germination of *Coynza canadensis*. Effects of smoke on germination and seedling growth depend on species and germination conditions.

5.1 Introduction

Fire is a selective pressure in the evolution of seedling traits after burning (Keeley *et al.*, 2012). Different fire-related cues, including heat, ash, and smoke, have different mechanisms in triggering seed germination and seedling establishment (Baldwin, 1994; Henig-Sever *et al.*, 1996; Keeley and Fotheringham, 2000). Historically, Fescue Prairie was subjected to fire with intervals of 5 to 10 years as estimated by Wright and Bailey (1982) and 10 to 26 years as estimated by Barrett (1999). The frequent fires occurring in Fescue Prairie suggests that species in this

grassland may have well adapted to fire (Wright and Bailey, 1982). Although responses in species coverage, richness, diversity, and composition to different fire regimes have been well studied in Fescue Prairie (Bailey and Anderson, 1978; Anderson and Bailey, 1980; Romo, 2003; Gross and Romo, 2010), little is known about how different fire-related factors, smoke specifically, affect seed germination and regeneration ecology of species in Fescue Prairie.

The stimulative effects of plant-derived smoke on seed germination have been extensively studied for species from different taxa (Brown, 1993; Brown *et al.*, 1993; Baxter and Van Staden, 1994; Brown *et al.*, 1994), including species from fire-prone and fire-free habitats (Van Staden *et al.*, 2000). The promotive effects of smoke are independent of seed size, shape, and/or life form (Dixon *et al.*, 1995). Active components in smoke are water soluble (Van Staden *et al.*, 2000). Smoke solutions have been shown to stimulate seed germination for many species, although the magnitude of stimulation varies among concentrations and duration of application (Roche *et al.*, 1997; Lloyd *et al.*, 2000). The mechanisms of smoke in stimulating germination vary among species. Smoke acted as chemical scarification and increased germination of *Emmenanthe penduliflora* by increasing seed coat permeability (Egerton-Warburton, 1998). Smoke may also induce germination by altering the sensitivity of seeds to phytohormones (Schwachtje and Baldwin, 2004; Nelson *et al.*, 2009). Seedling growth can also be positively affected by smoke. Exposing seeds to smoke solutions increased root length in tomato (*Lycopersicon esculentum*) seedlings by 10-fold (Jain *et al.*, 2006).

The effects of aqueous smoke solutions on seed germination are temperature and light dependent (Brown *et al.*, 1994; Ghebrehiwot *et al.*, 2009,). Ghebrehiwot *et al.* (2009) reported that the positive effects of smoke solutions on seed germination of *Aristida junciformis*, *Hyparrhenia hirta*, and *Panicum maximum* were more notable with increasing temperature. Aqueous smoke solutions also interact with light (Brown and Van Staden, 1997). With application of smoke solutions, negatively photoblastic seeds can germinate in light (Brown, 1993; Brown and Van Staden, 1997), whereas photoblastic seeds germinate in darkness (Drewes *et al.*, 1995; Thomas and Van Staden, 1995).

It is still arguable whether effects of smoke originated from different plant materials have similar germination responses. For example, Jager *et al.* (1996) reported effects of smoke solutions made from *Acacia mearnsii*, *Eucalyptus grandis*, *Hypoxis colchicifolia*, *Pinus patula*, and *Themeda triandra* on germination of Grand Rapid lettuce (*Lactuca sativa*) did not vary

among different smoke types. However, Van Staden *et al.* (1995) found possible stimulants varied between the smoke solutions made from *Passerina vulgaris* and *Themeda triandra*. In this study, besides prairie hay collected from a Fescue Prairie dominated by plains rough fescue (*Festuca hallii* [Vasey] Piper), smoke solutions produced from wheat straw (*Triticum aestivum* cv. Unity) and alfalfa (*Medicago sativa*) were used to determine whether germination responses vary among smoke originating from different plant materials.

Although the significance of plant-derived smoke as a crucial germination and seedling growth cue has been reported in different ecosystems, including South African fynbos (Brown, 1993), Californian chaparral (Keeley and Fotheringham, 1998), and Australian native grasslands (Dixon *et al.*, 1995; Read and Bellairs, 1999; Clarke and French, 2005), it is still little known in Fescue Prairie, which is well adapted to burning (Wright and Bailey, 1982). Disentangling the effects of fire-related cues, smoke specifically, on the germination and seedling growth of species in Fescue Prairie can help us better understand the mechanisms of fire in shaping species composition in Fescue Prairie. The main objective of this study was to determine how smoke derived from different plant materials interacts with temperature and light in affecting seed germination and seedling growth. It was hypothesized that 1) smoke stimulates seed germination and seedling growth differently depending on plant species; 2) smoke derived from different plant materials affects seed germination and seedling growth differently, and; 3) promotive effects of plant-derived smoke vary with temperature and light during germination.

5.2 Material and Methods

5.2.1 Species studied

Four species from Kernen Prairie in which total seedling densities were significantly affected by burning (Chapters 3 and 4) were chosen for this study, including fringed sage (*Artemisia frigida* Willd.), white sagebrush (*Artemisia ludoviciana* Nutt.), Canada thistle (*Cirsium arvense* (L.) Scop.), and Canadian horseweed (*Conyza canadensis* (L.) Cronquist). *Artemisia frigida*, *Artemisia ludoviciana*, and *Conyza canadensis* are native to North America and produce a prolific amount of seeds (Bai *et al.*, 1996; Noyes, 2000; Karlsson and Milberg, 2007). *Cirsium arvense* is an introduced species in Canada and native to Europe and Northern Asia (Bochenek *et al.*, 2009). Except *Conyza canadensis*, which does not have

dormancy, all species studied have physiological dormancy (Pelton, 1956; Bai and Romo, 1994; Karlsson and Milberg, 2007; Bochenek *et al.*, 2009).

5.2.2 Seed collection and storage

Seeds of *Artemisia frigida*, *Artemisia ludoviciana*, and *Cirsium arvense* were collected from at least 50 plants for each species on 10 October 2012 at the Kernen Prairie, located 1 km to the northeast of Saskatoon, SK (52°10′N, 106°33′W, elevation 510 m). Environmental conditions of Kernen Prairie can be found in 3.2.1. Seeds of *Conyza canadensis* were collected from mature plants at Round Prairie, which is near Dundrun, SK, on 23 August 2013. After at least 1 month for after-ripening at room temperature, seeds of *Artemisia frigida*, *Artemisia ludoviciana*, and *Conyza canadensis* were hand-stripped from plants. Seeds of *Cirsium arvense* were separated from the plants using a seed thresher. Seeds were then cleaned and separated using metal test sieves. Cleaned seeds were kept in sealed plastic bags and stored at -20°C until they were used for germination and seedling growth experiments.

5.2.3 Preparation of smoke solutions

The apparatus in producing smoke solutions included a wooden board with an electric ring heater firmly fixed on a 75L metal garbage can, a pot for containing plant material, and two weights (Figure 5.1). Enough room was left for the power cord of the ring heater to exit from under the board. Two opposite holes attached with pressure gauges on the metal garbage can, connecting an air supplying hose on one side and a silicon tube on the other side. Each smoke stock solution was produced by smouldering 1.5 kg wheat straw or prairie hay or alfafa. Each of the 1.5 kg samples was divided into 7-8 subsamples, which were put into container and being smouldered one by one. The 75L metal garbage can was inverted placed on the wooden board, enclosing the ring heater and the metal container containing plant materials. Two weights were placed on the top of the garbage can to eliminate the leakage of the produced smoke. Air was forced into the combustion chamber at a pressure of 70-100 kPa and the smoke produced was continuously passed through the silicon tube and bubbled into 4 L of distilled water in a flask. Four replicates of stock solutions (1/1 v/v dilution) for wheat straw, prairie hay, and alfalfa were produced for each run of the experiments, respectively.

Figure 5.1 Apparatus used for producing stock smoke solution. Wheat straw, prairie hay, or alfafa (1.5 kg) was smouldered in a 10 L metal container placed on an electric heater fitted to a wooded board. A 75 L metal garbage can is inversely placed on the wooded board, enclosing the ring heater and the 10L metal container containing plant materials. Air at 70-100 kPa was forced into the combustion chamber. Smoke produced was continuously passed through the silicon pipe and bubbled into 4 L of distilled water.

5.2.4 Seed germination tests

Stock smoke solutions (1/1 v/v) produced by smouldering wheat straw, prairie hay, or alfalfa as mentioned above were made into 3 serial dilutions, including 1/1000 v/v, 1/100 v/v and 1/10 v/v. Seeds primed in distilled water (0/1 v/v) and non-primed dry (0/0 v/v) seeds were also included in the experiment. For each species, 50 seeds were counted and placed in a 50 mL centrifuge tube which was vertically held in perforated paper boxes. After adding 10 mL of distilled water, 1/1000 v/v, 1/100 v/v, 1/10 v/v or 1/1 v/v aqueous smoke dilutions, together with non-primed dry seeds, each centrifuge tube was sealed with a cap and kept in darkness for 24 h at 20 °C. Seeds were then transferred to 10 cm Petri dishes lined with two layers of Whatman #1 filter paper and dried for 1 week at 20 °C in darkness. Seeds in each petri dish were then moistened with 5 mL of distilled water and incubated at 10/0 °C or 25/15 °C regimes under 12 h light/12 h darkness or 24 h darkness. Petri dishes were placed in transparent zip-lock bags for those incubated in 12 h light/12 h darkness. Zip-lock bags wrapped with 2 layers of aluminum foil were used to accommodate petri dishes for seeds incubated in 24h darkness.

Germination of *Artemisia frigida*, *Artemisia ludoviciana*, *Cirsium arvense*, and *Conyza canadensis* was counted every week for 7 weeks. Seeds of *Artemisia frigida*, *Artemisia ludoviciana*, and *Conyza canadensis* with a radicle ≥1 mm were considered germinated. Seeds of

Cirsium arvense with a radical ≥2 mm were considered germinated. Distilled water was added to keep the filter paper moist. Germination of seeds in the 24h darkness was checked under a green safe light (Drewes *et al.*, 1995). Germinated seeds were counted, transferred to a new petri dish and seedlings allowed to grow for 7 d under 12 h:12 h light/darkness at the same temperature for seed germination. Lengths of radicle and hypocotyl were measured after 7 d. Time to 50% germination was used as an indicator of germination rate.

A factorial experiment with a randomized-complete-block-design (RCBD) was conducted for each species with six priming treatments (1/1000v/v, 1/100v/v, 1/10v/v, 1/1v/v, distilled water, and dry), three smoke types (wheat straw, prairie hay and alfalfa), and four replicates within each of four germination conditions, which included 12 h light/12 h darkness at 10/0 °C, 24 h darkness at 10/0 °C, 12 h light/12 h darkness at 25/15 °C, and 24 h darkness at 25/15 °C. The experiment was repeated with four new replicates of stock solutions as the second run.

5.2.5 Procedure used for testing viability of seeds

Due to the small seed size of *Artemisia frigida*, *Artemisia ludoviciana*, and *Conyza canadensis*, seed viability at the end of germination tests was determined by pinching the seeds to determine whether they had decayed. Seeds were regarded as viable if they were still firm. Viability of ungerminated seeds of *Cirsium arvense* at the end of germination tests was determined using Tetrazolium Chloride (TZ) tests. Seeds were submerged in distilled water for 5 h then cut laterally to expose their embryos. TZ solution (0.1%) was used to stain the embryo for 18h at room temperature. Seed viability was determined by observing the coloration of the stained embryo (Hampton *et al.*, 1999). Seeds were regarded as viable if they were stained evenly.

5.2.6 Data analysis

Data on total germination, germination rate, radical length, hypocotyl length, and total seedling length were analyzed as a RCBD with factorial treatments with 2 runs (4 replications for each run) using the mixed model procedure in SAS version 9.3 (SAS Institute Inc., USA). For each species, differences in seed germination and seedling length among treatments were tested within each of the four germination conditions, which were 10/0 °C with 12 h light/12 h darkness, 10/0 °C with 24 h darkness, 25/15 °C with 12 h light/12 h darkness, and 25/15 °C with 24 h darkness. Total germination, germination rate, radical length, hypocotyl length, or total

seedling length were taken as the dependent variable, and in each case, the main effects and possible interactions of priming and smoke type were used as independent variables. Replicates, blocks, and runs were factored into the model as random effects. When the interaction of priming x smoke type was significant ($P \leq 0.05$), data were analyzed within each factor separately. Total germination data were arcsine square root transformed before subjected to Analysis of Variance (ANOVA). The non-normalized data of germination rate, radical length, hypocotyl length, and total seedling length were log-transformed and subjected to ANOVA. Shapiro-Wilk test was used to test normality. Treatment means were separated using Tukey test at $P \leq 0.05$.

5.3 Results

5.3.1 Effects of smoke on seed germination and seedling growth of *Artemisia frigida*

Seed germination of *Artemisia frigida* was affected by the interaction of priming × smoke type in all tested germination conditions (Figure 5.2). In 12 h light/12 h darkness at 10/0 °C, priming seeds in 1/1 v/v dilution made from prairie hay reduced total germination by 52% compared with the non-primed control, the 1/1 v/v dilution made from wheat straw reduced total germination by 96% relative to the non-primed control and distilled water. Compared with the non-primed control and distilled water, priming seeds in 1/1 v/v dilutions made from prairie hay and wheat straw reduced total germination in 24 h darkness at 10/0 °C and 12 h light/12 h darkness at 25/15 °C. In 24 h darkness at 25/15 °C, priming seeds in 1/100 v/v and 1/10 v/v smoke dilutions made from all three plant materials, together with 1/1000 v/v dilution produced from alfalfa, increased total germination compared with the non-primed control. Total germination in 1/1 v/v dilution made from prairie hay and wheat straw was reduced by 67% and 91%, respectively, relative to distilled water. Priming seeds in 1/1 v/v wheat straw also reduced total germination by 82% compared with the non-primed control. The high concentration of smoke solution made from alfalfa had no negative effects on seed germination in all tested conditions compared with distilled water and non-primed control.

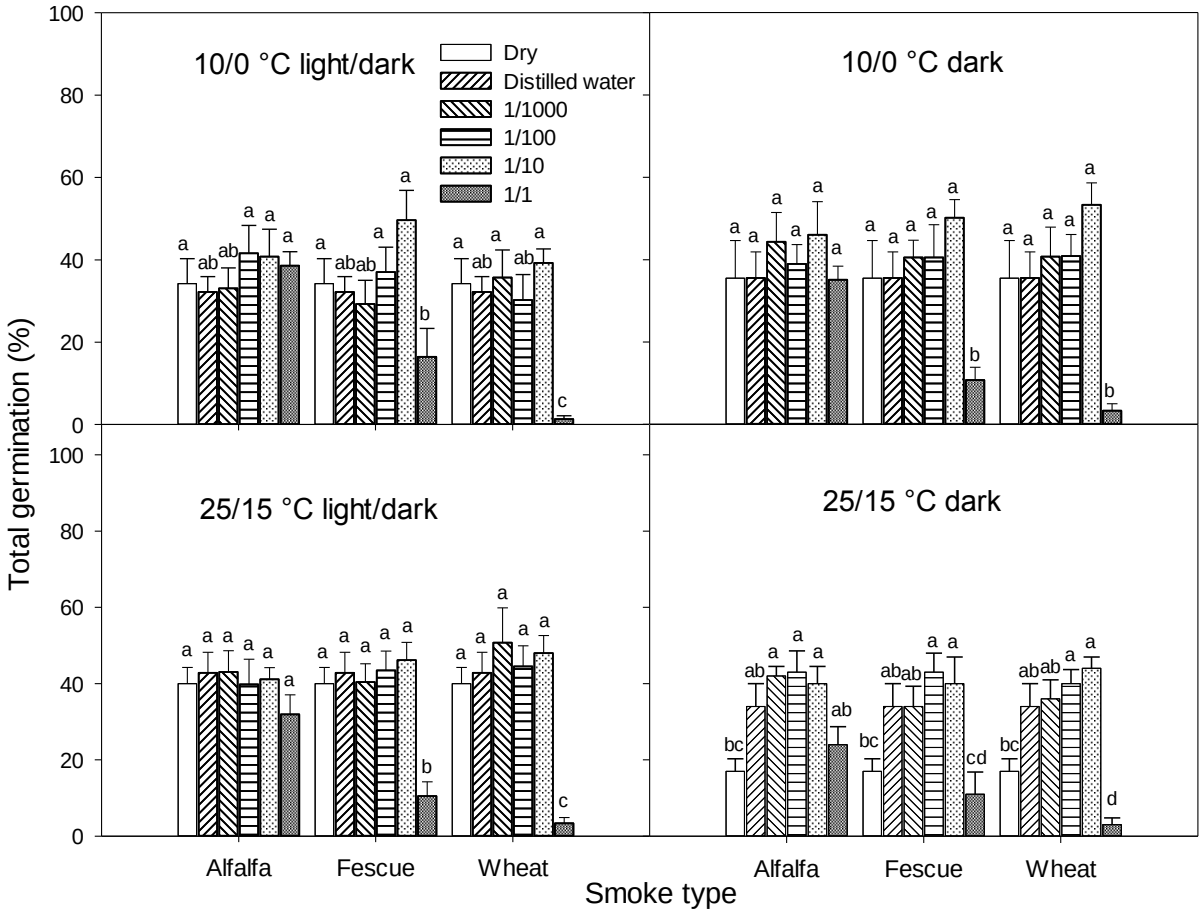

Figure 5.2 Total germination of *Artemisia frigida* seeds after priming in serial dilutions of aqueous smoke solutions made from alfalfa, prairie hay, or wheat straw and incubating at 10/0 ^{o}C or 25/15 ^{o}C in 24 h darkness or in 12 h light/12 h darkness. Means with different letters indicate total germination of primed seeds were significantly different ($P \leq 0.05$) within germination conditions. Bars represent (±) standard error.

Germination rate was affected by priming in 24 h darkness at 10/0 °C and 25/15 °C (Figure 5.3). Under 24 h darkness at 10/0 °C, germination was slower after priming in the 1/1 v/v dilution as compared with the non-primed control and distilled water. In 24 h darkness at 25/15 °C, seeds germinated faster in distilled water relative to the non-primed control and 1/1 v/v dilution. Radical, hypocotyl, and total seedling lengths for *Artemisia frigida* were not affected by priming with different smoke solutions under all four germination conditions (data now shown).

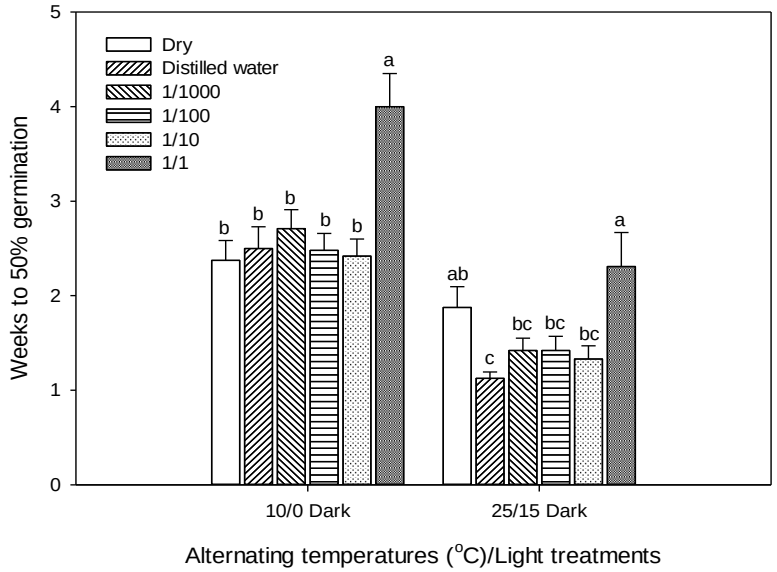

Figure 5.3 Germination rate (weeks to 50% total germination) for *Artemisia frigida* seeds after priming in serial dilutions from aqueous smoke solutions made from alfalfa, prairie hay, or wheat straw and incubating at 10/0 °C or 25/15 °C in 24 h darkness or in 12 h light/12 h darkness. Means with different letters indicate weeks to 50% total germination of primed seeds were significantly different ($P \leq 0.05$) within germination conditions. Bars represent (±) standard error.

5.3.2 Effects of smoke on seed germination and seedling growth of *Artemisia ludoviciana*

Total germination of *Artemisa ludoviciana* was affected by priming in 12 h light/12 h darkness, 24 h darkness at 10/0 °C, and 24 h darkness at 25/15 °C (Figure 5.4). In 12 h light/12 h darkness at 10/0 °C, priming seeds in 1/100 v/v, 1/10, and 1/1 v/v dilutions increased seed germination by 53%, 60%, and 89% compared with distilled water, respectively. Total germination was increased after priming in 1/10 v/v and 1/1 v/v dilutions in 24 h darkness at 10/0 °C and 25/15 °C as compared with distilled water and the non-primed control. Priming seeds in 1/1 v/v dilution increased total germination by 110% and 93% as compared with the non-primed control and distilled water, respectively, in 24 h darkness at 25/15 °C. Seed germination rate for *Artemisia ludoviciana* was not affected by priming with different smoke solutions under all four germination conditions (data not shown).

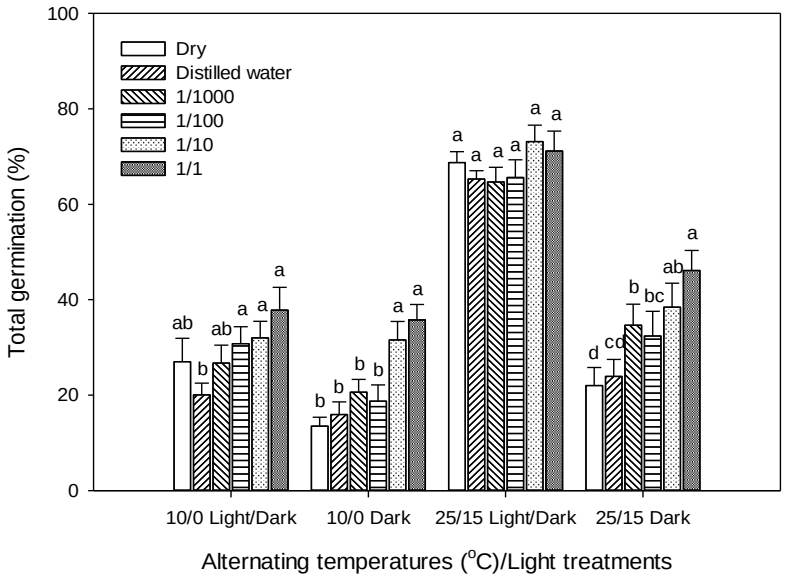

Figure 5.4 Total germination of *Artemisia ludoviciana* seeds after priming in serial dilutions of aqueous smoke solutions made from alfalfa, prairie hay, or wheat straw and incubating at 10/0 ^{o}C or 25/15 ^{o}C in 24 h darkness or in 12 h light/12 h darkness. Means with different letters indicate total germination of primed seeds were significantly different ($P \leq 0.05$) within germination conditions. Bars represent (±) standard error.

Radical and hypocotyl lengths of *Artemisia ludoviciana* were affected by priming in 24 h darkness at 25/15 °C (Figure 5.5). Priming seeds in 1/1000 v/v dilution increased radical lengths by 45% and 40% as compared with the non-primed control and distilled water, respectively. Hypocotyl lengths were 26% shorter after priming seeds in the 1/1 v/v smoke solution compared with distilled water. Total seedling lengths were not affected by priming with different smoke solutions under four germination conditions.

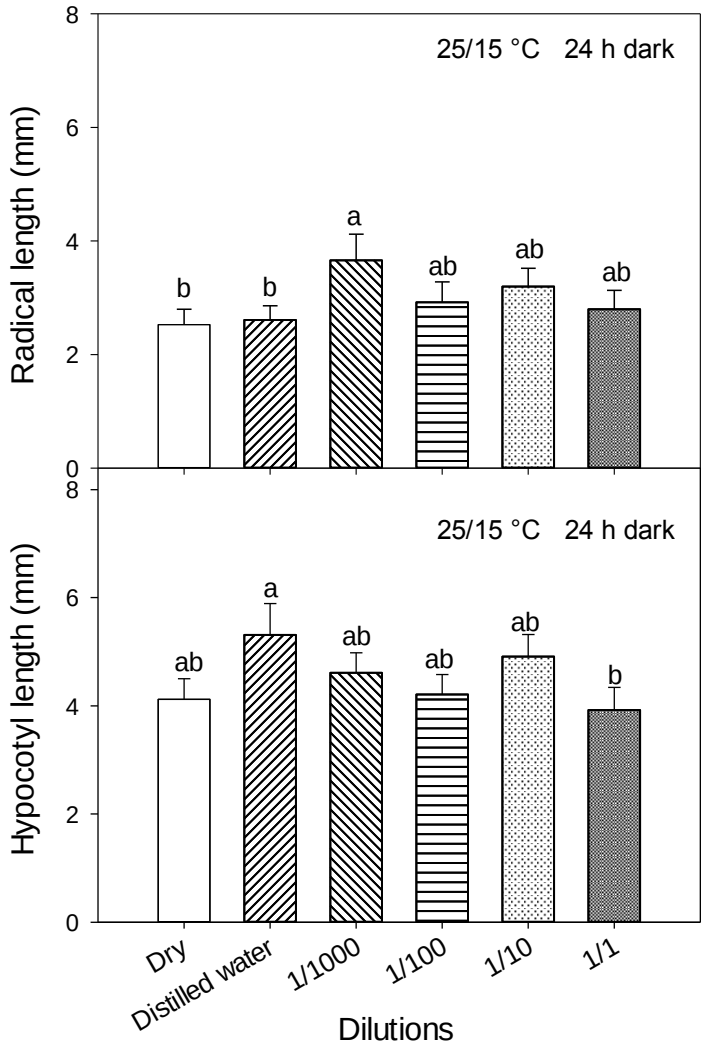

Figure 5.5 Radical and hypocotyl lengths for *Artemisia ludoviciana* seeds after priming in serial dilutions from aqueous smoke solutions made from alfalfa, prairie hay, or wheat straw and incubating at 10/0 ^{o}C or 25/15 ^{o}C in 24 h darkness or in 12 h light/12 h darkness. Means with different letters indicate radical or hypocotyl lengths of primed seeds were significantly different ($P \leq 0.05$) within germination conditions. Bars represent (±) standard error.

5.3.3 Effects of smoke on seed germination and seedling growth of *Cirsium arvense*

Total germination of *Cirsium arvense* was affected by priming in 12 h light/12 h darkness and 24 h darkness at 25/15 °C (Figure 5.6). Germination percentage was less than 5% at 10/0 °C regime. Priming seeds in 1/1 v/v dilution reduced seed germination by 60% and 55%, respectively, as compared with non-primed control and distilled water in 12 h light/12 h darkness at 25/15 °C. Under 24 h darkness at 25/15 °C, total germination increased by 47% after priming in 1/100 v/v dilution relative to the non-primed control. Priming seeds in 1/1 v/v smoke dilution reduced total germination by 57% and 66% as compared with non-primed control and distilled water, respectively.

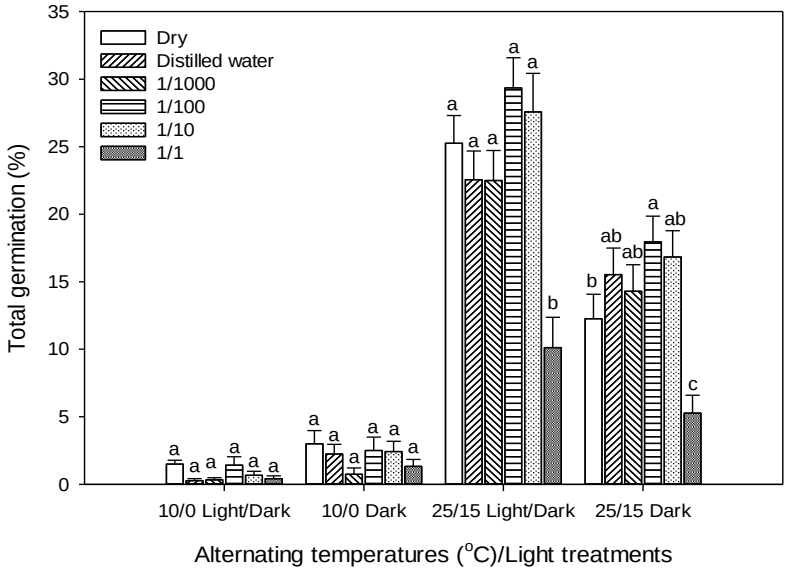

Figure 5.6 Total germination of *Cirsium arvense* seeds after priming in serial dilutions of aqueous smoke solutions made from alfalfa, prairie hay, or wheat straw and incubating at 10/0 °C or 25/15 °C in 24 h darkness or in 12 h light/12 h darkness. Means with different letters indicate total germination of primed seeds were significantly different (P≤0.05) within germination conditions. Bars represent (±) standard error.

Germination rate varied with priming and smoke type in 12 h light/12 h darkness and 24 h darkness at 25/15 °C (Figure 5.7). Seeds germinated faster after priming in smoke solutions made from alfalfa compared with wheat straw in 12 h light/12 h darkness at 25/15 °C and prairie hay in 24 h darkness at 25/15 °C. Germination was more rapid after priming seeds in distilled water as compared with priming seeds in 1/1 v/v dilution and the non-primed control in 24 h darkness at 25/15 °C.

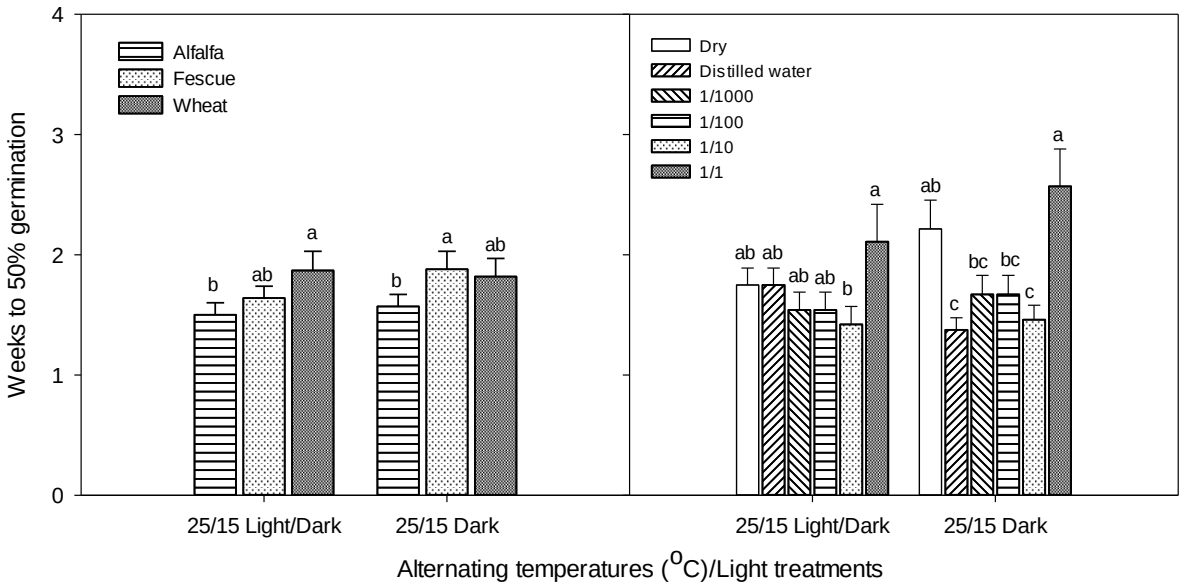

Figure 5.7 Germination rate (weeks to 50% total germination) for *Cirsium arvense* seeds after priming in serial dilutions from aqueous smoke solutions made from alfalfa, prairie hay, or wheat straw and incubating at 10/0 °C or 25/15 °C in 24 h darkness or in 12 h light/12 h darkness. Means with different letters indicate weeks to 50% total germination were significantly different ($P \leq 0.05$) within germination conditions. Bars represent (±) standard error.

Radical, hypocotyl, and total seedling lengths responded to priming in 12 h light/12 h darkness and 24 h darkness at 25/15 °C (Figure 5.8). Under 12 h light/12 h darkness at 25/15 °C, priming seeds in distilled water increased radical lengths by 45% and 61% as compared with the non-prime control and priming in 1/1 v/v dilution, respectively. Hypocotyl lengths were longer after priming in distilled water compared with priming in different aqueous smoke dilutions and the non-primed control. Total seedling lengths were longer after priming in distilled water as compared with priming in 1/1000 v/v, 1/10 v/v, and 1/1 v/v dilutions and the non-primed control. Under 24 h darkness at 25/15 °C, radical lengths were longer after priming in 1/1000 v/v, 1/100 v/v, 1/10 v/v, and distilled water relative to the non-primed control. Priming seeds in 1/1 v/v reduced radical lengths by 24% and 54% compared with the non-primed control and distilled water, respectively. Hypocotyl lengths were 52% longer after priming in 1/1000 v/v dilution relative to the non-primed control. Hypocotyl lengths were 43% shorter after priming in 1/1 v/v dilution as compared with distilled water. Total seedling lengths were longer after priming in 1/1000 v/v and 1/100 v/v dilutions compared with the non-primed control, but were 48% shorter after priming in 1/1 v/v dilution compared with distilled water.

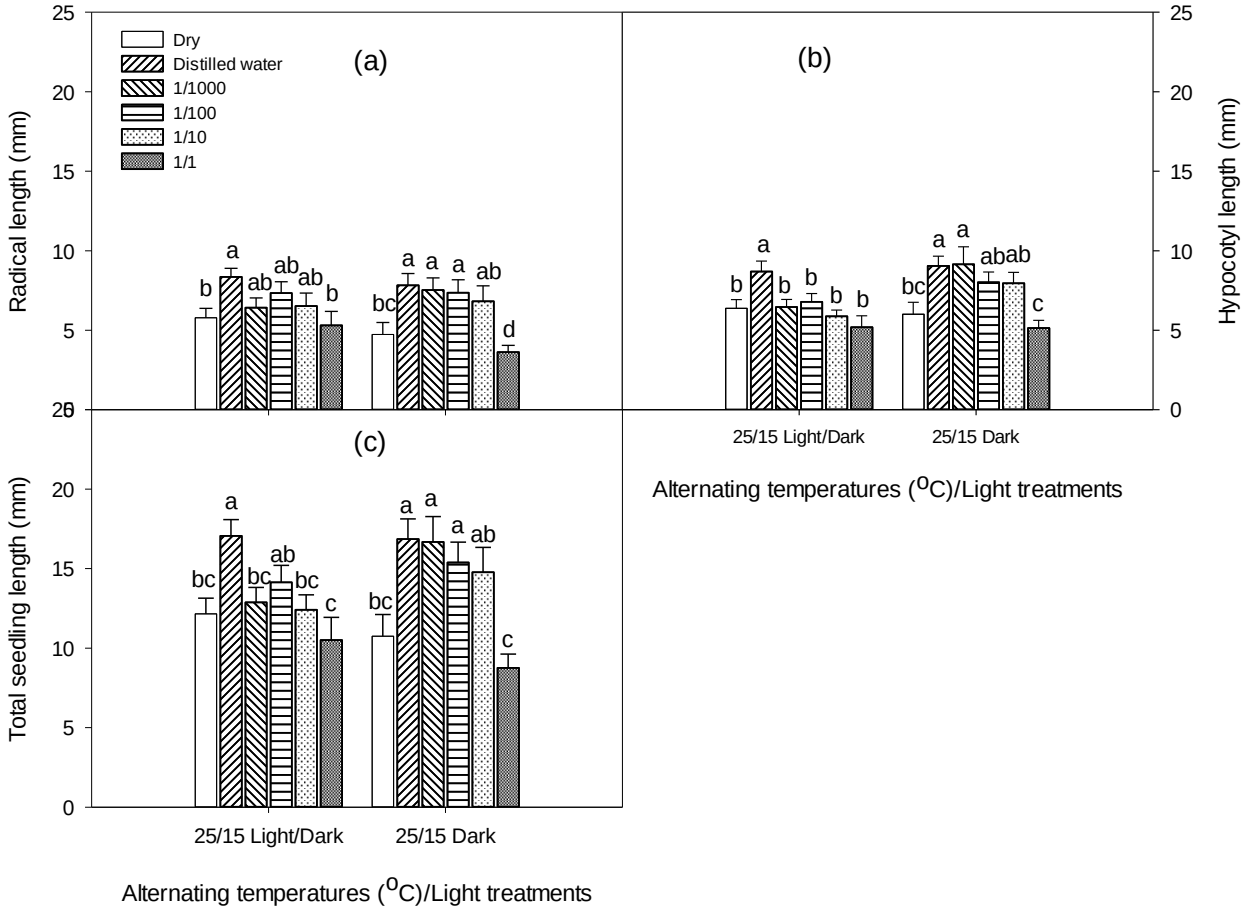

Figure 5.8 Radical lengths (a), hypocotyl lengths (b), and total seedling lengths (c) for *Cirsium Arvense* seeds after priming in serial dilutions from aqueous smoke solutions made from alfalfa, prairie hay, or wheat straw and incubating at 25/15 ^{o}C in 24 h darkness or in 12 h light/12 h darkness. Means with different letters indicate radical lengths, hypocotyl lengths, or total seedling lengths of primed seeds were significantly different ($P \leq 0.05$) within germination conditions. Bars represent (±) standard error.

5.3.4 Effects of smoke on seed germination and seedling growth of *Conyza canadensis*

Total germination of *Conyza canadensis* was affected by the interaction of priming × smoke type under all four germination conditions (Figure 5.9). Priming seeds in 1/10 v/v dilution made from prairie hay increased total germination by 192% compared with non-primed control in 24 h darkness at 10/0 °C. Under 12 h light/12 h darkness at 25/15 °C, priming seeds in 1/1000 v/v and 1/100 v/v dilutions of all three smoke types significantly reduced total germination as compared with the non-primed control. Total germination was reduced after priming in 1/1 v/v dilution made from prairie hay and wheat straw relative to the non-primed control and distilled water. Priming seeds in 1/10 dilutions of all three smoke solutions and 1/1 v/v dilution produced from alfalfa increased total germination compared with priming in distilled water. In 24 h darkness at 25/15 °C, total germination was increased by 90% and 104% after priming in 1/1 v/v dilution made from alfalfa compared with the non-primed control and distilled water, respectively. Priming seeds in 1/1 v/v dilutions made from prairie hay and wheat straw reduced total germination relative to the non-primed control and distilled water.

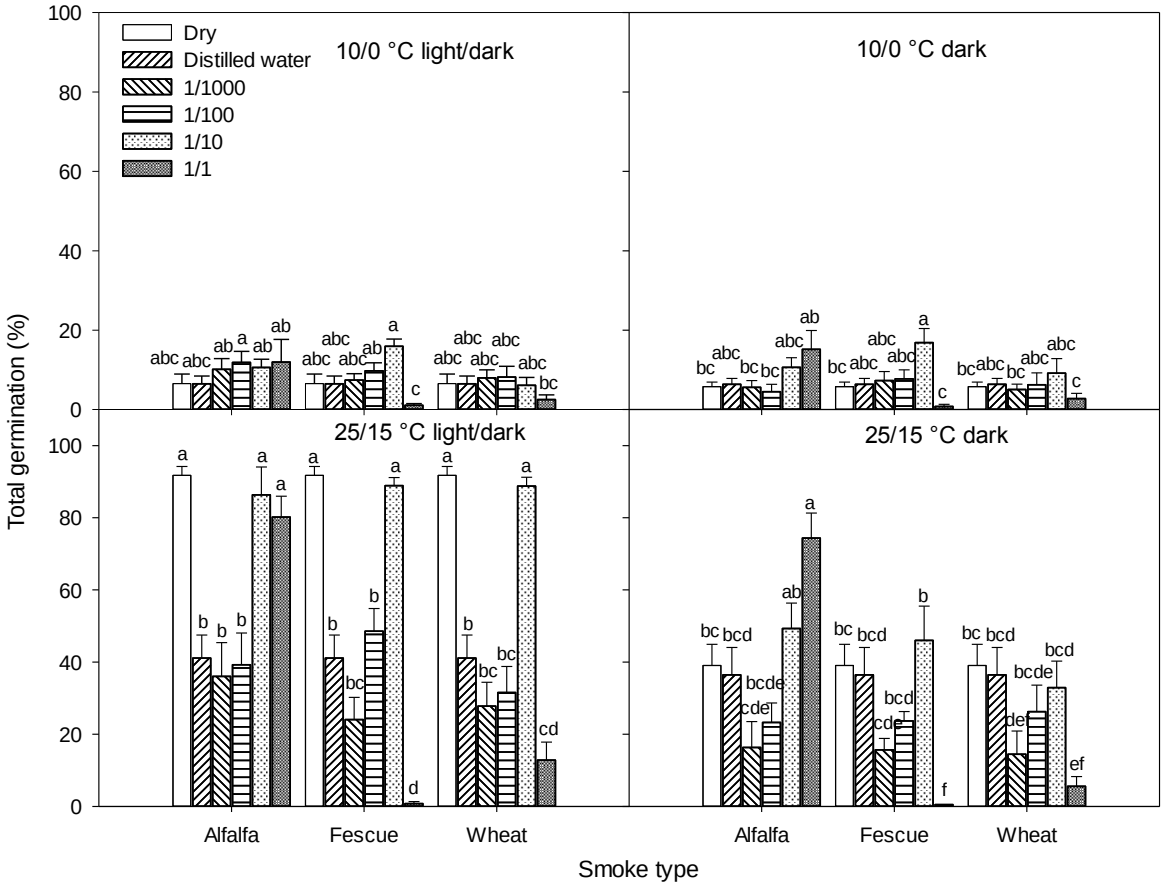

Figure 5.9 Total germination of *Conyza canadensis* seeds after priming in serial dilutions of aqueous smoke solutions made from alfalfa, prairie hay, or wheat straw and incubating at 10/0 ^{o}C or 25/15 ^{o}C in 24 h darkness or in 12 h light/12 h darkness. Means with different letters indicate total germination of primed seeds were significantly different ($P \leq 0.05$) within germination conditions. Bars represent (±) standard error.

Germination rate was affected by priming in 12 h light/12 h darkness and 24 h darkness at 10/0 °C (Figure 5.10). Seeds germinated faster after priming in distilled water compared with the non-primed control in both conditions. Compared with the non-primed control, seeds germinated faster after priming in 1/1000 v/v and 1/100 v/v dilutions, but slower after priming in 1/1 v/v dilution in 12 h light/12 h darkness at 10/0 °C. Germination was slower after priming in 1/10 v/v and 1/1 v/v dilutions relative to distilled water in 12 h light/12 h darkness and 24 h darkness at 10/0 °C.

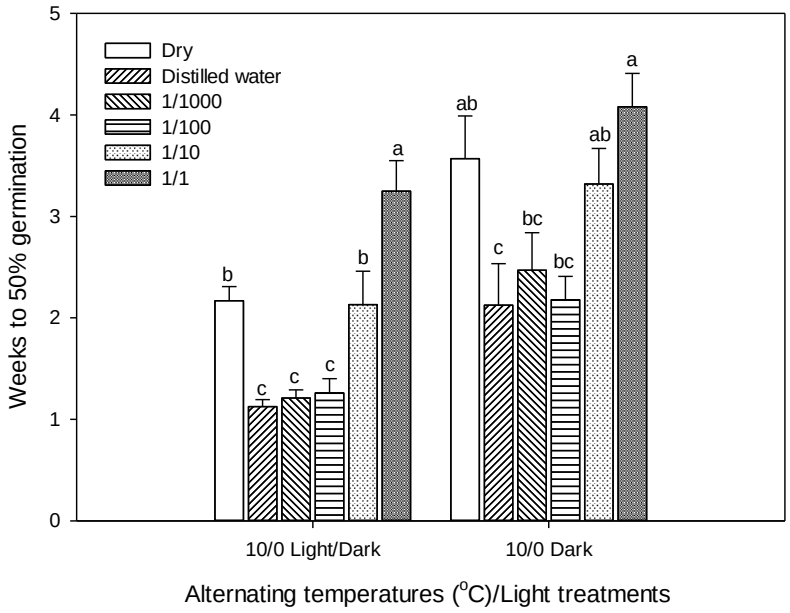

Figure 5.10 Germination rate (weeks to 50% total germination) for *Conyza canadensis* seeds after priming in serial dilutions from aqueous smoke solutions made from alfalfa, prairie hay, or wheat straw and incubating at 10/0 ^{o}C or 25/15 ^{o}C in 24 h darkness or in 12 h light/12 h darkness. Means with different letters indicate days to 50% total germination of primed seeds were significantly different ($P \leq 0.05$) within germination conditions. Bars represent (±) standard error.

Radical lengths were affected by the interaction of priming × smoke type in 12 h light/12 h darkness and 24 h darkness at 25/15 °C (Figure 5.11). Under 12 h light/12 h darkness at 25/15 °C, except for the 1/10 v/v dilution made from wheat straw, priming seeds in different aqueous smoke dilutions made from prairie hay and wheat straw and 1/1000 v/v dilution made from alfalfa reduced radical lengths compared with non-primed control. Radical lengths were shorter after priming in 1/1 v/v dilution made from wheat straw relative to distilled water. In 24 h darkness at 25/15 °C, radical lengths were longer after priming in 1/1 v/v dilution produced from alfalfa compared with the non-primed control and distilled water. Seeds primed in 1/1 v/v dilution made from prairie hay produced shorter radicals as compared with non-primed control.

Hypocotyl lengths were affected by the interaction of priming × smoke type in 24 h darkness at 25/15 °C (Figure 5.12). Priming seeds in 1/100 v/v dilution made from alfalfa, 1/1000 and 1/1 v/v dilutions made from prairie hay and all four smoke dilutions made from wheat straw reduced hypocotyl lengths compared with the non-primed control. Hypocotyl lengths were 69% shorter after priming in 1/1 v/v smoke solution produced from prairie hay compared with distilled water.

Total seedling lengths were affected by the interaction of priming × smoke type in 12 h light/12 h darkness and 24 h darkness at 25/15 °C (Figure 5.13). Under 12 h light/12 h darkness at 25/15 °C, priming with 1/1000 v/v smoke solution produced from alfalfa, 1/1000 and 1/1 v/v smoke solution produced from wheat straw and all four smoke solutions produced from prairie hay significantly reduced total seedling lengths relative to the non-primed control. Seedlings were 41% and 45% shorter after priming in 1/1 v/v smoke solution made from prairie hay and wheat straw, respectively, as compared with distilled water. In 24 h darkness at 25/15 °C, seedlings were shorter after priming in 1/1000 and 1/1 v/v smoke dilutions produced from wheat straw compared with the non-primed control. As compared with distilled water, only 1/1 v/v smoke solution produced from prairie hay significantly reduced total seedling lengths by 72%.

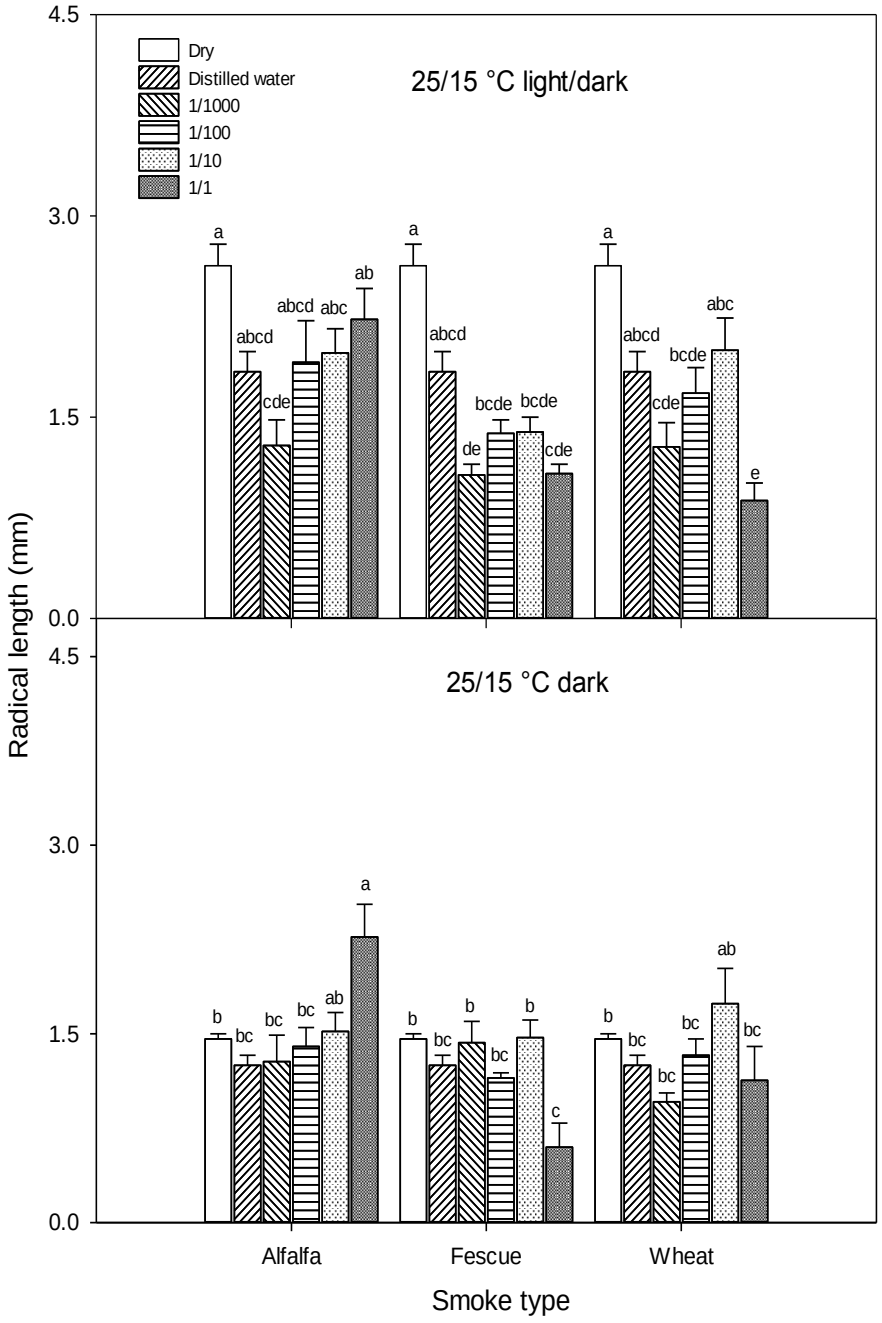

Figure 5.11 Radical lengths for *Conyza canadensis* seeds after priming in serial dilutions from aqueous smoke solutions made from alfalfa, prairie hay, or wheat straw and incubating at 10/0 ^{o}C or 25/15 ^{o}C in 24 h darkness or in 12 h light/12 h darkness. Means with different letters indicate radical lengths of primed seeds were significantly different ($P \leq 0.05$) within germination conditions. Bars represent (±) standard error.

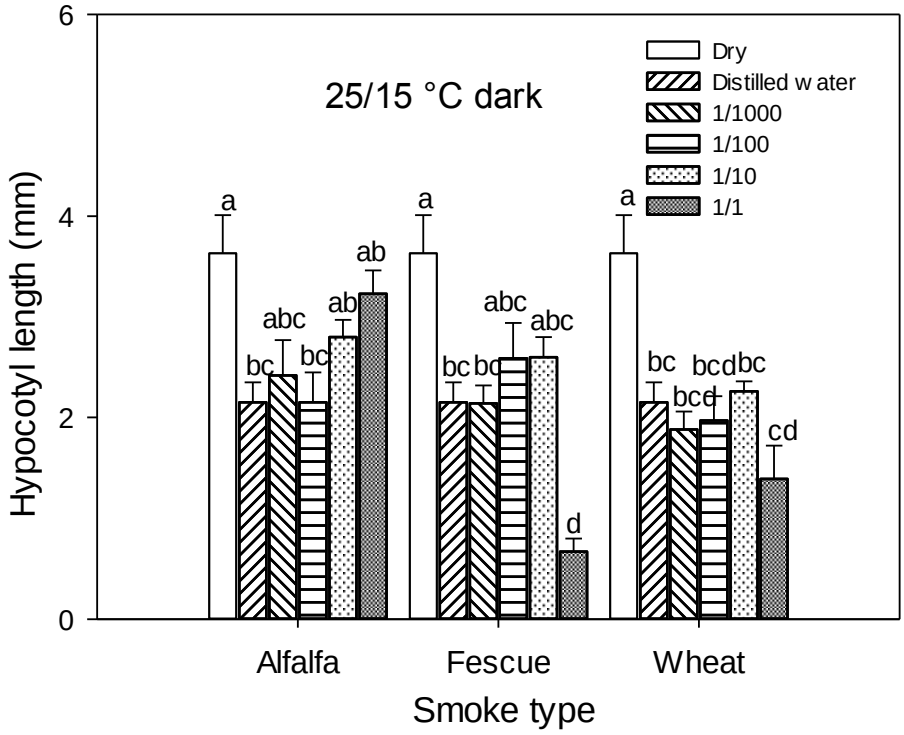

Figure 5.12 Hypocotyl lengths for *Conyza canadensis* seeds after priming in serial dilutions from aqueous smoke solutions made from alfalfa, prairie hay, or wheat straw and incubating at 10/0 ^{o}C or 25/15 ^{o}C in 24 h darkness or in 12 h light/12 h darkness. Means with different letters indicate hypocotyl lengths of primed seeds different among the interaction of fuel type and priming dilutions ($P \leq 0.05$) within germination conditions. Bars represent (±) standard error.

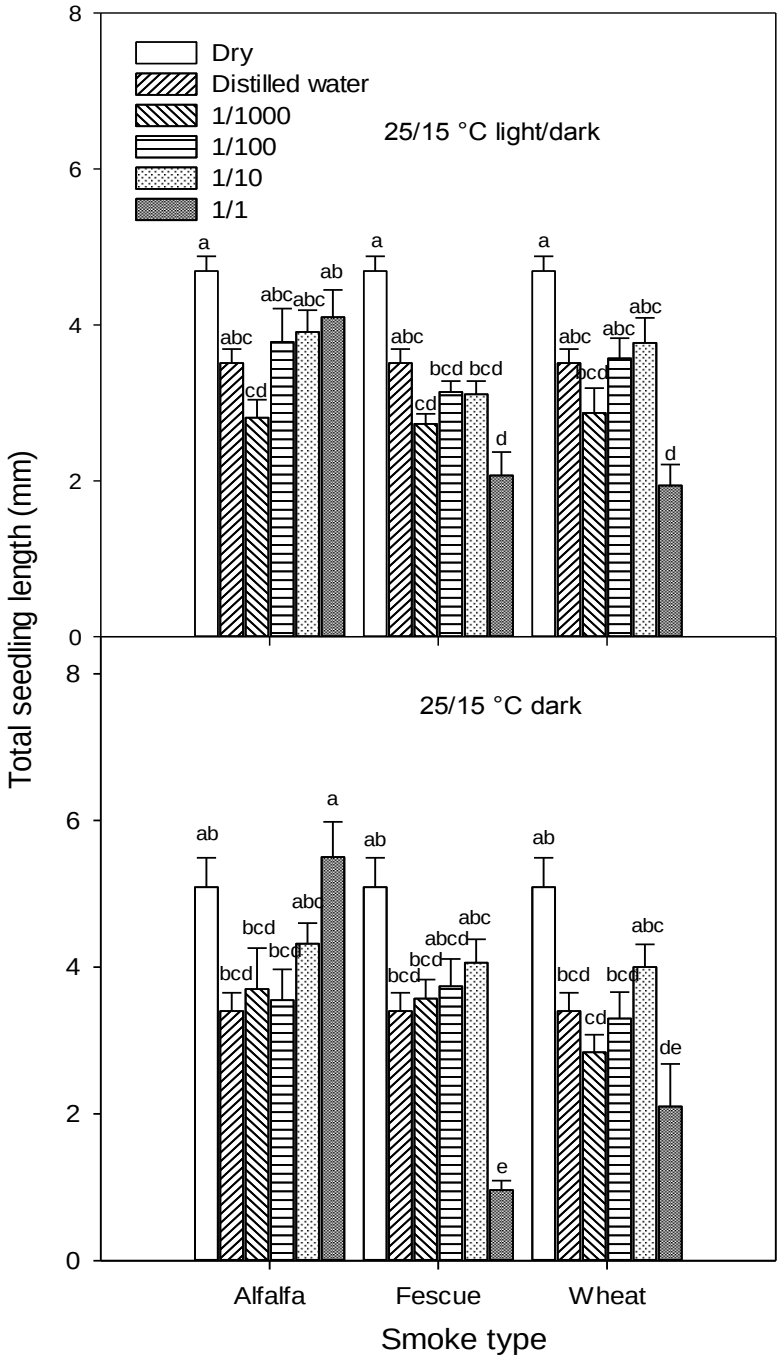

Figure 5.13 Total seedling lengths for *Conyza canadensis* seeds after priming in serial dilutions from aqueous smoke solutions made from alfalfa, prairie hay, or wheat straw and incubating at 10/0 ^{o}C or 25/15 ^{o}C in 24 h darkness or in 12 h light/12 h darkness. Means with different letters indicate seedling lengths of primed seeds were significantly different ($P \leq 0.05$) within germination conditions. Bars represent (±) standard error.

5.4 Discussion

5.4.1 Effects of aqueous smoke solutions on seed germination and seedling growth

Regardless of smoke type, germination of *Artemisia ludoviciana* and *Cirsium arvense* was significantly affected after priming in aqueous smoke solutions. Total germination of *Cirsium arvense* was not affected for seeds primed in low to moderate concentrated smoke solutions, but was reduced in high concentrated smoke dilutions. Lack of the responses of germination of *Cirsium arvense* to low and moderate concentrated smoke solutions may be due to its relatively hard seed coat, which impedes the entrance of active compounds in smoke solutions (Jain *et al.*, 2008). Adkins and Peters (2001) stated that smoke solutions were more effective in stimulating germination of seeds with weak seed coats compared with thicker seed coats. Reduced effects of highly concentrated smoke solutions on seed germination were observed in many species, including *Lactuca sativa* and *Acacia robusta* (Drewes *et al.*, 1995; Kulkarni *et al.*, 2007). This negative effect could be possibly due to the presence of the inhibiting or toxic compounds involved in high concentrated smoke solutions (Dixon and Roche, 1995), which may alter seed permeability, water uptake capability and mobilization of energy reserves (Van Staden *et al.*, 1995). In addition, the low pH (normally around 3.0) of high concentrated smoke solutions (Brown and van Staden, 2000; Flematti *et al.*, 2008) may also reduce germination of *Cirsium arvense*, whose germination was greatly reduced at a pH below 5.8 (Wilson, 1979). Total germination of *Artemisia ludoviciana* was not affected for seeds primed in low concentrated smoke solutions, but was increased in high concentrated smoke dilutions. Different species have varying sensitivities to aqueous smoke concentrations (Adkins and Peters, 2001). Although high concentrated smoke solutions exerted inhibiting effects on seed germination of many species (Drewes *et al.*, 1995; Kulkarni *et al.*, 2007; Lara Vanessa *et al.*, 2014), it stimulates seed germination of *Artemisia ludoviciana* (Lara Vanessa *et al.*, 2014), indicating *Artemisia ludoviciana* is insensitive or tolerant to toxic compounds in highly concentrated smoke solutions.

Low to moderate concentrated smoke solutions had no effects on germination rate of the species studied. Lack of the responses of germination rate to smoke cue were also observed in 19 Mediterranean woody and herbaceous species (Reyes and Trabaud, 2009; Catav *et al.*, 2012). Seeds of *Artemisia frigida*, *Cirsium arvense*, and *Conyza canadensis* primed in highly concentrated aqueous smoke solutions, however, germinated slower. The toxic compounds in

high concentrated smoke solutions may slow germination by either inhibiting physiological processes or deteriorating the seeds.

Seedling growth of different species responds differently to smoke solutions (Blank and Young, 1998; Moreira *et al.*, 2010). In the present study, priming seeds in high concentrated smoke solutions reduced radical lengths of *Cirsium arvense*, hypocotyl lengths of *Artemisia ludoviciana* and *Cirsium arvense*, and total seedling lengths of *Cirsium arvense* in certain germination conditions, supporting the inhibiting effects of high concentrated aqueous smoke solutions potentially due to the presence of inhibiting compounds (Light *et al.*, 2002). Priming in diluted smoke solutions increased radical lengths of *Artemisia ludoviciana* and radical, hypocotyl, and total seedling length of *Cirsium arvense*. The stimulant effects of diluted smoke solutions on seedling length were supported by previous studies (Sparg *et al.*, 2005, Kulkarni *et al.*, 2006). The physiological mechanisms of smoke resulting in increased seedling lengths are unknown, but may be due to the fact that smoke can alter the endogenous levels of hormones in seeds (van Staden *et al.*, 2000; Kulkarni *et al.*, 2006). Radical lengths, hypocotyl lengths and total seedling lengths of *Artemisia ludoviciana* and *Cirsium arvense* had varied responses to the same concentrated smoke solution, which agrees with Ghebrehiwot *et al.* (2009), where the same concentrated smoke solution reduced radical lengths but enhanced hypocotyl lengths of *Eragrostis curvula*. This may be due to the fact that radical and hypocotyl growth have different sensitivity to plant hormones, which can be altered by smoke. Aspinnall *et al.* (1967) reported that radicals of lettuce (*Lactuca sativa* cv. Great Lakes) and cucumber (*Cucumis sativus* cv. Long Green) seeds were more sensitive to gibberellic acid (GA) compared with hypocotyls. Exposing seeds to smoke alters endogenous GA in *Lactuca sativa* and *Nicotiana attenuate* (Gardner *et al.*, 2001; Schwachtje and Baldwin, 2004).

5.4.2 Interactive effect of priming and smoke type on seed germination and seedling growth

Low to moderate concentrated smoke solutions made from alfalfa, prairie hay, and wheat straw did not affect total seed germination of *Artemisia frigida* and *Conyza canadensis*. Priming seeds in 1/1 v/v smoke solutions produced from prairie hay and wheat straw significantly reduced total germination of *Artemisia frigida* and *Conyza canadensis* in certain germination conditions. However, priming seeds in the 1/1 v/v smoke solution produced from alfalfa had less negative effects on total germination of *Artemisia frigida*, and neutral or positive effects on germination of *Conyza canadensis* in the same tested conditions, indicating different compounds,

toxic components specifically, maybe involved in smoke solutions produced from alfalfa as compared with those made from prairie hay and wheat straw. This is contrary to most of the former studies which indicated smoke originating from different plant materials did not vary in their effect on germination (Baxter *et al.*, 1995; Jager *et al.*, 1996; Catav *et al.*, 2012). The possible explanation could be that those plant-derived smokes were coming from a mixture of plant species (Dixon *et al.*, 1995; Perez-Fernandez and Rodriguez-Echevarria, 2003; Thomas *et al.*, 2010) or from a single species abundant in the study region (Reyes and Trabaud, 2009; Moreira *et al.*, 2010) to highlight the importance of fire in affecting germination of species *in situ*. However, based on our knowledge, until the current study, smoke solutions made from herbaceous Leguminosae have never been tested before. Although the proposed role of NO_x as a cue in breaking seed dormancy in smoke is not clear, Keeley and Fotheringham (1997) reported that NO_2 or NO were possibly responsible for increased seed germination of *Emmenanthe penduliflora Benth.*. Species in the family of Leguminosae may have different quality and quantity nitrogen oxides compared with plants in other families due to the unique metabolic approaches in fixing N_2. This may explain why effects of smoke made from alfalfa were different from that of smoke made from prairie hay and wheat straw. Radical, hypocotyl, and total seedling lengths of *Coynza canadensis* were affected by the interaction of smoke type and dilutions in certain germination conditions. Compared with undiluted smoke solutions produced from prairie hay and wheat straw, the one produced from alfalfa had more positive effects on seedling growth, which further suggest different compounds involved in alfalfa smoke solution as compared with the smoke solution made from prairie hay and wheat straw.

5.4.3 Effects of smoke solutions on seed germination and seedling growth are temperature and light dependent

Light is a critical factor for germination of *Artemisia ludoviciana* and *Conyza canadensis* (Nandula *et al.*, 2006). In the present study, concentrated smoke solutions increased seed germination of *Artemisia ludoviciana* by 133% in darkness. Concentrated smoke solutions made from alfalfa increased germination of *Conyza canadensis* by 100% in darkness. This observation agrees with former studies indicating smoke solutions stimulate germination of positively photoblastic seeds, such as *Lactuca sativa* and *Apium graveolens*, in darkness (Drewes *et al.*, 1995; Thomas and Van Staden, 1995). Active components in smoke solutions may substitute for light in breaking dormancy and affect the conversion of P_r to P_{fr} in the phytochrome response

(Drewes *et al.*, 1995). In addition, metabolism and perception of GAs and abscicic acid (ABA) to seeds can be altered by smoke solutions (Nelson *et al.*, 2009), which in turn affects seed germination (Grappin *et al.*, 2000). Exposure to smoke solutions increased endogenous GA levels and decreased endogenous ABA levels for two positively photoblastic species, *Lactuca sativa* and *Nicotiana attenuate* (Gardner *et al.*, 2001; Schwachtje and Baldwin 2004). Increased germination in darkness indicates germination of buried seeds of *Artemisia ludoviciana* and *Conyza canadensis* can be favored by smoke, at least partly explaining the observed increase in coverage of these two species after burning in Fescue Prairie and tallgrass prairie (Bailey and Anderson, 1978; Anderson and Bailey, 1980; Collins, 1987).

Effects of aqueous smoke solutions on seed germination are also temperature dependent (Brown *et al.*, 1994; Ghebrehiwot *et al.*, 2009). Germination of *Cirsium arvense* and *Coynza canadensis* responded to various smoke solutions at 25/15°C, but not at 10/0°C. Optimum temperatures for germination of *Cirsium arvense* and *Coynza canadensis* are 30°C constant and 24/20°C (12 h-12 h alternation), respectively (Wilson, 1979; Nandula *et al.*, 2006). Neither of these two species germinates under 10°C (Wilson, 1979; Nandula *et al.*, 2006). Ghebrehiwot *et al.* (2009) showed that the stimulative effects of smoke solution on seed germination of *Aristida junciformis, Hyparrhenia hirta*, and *Panicum maximum* were more notable with increasing temperatures. Total germination of *Artemisia ludoviciana* was increased after priming in highly concentrated smoke solutions in 12 h light/12 h darkness at 10/0 °C. This may be due to the fact that smoke could widen the temperature range for germination and seedling growth (Jain *et al.*, 2006). Increased germination could be attributed to the altered dormancy by smoke. Seeds with less dormancy can germinate over a broader range of temperatures than those with deeper dormancy (Batlla *et al.*, 2003). The positive effects of smoke on germination of *Artemisia ludoviciana* at low temperatures indicate establishment of this species can be favored by early spring burning when ambient temperatures are still low.

The inhibitory effects of concentrated smoke solution on seed germination rates are temperature and light dependent. Seeds of *Artemisia frigida* and *Cirsium arvense* germinated slower after priming in concentrated smoke solution in darkness but not light. Priming seeds of *Coynza canadensis* in concentrated smoke solutions reduced germination rate at 10/0 °C but not 25/15 °C. Ghebrehiwot *et al.* (2009) reported effects of smoke solutions on seed germination rate can be positive, negative or neutral among different temperature regimes.

5.5 Conclusions

Priming in different concentrated aqueous smoke solutions independently, or interactively with different types of smoke (alfalfa, prairie hay and wheat straw) affected germination and seedling growth. Except for the total germination of *Artemisia ludoviciana*, priming in undiluted smoke solutions made from prairie hay and wheat straw had negative effects on total germination, germination rate, and seedling growth of tested species. Priming seeds in highly concentrated solution produced from alfalfa had less negative or positive effects on total germination of *Artemisia frigida* and *Conyza canadensis*, compared with same concentrated dilutions made from prairie hay and wheat straw, indicating less toxic or different active compounds in the smoke solutions produced from alfalfa as compared with those made from prairie hay and wheat straw. Seed priming in aqueous smoke solutions substituted light requirement for germination of *Artemisia ludoviciana* and *Conyza canadensis*. Germination of *Cirsium arvense* and *Coynza canadensis* responded to various smoke solutions at high temperature regime. Germination rate of *Coynza canadensis* responded to concentrated smoke solutions at low temperature. Low concentrated smoke solution increased radical length of *Artemisia ludoviciana*. These results suggest that possible unidentified active compounds may be involved in the smoke made from alfalfa. In addition, effects of smoke on germination and seedling growth of different species were dependent on environmental conditions.

6.0 ACTIVE COMPOUNDS IN PLANT-DERIVED SMOKE AND THEIR EFFECTS ON SEED GERMINATION AND SEEDLING GROWTH FOR SPECIES FROM THE FESCUE PRAIRIE

Abstract

Smoke solutions produced from different plant materials may have different compounds, which in turn may affect germination and seedling growth of species in Fescue Prairie differently. Salad Bowl lettuce (*Lactuca sativa*) was used as a quick bioassay to trace the active compounds in smoke solutions made from alfalfa (*Medicago sativa*), prairie hay (*Festuca hallii*), and wheat straw (*Triticum aestivum*). Each smoke solution was extracted with ethyl acetate followed by washing with aqueous NaOH. Normal and reverse phase column chromatography and High Performance Liquid Chromatography (HPLC) were used for further fractionation. Karrikinolide (KAR_1) was in the smoke made from prairie hay, and wheat straw, but was not in the smoke made from alfalfa. Seeds of four forbs from Fescue Prairie were primed for 24 h in darkness using serial dilutions (1/1000v/v, 1/100v/v, 1/10v/v and 1/1v/v) of separated fractions from alfalfa, prairie hay, and wheat straw, as well as KAR_1 solutions. After priming, seeds were dried at 20°C in darkness for 7d before distilled water was applied. Seeds were incubated at 10/0°C or 25/15°C in 12h light /12h darkness or 24 h darkness for 49 d. Seeds primed in distilled water was used as control. Priming in KAR_1 solutions and active fractions obtained from prairie hay and wheat straw increased germination of *Artemisia frigida*, *Artemisia ludoviciana*, and *Conyza canadensis*. Results suggest different compounds exist in smoke solutions made from alfalfa as compared with those made from prairie hay and wheat straw. Active compounds in smoke favor germination of certain species depending on environmental conditions, indicating their potential in shaping species composition in Fescue Prairie.

6.1 Introduction

As a natural and widespread selective force, fire regulates plant communities in Fescue Prairie (Bailey and Anderson, 1978; Anderson and Bailey, 1980; Wright and Bailey, 1982). Seedling recruitment of many species in Fescue Prairie can be favored by fire (Romo and Gross, 2011). In fire-prone habitats, many propagules, seeds in particular, have evolved strategies to various factors associated with fire (Van Staden *et al.*, 2000). Heat, the most important physical

fire cue, can fracture the hard seed coat (Brits *et al.*, 1993) or stimulate the embryo (Van de Venter and Esterhuizen, 1988; Musil and De Witt, 1991). Seed germination can also be affected by different chemical fire cues, including ethylene and ammonia (Van de Venter and Esterhuizen, 1988), nitrogen oxides (Keeley and Fotheringham, 1997), ash (Henig-Sever *et al.*, 1996; Ne'eman & Izhaki, 1998), and smoke (De Lange and Boucher, 1990; Brwon, 1993). Among these chemical fire cues, smoke is the most striking one, which stimulates seed germination of species from fire-prone and fire-free habitats with various seed size, shape, or life form (Dixon *et al.*, 1995; Van Staden *et al.*, 2000). However, limited attention was given to test the stimulant effects of smoke on seed germination for species from Fescue Prairie (Abu, 2014).

The major active compound in plant-derived smoke is 3-methyl-2*H*-furo [2,3-c]-pyran-2-one (Van Staden *et al.*, 2004; Flematti *et al.*, 2004), known as karrikinolide (KAR_1) (Commander *et al.*, 2009). The promoting effects of KAR_1 on seed germination have been reported in various species (Kulkarni *et al.*, 2007; Merritt *et al.*, 2006; Daws *et al.*, 2007; Stevens *et al.*, 2007). KAR_1 can be active in stimulating seed germination at very low concentration. Germination of *Lactuca sativa* and *Stylidium affine* can be increased after treating with KAR_1 solutions concentrated at 10^{-9}M and 10^{-7}M, respectively (Flematti *et al.*, 2004). KAR_1 can also widen the environmental conditions under which seeds can germinate. KAR_1 favors germination of tomato (*Lycopersicon esculentum*) seeds at sub- and supra-optimal temperatures (Jain *et al.*, 2006). In addition, KAR_1 plays a positive role in enhancing seedling growth of weeds (Daws *et al.*, 2007) and medical plants (Kulkarni *et al.*, 2007).

Five KAR_1 analogs (KAR_2-KAR_6), also known as karrikins, were discovered and confirmed by chemical synthesis from smoke solutions (Flematti *et al.*, 2009). Although KAR_1 is the most important stimulant for most species, germination of some species can be affected more by other analogs. For example, KAR_2 is the most active stimulant for germination of *Arabidopsis* (Nelson *et al.*, 2009). Besides karrikins, cyanohydrin glyceronitrile is another active compound in plant-derived smoke (Flematti *et al.*, 2011). Germination of *Tersonia cyathiflora* responded positively to cyanohydrin glyceronitrile but not to karrikins (Downes *et al.*, 2010). Because over 5000 compounds may exist in smoke (Smith *et al.*, 2003) and the stimulating effects of smoke on seed germination vary among different species, it is quite possible some active compounds that can stimulate seed germination of certain species have not been identified.

It is not conclusive whether effects of smoke originating from different plant materials have similar germination responses. Several studies showed that smoke produced from different plant materials had similar effects on germination response (Baxter *et al.*, 1995; Jager *et al.*, 1996; Catav *et al.*, 2012). However, Van Staden *et al.* (1995) found possible stimulants varied between the smoke solutions made from *Passerina vulgaris* and *Themede triandra*. It is possible that plant materials with contrasting chemical compositions produce smoke that has different effects on seed germination. As far as we know, smoke produced from herbaceous Leguminosae has never been tested before. In the current study, active compounds in smoke solution produced from alfalfa (*Medicago sativa*) were used to compare with those in smoke made from prairie hay collected from a Fescue Prairie dominated by plains rough fescue (*Festuca hallii* [Vasey] Piper) and wheat straw (*Triticum aestivum* cv. Unity).

Fescue Prairie is well known for its adaptation to burning (Wright and Bailey, 1982), indicating seeds of species in it may have evolved adapted strategies to the smoke cue. However, to our knowledge, germination responses to the active compounds in smoke have never been tested before for species in Fescue Prairie. The objectives of this study were to: 1) determine whether different active compounds exist in smoke originating from different plant materials and 2) how active compounds in smoke derived from different plant materials interact with temperature and light in affecting seed germination and seedling growth of species in Fescue Prairie. It was hypothesized that: 1) different active compounds exist in smoke solutions produced from different plant material; 2) effects of separated fractions obtained from different smoke solutions on seed germination and seedling growth vary among species, and; 3) effects of separated fractions obtained from different smoke solutions on seed germination and seedling growth are temperature and light dependent.

6.2 Material and methods

6.2.1 Selection of *Lactuca sativa* cultivar as a standard for testing the effects of smoke on seed germination

Eighteen different cultivars of *Lactuca sativa* seeds were purchased from Early's Home and Garden Center in Saskatoon, SK. Cleaned seeds were kept in sealed plastic bags and stored at -20°C prior to germination experiments. A completely randomized design (CRD) with five replications was used for germination tests. Thirty seeds of each cultivar of *Lactuca sativa* were

placed in 10 cm Petri dishes lined with two layers of Whatman #1 filter paper and moistened with 5 mL of distilled water under a green safe light in the darkness, and subsequently incubated at 25 °C under 12 h light/12 h darkness or 24 h darkness. Petri dishes were placed in transparent zip-lock bags for those incubated in 12 h light/12 h darkness. Zip-lock bags wrapped with 2 layers of aluminum foil were used to accommodate petri dishes for seeds incubated in 24h darkness. Germination was recorded after 1 day of incubation. The cultivar (*Lactuca sativa* L. cv. Salad Bowl) which showed biggest difference of germination between light and darkness was selected as studied cultivar.

6.2.2 Salad Bowl lettuce bioassay

For each bioassay test, a completely randomized design (CRD) with five replications was used. Thirty seeds of Salad Bowl lettuce were placed in 10 cm Petri dishes lined with two layers of Whatman #1 filter paper and moistened with 5 mL of test solutions under a green safe light in darkness. Distilled water was used as the control for each experiment, and various dilutions of each separated fraction were used to ensure the optimum concentration range for activity. Petri dishes were sealed in plastic bags wrapped with 2 layers of aluminum foil and incubated in the darkness at 25 °C. Germination was recorded after 1 day of incubation.

6.2.3 Identification of active compounds in smoke solutions

The methodology in producing stock smoke solution (1/1 v/v) made from alfalfa, prairie hay, or wheat straw can be found in 5.2.3. The procedures in separating the active compounds involved in smoke solutions produced from alfalfa, prairie hay, and wheat straw were based on Flematti *et al.* (2008) with some modifications. In total, 2 L of each stock smoke solution produced from alfalfa, prairie hay, or wheat straw were filtered (32 cm, Whatman #1 filter papers) and separated. Each liter of smoke solution was exhaustively extracted with ethyl acetate (3 × 200 ml), followed by washing 5 times with 1% (w/v) aqueous NaOH of the combined organic extract to remove the acid compounds. The resulting extract was dried with Na_2SO_4, filtered, and evaporated in vacuum to give the neutral fractions (452 mg for alfalfa, 422 mg for prairie hay, and 428 mg for wheat).

Concentrated neutral fractions were subjected to column chromatography using a 2.5 × 30 cm column packed with 50g silica gel 60 (Merck, 0.040-0.063 mm) and eluted with a hexane : ethyl acetate gradient (hexane proportion: 100%, 90%, 80%, 70%, 60%, 50%, 40%, 30%, 20%,

10%, and 0% (v/v); 150 mL aliquots of each mixture). The active fraction was evaporated in vacuum and then subjected to reverse-phase (RP) C18 column (waters sep-pak vac 12 cc Cartrige), eluted with a water: methanol gradient (methanol proportion: 0%, 10%, 15%, 20%, 25%, and 100% (v/v); 50 ml aliquots of each mixture). The active fraction was evaporated to 1.5 mL in vacuum.

Part of the active fraction (20 µL) from alfalfa, prairie hay, or wheat straw smoke solution was analyzed with a C18-RP High Performance Liquid Chromatography (HPLC) column (Chromolith ® Performance RP-18e 100-4.6), eluted with a acetonitrile: water gradient (7%-14%-95%-7%-7% acetonitrile/water over 0-14-15-16-20 min at 2 ml/min) for further fractionation. UV absorbance was measured at the wavelength of 330 nm. Fractions were collected between 3 and 12.5 min (3-5.7, 5.7-6.6, 6.6-9, 9-10, 10-11, and 11-12.5 min) based on the elution pattern. A sample of 20 µL of pure KAR_1 (0.1mg/ ml) (Toronto Research Chemicals Inc.) was eluted with the same acetonitrile based method as standard. More active fraction was obtained by applying 80 µL of the fraction obtained after (RP) C18 column chromatography to the C18-RP HPLC each time, using the same methodology mentioned above for fractionation for 10 times. In total, 18 mL (2 mL/min, 0.9 min for each run, and 10 runs) of active fractions from the smoke solutions produced from alfalfa, prairie hay, and wheat straw were obtained.

6.2.4 Priming effects of active fractions on germination and seedling growth of *Artemisa frigida*, *Artemisia ludoviciana*, *Circium arvense*, and *Conyza canadensis*.

Descriptions about seed collection and storage of *Artemisa frigida*, *Artemisia ludoviciana*, *Circium arvense*, and *Conyza canadensis* can be found in 5.2.2. Each active fraction (18 mL) obtained above from smoke solutions produced from alfalfa, prairie hay, or wheat straw was dissolved in 1.07 L (2 L times 0.8 divided by 1.5, because for each type of smoke solution, in total 1.5 mL fraction was obtained after reverse phase chromatography and only 0.8 mL of them were subjected to the HPLC for further separation) of distilled water. The concentration of the active compounds in each 1.07 L solution was equivalent to that in each 2 L of 1/1 v/v stock smoke solution. Each fraction (18 mL) had 10% acetonitrile. In order to determine and eliminate the effects of acetonitrile on seed germination, 1.8 mL acetonitrile was dissolved in 1.07 L distilled water and was regarded as 1/1 v/v acetonitrile solution. In addition, in order to determine the effects of KAR_1 on seed germination, a concentration of 10^{-6} M of KAR_1 was dissolved in distilled water and was regarded as the 1/1 v/v KAR_1 solution. Each of the five

different solutions (1/1 v/v), including active fractions obtained from alfalfa, prairie hay, wheat straw, acetonitrile solution, and KAR_1 solution was made into 3 serial dilutions, including 1/1000 v/v, 1/100 v/v and 1/10 v/v. Seeds primed in distilled water were used as the control. The priming procedure and procedures for seed germination, seedling growth, and seed germination rate test can be found in 5.2.4.

A randomized-complete-block-design (RCBD) was used for each species with five priming treatments (1/1000v/v, 1/100v/v, 1/10v/v, 1/1v/v and distilled water) for each of the five different types (alfalfa, prairie hay, wheat straw, acetonitrile, and KAR_1), and four replicates within each of the 4 germination conditions, which included 12 h light/12 h darkness at 10/0 °C, 24 h darkness at 10/0 °C, 12 h light/12 h darkness at 25/15 °C and 24 h darkness at 25/15 °C. The experiment was repeated with four new replicates of five different solutions as the second run.

6.2.5 Data analysis

A t test was used to compare the significant difference between germination of different *Lactuca sativa* cultivars under light and darkness conditions at 25 °C. One way Analysis of Variance (ANOVA) was used to determine the effects of various fractions on germination of Salad Bowl lettuce seeds within each smoke solution after separating by normal and reverse phase chromatography and HPLC. One way ANOVA was also used to determine the effects of smoke types on germination of Salad Bowl lettuce seeds within each separated fraction after separating by ethyl acetate and NaOH. Treatment means were separated using Tukey test at $P \leq 0.05$.

For the effects of separated fractions and KAR_1 on seed germination and seedling growth, data of total germination percentage, germination rate (time to 50% germination), length of radical, hypocotyl and total root were analyzed with a RCBD design with 2 runs (4 replication for each run) using the mixed model procedure in SAS version 9.3 software (SAS Institute Inc., USA). For each species in each of the tested conditions, priming effects for each of the studied types were used as independent variables. Replicates, blocks and runs were treated as random effects. Germination data were arcsine square root transformed before subjected to ANOVA. Data normality was tested using the Shapiro-Wilk test before each analysis. Data not meeting normality requirement were log-transformed before analyzing. Treatment means were separated using Tukey test at $P \leq 0.05$.

6.3 Results

6.3.1 Effects of light on seed germination of *Lactuca sativa*

Seed germination of 18 different cultivars of *Lactuca sativa* was tested in 12h light/12 darkness and 24h darkness at constant 25 °C (Figure 6.1). Germination of Salad Bowl showed the biggest difference in germination between light and darkness at 25 °C ($P<0.01$), which was subsequently chosen as the cultivar to test the effects of separated active compounds in smoke solutions on seed germination.

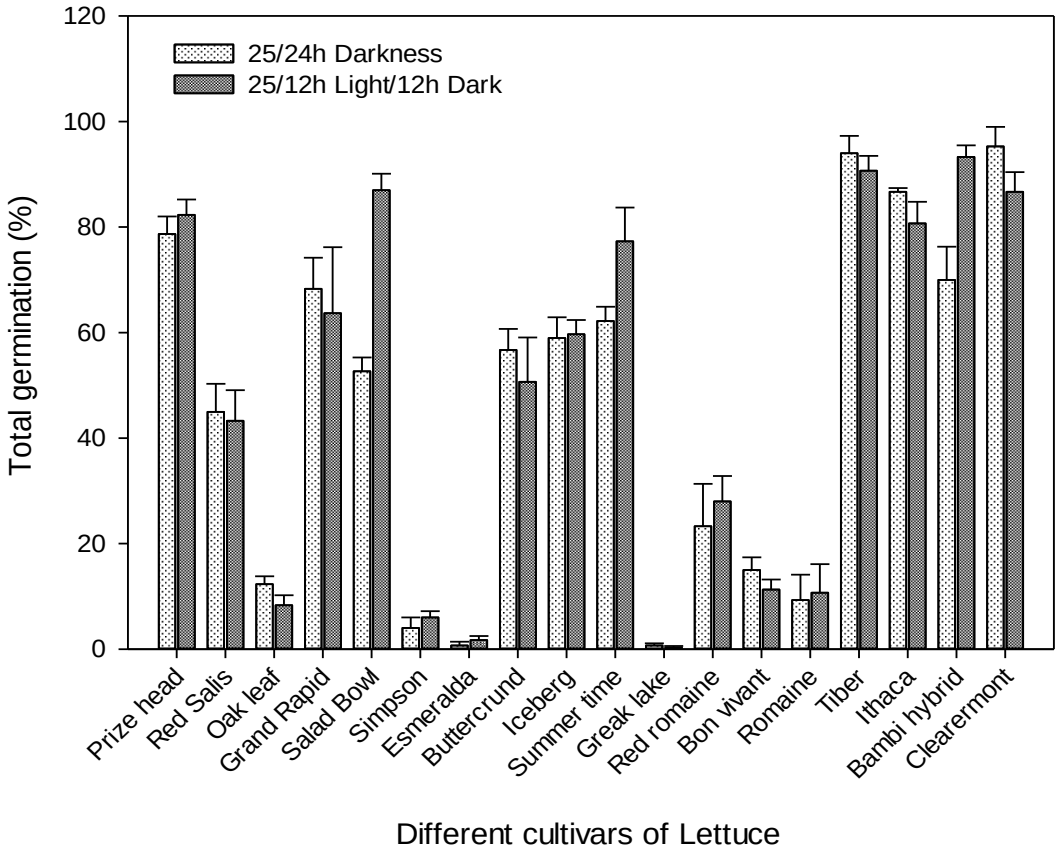

Figure 6.1 Total germination of various cultivars of *Lactuca sativa* seeds incubating at 25 ^{o}C in 24 h darkness or in 12 h light/12 h darkness for 1 day. Bars represent means ± SE of 5 replicates with 30 seeds each.

6.3.2 Effects of different smoke solutions on seed germination of Salad Bowl lettuce

The stimulant effects of smoke solutions on germination of Salad Bowl lettuce varied with different smoke types and different dilutions (Figure 6.2). Compared with the control, germination of Salad Bowl lettuce after treating with 1/5000 v/v smoke dilutions produced from prairie hay and wheat straw was increased by 46% ($P<0.01$) and 43% ($P=0.01$), respectively. Different dilutions of smoke made from alfalfa had no effects on germination of Salad Bowl lettuce compared with the control ($P=0.44$).

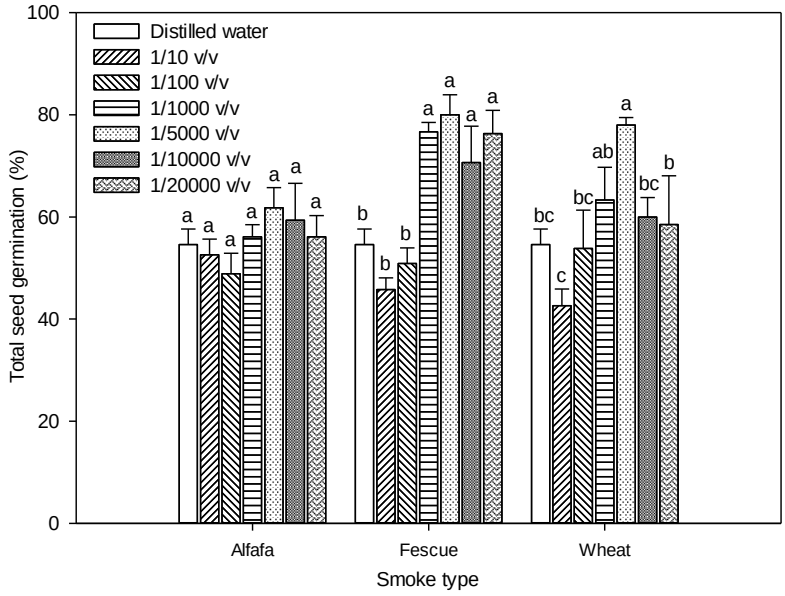

Figure 6.2 Total germination of Salad Bowl lettuce (*Lactuca sativa*) seeds after treating with serial dilutions of smoke solutions made from alfalfa, prairie hay, or wheat straw and incubating at 25 ^{o}C in 24 h darkness for 1 day. Means with different letters indicate total germination of treated seeds were significantly different ($P\leq0.05$) within smoke types. Bars represent means ± SE of 5 replicates with 30 seeds each.

6.3.3 Tracing the active compounds involved in plant-derived smoke using Salad Bowl bioassay

Ethyl acetate separated stock smoke solution made from alfalfa, prairie hay, or wheat straw into water (inorganic) fraction and ethyl acetate (organic) fraction (Figure 6.3). The two fractions were both tested with the Salad Bowl lettuce bioassay at a series of dilutions to ensure the right concentration range for activity (data not shown). Ethyl acetate fractions from prairie hay and wheat straw solutions significantly increased seed germination by 46% and 30%, respectively, as compared with the control ($P<0.01$). Ethyl acetate fraction from alfalfa solution had no effects on seed germination of Salad Bowl lettuce. None of the three water fractions from different fuel types had effects on seed germination of Salad Bowl lettuce compared with the control ($P=0.70$).

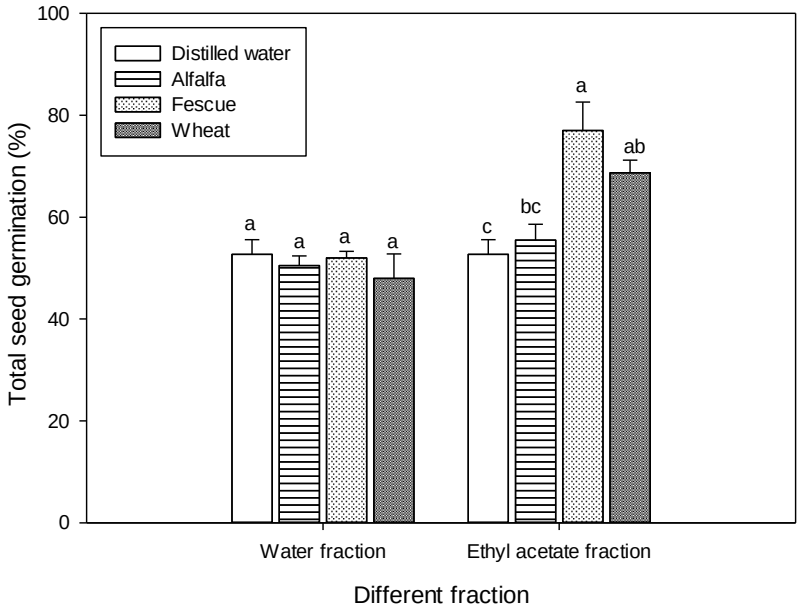

Figure 6.3 Total germination of Salad Bowl lettuce (*Lactuca sativa*) seeds after treating with water or ethyl acetate fraction derived from smoke solutions made from alfalfa, prairie hay, and wheat straw and incubating at 25 ^{o}C in 24 h darkness for 1 day. Means with different letters indicate total germination of treated seeds were significantly different ($P\leq0.05$) within fractions. Bars represent means ± SE of 5 replicates with 30 seeds each.

NaOH was used to fractionate organic extract obtained above into acid and neutral fractions (Figure 6.4). The two fractions were both tested with the Salad Bowl lettuce bioassay at a series of dilutions to ensure the right concentration range for activity (data not shown). NaOH fractions from prairie hay and wheat straw solutions significantly increased seed germination compared with the control ($P<0.01$). However, NaOH fraction from alfalfa had no effects on seed germination relative to the control. None of the 3 acid fractions from different fuel types had effects on seed germination of Salad Bowl lettuce compared with the control ($P=0.10$).

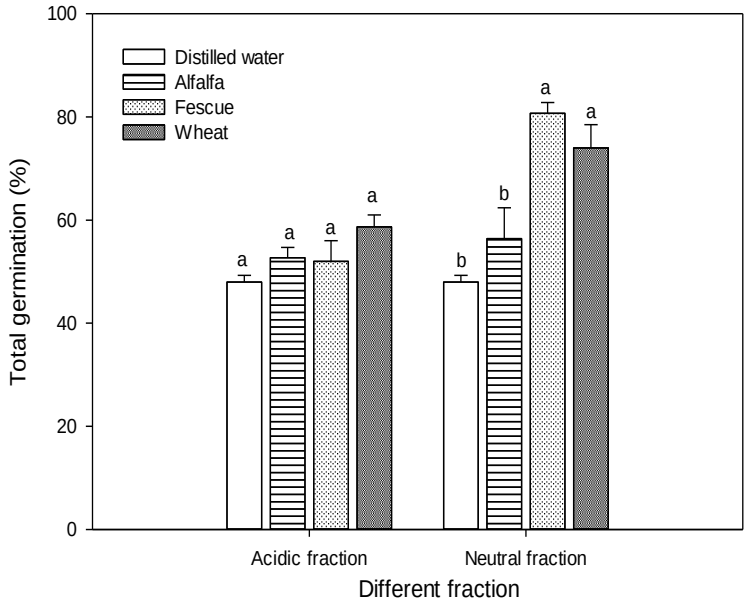

Figure 6.4 Total germination of Salad Bowl lettuce (*Lactuca sativa*) seeds after treating with neutral or acidic fraction derived from smoke solutions made from alfalfa, prairie hay, and wheat straw and incubating at 25 ^{o}C in 24 h darkness for 1 day. Means with different letters indicate total germination of treated seeds were significantly different ($P\leq0.05$) within fractions. Bars represent means ± SE of 5 replicates with 30 seeds each.

An important consideration was given that active compounds in smoke may be volatile. After evaporating the solvent involved in the neutral fraction obtained above, part of the concentrated compounds were resolved and a series of different concentrated solutions were tested with the Salad Bowl lettuce bioassay (data not shown). After evaporation, stimulant compounds stilled involved in the neutral fractions gained from prairie hay and wheat straw, because they significantly increased seed germination of Salad Bowl lettuce compared with the control ($P<0.01$) (Figure 6.5). Neutral fraction from alfalfa had no effects on seed germination of Salad Bowl lettuce compared with the control.

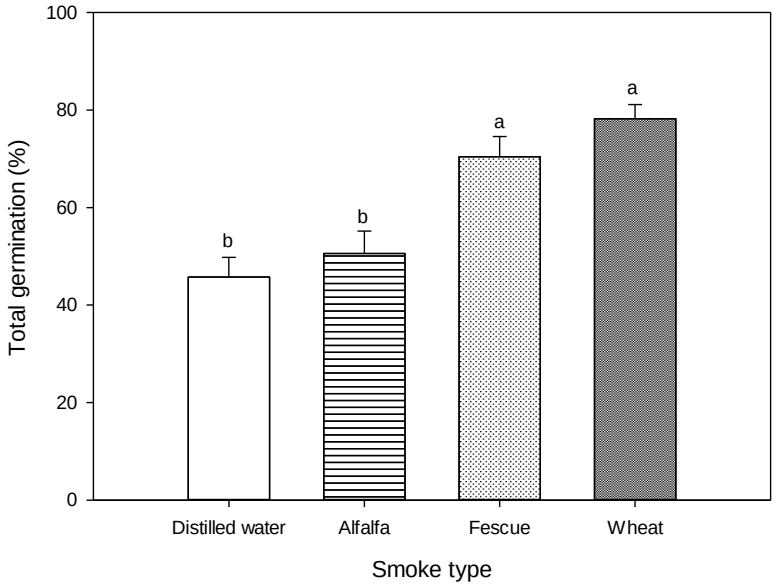

Figure 6.5 Total germination of Salad Bowl lettuce (*Lactuca sativa*) seeds treated in appropriate concentrated neutral fractions derived from smoke solutions made from alfalfa, prairie hay, and wheat straw after concentrating by evaporation and incubating at 25 ^{o}C in 24 h darkness for 1 day. Means with different letters indicate total germination of treated seeds were significantly different ($P\leq0.05$). Bars represent means ± SE of 5 replicates with 30 seeds each.

Concentrated neutral fraction was separated by chromatography, eluted with a hexane: ethyl acetate gradient (hexane proportion: 100%, 90%, 80%, 70%, 60%, 50%, 40%, 30%, 20%, 10%, and 0%). Different fractions were tested with the Salad Bowl lettuce bioassay at a series of dilutions to ensure the right concentration range for activity (data not shown). Fraction with germination stimulating activity eluted in 70: 30 ethyl acetate: hexane fraction occurred in all three fuel types (Figure 6.6). Active fractions were concentrated using a rotary evaporator.

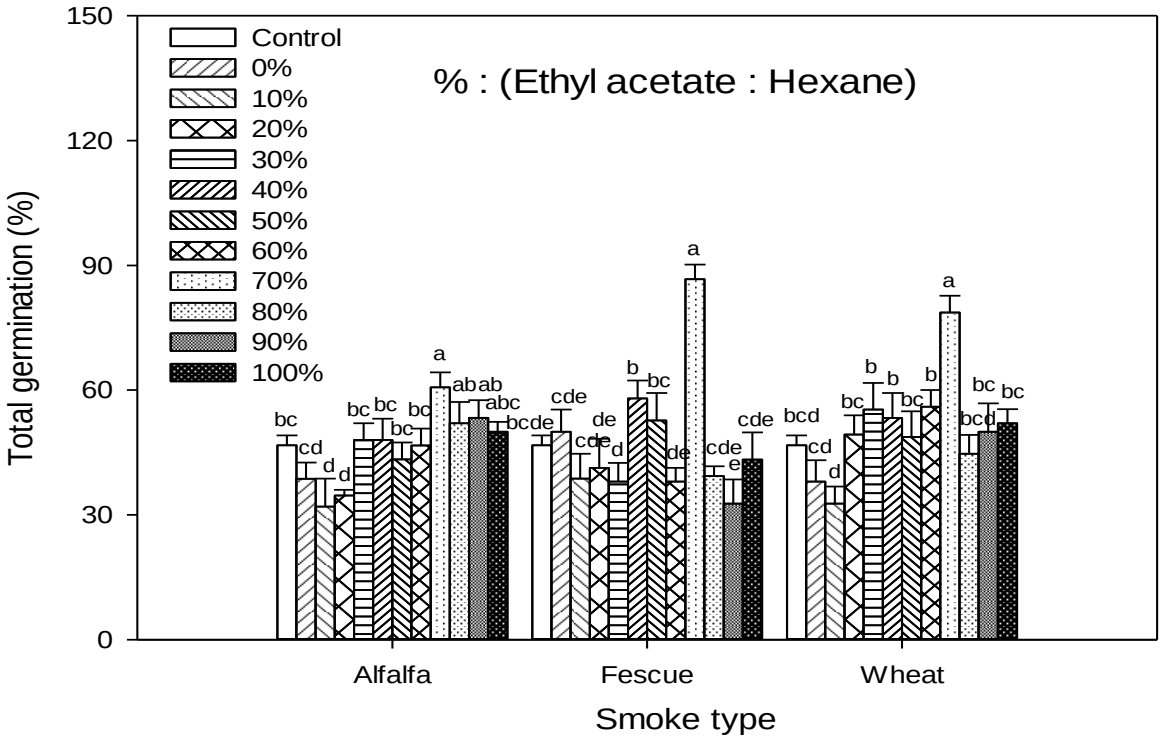

Figure 6.6 Total germination of Salad Bowl lettuce (*Lactuca sativa*) seeds after treating with different fractions derived from the normal phase chromatography of the neutral fraction from alfalfa, prairie hay, and wheat straw or distilled water and incubating at 25 ^{o}C in 24 h darkness for 1 day. Means with different letters indicate total germination of treated seeds were significantly different ($P \leq 0.05$) within smoke types. Bars represent means ± SE of 5 replicates with 30 seeds each.

The 70: 30 hexane: ethyl acetate fraction was separated by reverse-phase chromatography, eluted with a water: methanol gradient (methanol proportion: 0%, 10%, 15%, 20%, 25%, and 100%). Different fractions were then tested with the Salad Bowl lettuce bioassay at a series of dilutions to ensure the right concentration range for activity. Fractions with germination stimulating activity was eluted in 10: 90 methanol: water obtained from prairie hay and wheat straw (Figure 6.7). Active fraction was concentrated using rotary evaporator. None of the fractions from alfalfa had effects on seed germination of Salad Bowl lettuce compared with the control (P=0.31).

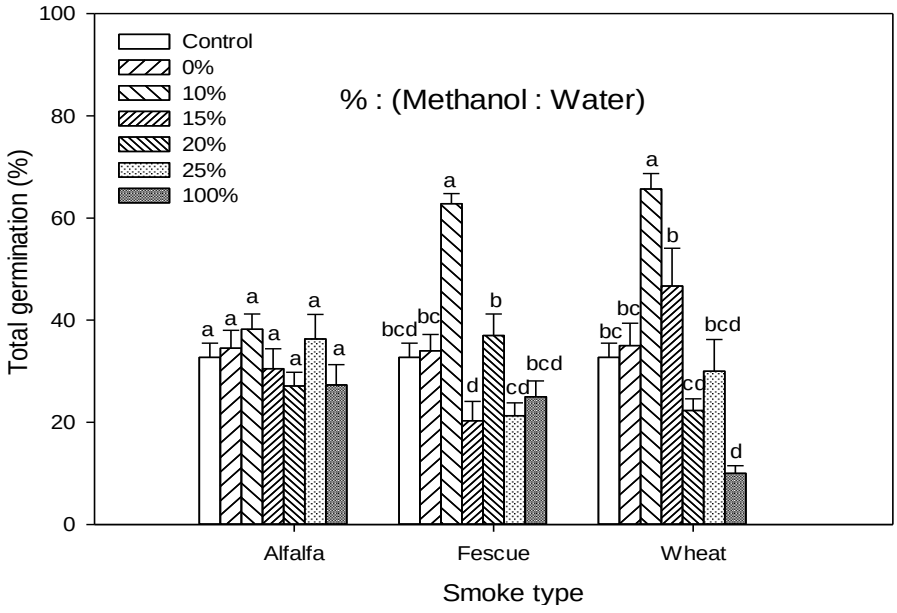

Figure 6.7 Total germination of Salad Bowl lettuce (*Lactuca sativa*) seeds after treating with different fractions derived from the reverse phase chromatography of the 70:30 hexane: ethyl acetate fraction from alfalfa, prairie hay, and wheat straw or distilled water and incubating at 25 ^{o}C in 24 h darkness for 1 day. Means with different letters indicate total germination of treated seeds were significantly different (P≤0.05) within smoke types. Bars represent means ± SE of 5 replicates with 30 seeds each.

The 90: 10 water: methanol fraction was separated using HPLC. Six parts were collected between 3 to 12.5 min (3-5.7, 5.7-6.6, 6.6-9, 9-10, 10-11 and 11-12.5 min) based on the elution pattern and were regarded as parts 1 to 6, respectively. Different parts were tested with the Salad Bowl lettuce bioassay at a series of dilutions to ensure the right concentration range for activity (data not shown). Treating seeds with the 2nd part from prairie hay and wheat straw significantly increased seed germination by 97% and 81%, respectively, as compared with the control ($P<0.01$) (Figure 6.8). None of the six parts from alfalfa had effects on seed germination of Salad Bowl lettuce compared with the control ($P=0.61$).

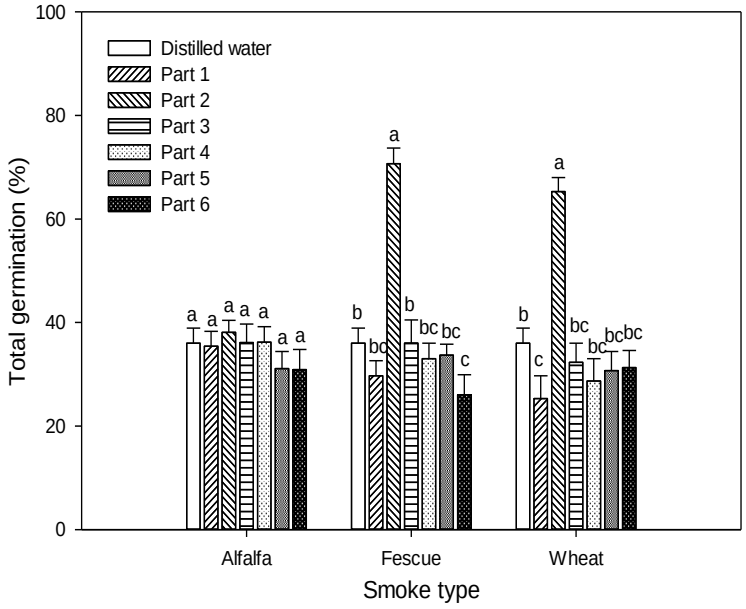

Figure 6.8 Total germination of Salad Bowl lettuce (*Lactuca sativa*) seeds after treating with different fractions derived from the HPLC of the 90:10 water: methanol fraction from alfalfa, prairie hay, and wheat straw or distilled water and incubating at 25 ^{o}C in 24 h darkness for 1 day. Means with different letters indicate total germination of treated seeds were significantly different ($P\leq0.05$) within smoke types. Bars represent means ± SE of 5 replicates with 30 seeds each.

Chromatogram of the 90: 10 water: methanol fraction from alfalfa, prairie hay, wheat straw, and KAR_1 as the standard (Figure 6.9). Red arrows indicated the possible KAR_1 involved in the smoke solutions produced from prairie hay and wheat straw. KAR_1 was not in the smoke solution made from alfalfa.

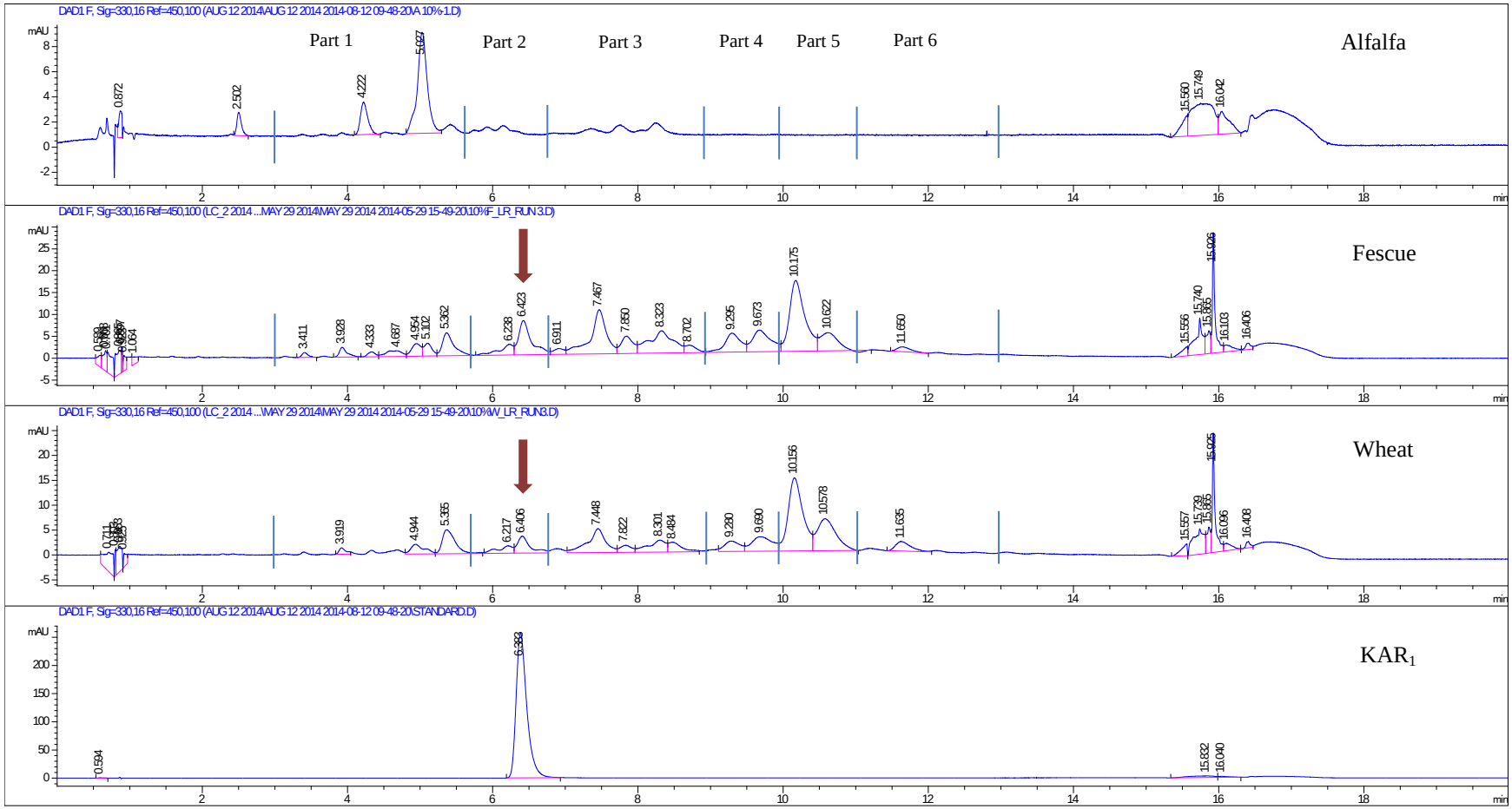

Figure 6.9 Chromatograms of the 90:10 water: methanol fraction from alfalfa (top), fescue (second), and wheat (third), together with KAR_1 standard (bottom). Six parts were separated for alfalfa, fescue and wheat chromatograms. Red arrows indicate possible KAR_1 in those two smoke solutions.

Chromatogram of the 2nd part for smoke solutions produced from alfalfa, prairie hay, wheat straw were compared with that of KAR_1(Figure 6.10). KAR_1 was in the smoke solutions produced from prairie hay and wheat straw, but was not in the smoke solution made from alfalfa.

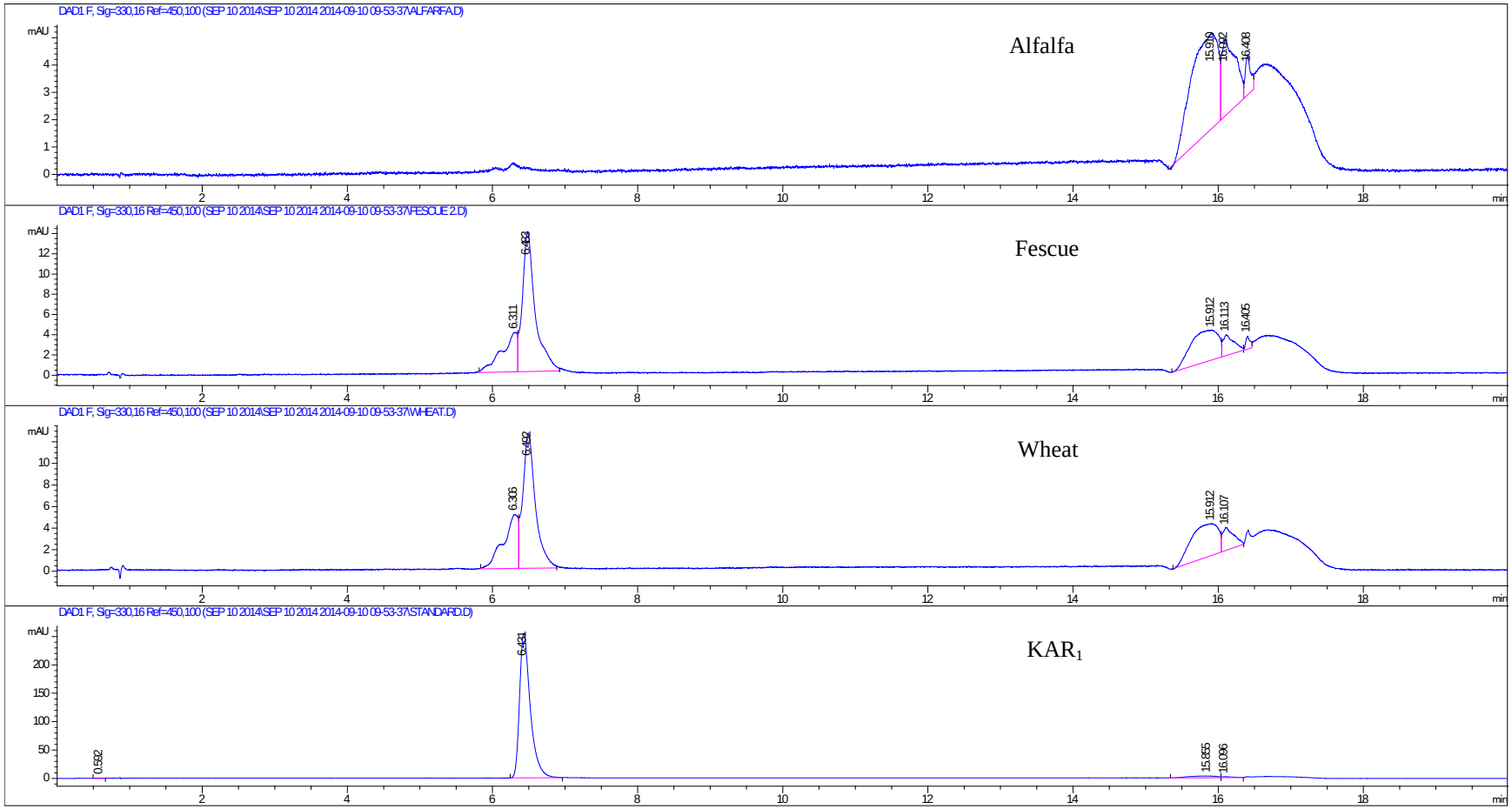

Figure 6.10 Chromatograms of the active fraction (2nd part in Figure 6.9) from alfalfa (top), fescue (second), and wheat (third), together with KAR_1 standard (bottom).

Treating with 1/5000 v/v of the 2nd part for smoke solutions produced from prairie hay and wheat significantly increased seed germination for Salad Bowl lettuce in the 24 h darkness at 25 °C (Figure 6.11). Priming seeds with the 2nd part for smoke solution made from alfalfa had no effect on seed germination. Priming seeds with 10^{-6} to 10^{-9} M KAR_1 solutions significantly increased seed germination for Salad Bowl in the 24 h darkness at constant 25 °C.

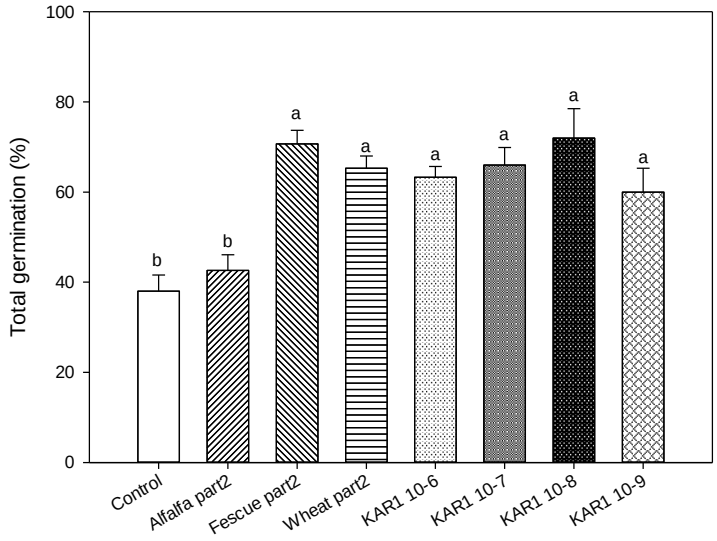

Figure 6.11 Total germination of Salad Bowl lettuce (*Lactuca sativa*) seeds after treating with active fractions from alfalfa, prairie hay, and wheat straw, and different concentrated KAR_1, and incubating at 25 ^{o}C in 24 h darkness for 1 day. Means with different letters indicate total germination of treated seeds were significantly different ($P \leq 0.05$). Bars represent means ± SE of 5 replicates with 30 seeds each.

6.3.4 Effects of active fractions and KAR_1 on seed germination and seedling growth of *Artemisia frigida*

Total seed germination of *Artemisia frigida* was affected by primings effect of active fraction from prairie hay solution ($P<0.01$) and KAR_1 ($P<0.01$) in 12 h light/12 h darkness at 10/0 °C (Table 6.1). Priming with 1/1 v/v active fraction from prairie hay significantly increased seed germination by 76% compared with the control. Seed germination was 62% and 67% higher for the seeds primed with 1/10 v/v and 1/1 v/v KAR_1 solutions, respectively, as compared with the control. Priming in various dilutions of fractions from wheat straw ($P=0.09$) and alfalfa ($P=0.33$), as well as acetonitrile solutions ($p=0.01$) had no effect on germination of *Aretemisia frigida* compared with the control. Seed germination rates, radical, hypocotyl, and total seedling lengths of *Artemisia frigida* were not affected by priming effects of any studied solutions in any studied germination conditions (data not shown).

Table 6.1 Total germination (%) of *Artemisia frigida* seeds after priming in serial dilutions of different separated fractions and incubating at 10/0 ^{o}C in 12 h light/12 h dark.

		Types				
Condition	Dilution	ACE	Alfalfa	Fescue	Wheat	KAR_1
	DW	28 ± 5 ab	28 ± 5 a	28 ± 5 b	28 ± 5 a	28 ± 5 b
	1/1000	22 ± 4 b	33 ± 4 a	33 ± 5 ab	28 ± 4 a	34 ± 4 ab
10/0 °C	1/100	35 ± 3 ab	25 ± 5 a	27 ± 5 b	28 ± 2 a	38 ± 5 ab
Light/Darkness	1/10	35 ± 3 a	29 ± 4 a	40 ± 3 ab	37 ± 5 a	45 ± 4 a
	1/1	36 ± 2 a	32 ± 4 a	49 ± 6 a	39 ± 6 a	46 ± 3 a

Means with different letters indicate total germination of primed seeds were significantly different ($P\leq0.05$) within fractions. DW=Distilled water. Values represent means ± SE. ACE= acetonitrile.

6.3.5 Effects of separated fractions and KAR_1 on seed germination and seedling growth for *Artemisia ludoviciana*

Total seed germination of *Artemisia ludoviciana* was affected by priming effects of active fractions from prairie hay (P=0.04) and wheat straw (P=0.04) and KAR_1 solutions (P=0.02) in 24 h darkness at 25/15 °C (Table 6.2). Priming with 1/1 v/v fractions from prairie hay and wheat straw increased seed germination by 37% and 27%, respectively, as compared with the control. Seed germination was 38% higher for seeds primed with 1/1 v/v KAR_1 solution relative to the control. Priming in various dilutions of fractions from alfalfa (P=0.25) and acetonitrile solutions (P=0.34) had no effect on germination of *Artemisia ludoviciana* compared with the control. Seed germination rates, radical, hypocotyl, and total seedling lengths for *Artemisia ludoviciana* were not affected by any priming effect of any studied solutions in any studied germination conditions (data not shown).

Table 6.2 Total germination (%) of *Artemisia ludoviciana* seeds after priming in serial dilutions of different separated fractions and incubating at 25/15 ^{o}C in 24 h dark.

		Types				
Condition	Dilution	ACE	Alfalfa	Fescue	Wheat	KAR_1
	DW	47 ± 4 a	47 ± 4 a	47 ± 4 b	47 ± 4 b	47 ± 4 b
	1/1000	56 ± 6 a	55 ± 5 a	55 ± 5 ab	58 ± 6 ab	52 ± 6 ab
25/15 °C	1/100	55 ± 5 a	56 ± 4 a	51 ± 3 ab	57 ± 4 ab	52 ± 6 ab
Darkness	1/10	55 ± 4 a	50 ± 4 a	53 ± 4 ab	52 ± 7 ab	55 ± 4 ab
	1/1	53 ± 5 a	56 ± 5 a	64 ± 5 a	59 ± 4 a	64 ± 5 a

Means with different letters indicate total germination of primed seeds were significantly different (P≤0.05) within studied germination conditions. DW=Distilled water. Values represent means ± SE. ACE represents acetonitrile.

6.3.6 Effects of separated fractions and KAR_1 on seed germination and seedling growth for *Cirsium arvense*

Total seed germination, germination rates, hypocotyl, and total seedling lengths of *Cirsium arvense* were not affected by any priming effect of any studied solutions in any studied germination conditions (data not shown). Radical length for *Cirsium arvense* was affected by priming effect of KAR_1 solution in 12 h light/12 h darkness at 25/15 °C (Table 6.3). Priming with 1/1 v/v KAR_1 solution ($P<0.01$) significantly reduced radical length by 38% as compared the control. Priming in various dilutions of fractions from alfalfa ($P=0.28$), prairie hay ($P=0.06$), and wheat straw ($P=0.46$), as well as acetonitrile solutions ($p=0.74$) had no effect on radical length of *Cirsium arvense* compared with the control.

Table 6.3 Radical length (mm) for *Cirsium arvense* seeds after priming in serial dilutions of different separated fractions and incubating at 25/15 ^{o}C in 12 h light/12 h darkness.

		Types				
Condition	Dilution	ACE	Alfalfa	Fescue	Wheat	KAR_1
	DW	12 ± 1 a	12 ± 1 a	12 ± 1 a	12 ± 1 a	12 ± 1 a
25/15 °C	1/1000	11 ± 1 a	13 ± 1 a	9 ± 1 a	12 ± 1 a	11 ± 1 ab
Light/Darkness	1/100	12 ± 1 a	11 ± 1 a	10 ± 1 a	10 ± 1 a	11 ± 1 ab
	1/10	13 ± 2 a	10 ± 1 a	12 ± 1 a	11 ± 1 a	9 ± 1 ab
	1/1	12 ± 1 a	10 ± 1 a	12 ± 1 a	10 ± 1 a	7 ± 1 b

Means with different letters indicate radical length of primed seeds were significantly different ($P\leq0.05$) within studied germination conditions. DW=Distilled water. Values represent (±) standard error. ACE represents acetonitrile.

6.3.7 Effects of separated fractions and KAR_1 on seed germination and seedling growth for *Conyza canadensis*

Total seed germination of *Conyza canadensis* was affected by priming effects of active fractions from prairie hay (P=0.02) and wheat straw (P=0.03) and KAR_1 solution (P=0.02) in 12 h light/12 h darkness at 25/15 °C (Table 6.4). Priming with 1/10 v/v fraction from prairie hay increased seed germination by 96% as compared with the control. Priming with 1/1000 v/v fraction from wheat increased seed germination by 114% relative to the control. Seed germination was 73% and 68% higher for seeds primed with 1/1000 v/v and 1/100 v/v of KAR_1 solution, respectively, as compared with the control. Priming in various dilutions of fractions from alfalfa (P=0.43) and acetonitrile solutions (P=0.72) had no effect on germination of *Conyza canadensis* compared with the control. Seed germination rates, radical, hypocotyl, and total seedling lengths for *Conyza canadensis* were not affected by any priming effect of any studied solutions in any studied germination conditions (data not shown).

Table 6.4 Total germination (%) of *Conyza canadensis* seeds after priming in serial dilutions of different separated fractions and incubating at 25/15 ^{o}C in 12 h light/12 h darkness.

		Types				
Condition	Dilutions	ACE	Alfalfa	Fescue	Wheat	KAR_1
	DW	38 ± 8 a	38 ± 8 a	38 ± 8 b	38 ± 8 b	38 ± 8 bc
25/15 °C	1/1000	42 ± 8 a	57 ± 8 a	61 ± 10 ab	80 ± 8 a	65 ± 3 a
Light/Darkness	1/100	47 ± 8 a	49 ± 8 a	38 ± 10 b	76 ± 9 a	63 ± 2 a
	1/10	55 ± 13 a	57 ± 10 a	74 ± 7 a	58 ± 12 ab	60 ± 8 ab
	1/1	46 ± 9 a	44 ± 10 a	60 ± 10 ab	62 ± 10 ab	33 ± 9 c

Means with different letters indicate total germination of primed seeds were significantly different (P≤0.05) within studied germination conditions. DW=Distilled water. Values represent means ± SE. ACE represents acetonitrile.

6.4 Discussion

6.4.1 Salad Bowl lettuce (*Lactuca sativa*) was used as a quick bioassay

Active compounds in smoke solutions can substitute light effects and promote germination of light sensitive *Lactuca sativa* seeds incubated in the darkness (Drewes *et al.*, 1995). In the present study, Salad Bowl lettuce (*Lactuca sativa*) was used as a rapid bioassay for the detection of germination promoting compounds in smoke solutions because it germinated consistently much higher in the light compared with that in the darkness. Previous studies used Grand Rapid lettuce as a quick bioassay to detect and separate active compounds in smoke solutions (Flematti *et al.*, 2004; Van Staden *et al.*, 2004). However, in the present study, germination of Grand Rapid lettuce seeds did not differ between light and darkness. This may be due to the fact that seeds from different sources have different germination characteristics. Drewes *et al.* (1995) reported germination of Grand Rapid lettuce seeds from Stokes Seeds Inc. was much higher in 24 h darkness at constant 25 ^{o}C relative to that of the seeds collected from other five different sources.

6.4.2 Different compounds involved in smoke solutions made from different plant materials

Priming in low concentrated smoke solutions made from prairie hay and wheat straw increased germination of Salad Bowl lettuce seeds in the darkness. This agrees with the former study that germination of photoblastic seeds of *Apium graveolens* was increased by diluted smoke solutions (Thomas and Van Staden, 1995). Active components involved in smoke solutions may substitute for light effects and affect the conversion of P_r to P_{fr} in the phytochrome response (Drewes *et al.*, 1995). In addition, Karrikinolide (KAR_1), the major active compound in smoke (Flematti *et al.*, 2004; Van Staden *et al.*, 2004), may act like cytokinin and auxin plant hormones when applied at low concentrations (Jain *et al.*, 2008). Although smoke solutions produced from different plant species tested before all exerted significant promotive, but variable effects on germination of *Lactuca sativa* L. (Jager *et al.*, 1996; Gardner *et al.*, 2001; Van Staden *et al.*, 2004), priming in various dilutions of smoke solution made from alfalfa did not affect seed germination of Salad Bowl lettuce in the present study, indicating previous founded active compounds were not present in the smoke solution made from alfalfa. Arruda *et al.* (2012) stated promotive effects on seed germination and seedling vigor of tomato (*Lycopersicon esculentum*) seeds varied among smoke solutions gained from different plant materials. In addition, Van

Staden *et al.* (1995) found possible stimulants varied between the smoke solutions made from *Passerina vulgaris* and *Themede triandra*.

Various KAR_1 dilutions stimulated germination of Salad Bowl lettuce in the darkness, which agrees with the former study that KAR_1 enhanced germination of *Lactuca sativa* seeds incubated in darkness (Flematti *et al.*, 2004; Van Staden *et al.*, 2004). KAR_1 was found in the smoke solutions made from prairie hay and wheat straw, but not in the one made from alfalfa. Lack of KAR_1 may explain the neutral responses of germination of Salad Bowl lettuce seeds to the smoke made from alfalfa.

Different active compounds may exist in different steps of separation. Priming in 70: 30 hexane: ethyl acetate fraction from alfalfa after normal phase chromatography increased germination of Salad Bowl lettuce compared with the control. Lack of the responses of different fractions on germination of Salad Bowl lettuce ahead of this fraction may be due to inhibitors. Lack of the responses of different fractions on germination of Salad Bowl lettuce after this fraction indicated that maybe synergistic effects of more than one compound, which were separated into different fractions afterwards, accounted for increased germination.

6.4.3 Effects of active fractions and KAR_1 on seed germination and seedling growth of species from Fescue Prairie

Effects of KAR_1 on germination are temperature dependent (Ghebrehiwot *et al.*, 2009). In the present study, priming in high concentrated KAR_1 solutions and active fractions from prairie hay increased germination of *Artemisia frigida* compared with distilled water at 10/0 °C in 12h Light/ 12h darkness. Germination of *Artemisia frigida* occurred in a wide temperature range, with 20/10 °C as the optimum temperature (Wilson, 1979). Increased germination of *Artemisia frigida* in sub-optimum temperatures observed in this study was in line with Jain *et al.* (2006), which reported that KAR_1 can improve seed germination of *Lycopersicon esculentum* under sub-optimum temperatures. Priming in various concentrated smoke solutions made from prairie hay had no promoting effects on seed germination of *Artemisia frigida* in all tested germination conditions (Chapter 5). This may be due to the fact that germination inhibitors exist in smoke solutions. A related butenolide, named as 3,4,5-trimethylfuran-2(5*H*)-one is an germination inhibitor isolated from plant-derived smoke. When applying simultaneously, it significantly reduced the effect of KAR_1 on seed germination (Light *et al.*, 2010).

KAR_1 can partly substitute light effects for the germination of photoblastic species (Thomas and Van Staden, 1995). In the present study, priming in concentrated KAR_1 solutions and active fractions made from prairie hay and wheat straw increased germination of *Artemisia ludoviciana* compared with distilled water at 25/15 ^{o}C in 24 h darkness. This observation agrees with the former studies that KAR_1 stimulated seed germination of light-sensitive seeds, including *Angianthus tomentosus*, *Gnephosis tenuissima*, *Myriocephalus guerinae*, *Podolepis canescens*, and *Rhodanthe citrina* in the suboptimal light or darkness (Merritt *et al.*, 2006). As mentioned above, active components involved in smoke solutions may substitute for light effect and affect the conversion of P_r to P_{fr} in the phytochrome response (Drewes *et al.*, 1995). In addition, KAR_1 can alter the metabolism and perception of gibberellins (GAs) and abscicic acid (ABA) to the seeds (Nelson *et al.*, 2009). For example, exposure to smoke solutions increased endogenous GA levels and decreased endogenous ABA levels for two positively photoblastic species, *Lactuca sativa* and *Nicotiana attenuate* (Gardner *et al.*, 2001; Schwachtje and Baldwin 2004). Concentrated smoke solutions also increased germination of *Artemisia ludoviciana* in light and darkness at 10/0 ^{o}C (Chapter 5). Various concentrated active fractions and KAR_1 solutions had no effect on seedling lengths of *Artemisia ludoviciana*. However, low concentrated smoke solutions increased radical length of *Artemisia ludoviciana* (Chapter 5). The different effects between smoke solutions and separated active fractions indicated other chemicals that promote germination and radical length of *Artemisia ludoviciana* may be present in the smoke solutions. Downes *et al.* (2013) reported germination of *Gyrostemon racemiger*, *G. ramulosus*, and *Anigozanthos. flavidus* were stimulated by smoke water, but not by KAR_1.

Effects of KAR_1 on germination are concentration dependent. Although the active fractions produce from prairie hay and wheat straw had KAR_1, priming in concentrated active fraction from prairie hay but not wheat straw increased germination of *Artemisia frigida* compared with distilled water at 10/0 °C in 12h Light/ 12h darkness. This is due to the fact that more KAR_1 was contained in the active fraction from prairie hay as compared with that in the wheat straw, as shown by the chromatograms of these two active fractions in the present study. This indicates not only qualitative but also quantitative variations existed in smoke solutions made from different material, which in turn may affect germination of species differently. Priming in low, but not high concentrated active fractions made from prairie hay and wheat straw and KAR_1 solutions stimulated seed germination of *Conyza canadensis* in 12 h light/12 h

darkness at 25/15 ^{o}C. Different seeds have different sensitivity to KAR_1 solutions. For example, germination of *Lactuca sativa* and *Stylidium affine* can be increased after treating with KAR_1 solutions concentrated at 10^{-9}M and 10^{-7}M, respectively (Flematti *et al.*, 2004). Priming in 1/10 v/v smoke solution made from prairie hay increased germination of *Conyza canadensis* in 24 h darkness at 10/0 ^{o}C (Chapter 5), further supporting the notion that other chemicals that promote germination may be present in the smoke solutions, but not in the fractions separated in this study.

Promoting effects of KAR_1 on seed germination vary among species. Germination of *Cirsium arvense* was not affected by priming of either fractions from smoke solutions made from the 3 tested species, or KAR_1. Not all the species responded to smoke solutions or KAR_1. Zhou *et al.* (2014) tested the germination responses of 13 species from South China to smoke solutions and KAR_1, and only *Aristolochia debilis* showed positive response to commercial smoke solutions and KAR_1. Among the 18 tested species, KAR_1 was effective in enhancing the germination of only 8 species (Daws *et al.*, 2007). Concentrated smoke solutions reduced germination of *Cirsium arvense* at 25/15 ^{o}C (Chpater 5), indicating the presence of the inhibiting or toxic compounds involved in high concentrated smoke solutions (Dixon and Roche, 1995)

Priming in 1/1 v/v KAR_1 reduced radical length of *Cirsium arvense* compared with distilled water in 12 h light/12 darkness at 25/15 ^{o}C. Although most former studies showed the positive or neutral effect of KAR_1 on the seedling growth of different species (Daws *et al.*, 2007; Kulkarni *et al.*, 2007; Stevens *et al.*, 2007), radical length of *Eragrostis curvula* was reduced by KAR_1 at constant 30 and 35 °C regimes, but not at constant 15, 20 and 25 °C (Ghebrehiwot *et al.*, 2009), indicating effects of KAR_1 on seedling length may be temperature and species dependent.

Fractions from smoke solution made from alfalfa had no effects on seed germination and seedling length for all 4 tested species under any studied conditions. However, smoke solutions produced from alfalfa increased seed germination of *Artemisia ludoviciana* and *Conyza conadensis*, as well as the radical length of *Conyza conadensis* at certain conditions (Chapter 5), indicating other stimulants, rather than KAR_1 may be involved in the smoke solution made from alfalfa. Recently, another active compound, named glyceronitrile, was isolated from smoke solution made from the mixture of *Eucalyptus*, *Adenanthos* and *Banksia* species, which promoted germination of *Anigozanthos* species (Flematti *et al.*, 2011; Downes *et al.*, 2013). In the present

study, effects of this potential active compound were not tested. Effects of glyceronitrile on seed germination and seedling growth of species in Fescue Prairie need further studies to determine.

6.5 Conclusions

KAR_1 was the major stimulant in the smoke solutions made from prairie hay and wheat straw, but was not in the smoke solution made from alfalfa, indicating different compounds involved in the smoke solutions originating from different plant materials. Effects of active fractions obtained from smoke solutions produced from alfalfa, prairie hay, and wheat straw, together with KAR_1 solutions on germination and seedling growth varied among species. Priming in concentrated KAR_1 solutions and active fractions obtained from prairie hay and wheat straw increased germination of *Artemisia frigida* and *Artemisia ludoviciana* in 12 h light/12 h darkness at 10/0 °C and 24 h darkness at 25/15 °C, respectively. In 12 h light/12 h darkness at 25/15 °C, priming in diluted KAR_1 solutions and active fractions obtained from prairie hay and wheat straw increased germination of *Conyza canadensis*. In contrast, priming in high concentrated KAR_1 solutions reduced radical length of *Cirsium arvense*. These results suggest active compounds in smoke, which is an important fire cue, has the potential to affect the species composition in Fescue Prairie through their various effects on germination and seedling growth of different species. Understanding the responses of different species to active compounds in smoke and other environmental factors can help us better understand the fire ecology of Fescue Prairie.

7.0 GENERAL DISCUSSION AND CONCLUSIONS

Regeneration after burning in grasslands occurs with vegetative reproduction and seedling emergence from seeds (Everson and Tainton, 1984). Although the importance of fire in regulating plant communities in Fescue Prairie has been well reported (Bailey and Anderson, 1978; Anderson and Bailey, 1980; Wright and Bailey, 1982), little attention has been given to the contribution of different regeneration strategies to altered species composition after burning. Seeds in fire-prone habitat may have evolved adapted strategies to various factors associated with fire (Van Staden *et al.*, 2000). The studies included in this thesis provide link between physiological mechanisms of seed responses to fire cues and seedling recruitment in Fescue Prairie at both the species and community levels.

7.1 Burning modified seedling emergence in the field and from the soil seed bank

Burning increased seedling densities of native forbs, incuding *Artemisia frigida*, *Artemisia ludoviciana*, and *Conyza canadensis*, non-native forbs, such as *Circium arvense*, and *Taraxacum officinale*, and total seedlings emerging in the field compared with the control, and had no negative effects on seedling densities of any other emerged species. Species richness and diversity of seedlings emerging in the field were increased by burning. The density and richness of native forbs emerging from the soil seed bank were increased by burning as functional groups, but that of non-native graminoids were decreased by burning. Field and seed bank studies favored that notion that species composition shifted in favor of perennial forbs after a burn, regardless of the time of burning in Fescue Prairie (Daubenmire, 1968; Bailey and Anderson, 1978; Anderson and Bailey, 1980). Fire has the potential to restore native species and control non-native species in Fescue Prairie, due to its positive effects on seedling densities of native species and negative effects on seedling densities of non-native species emerging from the soil seed bank.

7.2 Direct fire cues played more important role in regulating seedling emergence in Fescue Prairie

The promotive effects of direct fire cues, ash and smoke plus ash specifically, on seedling emergence were supported by testing their effects on seedlings emerging from the soil seed bank. Ash increased densities of *Artemisia frigida* and *Conyza canadensis* seedlings emerging from the

soil seed bank. This was possibly due to the fact that increased soil pH by ash created a better germination condition for these two species. Smoke plus ash increased seedling densities of *Artemisia ludoviciana* from the soil seed bank. Although the possible interactive effect of smoke and ash could be attributed to the fact that the stimulant effects of smoke on germination varied in different pH conditions (Keeley and Fotheringham, 1998), the underlying mechanisms are still vague and further studies are needed. Ash and smoke plus ash had positive effects on density and richness of forbs emerging from the soil seed bank, indicating that improved soil pH conditions is perhaps the main reason for the shift in species composition in favor of forbs after burning in Fescue Prairie.

7.3 Smoke and active compounds involved in were important germination cues for species in Fescue Prairie

There was a lack of positive effects of smoke on density, richness and emergence rate of seedlings emerging from the soil seed bank. This was possibly due to the high concentrated smoke solutions applied. Except for *Artemisia ludoviciana*, priming in highly concentrated smoke solutions made from prairie hay did not affect or reduced seed germination and seedling growth of tested species. Highly concentrated smoke solutions increased seed germination of *Artemisia ludoviciana* by 133% in darkness. The stimulant effects of smoke on germination of positively photoblastic seeds, such as *Lactuca sativa* and *Apium graveolens*, in darkness have been reported (Drewes *et al.*, 1995; Thomas and Van Staden, 1995). In addition, it indicates that *Artemisia ludoviciana* is insensitive or tolerant to the toxic compounds in highly concentrated smoke solutions. Diluted smoke solutions increased radical length of *Artemisia ludoviciana*. Germination of *Cirsium arvense* and *Coynza canadensis* responded to various smoke solutions at 25/15 °C, but not at 15/5 °C, indicating effects of smoke on seed germination and seedling growth depend on germination conditions.

The promotive effects of smoke on germination vary among ecosystems. Germination of 12 out of 28 species was positively affected by smoke in South African fynbos (Brown, 1993). Smoke increased germination of *Syncarpha vestita* by 1000% (Brown, 1993). Vegetation of Mediterranean-type ecosystems also adapt well to the smoke cue. Dixon *et al.* (1995) reported smoke increased germination of 45 out of 94 species of native Western Australian plants. Smoke increased germination of certain species up to 400% (Dixon *et al.*, 1995). In the present study, smoke increased germination of *Artemisia ludoviciana* and *Conyza canadensis* by 100% in

certain germination conditions. Abu (2013) found germination of 4 out of 10 species in Fescue Prairie was positively affected by smoke, with the highest increased by 50%. The various responses of smoke on seed germination among different ecosystems may be due to the different fire regimes, such as fire frequency occurring in different ecosystems, which in turn lead to the different adaptation of seeds to the fire cues.

7.4 Different active compounds existed in smoke made from different materials, which in turn affected germination and seedling growth differently.

Karrikinolide (KAR_1), the most common active compounds in the karrikins family, was the major stimulant in smoke solutions made from two graminoids, namely prairie hay and wheat straw, but was not in the smoke solution made from alfalfa, a herbaceous Leguminosae. However, smoke solutions produced from alfalfa increased seed germination of *Artemisia ludoviciana* and *Conyza conadensis*, as well as the radical length of *Conyza conadensis* at certain conditions, indicating other active stimulants, rather than KAR_1 may be involved in the smoke solution made from alfalfa. This was the first time to provide direct evidence that different compounds existed in smoke made from different materials. The unique metabolic approaches in fixing N_2 in the Leguminosae may account for the different compounds created in smoke compared with plants in other families. Different effects of smoke solutions originated from different plant material on germination. For example, at 25/15 °C in 24 h darkness, concentrated smoke solutions made from alfalfa increased germination of *Conyza canadensis*, while same concentrated smoke solutions made from prairie hay and wheat straw reduced germination of *Conyza canadensis*. More KAR_1 contained in the active fraction from prairie hay as compared with that in the wheat straw. Priming in concentrated active fraction from prairie hay but not wheat straw increased germination of *Artemisia frigida* compared with distilled water at 10/0 °C in 12h Light/ 12h darkness. This result indicates not only qualitative but also quantitative variations existed in smoke solutions made from different material, which in turn may affect germination of species differently. Priming in high concentrated active fractions from prairie hay, but not the smoke solution made from prairie hay increased germination of *Artemisia frigida* compared with distilled water in certain germination conditions, indicating germination inhibitors may exist in the crude smoke solution, which reduced the effect of KAR_1 on seed germination (Light *et al.*, 2010). Concentrated smoke solutions, but not KAR_1, increased germination of *Artemisia ludoviciana* in light and darkness at 10/0 ^{o}C, indicating other active

compounds that promote germination of *Artemisia ludoviciana* may be present in the smoke solutions. Germination and seedling growth of tested species responded only to active fractions and KAR_1 under certain condition. For example, priming in low concentrated active fractions made from prairie hay and wheat straw and KAR_1 solutions only stimulated seed germination of *Conyza canadensis* in 12 h light/12 h darkness at 25/15 ^{o}C, indicating effects of KAR_1 and active fractions on germination and seedling growth were dependent on environmental conditions.

In conclusion, fire and direct fire cues, smoke and ash specifically, stimulate seedling recruitment of some species, especially early seral species and native forbs, having the potential in changing species composition and restoring native species of the Fescue Prairie. For the first time, it was shown that different compounds involved in smoke solutions originated from legumes as compared with that made from graminoids. KAR_1, the major active compounds found in smoke previously exist in smoke solutions made from prairie hay and wheat straw, but not in that made from alfalfa. Other active compounds, not in the group of karrikins, existed in the smoke solutions. Effects of smoke and active compounds involved in seed germination and seedling growth are species and temperature dependent.

8.0 LITERATURE CITED

Abella, S. R. (2009) Smoke-cued emergence in plant species of ponderosa pine forest: contrasting greenhouse and field results. *Fire Ecology* **5**, 22-37.

Abu, Y. (2014) Priming seeds in aqueous smoke solutions improves germination of *Agropyron dasystachyum, Dactylis glomerata*, *Elymus angustus*, *Elymus junceus*, and *Festuca hallii*. Msc thesis, University of Saskachewan, Saskatoon, Canada.

Acton, D. F. and Ellis, J. G. (1978) The soils of the Saskatoon map area. Saskatchewan Institute of Pedology, University of Saskatchewan, Saskatoon, Sask. Publication S4.

Adkins, S. W. and Peters, M. C. B. (2001) Smoke derived from burnt vegetation stimulates germination of arable weeds. *Seed Science Research* **11**, 213-222.

Alberta Prairie Forum, (2007) Prairie conservation (http://www.albertapcf.org/Prairie_Conservat-ion.html) (accessed November 5, 2014)

Anderson, H. G. and Bailey, A. W. (1980) Effects of annual burning on grassland in the Aspen parkland of east-central Alberta. *Canidian Journal of Botany* **58**, 985-996.

Anderson, T. M., Schutz, M. and Risch, A. C. (2012) Seed germination cues and the importance of the soil seed bank across environmental gradient in the Serengeti. *Oikos* **121**, 306-312.

Archibold, O. W. (1981) Buried viable propagules in native prairie and adjacent agricultural sites in central Saskatchewan. *Canadian Journal of Botany* **59**, 701-706.

Archibold, O. W., Nelson, L. J., Ripley, E. A. and Delanoy, L. (1998) Fire temperatures in plant communities of the Northern Mixed Prairie. *Canadian Field-Naturalist* **112**, 234-240.

Archibold, O. W., Ripley, E. A. and Delanoy, L. (2003) Effects of season of burning on the microenvironment of fescue prairie in Central Saskatchewan. *Canadian Field-Naturalist* **117**, 257-266.

Arruda, Y. M. B. C., Ferraz, I. D. K. and Albuquerque, M. C. F. (2012) Sources and concentrations of smoke-water on the seed germination and seedling vigor of tomato. *Horticultura Brasileira* **30**, 293-299.

Ashton, D. H. (1986) Viability of seeds of *Eucalyptus obliqua* and *Leptospermum juniperinum* from capsules subjected to a crown fire. *Australian Forestry* **49**, 28-35.

Auld, T. D. and Bradstock, R. A. (1996) Soil temperature after the passage of fire: Do they influence the germination of buried seeds? *Australian Journal of Ecology* **21**, 106-109.

Auld, T. D. and O'Connell, M. A. (1991) Predicting patterns of postfire germination in 35 Eastern Australian Fabaceae. *Australian Journal of Ecology* **16**, 53-70.

Bai, Y. and Romo, J. T. (1997) Seed production, seed rain, and the seedbank of fringed sagebrush. *Journal of Range Management* **50**, 151-155.

Bai, Y. G. and Romo, J. T. (1994) Germination of previously buried seeds of fringed sage (*Artemisia frigida*). *Weed Science* **42**, 390-397.

Bai, Y. G., Romo, J. T. and Hou, J. Q. (1996) Phytochrome action in seed germination of fringed sage (*Artemisia frigida*). *Weed Science* **44**, 109-113.

Bailey, A. W. and Anderson, H. G. (1978) Prescribed burning of a Festuca-Stipa grassland. *Journal of Range Management* **31**, 446-449.

Bailey, A. W. and Anderson, M. L. (1980) Fire temperatures in grass, shrub and aspen forest communities of central Alberta. *Journal of Range Management* **33**, 37-40.

Bailey, A. W. and Wroe, R. A. (1974) Aspen invasion in a protion of Alberta parklands. *Journal of Range Management* **27**, 263-266.

Baldwin, I. T., Staszak-Kosinski, R. and Davidson, R. (1994) Up in Smoke I. Smoke derived germination cues for post fire annual, *Nicotiana attenuata* Torr. Ex. Watson. *Journal of Chemical Ecology* **20**, 2345-2371.

Banting, J. D. (1966) Studies on the persistence of *Avena fatua*. *Canadian Journal of Plant Science* **46**, 129-140.

Bargmann, T., Maren, I. E. and Vandvid, V. (2014) Life after fire: smoke and ash as germination cues in ericads, herbs and graminoids of northern heathlands. *Applied Vegetation Science* **17**, 670-679.

Barrett, S. W. (1999) Fire regimes on the Pine Butte Swamp Preserve, Montana. Final Report for the Nature Conservancy, Helena, Montana.

Baskin, C. C. and Baskin, J. M. (1998) Seeds: Ecology, biogeography, and evolution of dormancy and germination, Academic Press, San Diego, California.

Batlla, D., Verges, V. and Benech-Arnold, R. L. (2003) A quantitative analysis of seed responses to cycle-does of fluctuating temperatures in relation to dormancy: Development of a thermal time model for *Polygonum aviculare* L. seeds. *Seed Science Research* **13**, 197-207.

Baxter, B. J. M. and Van Staden, J. (1994) Plant derived smoke an effective seed pre-treatment. *Plant Growth Regulation* **14**, 279-282.

Baxter, B., Granger, J. and Van Staden, J. (1995) Plant-derived smoke and seed germination: is all smoke good smoke? That is the burning question. *South African Journal of Botany* **61**, 275-277.

Becker, D. A. (1989) Five years of annual prairie burns, pp. 163-168 in Bragg, T. B. and Stubbendieck, J. (eds.), *Proceedings of the Eleventh North American Prairie Conference*. University of Nebraska, Lincoln, Nebraska.

Bell, D. T. (1994) Interaction of fire, temperature and light in the germination response of 16 species from the *Eucalyptus marginata* forest of South-western Western Australia. *Australian Journal of Botany* **42**, 501-509.

Bell, D. T. (2001) Ecological response syndromes in the flora of southwestern Western Australia: Fire resprouters versus reseeders. *Botanical Review* **67**, 417-440.

Benech-Arnold, R. L., Sanchez, R. A., Forcella, F., Kruk, B. C. and Ghersa, C. M. (2000) Environmental control of dormancy in weed seed banks in soil. *Field Crops Research* **67**, 105-122.

Benoit, D. L., Kenkel, N. C. and Cavers, P. B. (1989) Factors influencing the precision of soil seed bank estimates. *Canadian Journal of Botany* **67**, 2833-2840.

Bewley, J. D. (1997) Seed germination and dormancy. *Plant Cell* **9**, 1055-1066.

Blank, R. R. and Young, J. A. (1998) Heated substrate and smoke: Inluence on seed emergence and plant growth. *Journal of Range Management* **51**, 577-583.

Biswell, H. H. (1974) Effects of fire on chaparral. pp. 321-364. In Kozlowski, T. T. and Ahlgren, C. E. *Fire and Ecosystems (Physiological Ecology)*, Academic press, New York, NY.

Bochenek, A., Golaszewski, J. and Piotrowicz-Cieslak, A. I. (2009) The effects of temperature on the dormancy and germination of *Cirsium arvense* (L.) Scop. seeds. *Acta Societatis Botanicorum Poloniae* **78**, 105-114.

Bond, W. J. and Keeley, J. E. (2005) Fire as a global 'herbivore': the ecology and evolution of flammable ecosystems. *Trends in Ecology and Evolution* **20**, 387-394.

Bond, W. J. and Midgley, J. J. (2001) Ecology of sprouting in woody plants: the persistence niche. *Trends in Ecology and Evolution* **16**, 45-51.

Bossuyt, B. and Honnay, O. (2008) Heat shock increases the reliability of a temperate calcareous grassland seed bank study. *Plant Ecology* **199**, 1-7.

Bradstock, R. A., Gill, A. M., Hastings, S. M. and Moore, P. H. R. (1994) Survival of serotinous seedbanks during bushfires: Comparative studies of *Hakea* species from southeastern Australia. *Australian Journal of Ecology* **19**, 276-282.

Brits, G. J. (1986) Influence of fluctuating temperatures and H_2O_2 treatment on the germination of *Leucospermum cordifolium* and *Serruria florida* (Proteaceae) seeds. *South African Journal of Botany* **123**, 286-290.

Brits, G. J., Calitz, F. L., Brown, N. A. C. and Manning, J. C. (1993) Desiccation as the active principle in heat-stimulated seed germination of *Leucospermum* R. Br. (Proteaceae) in fynbos. *New Phytologist* **125**, 397-403.

Brown, N. A. C. (1993) Promotion of germination of fynbos seeds by plant-derived smoke. *New Phytologist* **123**, 575–583.

Brown, N. A. C. and Van Staden, J. (1997) Smoke as a germination cue: A review. *Plant Growth Regulation* **22**, 115-124.

Brown, N. A. C., Jamieson, H. and Botha, P. A. (1994) Stimulation of germination in South African species of Restionaceae by plant derived smoke. *Plant Growth Regulation* **15**, 93-100.

Brown, N. A. C., Kotze, G. and Botha, P. A. (1993) The promotion of seed germination of cape Erica species by plant-derived smoke. *Seed Science Technology* **21**, 179-185.

Brown, N. A. C., Van Staden, J., Daws, M. I. and Johnson, T. (2003) Patterns in the seed germination response to smoke in plants from the Cape Floral Region. *South Africa Journal of Botany* **69**, 514–525.

Bullowa, S., Negbi, M. and Ozeri, Y. (1975) Role of temperature light and growth regulators in germination in *Anemone coronaria*. *Australian Journal of Plant Physiology* **2**, 91-100.

Bushey, C. L. (1985) Summary of results from the galena gulch 1982 spring burns (units 1b). Systems for Environmental Management, Missoula, MT

Catav, S. S., Bekar, I., Ates, B. S., Ergan, G., Oymak, F., Ulker, E. D. and Tavsanoglu, C. (2012) Germination response of five eastern Mediterranean woody species to smoke solution derived from various plant. *Turkish Journal of Botany* **36**, 480-487.

Cespedes, B., Torres, I., Luna, B., Perez, B. and Moreno, J. M. (2012) Soil seed bank, fire season, and temporal patterns of germination in a seeder-dominated Mediterranean shrubland. *Plant Ecology* **213**, 383-393.

Champness, S. S. and Morris, K. (1948) The population of buried viable seeds in relation to contrasting pasture and soil types. *Journal of Ecology* **36**, 149-173.

Chauvel, B., Gasquez, J. and Darmency, H. (1989) Changes of weed seed bank parameters according to species, time and environment. *Weed Research* **29**, 213-219.

Chen, H., Cao, M., Baskin, J. M. and Baskin, C. C. (2013) Temperature regulates positively photoblastic seed germination in four *Ficus* (Moraceae) tree species from contrasting habitats in a seasonal tropical rainforest. *American Journal of Botany* **100**, 1683-1687.

Chiwocha, S. D. S., Dixon, K. W., Flematti, G. R., Ghisalberti, E. L., Merritt, D. J., Nelson, D. C., Riseborough, J. M., Smith, S. M. and Stevens, J. C. (2009) Karrikins: A new family of plant growth regulators in smoke. *Plant Science* **177**, 252-256.

Clarke, S. and French, K. (2005) Germination response to heat and smoke of 22 Poaceae species from grassy woodlands. *Australian Journal of Botany* **53**, 445-454.

Cochrane, A., Monks, L. and Lally, T. (2007) Response of the germinatble soil-stored seed bank of a remnant reserve in the southern Western Australia agricultural zone to smoke and fire treatment. *Journal of the Royal Society of Western Australia* **90**, 47-52.

Collins, S. L. (1987) Interaction of disturbances in tallgrass prairie: a field experiment. *Ecology* **68**, 1243-1250.

Commander, L. E., Merritt, D. J., Rokich, D. P. and Dixon, K. W. (2009) Seed biology of Australian arid zone species: germination of 18 species used for rehabilitation. *Journal of Arid Environments* **73**, 617-625.

Commander, L. E., Merritt, D. J., Rokich, D. P., Flematti, G. R. and Dixon, K. W. (2008) Seed germination of *Solanum* spp. (Solanaceae) for use in rehabilitation and commercial industries. *Australian Journal of Botany* **56**, 333-341.

Coupland, R. T. (1950) Ecology of Mixed Prairie in Canada. *Ecologiccal Monographs* **20**, 271-315.

Coupland, R. T. (1961) A reconsideration of grassland classification in the northern great plains of North-America. *Journal of Ecology* **49**, 135-167.

Coupland, R. T. and Brayshaw, T. C. (1953) The fescue grassland in Saskatchewan. *Ecology* **34**, 386-405.

Crandall, R. M. and Platt, W. J. (2012) Habitat and fire heterogeneity explain the co-occurrence of congeneric resprouter and reseeder *Hypericum* spp, along a Florida pine savanna ecocline. *Plant Ecology* **213**, 1634-1654.

Crosti, R., Ladd, P. G., Dixon, K. W. and Piotto, B. (2006) Post germination: The effect of smoke on seeds of selected species from the central Mediterranean Basin. *Forest Ecology and Management* **221**, 306-312.

Csontos, P. (2007) Seed banks: ecological definitions and sampling considerations. *Community Ecology* **8**, 75-85.

Cumming, B. G. and Hay, J. R. (1958) Light and dormancy in wild oats (*Avena fatua* L.). *Nature* **182**, 60-610.

Curtin, D. and Ukrainetz, H. (1997) Acidification rate of limed soil in a semiarid environment. *Canadian Journal of Soil Science* 77, 415-420.

Daubenmire, R. (1968) Ecology of fire in grasslands. *Advances in Ecological Research* **5**, 209-266.

Daws, M. I., Davies, J., Pritchard, H. W., Brown, N. A. C. and Van Staden, J. (2007) Butenolide from plant-derived smoke enhances germination and seedling growth of arable weed species. *Plant Growth Regulation* **51**, 73-82.

De Lange, J. H. and Boucher, C. (1990) Autecological studies on *Audouinia-capitata* Bruniaceae I. Plant-derived smoke as a seed germination cue. *South African Journal of Botany* **56**, 700-703.

De Luis, M., Verdu, M. and Raventos, J. (2008) Early to rise makes a plant healthy, wealthy, and wise. *Ecology* **89**, 3061-3071.

Dejong, E. and Macdonald, K. B. (1975) Soil-moisture regime under native grassland. *Geoderma* **14**, 207-221.

Dessaint, F., Chadoeuf, R. and Barralis, G. (1991) Spatial pattern analysis of weed seeds in the cultivated soil seed bank. *Journal of Applied Ecology* **28**, 721-730.

DiTomaso, J. M., Kyser, G. B. and Hastings, M. S. (1999) Prescribed burning for control of yellow starthistle (*Centaurea solstitialis*) and enhanced native plant diverisity. *Weed Science* **47**, 233-242.

Dixon, K. W., Roche, S., and Pate, J. S. (1995) The promotive effect of smoke derived from burnt native vegetation on seed germination of western Australian plants. *Oecologia* **101**, 185-192.

Dixon, K. W. and Roche, S. (1995) The role of combination products (smoke) in stimulating *ex-situ* and *in-situ* germination of western Australian plants. *Proceedings of International Plant Propagation Society* **45**, 53-56.

Donald, W. W. (1994) The biology of Canada thistle (*Cirsium arvense*). *Reviews of Weed Science* **6**, 77-101.

Downes, K. S., Lamont, B. B., Light, M. E. and Van Staden, J. (2010) The fire ephemeral *Tersonia cyathiflora* (Gyrostemonaceae) germinates in response to smoke but not the butenolide 3-methyl-2H-furo[2,3-c]pyran-2-one. *Annals of Botany* **106**, 381-384.

Downes, K. S., Light, M. E., Posta, M., Kohout, L. and Van Staden, J. (2013) Comparison of germination responses of *Anigozanthos flavidus* (Haemodoraceae), *Gyrostemon racemiger* and *Gyrostemon ramulosus* (Gyrostemonaceae) to smoke-water and the smoke-derived compounds karrikinolide (KAR_1) and glyceronitrile. *Annals of Botany* **111**, 489-497.

Drewa, P. B., Platt, W. J. and Moser, E. B. (2002) Fire effects on resprouting of shrubs in headwaters of southeastern longleaf pine savannas. *Ecology* **83**, 755-767.

Drewes, F., Smith, M. and Staden, J. (1995) The effect of a plant-derived smoke extract on the germination of light-sensitive lettuce seed. *Plant Growth Regulation* **16**, 205-209.

Dufrene, M. and Legender, P. (1997) Species assemblages and indicator species: the need for a flexible asymmetrical approach. *Ecological Monographs* **67**, 345-366.

Dunn, P. H. and Debano, L. F. (1977) Fire's effect on biological and chemical properties of chaparral soils. pp. 75-84. In: Mooney, H. A. and Conrad, C. E. *Proceedings of the symposium on the environmental consequences of fire and fuel management in Mediterranean ecosystems*, Palo Alto, Ca, USA.

Dziewanowska, K., Niedzwiedz, I. and Chodelska, I. (1979) Hydrogen-cyanide and cyanogenic compounds in seeds. *Physiologie Vegetale* **17**, 297-303.

Egerton-Warburton, L. M. (1998) A smoke-induced alteration of the sub-testa cuticle in seeds of the post-fire recruiter, *Emmenanthe penduliflora* Benth. (Hydrophyllaceae). *Journal of Experimental Botany* **49**, 1317-1327.

Eichhorn, L. C. and Watts, C. R. (1984) Plant succession on burns in the river breaks of central Montana. *Proceedings of the Montana Academy of Sciences* **43**, 21-34.

Enright, N. J., Fontaine, J. B., Westcott, V. C., Lade, J. C. and Miller, B. P. (2011) Fire interval effects on persistence of resprouter species in Mediterranean-type shrublands. *Plant Ecology* **212**, 2071-2083.

Enright, N. J. and Goldblum, D. (1999) Demography of a non-sprouting and resprouting *Hakea* species (Proteaceae) in fire prone *Eucalyptus* woodlands of southeastern Australia in relation to stand age, drought and disease. *Plant Ecology* **144**, 71-82.

Enright, N. J., Goldblum, D., Ata, P. and Ashton, D. H. (1997) The independent effects of heat, smoke and ash on emergence of seedlings from the soil seed bank of heathy *Eucalyptus* Woodland in Gramipians (Gariwerd) National Park, western Victoria. *Australian Journal of Ecology* **22**, 81-88.

Enright, N. J. and Kintrup, A. (2001) Effects of smoke, heat and charred wood on the germination of dormant soil-stored seeds from a *Eucalyptus baxteri* heathy-woodland in Victoria, SE Australia. *Austral Ecology* **26**, 132-141.

Environment Canada (2012) Daily data report (http://climate.weather.gc.ca/climateData/dailydata_e.html?StationID=47707&timeframe=2&cmdB1=Go&Year=2012&Month=8&cmdB1=Go#) (accessed March 15, 2013)

Environment Canada (2013) Climate normals, 1971-2000 station data (http://climate.weather.gc.ca/climate_normals/results_e.html?stnID=3328&lang=e&dCode=1&province=ALL&provBut=Go&month1=0&month2=12Annual temp and pre average) (accessed November 15, 2013)

Environment Canada (2013) Daily data report (http://climate.weather.gc.ca/climateData/dailydata_e.html?StationID=47707&timeframe=2&cmdB1=Go&Year=2013&Month=8&cmdB1=Go#) (accessed April 10, 2014)

Environment Canada (2014) Daily data report (http://climate.weather.gc.ca/climateData/dailydata_e.html?StationID=47707&timeframe=2&cmdB1=Go&Year=2014&Month=8&cmdB1=Go#) (accessed April 10, 2014)

Everson, C. S. and Tainton, N. M. (1984) The effect of thirty years of burning on the highland sourveld of Natal. *Journal of the Grassland Society of Southern Africa* **1**, 15-20.

Everson, T. (1994) Seedling establishment of *Themeda triandra* Forssk. In the Montane grasslands of Natal. PhD thesis. University of Natal, Pietermaritzburg, South Africa.

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

Ferrandis, P., Herranz, J. M. and Martinez-Sanchez, J. J. (2001) Response to fire of a predominantly transient seed bank in a Mediterranean weedy pasture (eastern-central Spain). *Ecoscience* **8**, 211-219.

Figueroa, J. A. and Cavieres, L. A. (2012) The effect of heat and smoke on the emergence of exotic and native seedlings in a Mediterranean fire-free matorral of central Chile. *Revista Chilena De Historia Natural* **85**, 101-111.

Finch-Savage, W. E. and Leubner-Metzger, G. (2006) Seed dormancy and the control of germination. *New Phytologist* **171**, 501-523.

Flematti, G. R., Ghisalberti, E. L., Dixon, K. W. and Trengove, R. D. (2004) Compound from smoke that promotes seed germination. *Science* **305**, 977.

Flematti, G. R., Ghisalberti, E. L., Dixon, K. W. and Trengove, R. D. (2009) Identification of alkyl substituted *2H*-Furo[2,3-c]pyran-2-ones as germination stimulants present in smoke. *Journal of Agricultural and Food Chemistry* **57**, 9475-9480.

Flematti, G. R., Ghisalberti, E. L., Dixon, K. W. and Trengove, R. D. (2008) Germination stimulant in smoke: isolation and identification. pp. 531-544 in Colegate, S. M. and Molyneux, R. J. *Bioactive natural products: detection, isolation and structural determination*, 2nd ed, CRC Press, Boca Raton.

Flematti, G. R., Merritt, D. J., Piggott, M. J., Trengove, R. D., Smith, S. M., Dixon, K. W. and Ghisalberti, E. L. (2011) Burning vegetation produces cyanohydrins that liberate cyanide and stimulate seed germination. *Nature Communications* **2**, 360.

Floyd, A. G. (1976) Effect of burning on regeneration from seeds in wet sclerophyll forest. *Australian Forestry* **39**, 210-220.

Fukumi, N. and Nakata, N. (2008) Emergence pattern and seed dormancy of *Aeschynomene indica* L. in paddy field. *Journal of Weed Science and Technology* **53**, 185-191.

Gardner, M. J., Dalling, K. J. and Light, M. E. (2001) Does smoke substitute for red light in the germination of light-sensitive lettuce seeds by affecting gibberellin metabolism? *South African Journal of Botany* **67**, 636-640.

Gerling, H. S., Bailey, A. W. and Willms, W. D. (1995) The effects of burning on *Festuca hallii* in the praklands of central Alberta. *Canadian Journal of Botany* **73**, 937-942.

Ghebrehiwot, H. M., Kulkarni, G. and Kirkman, K. P. (2009) Smoke solutions and temperature influence the germination and seedling growth of South African mesic grassland species. *Rangeland Ecology and Management* **62**, 572-578.

Ghebrehiwot, H. M., Kulkarni, M. G. and Szalai, G. (2013) Karrikinolide residues in grassland soils following fire: Implications on germination activity. *South African Journal of Botany* **88**, 419-424.

Ghebrehiwot, H. M., Kulkarni, M. G., Kirkman, K. P. and Van Staden, J. (2009) Smoke solutions and temperature influence the germination and seedling growth of South African mesic grassland species. *Rangeland Ecology and Management* **62**, 572-578.

Ghebrehiwot, H. M., Kulkarni, M. G., Kirkman, K. P. and Van Staden, J. (2012) Smoke and heat: influence on seedling emergence from the germinable soil seed bank of mesic grassland in South Africa. *Plant Growth Regulation* **66**, 119-127.

Ghebrehiwot, H. M., Kulkarni, M. G., Kirkman, K. P. and Van Staden, J. (2011) Germination activity of smoke residues in soils following a fire. *South African Journal of Botany* **77**, 718-724.

Ghermandi, L., Gonzalez, S., Natalia Lescano, M. and Oddi, F. (2013) Effects of fire severity on early recovery of Patagonian steppes. *International Journal of Wildland Fire* **22**, 1055-1062.

Gilmour, C. A., Crowden, R. K. and Koutoulis, A. (2000) Heat shock, smoke and darkness: partner cues in promoting seed germination in *Epacris tasmanica* (Epacridaceae). *Australian Journal of Botany* **48**, 603-609.

Gonzalez-Rabanal, F. and Casal, M. (1995) Effect of high temperatures and ash on germination of ten species gorse shrubland. *Vegetatio* **116**, 123-131.

Gonzalez, S. and Ghermandi, L. (2008) Postfire seed bank dynamics in semiarid grasslands. *Plant Ecolgoy* **199**, 175-185.

Grant, C. D. and Koch, J. M. (1997) Ecological aspects of soil seed banks in relation to bauxite mining. 2. Twelve year old rehabilitated mines. *Australian Journal of Ecology* **22**, 177-184.

Grappin, P., Bouinot, D., Sotta, B., Miginiac, E. and Jullien, M. (2000) Control of seed dormancy in *Nicotiana plumbaginifolia*: Post-imbibition abscisic acid synthesis imposes dormancy maintenance. *Planta* **210**, 279–285.

Grilz, P. L. and Romo, J. T. (1994) Water relations and growth of *Bromus inermis* Leyss. (smooth brome) following spring or autumn burning in a fescue prairie. *American Midland Naturalist* **132**, 340-348.

Grilz, P. L. and Romo, J. T. (1995) Management considerations for controlling smooth brome on Fescue Prairie. *Natural Areas Journal* **15**, 148-156.

Grime, J. P. (2006) Trait convergence and trait divergence in herbaceous communities: Mechanisms and consequences. *Journal of Vegetation Science* **17**, 255-260.

Gross, D. V. and Romo, J. T. (2010) Temporal changes in species composition in Fescue Prairie:relationships with burning history, time of burning, and environmental conditions. *Plant Ecology* **208**, 137-153.

Gross, K. L. (1990) A comparison of methods for estimating seed numbers in the soil. *Journal of Ecology* **78**, 1079-1093.

Guttridge, C. G. and Bright, S. E. (1978) Accelerating and synchronising germination of strawberry seeds by osmotic pre-treatment. *Euphytica* **27**, 843-848.

Hampton, J. G., Martinelli, A. H., Farrant, J. M., Schmiermann, H. M. J., Powell, A. A., Abdelmonem, A. M., Mtindi, M., Come, D. and Ednie, A. B. (1999) Seed technology-past, present and future. *Seed Science and Technology* **27**, 681-702.

Harper, J. L. (1977) Population biology of plants, Academic Press, London, UK.

He, T., Pausas, J. G., Belcher, C. M., Schwild, D. W. and Lamont, B. B. (2012) Fire-adapted traits of *Pinus* arose in the fiery Cretaceous. *New Phytologist* **194**, 751-759.

Heimann, B. and Cussans, G. W. (1996) The importance of seeds and sexual reproduction in the population biology of *Cirsium arvense* – a literature review. *Weed Research* **36**, 493-503.

Hendricks, S. B. and Taylorso, R. B. (1972) Promotion of seed germination by nitrates and cyanides. *Nature* **237**, 169.

Henig-Server, N., Eshel, A. and Ne'eman, G. (1996) pH and osmotic potential of pine ash as post-fire germination inhibitors. *Physiologia Plantarum* **96**, 71-76.

Hilton, J. R. (1982) An unusual effect of the far-red absorbing form of phytochrome-photoinhibition of seed germination in *Bromus sterilis* L. *Planta* **155**, 524-528.

Inouye, D. W., Saavedra, F. and Lee-Yang, W. (2003) Environmental influences on the phenology and abundance of flowering by *Androsace septentrionalis* (Primulaceae). *American Journal of Botany* **90**, 905-910.

Izhaki, I., Henig-Sever, N. and Ne'Eman, G. (2000) Soil seed bak in Mediterranean Aleppo pine forests: the effect of heat, cover and ash on seedling emergence. *Journal of Ecology* **88**, 667-675.

Jager, A. K., Light, M. E. and Van Staden, J. (1996) Effects of source of plant material and temperature on the production of smoke extracts that promote germination of light-sensitive lettuce seeds. *Environmental and Experimental Botany* **36**, 421-429.

Jain, N., Kulkarni, M. G. and Van Staden, J. (2006) A butenolide, isolated from smoke, can overcome the detrimental effects of extreme temperatures during tomato seed germination. *Plant Growth Regulation* **49**, 263-267.

Jain, N., Stirk, W. A. and Van Staden, J. (2008) Cytokinin and auxin-like activity of a butenolide isolated from plant-derived smoke. *South African Journal Botany* **74**, 327–331.

Jefferson, L. V., Pennacchio, M. and Havens-Young, K. (2014) Ecology of plant-derived smoke: its use in seed germination. Oxford University Press, New York, NY.

Johnston, A. and Smoliak, S. (1968) Reclaiming brushland in southwestern Alberta. *Journal of Range Management* **21**, 404-406.

Judd, T. S. (1993) Seed survival in small myrtaceous capsules subjected to experimental heating. *Oecologia* **93**, 576-581.

Jutila, H. M. and Grace, J. B. (2002) Effects of disturbance on germination and seedling establishment in a coastal prairie grassland: a test of the competitive release hypothesis. J*ournal of Ecology* **90**, 291-302.

Karlsson, L. M. and Milberg, P. (2007) Comparing after-ripening response and germination requirements of *Conyza Canadensis* and *C. bonariensis* (Asteraceae) through logistic functions. *Weed Research* **47**, 433-441.

Keeley, J. E. (1991) Seed germination and life history syndromes in the California chaparral. *Botanical Review* **57**, 81-116.

Keeley, J. E. (2009) Fire intensity, fire severity and burn severity: a brief review and suggested usage. *International Journal of Wildland Fire* **18**, 116-126.

Keeley, J. E. (1986) Resilience of Mediterranean shrub communities to fire, pp. 95-112 in Dell, B., Hopkins, A. J. M. and Lamont, B. B. (eds.), *Resilience in Mediterranean-type ecosystems*. Dr. W. Junk Publishers, Dordrecht, Netherlands.

Keeley, J. E. and Brenna, T. J. (2012) Fire-driven alien invasion in a fire-adapted ecosystem. *Oecologia* **169**, 1043-1052.

Keeley, J. E. and Fotheringham, C. J. (1997) Trace gas emissions and smoke-induced seed germination. *Science* **276**, 1248-1250.

Keeley, J. E. and Fotheringham, C. J. (1998) Smoke-induced seed germination in California chaparral. *Ecology* **79**, 2320-2336.

Keeley, J. E., Fotheringham, C. J. and Morais, M. (1999) Reexamining fire suppression impacts on brushland fire regimes. *Science* **5421**, 1829-1832.

Keeley, J. E. and Fotheringham, C. J. (2000) Role of fire in regeneration from seed. pp. 311-330. In Fenner, M. *Seeds: the ecology of regeneration in plant communities*, 2nd ed, CAB International, Oxon, UK.

Keeley, J. E. and Zedler, P. H. (1978) Reproduction of chaparral shrubs after fire – comparison of sprouting and seedling strategies. *American Midland Naturalist* **99**, 142-161.

Keeley, J. E., Pausas, J. G., Rundel, P. W., Bond, W. J. and Bradstock, R. A. (2011) Fire as an evolutionary pressure shaping plant traits. *Trends in Plant Science* **16**, 406-411.

Keeley, J. E., Bond, W. J., Bradstock, R. A., Pausas, J. G. and Rundel, P. W. (2012) Fire in Mediterranean ecosystems: ecology, evolution and management. Cambridge University Press, Cambridge, UK.

Keeley, J. E., Morton, B. A. and Pedrosa, A. (1985) Role of allelopathy, heat and charred wood in the germination of chaparral herbs and suffrutescents. *Journal of Ecology* **73**, 445-458.

Keith, D. A. (1997) Combined effects of heat shock, smoke and darkness on germination of *Epacris stuartii* Stapf., an endangered fire-prone Australian shrub. *Oecologia* **112**, 340-344.

Kenny, B. J. (2000) Influence of multiple fire-related germination cues on three Sydney *Grevillea* (Proteaceae) species. *Austral Ecology* **25**, 664-669.

Knapp, A. (2000) An overview of seed dormancy in native warm-season grasses. pp. 107-122 *in* Moore, K. J. and Anderson, B. E. (Eds.) *Native warm-season grasses: Research trends and issues*. CSSA Special publication no. 30. Crop Science Society of America, Madison, Wisconsin.

Kozlowski, T. T. (1972) Seed biology. Vol. 1-3. Academic Press, New York, NY.

Komarova, T. A. (1985) Role of forest fires in the germination of seeds dormant in the soil. *Ekologiya* **6**, 3-8.

Kucear, B., Cohn, M. A. and Leubner-Metzger, G. (2005) Plant hormone interactions during seed dormancy release and germination. *Seed Science Research* **15**, 281-307.

Kulkarni, M. G., Sparg, S. G. and Van Staden, J. (2007) Germination and post-germination response of Acacia seeds to smoke-water and butenolide, a smoke-derived compound. *Journal of Arid Environments* **69**, 177-187.

Kulkarni, M. G., Sparg, S. G., Light, M. E. and Van Staden, J. (2006) Stimulation of rice (*Oryza sativa* L.) seedling vigour by smoke-water and butenolide. *Journal of Agronomy and Crop Science* **192**, 395-398.

Lahti, D. C., Johnson, N. A., Ajie, B. C., Otto, S. P., Hendry, A. P., Blumstein, D. T., Coss, R. G., Donohue, K. and Foster, S. A. (2009) Relaxed selection in the wild. *Trends in Ecology and Evolution* **24**, 487-496.

Lara Vanessa, J., Marcello, P. and Kayri, H. (2014) Ecology of plant-derived smoke: its use in seed germination. Oxford University Press, New York.

Leck, M. A., Parker, V. T. and Simpson, R. L. (1989) Ecology of soil seed banks. Academic Press, San Diego.

Leroux, G. D., Benoit, D. L. and Banville, S. (1996) Effect of crop rotations on weed control, *Bidens cernua* and *Erigeron canadensis* populations, and carrot yields in organic soils. *Crop Protection* **15**, 171-178.

Light, M. E., Burger, B. V., Staerk, D., Kohout, L. and Van Staden, J. (2010) Butenolides from plant-derived smoke: Natural plant-growth regulators with antagonistic actions on seed germination. *Journal of Natural Products* **73**, 267-269.

Light, M. E., Gardner, M. J. and Van Staden, J. (2002) Dual regulation of seed germination by smoke solutions. *Plant Growth Regulation* **37**, 135-141.

Lloyd, M. V., Dixon, K. W. and Sivasithamparam, K. (2000) Comparative effects of different smoke treatments on germination of Australian native plants. *Austral Ecology* **25**, 610-615.

Long, R. L., Stevens, J. C., Griffiths, E. M., Adamek, M., Gorecki, M. J., Powles, S. B. and Merritt, D. J. (2011) Seeds of Brassicaceae weeds have an inherent or inducible response to the germination stimulant karrikinolide. *Annals of Botany* **108**, 933-944.

Looman, J. and Best, K. F. (1979) Budd's flora of the Canadian prairie provinces, Agriculture Canada, Ottawa, Ontario.

MacDonald, N. W., Scull, B. T. and Abella, S. R. (2007) Mid-spring burning reduces spotted knapweed and increases native grasses during a Michigan experimental grassland establishment. *Restoration Ecology* **15**, 118-128.

Major, J. and Pyott, W. T. (1966) Buried viable seeds in two California bunchgrass sites and their bearing on the definition of a flora. *Vegetatio* **13**:253–282.

Maloof, J. N., Borevitz, J. O., Weigel, D. and Chory, J. (2000) Natural variation in phytochrome signaling. *Seminars in Cell and Developmental Biology* **11**, 523-530.

Mamede, M. de A. and de Araujo, F. S. (2008) Effects of slash and burn practices on a soil seed bank of caatinga vegetation in Northeastern Brazil. *Journal of Arid Environments* **72**, 458-470.

Matlack, G. R. and Good, R. E. (1990) Spatial heterogeneity in the soil seed bank of a mature coastal-plain forest. *Bulletin of The Torrey Botanical Club* **117**, 143-152.

Mayer, A. M. and Poljakoff-Mayber, A. (1989) The germination of seeds. Pergamon Press, Oxford, UK.

McCune, B. and Grace, J. B. (2002) Analysis of ecological communities. MJM Software, Gleneden Beach, Ore.

McWilliams, J. (2003) *Artemisia frigida*. In: Fire Effects Information System, [Online]. U.S. Department of Agriculture, Forest Service, Rocky Mountain Research Station, Fire Sciences Laboratory (Producer). (http://www.fs.fed.us/database/feis/plants/shrub/artrig/all.html) (accessed May 25, 2015)

Merritt, D. J., Kristiansen, M., Flematti, G. R., Turner, S. R., Ghisalberti, E. L., Trengove, R. D. and Dixon, K. W. (2006) Effects of a butenolide present in smoke on light-mediated germination of Australian *Asteraceae*. *Seed Science Research* **16**, 29-35.

Mitchell, W. W. (1958) An ecological study of the grasslands in the region of Missoula, Montana. M. A. thesis. University of Montana, Missoula, USA.

Moore, R. J. (1975) The biology of Canadian weeds. 13. *Cirsium arvense* (L.) Scop. *Canadian Journal of Plant Science* **55**, 1033-1048.

Moreira, B. and Pausas, J. G. (2012) Tanned or burned: the role of fire in shaping physical seed dormancy. *Plos One* 7: e51523.

Moreira, B., Tormo, J. and Estrelles, E. (2010) Disentangling the role of heat and smoke as germination cues in Mediterranean Basin flora. *Annals of Botany* **105**, 627-635.

Morrison, D. A., McClay, K., Porter, C. and Rish, S. (1998) The role of the lens in controlling heat-induced breakdown of testa-imposed dormancy in native Australian legumes. *Annals of Botany* **82**, 35-40.

Moss, E. H. and Campbell, J. A. (1947) The fescue grassland of Alberta. *Canadian Journal of Research* **25**, 209-227.

Murray, B. D., Holmes, S. A., Webster, C. R. and Witt, J. C. (2012) Post-disturbance plant community dynamics following a rare natural-origin fire in a *Tsuga canadensis* forest. *Plos One* **7**, e43867.

Musil, C. F. and Dewitt, D. W. (1991) Heat-stimulated germination in two Restionaceae species. *South African Journal of Botany* **57**, 175-176.

Nandula, V. K., Eubank, T. W., Poston, D. H., Koger, C. H. and Reddy, K. N. (2006) Factors affecting germination of horseweed (*Conyza canadensis*). *Weed Science* **54**, 898-902.

Naveh, Z. (1974) Effects of fire in the Mediterranean region. pp. 401-434. In Kozlowski, T. T. and Ahlgren, C. E. *Fire and Ecosystems (Physiological Ecology)*, Academic press, New York, NY.

Ne'eman, G., Meir, I. and Ne'eman, R. (1993) The effect of ash on the germination and early growth of shoots and roots of *Pinus*, *Cistus* and annuals. *Seed Science and Technology* **21**, 339-349.

Ne'eman, G. and Izhaki, I. (1998) Stability of pre- and post-fire spatial structure of pine trees in Aleppo pine forest. *Ecography* **21**, 535-542.

Nelson, D. C., Flematti, G. R., Ghisalberti, E. L., Dixon, K. W. and Smith, S. M. (2012) Regulation of seed germination and seedling growth by chemical signals from burning vegetation. *Annual Review* of *Plant Biology* **63**, 107-130.

Nelson, D. C., Riseborough, J., Flematti, G. R., Stevens, J., Ghisalberti, E. L., Dixon, K. W. and Smith, S. M. (2009) Karrikins discovered in smoke trigger Arabidopsis seed germination by a mechanism requiring gibberellic acid synthesis and light. *Plant Physiology* **149**, 863-873.

Nimir, M. B. and Payne, G. F. (1978) Effects of spring burning on a mountain range. *Journal of Range Management* **31**, 259-263.

Noyes, R. D. (2000) Diplospory and parthenogenesis in sexual x agamospermous (apomictic) *Eirgeron* (Asteraceae) hybrids. *International Journal of Plant Sciences* **161**, 1-12.

Palit, R. (2013) Seed production in *Festuca hallii* (Vasey) Piper: Ecotypic variation and physiological mechanisms. Msc thesis. University of Saskatchewan, Saskatoon, Canada.

Pannangpetch, K. and Bean, E. W. (1984) Effects of temperature on germination in populations of *Dactylis glomerata* from NW Spain and Central Italy. *Annals of Botany* **53**, 633-639.

Parera, C. A. and Cantlife, D. J. (1994) Pre-sowing seed priming. *Horticultural Reviews* **16**, 109-141.

Paula, S. and Pausas, J. G. (2008) Burning seeds: germinative response to heat treatments in relation to resprouting ability. *Journal of Ecology* **96**, 543-552.

Pausas, J. G. and Keeley, J. E. (2009) A burning story: the role of fire in the history of life. *Bioscience* **59**, 593-601.

Pausas, J. G. and Keeley, J. E. (2014) Evolutionary ecology of resprouting and seeding in fire-prone ecosystems. *New Phytologist* **204**, 55-65.

Pausas, J. G. and Verdu, M. (2005) Plant persistence traits in fire-prone ecosystems of the Mediterranean basin: a phylogenetic approach. *Oikos* **109**, 196-202.

Pelton, J. (1956) A study of seed dormancy in eighteen species of high altitude Colorado plants. *Butler University Botanical Studies* **13**, 74-84.

Perez-Fernandez, M. A. and Rodriguez-Echeverria, S. (2003) Effect of smoke, charred wood, and nitrogenous compounds on seed germination of ten species from woodland in Central-Western Spain. *Journal of Chemical Ecology* **29**, 237-251.

Pierce, S. M., Esler, K. and Cowling, R. W. (1995) Smoke-induced germination of succulents (Mesembryanthemaceae) from fire-prone and fire-free habitats in South Africa. *Oecologia* **102**, 520-522.

Pierce, S. M. and Moll, E. J. (1994) Germination ecology of six shrubs in fire prone Cape fynbos. *Vegetatio* **110**: 25-41.

Pratt, D. W., Black, R. A. and Zamora, B. A. (1984) Buried viable seed in a ponderosa pine community. *Canadian Journal of Botany* **62**, 44-52.

Preston, C. A., Becker, R. and Baldwin, I. T. (2004) Is 'NO' news good news? Nitrogen oxides are not components of smoke that elicits germination in two smoke-stimulated species, *Nicotiana attenuate* and *Emmenanthe penduliflora*. *Seed Science Research* **14**, 73-79.

Prober, S. M., Thiele, K. R., Lunt, I. D. and Koen, T. B. (2005) Exotic annuals and native perennial grasses through carbon supplements and spring burns. *Journal of Applied Ecology* **42**, 1073-1085.

Pylypec, B. and Romo, J. T. (2003) Long-term effects of burning *Festuca* and *Stipa Agropyron* grasslands. *Journal of Range Management* **56**, 640-645.

Qiu, J., Bai, Y., Romo, J. T. and Coulman, B. (2008). Mechanisms regulating seedling emergence of orchardgrass (*Dactylis glomerata* L.) and western wheatgrass (*Pascopyrum smithii* [Rydb.] L.): Dormancy change, seed fate and seeding date. *Environmental and Experimental Botany* **62**, 185–194.

Rabinowitz, D. (1981) Buried viable seeds in a North American tall-grass prairie: the resemblance of their abundance and composition to dispersing seeds. *Oikos* **36**, 191-195.

Read, T. R. and Bellairs, S. M. (1999) Smoke affects the germination of native grasses of New South Wales. *Australian Journal of Botany* **47**, 563-576.

Read, T. R., Bellairs, S. M., Mulligan, D. R. and Lamb, D. (2000) Smoke and heat effects on soil seed bank germination for the re-establishment of a native forest community in New South Wales. *Austral Ecology* **25**, 48-57.

Redmann, R. E., Romo, J. T. and Pylypec, B. (1993) Impacts of burning on primary productivity of *Festuca* and *Stipa-Agropyron* grasslands in central Saskatchewan. *American Midland Naturalist* **130**, 262-273.

Reyes, O. and Casal, M. (2006) Seed germination of *Quercus robur*, *Q. pyrenaica* and *Q. ilex* and the effects of smoke, heat, ash and charcoal. *Annals of Forest Science* **63**, 205-212.

Reyes, O. and Trabaud, L. (2009) Germination behaviour of 14 Mediterranean species in relation to fire factors: smoke and heat. *Plant Ecology* **202**, 113-121.

Rivas, M., Reyes, O. and Casal, M. (2006) Influence of heat and smoke treatments on the germination of six leguminous shrubby species. *International Journal of Wildland Fire* **15**, 73-80.

Roberts, H. A. (1981) Seed banks in soils. *Advances in Applied Biology* **6**, 1-55.

Roche, S., Koch, J. M. and Dixon, K. W. (1997) Smoke enhanced seed germination for mine rehabilitation in the southwest of Western Australia. *Restoration Ecology* **5**, 191-203.

Romme, W. H., Bohland, L., Persichetty, C. and Caruso, T. (1995) Germination ecology of some common forest herbs in Yellowstone National Park, Wyoming, USA. *Arctic and Alpine Research* **27**, 407-412.

Romo, J. T. (2003) Reintroducing fire for conservation of Fescue Prairie Association remnants in the Northern Great Plains. *Canadian Field-Naturalist* **117**, 89-99.

Romo, J. T. and Bai, Y. (2004) Seed bank and plant community composition, Mixed Prairie of Saskatchewan. *Journal of Range Management* **57**, 300-304.

Romo, J. T., Grilz, P. L., Redmann, R. E. and Driver, E. A. (1993) Standing crop, biomass allocation patterns and soil-plant water relations in *Symphoricarpos occidentalis* Hook. following autumn or spring burning. *American Midland Naturalist* 130, 106-115.

Romo, J. T. and Gross, D. V. (2011) Preburn history and seasonal burning effects on the soil seed bank in the Fescue Prairie. *American Midland Naturalist* **165**, 74-90.

Romo, J. T., Grilz, P. L., Bubar, C. J. and Young, J. A. (1991) Influence of temperature and water stress on germination of plains rough fescue. *Journal of Range Management* **44**, 75-81.

Reyes, O. and Trabaud, L. (2009) Germination behaviour of 14 Mediterranean species in relation to fire factors: smoke and heat. *Plant Ecology* **202**, 113-121.

Santana, V. M., Baeza, M. J. and Blanes, M. C. (2013) Clarifying the role of fire heat and daily temperature fluctuations as germination cues for Mediterranean Basin obligate seeders. *Annals of Botany* **111**, 127-134.

Santana, V. M., Alday, J. G. and Baeza, M. J. (2014) Effects of fire regime shift in Mediterranean Basin ecosystems: changes in soil seed bank composition among functional types. *Plant Ecology* **215**, 555-566.

Schwachtje, J. and Baldwin, I. T. (2004) Smoke exposure alters endogenous gibberellin and abscisic acid pools and gibberellin sensitivity while eliciting germination in the post-fire annual, *Nicotiana attenuate*. *Seed Science Research* **14**, 51-60.

Schwilk, D. W. and Ackerly, D. D. (2001) Flammability and serotiny as strategies: correlated evolution in pines. *Oikos* **94**, 326-336.

Schwilk, D. W. and Zavala, N. (2012) Germination response of grassland species to plant-derived smoke. *Journal of Arid Environments* **79**, 111-115.

Shea, S. R., Mccormick, J. and Portlock, C. C. (1979) The effect of fires on regeneration of leguminous species in the northern jarrah (*Eucalyptus marginata* Sm.) forest of Western Australia. *Australian Journal of Ecology* **4**, 195-205.

Siddiqui, M. Y., Carolin, R. C. and Myerscough, P. J. (1976) Studies in the ecology of coastal heath in New South Wales. III. Regrowth of vegetation after fire. *Proceedings of the Linnean Society of New South Wales* **101**, 53-63.

Sinton, H. M. (1980) Effect of burning and mowing on *Festuca hallii* (Vassey) Piper (*Festuca scabrella* Torr.). Msc thesis. University of Alberta, Edmonton, Canada.

Smith, C. J., Perfetti, T. A., Garg, R. and Hansch, C. (2003) IARC carcinogens reported in cigarette mainstream smoke and their calculated log P values. *Food and Chemical Toxicology* **41**, 807-817.

Snyman, H. A. (2005) The effect of fire on the soil seed bank of a semi-arid grassland in South Africa. *South African Journal of Botany* **71**, 53-60.

Sparg, S. G., Kulkarni, M. G., Light, M. E. and Van Staden, J. (2005) Improving seedling vigour of indigenous medicinal plants with smoke. *Bioresource Technology* **96**, 1323-1330.

Sparg, S. G., Kulkarni, M. G. and Van Staden, J. (2006) Aerosol some and smoke-water stimulation of seedling vigor of a commercial maize cultivar. *Crop Science* **46**, 1336-1340.

Standifer, L. C. and Wilson, P. W. (1988) A high-temperature requirement for after ripening of imbibed dormany *Poa annua* L. seeds. *Weed Research* **28**, 365-371.

Stevens, J. C., Merritt, D. J., Flematti, G. R., Ghisalberti, E. L. and Dixon, K. W. (2007) Seed germination of agriculture weeds is promoted by the butenolide 3-methyl-2*H*-furo[2,3-c]pyran-2-one under laboratory and field conditions. *Plant Soil* **298**, 113-124.

Stevens, N., Seal, C., Archibald, S. and Bond, W. (2014) Increasing temperatures can improve seedling establishment in arid-adapted savanna trees. *Oecologia* **175**, 1029-1040.

Stranathan, S. E. and Monsen, S. B. (1986) Selection of a cultivar of *Artemisia ludoviciana* for disturbed land plantings, pp. 108-113 in McArthur, E. D. and Welch, B. L. (eds.), *Proceedings-symposium of the biology of Artemisia and Chrysothamnus*. Intermountain Research Station, Ogden, Utah.

Sweeney, J. R. (1956) Responses of vegetation to fire. A study of the herbaceous vegetation following chaparral fires. *University of California publications in botany* **28**, 143-250.

Tavili, A., Zare, S., Moosavi, S. A. and Enayati, A. (2010) Effects of priming techniques on seed germination and early growth characteristics of *Bromus tomentellus* L. and *Bromus inermis* L. *Notulae Scientia Biologicae* **2**, 104-108.

Taylor, J. L. S. and Van Staden, J. (1996) Root initiation in *Vigna radiate* (L.).Wilczek hypocotyl cuttings is stimulated by smoke derived extracts. *Plant Growth Regulation* **18**, 165-168.

Tieu, A., Dixon, K. W., Meney, K. A. and Sivasithamparam, K. (2001) Interaction of soil burial and smoke on germination patterns in seeds of selected Australian native plants. *Seed Science Research* **11**, 69-76.

Tieu, A., Dixon, K. A. and Sivasithamparam, K. (1999) Germination of four species of native western Australian plants using plant-derived smoke. *Australian Journal of Botany* **47**, 207-219.

Thomas, P. B., Morris, E. C. and Auld, T. D. (2003) Interactive effects of heat shock and smoke on germination of nine species forming soil seed banks within the Sydney region. *Austral Ecology* **28**, 674-683.

Thomas, P. B., Morris, E. C. and Auld, T. D. (2007) Response surfaces for the combined effects of heat shock and smoke on germination of 16 species forming soil seed banks in south-east Australia. *Austral Ecology* **32**, 605-616.

Thomas, P. B., Morris, E. C., Auld, T. D. and Haigh, A. M. (2010) The interaction of temperature, water availability and fire cues regulates seed germination in a fire-prone landscape. *Oecologia* **162**, 293-302.

Thomas, T. H. and Van Staden, J. (1995) Dormancy break of celery (*Apium graveolens* L.) seeds by plant-derived smoke extract. *Plant Growth Regulation* **17**, 195-198.

Thomas, P. A. and Wein, R. W. (1990) Jack pine establishment on ash from wood and organic soil. *Canadian Journal of Forest Research* **20**, 1926-1932.

Thomas, P. A. and Wein, R. W. (1994) Amelioration of wood ash toxicity and jack pine establishment. *Canadian Journal of Forest Research* **24**, 748-755.

Thompson, K. (1978) Occurrence of buried viable seeds in relation to environmental gradients. *Journal of Biogeography* **5**, 425-430.

Thompson, K. and Grime, J. P. (1979) Seasonal variation in the seed banks of herbaceous species in ten contrasting habitats. *Journal of Ecology* **67**, 893-921.

Thompson, K. and Grime, J. P. (1983) A comparative study of germination responses to diurnally fluctuating temperatures. *Journal of Applied Ecology* **20**, 141-156.

Thompson, K. (1986) Small scale heterogeneity in the seed bank of an acidic grassland. *Journal of Ecology* **74**, 733-738.

Tormo, J., Moreira, B. and Pausas, J. G. (2014) Field evidence of smoke-stimulated seedling emergence and establishment in Mediterranean Basin flora. *Journal of Vegetation Science* **25**, 771-777.

Towne, G. and Owensby, C. (1984) Long-term effects of annual burning at different date in ungrazed Kansas tallgrass prairie. *Journal of Range Management* **37**, 392-397.

Travnicek, A. J., Lym, R. G. and Prosser, C. (2005) Fall-prescribed burn and spring-applied herbicide effect on Canada thistle control and soil seedbank in a northern mixed-grass prairie. *Rangeland Ecology and Management* **58**, 413-422.

Tucker, C. M. and Cadotte, M. W. (2013) Fire variability, as well as frequency, can explain coexistence between seeder and resprouter life histories. *Journal of Applied Ecology* **50**, 594-602.

Uchytil, R. (1993) *Poa pratensis* (Kentucky bluegrass). In: Fire Effects Information System, [Online]. U.S. Department of Agriculture, Forest Service, Rocky Mountain Research Station, Fire Sciences Laboratory (Producer). (http://www.fs.fed.us/database/feis/plants/graminoid/poapra/all.html) (access May 25, 2015)

Van De Venter, H. A. and Esterhuizen, A. D. (1988) The effects of factors associated with fire on seed germination of *Erica sessiliflora* and *E. herbacalyx* (Ericaceae). *South African Journal of Botany* **54**, 301-304.

Van Staden, J., Brown, N. A. C., Jäger, A. K. and Johnson, T. A. (2000) Smoke as a germination cue. *Plant Species Biology* **15**, 167-178.

Van Staden, J., Drewes, F. E. and Brown, N. A. C. (1995) Some chromatographic characteristics of germination stimulants in plant-derived smoke extracts. *Plant Growth Regulation* **17**, 241-249.

Van Staden, J., Jager, A. K. and Light, M. E. (2004) Isolation of the major germination cue from plant-derived smoke. *South African Journal of Botany* **70**, 654-659.

Van Staden, J., Jager, A. K. and Strydom, A. (1995) Interaction between a plant-derived smoke extract, light and phytohormones on the germination of light-sensitive lettuce seeds. *Plant Growth Regulation* **17**, 213-218.

Van Staden, J. and Jain, N. (2006) Stimulation of tomato seed germination by 3-methyl-2H-furo[2,3-c] pyran-2-one: Some physiological aspects. *South African Jorunal of Botany* **72**, 334-335.

Walck, J. L., Baskin, J. M. and Baskin C. C. (1996) An ecologically and evolutionarily meaningful definition of a persistent seed bank in Solidago. *American Journal of Botany* **83**, 78-79.

Walck, J. L., Hidayati, S. N., Dixon, K. W., Thompson, K. and Poschlod, P. (2011) Climate change and plant regeneration from seed. *Global Change Biology* **17**, 2145-2161.

Wally, A. L., Menges, E. S. and Weekley, C. W. (2006) Comparison of three devices for estimating fire temperatures in ecological studies. *Applied Vegetation Science* **9**, 97-108.

Walter, H. and Lieth, H. (1960–1967) Klimadiagramm-Weltatlas. VEG Gustav Fischer-Verlag, Jena, DE. Vol. 3.

Wang, Y. R., Yang, L. and Hu, X. W. (2012) Seed dormancy release and soil seed bank of three arid desert plants under burial conditions. *Chinese Journal of Plant Ecology* **36**, 774-780.

Warcup, J. H. (1980) Effect of heat treatment of forest soil on germination of buried seed. *Australian Journal of Botany* **28**, 567-571.

Warr, S. J., Kent, M. and Thompson, K. (1994) Seed bank composition and variability in five woodlands in south-west England. *Journal of Biogeography* **21**, 151-168.

Watson, P. J., Bradstock, R. A. and Morris, C. (2009) Fire frequency influences composition and structure of the shrub layer in an Australian subcoastal temperate grassy woodland. *Austral Ecology* **34**, 218-232.

Weaver, S. E. (2001) The biology of Canadian weeds. 115. *Conyza canadensis*. *Canadian Journal of Plant Science* **81**, 867-875.

Weber, W. A. and Wittmann, R. C. (2001) Colrado flora: western slope. University Press of Colorado, Boulder, Colorado, USA.

Welsh, S. L., Atwood, N. D., Goodrich, S. and Higgins, L. C. (1987) A Utah Flora: Great Basin Naturalist Memoir, 9. Brigham Young University Press, Utah.

Wicklow, D. T. (1977) Germination response in *Emmenanthe penduliflora* (hydrophyllaceae). *Ecology* **58**, 201-205.

Wicklow, D. T. (1988) Parallels in the development of post-fire fungal and herb communities. *Biological Sciences* **94**, 87-95.

Williams, P. R., Congdon, R. A., Grice, A. C. and Clarke, P. J. (2005) Germinable soil seed banks in a tropical savanna: seasonal dynamics and effects of fire. *Austral Ecology* **30**, 79-90.

Wills, T. J. and Read, J. (2002) Effects of heat and smoke on germination of soil-stored seed in a south-eastern Australian sand heathland. *Australian Journal of Botany* **50**, 197-206.

Wills, T. J. and Read, J. (2007) Soil seed bank dynamics in post-fire heathland succession in south-eastern Australia. *Plant Ecology* **190**, 1-12.

Wilson, R. G. (1979) Germination and seedling development of Canada thistle (*Cirsium arvense*). *Weed Science* **27**, 146-151.

Wilson, R. G. (1982) Germination and seedling development of fringed sagebrush (*Artemisia frigida*). *Weed Science* **30**, 102-105.

Wood, D. M. and del Moral, R. (2000) Seed rain during early primary succession on Mount St. Helens, Washington. *Madrono* **47**, 1-9.

Wooller, S. J., Wooller, R. D. and Brown, K. L. (2002) Regeneration by three species of *Banksia* on the south coast of Western Australia in relation of fire interval. *Australian Journal of Botany* **50**, 311-317.

Wright, H. A. (1971) Why squirreltain is more tolerant to burning than needle-and-thread. *Journal of range management* **24**, 277-284.

Wright, H. A. and Bailey, A. W. (1982) Fire ecology: United States and southern Canada. Wiley-InterScience Publication, New York, NY.

Zhou, J. F., da Silva, J. A. T. and Ma, G. H. (2014) Effects of smoke water and karrikin on seed germination of 13 species growing in China. *Central European Journal of Biology* **9**, 1108-1116.

Zuloaga-Aguilar, S., Briones, O. and Orozco-Segovia, A. (2011) Seed germination of montane forest species in response to ash, smoke and heat shock in Mexico. *Acta Oecologica* **37**, 256-262.

APPENDIX 8.0

8.1 Appendix A: Analysis of variance (ANOVA) tables for the effects of year, mowing, and burning on seedling emergence in the field and from the soil seed bank

Table A1: ANOVA table for the effects of year (Y), treatment (mowing or burning) (T), and year ×treatment (Y × T) on densities of affected species and total seedlings emerging in the field

Seedlings emerging	Source of variation	numDF	denDF	F-value	P-value
Artemisia frigida	Y	1	20	1.42	0.25
	T	2	20	8.38	<0.01
	Y × T	2	20	0.52	0.60
Cirsium arvense	Y	1	20	0.69	0.42
	T	2	20	13.44	<0.01
	Y × T	2	20	0.03	0.97
Conyza canadensis	Y	1	20	0.47	0.50
	T	2	20	7.14	<0.01
	Y × T	2	20	0.04	0.96
Artemisia ludoviciana	Y	1	8	4.94	0.06
	T	2	16	19.31	<0.01
	Y × T	2	16	6.98	<0.01
Taraxacum officinale	Y	1	8	10.08	0.01
	T	2	16	3.74	0.05
	Y × T	2	16	5.38	0.02
Total seedlings	Y	1	24	0.17	0.69
	T	2	96	21.11	<0.01
	Y × T	2	96	4.06	0.02

Table A2: ANOVA table for the effects of year (Y), treatment (mowing or burning) (T), and year ×treatment (Y × T) on species richness and species diversity of seedlings emerging in the field

Richness and Diversity	Source of	numDF	denDF	F-value	P-value
Species richness	Y	1	48	13.57	<0.01
	T	2	96	41.28	<0.01
	Y × T	2	96	0.54	0.58
Shannon diversity index (H')	Y	1	120	23.01	<0.01
	T	2	120	21.27	<0.01
	Y × T	2	120	0.25	0.78

Table A3: ANOVA table for the effects of year (Y), treatment (mowing or burning) (T), and year ×treatment (Y × T) on seedlings densities of affected species, functional groups, and total seedlings emerging from different soil layers in the soil seed bank

Seedlings emerging	Soil layer	Source of variation	numDF	denDF	F-value	P-value
Agrostis scabra	litter	Y	1	24	0.24	0.63
		T	2	24	7.17	<0.01
		Y × T	2	24	1.15	0.33
	0-1 cm	Y	1	4	3.78	0.15
		T	2	16	2.30	0.13
		Y × T	2	16	1.45	0.18
	1-5 cm	Y	1	8	64.76	<0.01
		T	2	16	2.02	0.16
		Y × T	2	16	2.59	0.11
	0-5 cm	Y	1	4	4.71	0.09
		T	2	16	1.52	0.25
		Y × T	2	16	2.46	0.11
	all	Y	1	4	3.43	0.18
		T	2	16	2.48	0.13
		Y × T	2	16	1.74	0.23
Artemisia frigida	litter	Y	1	24	0.82	0.37
		T	2	24	9.03	<0.01
		Y × T	2	24	0.36	0.70
	0-1 cm	Y	1	4	27.93	<0.01
		T	2	16	0.86	0.44
		Y × T	2	16	0.24	0.79
	1-5 cm	Y	1	8	15.00	<0.01
		T	2	16	0.32	0.73
		Y × T	2	16	0.01	0.99
	0-5 cm	Y	1	24	47.55	<0.01
		T	2	24	0.87	0.43
		Y × T	2	24	0.14	0.87
	all	Y	1	24	30.14	<0.01
		T	2	24	0.54	0.59
		Y × T	2	24	0.04	0.96
Poa pratensis	litter	Y	1	20	1.89	0.18
		T	2	20	4.45	0.03
		Y × T	2	20	0.35	0.71
	0-1 cm	Y	1	4	3.58	0.13
		T	2	16	0.96	0.41
		Y × T	2	16	0.45	0.65
	1-5 cm	Y	1	4	5.26	0.08
		T	2	16	2.32	0.13
		Y × T	2	16	3.35	0.06
	0-5 cm	Y	1	4	4.51	0.10
		T	2	16	1.47	0.26

Table A3 Continued

Affected species	Soil layer	Source of variation	numDF	denDF	F-value	P-value
		Y × T	2	16	1.18	0.33
	all	Y	1	4	4.83	0.09
		T	2	16	2.58	0.11
		Y × T	2	16	0.57	0.58
Total seedling	litter	Y	1	24	4.71	0.04
		T	2	96	9.96	<0.01
		Y × T	2	96	0.76	0.47
	0-1 cm	Y	1	120	105.98	<0.01
		T	2	120	4.85	<0.01
		Y × T	2	120	3.36	0.04
	1-5 cm	Y	1	24	42.04	<0.01
		T	2	96	7.54	<0.01
		Y × T	2	96	9.17	<0.01
	0-5 cm	Y	1	24	89.86	<0.01
		T	2	96	7.89	<0.01
		Y × T	2	96	7.01	<0.01
	all	Y	1	24	76.17	<0.01
		T	2	96	5.99	<0.01
		Y × T	2	96	5.71	<0.01
Native	litter	Y	1	24	1.84	0.19
		T	2	96	7.01	<0.01
		Y × T	2	96	0.59	0.55
	0-1 cm	Y	1	24	85.22	<0.01
		T	2	96	4.29	0.02
		Y × T	2	96	3.19	0.05
	1-5 cm	Y	1	48	74.01	<0.01
		T	2	96	6.36	<0.01
		Y × T	2	96	8.70	<0.01
	0-5 cm	Y	1	24	122.97	<0.01
		T	2	96	6.69	<0.01
		Y × T	2	96	6.92	<0.01
	all	Y	1	48	1110.50	<0.01
		T	2	96	4.93	<0.01
		Y × T	2	96	6.86	<0.01
Non-native	litter	Y	1	24	2.75	0.11
		T	2	96	6.66	<0.01
		Y × T	2	96	0.60	0.55
	0-1 cm	Y	1	24	7.10	0.01
		T	2	96	3.41	0.04
		Y × T	2	96	1.71	0.19
	1-5 cm	Y	1	24	14.76	<0.01
		T	2	96	2.29	0.11
		Y × T	2	96	3.99	0.02

Table A3 Continued

Affected species	Soil layer	Source of variation	numDF	denDF	F-value	P-value
	0-5 cm	Y	1	24	11.66	<0.01
		T	2	96	3.66	0.03
		Y × T	2	96	3.03	0.06
	all	Y	1	24	7.57	0.01
		T	2	96	6.00	<0.01
		Y × T	2	96	1.25	0.29
Forbs	litter	Y	1	48	2.57	0.12
		T	2	96	7.56	<0.01
		Y × T	2	96	1.13	0.33
	0-1 cm	Y	1	24	66.22	<0.01
		T	2	96	4.12	0.02
		Y × T	2	96	3.42	0.04
	1-5 cm	Y	1	48	60.34	<0.01
		T	2	96	5.69	<0.01
		Y × T	2	96	8.55	<0.01
	0-5 cm	Y	1	24	98.95	<0.01
		T	2	96	6.21	<0.01
		Y × T	2	96	6.82	<0.01
	all	Y	1	24	90.23	<0.01
		T	2	96	4.01	0.02
		Y × T	2	96	7.18	<0.01
Graminoids	litter	Y	1	24	2.76	0.11
		T	2	96	7.38	<0.01
		Y × T	2	96	0.49	0.61
	0-1 cm	Y	1	24	16.35	<0.01
		T	2	96	3.94	0.02
		Y × T	2	96	2.34	0.10
	1-5 cm	Y	1	24	17.29	<0.01
		T	2	96	3.46	0.04
		Y × T	2	96	4.56	0.01
	0-5 cm	Y	1	24	17.85	<0.01
		T	2	96	4.80	0.01
		Y × T	2	96	4.02	0.02
	all	Y	1	24	12.46	<0.01
		T	2	96	7.29	<0.01
		Y × T	2	96	1.87	0.16

Table A4: ANOVA table for the effects of year (Y), treatment (mowing or burning) (T), and year ×treatment (Y × T) on species richness of functional groups and total seedlings emerging from different layers in the soil seed bank

Seedlings emerging	Soil layer	Source of variation	numDF	denDF	F-value	P-value
Total seedling	litter	Y	1	24	7.38	0.01
		T	2	96	21.04	<0.01
		Y × T	2	96	1.04	0.36
	0-1 cm	Y	1	120	107.22	<0.01
		T	2	120	2.12	0.12
		Y × T	2	120	1.16	0.32
	1-5 cm	Y	1	120	169.13	<0.01
		T	2	120	2.53	0.08
		Y × T	2	120	2.64	0.08
	0-5 cm	Y	1	120	204.83	<0.01
		T	2	120	4.08	0.02
		Y × T	2	120	2.59	0.08
	all	Y	1	120	106.93	<0.01
		T	2	120	2.09	0.13
		Y × T	2	120	2.54	0.08
Native	litter	Y	1	24	6.18	0.02
		T	2	96	19.88	<0.01
		Y × T	2	96	0.65	0.52
	0-1 cm	Y	1	120	118.27	<0.01
		T	2	120	2.85	0.06
		Y × T	2	120	1.33	0.27
	1-5 cm	Y	1	144	124.15	<0.01
		T	2	144	2.29	0.12
		Y × T	2	144	1.70	0.19
	0-5 cm	Y	1	120	164.42	<0.01
		T	2	120	4.13	0.02
		Y × T	2	120	2.80	0.06
	all	Y	1	144	90.61	<0.01
		T	2	144	1.30	0.28
		Y × T	2	144	2.10	0.21
Non-native	litter	Y	1	48	2.42	0.13
		T	2	96	17.80	<0.01
		Y × T	2	96	0.15	0.86
	0-1 cm	Y	1	120	7.38	<0.01
		T	2	120	0.17	0.84
		Y × T	2	120	0.06	0.94
	1-5 cm	Y	1	120	62.44	<0.01
		T	2	120	2.30	0.10
		Y × T	2	120	1.66	0.19
	0-5 cm	Y	1	120	32.25	<0.01
		T	2	120	0.49	0.61

Table A4 Continued

Seedlings emerging	Soil layer	Source of variation	numDF	denDF	F-value	P-value
		Y × T	2	120	0.08	0.93
	all	Y	1	120	13.04	<0.01
		T	2	120	0.76	0.47
		Y × T	2	120	0.07	0.94
Forbs	litter	Y	1	48	2.42	0.13
		T	2	96	17.80	<0.01
		Y × T	2	96	0.15	0.86
	0-1 cm	Y	1	120	115.22	<0.01
		T	2	120	1.83	0.16
		Y × T	2	120	1.62	0.20
	1-5 cm	Y	1	120	138.00	<0.01
		T	2	120	2.80	0.07
		Y × T	2	120	2.59	0.08
	0-5 cm	Y	1	120	199.47	<0.01
		T	2	120	3.38	0.04
		Y × T	2	120	2.94	0.06
	all	Y	1	120	139.59	<0.01
		T	2	120	1.16	0.32
		Y × T	2	120	2.91	0.06
Graminoids	litter	Y	1	120	13.47	<0.01
		T	2	120	13.76	<0.01
		Y × T	2	120	2.65	0.07
	0-1 cm	Y	1	120	28.40	<0.01
		T	2	120	1.18	0.31
		Y × T	2	120	0.32	0.73
	1-5 cm	Y	1	120	90.21	<0.01
		T	2	120	2.40	0.10
		Y × T	2	120	0.01	0.99
	0-5 cm	Y	1	120	46.18	<0.01
		T	2	120	1.73	0.18
		Y × T	2	120	0.68	0.51
	all	Y	1	120	0.97	0.33
		T	2	120	2.30	0.10
		Y × T	2	120	0.35	0.70

Table A5: ANOVA table for the effects of year (Y), treatment (mowing or burning) (T), and year ×treatment (Y × T) on the rate of seedling emergence of functional groups and total seedlings emerging from different soil layers in the soil seed bank.

Seedlings emerging	Soil layer	Source of variation	numDF	denDF	F-value	P-value
Total seedling	litter	Y	1	117	31.58	<0.01
		T	2	117	1.35	0.26
		Y × T	2	117	0.58	0.56
	0-1 cm	Y	1	140	30.87	<0.01
		T	2	140	4.59	0.01
		Y × T	2	140	0.35	0.70
	1-5 cm	Y	1	47	12.94	<0.01
		T	2	92	5.75	<0.01
		Y × T	2	92	0.93	0.40
	0-5 cm	Y	1	143	43.49	<0.01
		T	2	143	5.95	<0.01
		Y × T	2	143	0.04	0.96
	all	Y	1	24	22.71	<0.01
		T	2	96	6.26	<0.01
		Y × T	2	96	2.56	0.08
Native	litter	Y	1	106	8.16	<0.01
		T	2	106	1.83	0.16
		Y × T	2	106	0.09	0.91
	0-1 cm	Y	1	139	75.24	<0.01
		T	2	139	5.93	<0.01
		Y × T	2	139	0.43	0.65
	1-5 cm	Y	1	44	20.55	<0.01
		T	2	88	6.16	<0.01
		Y × T	2	88	0.96	0.39
	0-5 cm	Y	1	44	55.26	<0.01
		T	2	91	3.10	0.06
		Y × T	2	91	0.12	0.09
	all	Y	1	24	60.02	<0.01
		T	2	96	5.93	<0.01
		Y × T	2	96	0.20	0.82
Non-native	litter	Y	1	80	57.00	<0.01
		T	2	78	1.64	0.20
		Y × T	2	80	1.92	0.15
	0-1 cm	Y	1	108	0.91	0.34
		T	2	107	2.75	0.07
		Y × T	2	107	0.31	0.73
	1-5 cm	Y	1	38	1.33	0.26
		T	2	73	4.89	0.01
		Y × T	2	73	1.31	0.28
	0-5 cm	Y	1	38	1.33	0.26
		T	2	73	4.89	0.01

Table A5 Continued

Seedlings emerging	Soil layer	Source of variation	numDF	denDF	F-value	P-value
		Y × T	2	73	1.31	0.28
	all	Y	1	49	0.14	0.71
		T	2	93	2.85	0.07
		Y × T	2	93	2.64	0.08
Forbs	litter	Y	1	86	10.73	<0.01
		T	2	86	2.88	0.06
		Y × T	2	84	0.07	0.93
	0-1 cm	Y	1	138	33.01	<0.01
		T	2	138	4.88	<0.01
		Y × T	2	138	0.63	0.53
	1-5 cm	Y	1	25	36.20	<0.01
		T	2	91	3.0	0.06
		Y × T	2	91	0.16	0.85
	0-5 cm	Y	1	143	44.86	<0.01
		T	2	143	1.72	0.18
		Y × T	2	143	0.34	0.71
	all	Y	1	144	31.85	<0.01
		T	2	144	1.80	0.17
		Y × T	2	144	0.18	0.84
Graminoids	litter	Y	1	24	43.18	<0.01
		T	2	71	1.53	0.22
		Y × T	2	71	1.32	0.27
	0-1 cm	Y	1	131	7.56	<0.01
		T	2	131	5.81	<0.01
		Y × T	2	131	0.73	0.48
	1-5 cm	Y	1	36	0.41	0.85
		T	2	74	2.73	0.07
		Y × T	2	74	0.01	0.99
	0-5 cm	Y	1	44	2.36	0.13
		T	2	89	3.00	0.06
		Y × T	2	89	1.76	0.18
	all	Y	1	24	3.18	0.09
		T	2	96	9.53	<0.01
		Y × T	2	96	0.55	0.58

8.2 Appendix B: Analysis of variance (ANOVA) tables for the effects of year, smoke, and ash on seedling emergence from the soil seed bank

Table B1: ANOVA table for the effects of year (Y), treatment (T) (smoke, ash, or smoke plus ash), and year × treatment (Y × T) on seedlings densities of affected species, functional groups, and total seedlings emerging from different soil layers in the soil seed bank

Affected species	Soil layer	Source of variation	numDF	denDF	F-value	P-value
Artemisia frigida	litter	Y	1	4	6.11	0.07
		T	3	24	0.53	0.67
		Y × T	3	24	1.29	0.30
	0-1 cm	Y	1	28	9.34	<0.01
		T	3	28	3.62	0.03
		Y × T	3	28	1.62	0.21
	1-5 cm	Y	1	4	1.35	0.31
		T	3	24	1.79	0.18
		Y × T	3	24	3.45	0.06
	0-5 cm	Y	1	28	9.57	<0.01
		T	3	28	3.37	0.03
		Y × T	3	28	1.92	0.15
	all	Y	1	4	9.02	0.04
		T	3	24	1.10	0.37
		Y × T	3	24	1.76	0.18
Artemisia ludoviciana	litter	Y	1	8	0.13	0.73
		T	3	24	1.09	0.37
		Y × T	3	24	2.54	0.08
	0-1 cm	Y	1	4	0.50	0.52
		T	3	24	3.68	0.03
		Y × T	3	24	0.39	0.76
	1-5 cm	Y	1	8	0.92	0.37
		T	3	24	0.70	0.56
		Y × T	3	24	2.23	0.11
	0-5 cm	Y	1	4	0.56	0.49
		T	3	24	3.94	0.02
		Y × T	3	24	0.93	0.44
	all	Y	1	8	0.16	0.70
		T	3	24	3.18	0.04
		Y × T	3	24	2.40	0.09
Conyza canadensis	litter	Y	1	8	7.03	0.03
		T	3	24	3.66	0.03
		Y × T	3	24	0.77	0.52
	0-1 cm	Y	1	8	8.41	0.02
		T	3	24	2.73	0.07
		Y × T	3	24	1.42	0.26
	1-5 cm	Y	1	8	1.19	0.31
		T	3	24	0.17	0.91

Table B1 Continued

Affected species	Soil layer	Source of variation	numDF	denDF	F-value	P-value
		Y × T	3	24	2.93	0.06
	0-5 cm	Y	1	8	7.27	0.03
		T	3	24	2.03	0.14
		Y × T	3	24	1.93	0.15
	all	Y	1	8	9.03	0.02
		T	3	24	3.18	0.04
		Y × T	3	24	0.82	0.50
Total seedling	litter	Y	1	24	17.98	<0.01
		T	3	144	3.42	0.02
		Y × T	3	144	1.73	0.16
	0-1 cm	Y	1	24	9.19	<0.01
		T	3	144	2.41	0.07
		Y × T	3	144	2.71	0.05
	1-5 cm	Y	1	24	0.29	0.60
		T	3	144	1.42	0.24
		Y × T	3	144	0.83	0.48
	0-5 cm	Y	1	24	4.58	0.04
		T	3	144	2.03	0.11
		Y × T	3	144	2.50	0.06
	all	Y	1	24	9.65	<0.01
		T	3	144	3.22	0.02
		Y × T	3	144	3.11	0.03
Native	litter	Y	1	48	12.10	<0.01
		T	3	144	1.88	0.14
		Y × T	3	144	1.49	0.22
	0-1 cm	Y	1	48	6.06	0.02
		T	3	144	3.99	<0.01
		Y × T	3	144	3.17	0.03
	1-5 cm	Y	1	24	3.94	0.06
		T	3	144	0.80	0.50
		Y × T	3	144	0.99	0.40
	0-5 cm	Y	1	48	7.62	<0.01
		T	3	144	2.87	0.04
		Y × T	3	144	2.89	0.04
	all	Y	1	48	10.88	<0.01
		T	3	144	3.55	0.02
		Y × T	3	144	3.47	0.02
Non-native	litter	Y	1	24	10.28	<0.01
		T	3	144	2.11	0.10
		Y × T	3	144	0.90	0.44
	0-1 cm	Y	1	24	4.25	0.05
		T	3	144	0.57	0.64
		Y × T	3	144	0.91	0.44

Table B1 Continued

Affected species	Soil layer	Source of variation	numDF	denDF	F-value	P-value
	1-5 cm	Y	1	24	5.61	0.03
		T	3	144	1.16	0.33
		Y × T	3	144	0.47	0.70
	0-5 cm	Y	1	24	0.08	0.78
		T	3	144	0.79	0.50
		Y × T	3	144	0.93	0.43
	all	Y	1	24	2.23	0.15
		T	3	144	1.28	0.29
		Y × T	3	144	0.87	0.46
Forbs	litter	Y	1	48	13.46	<0.01
		T	3	144	1.99	0.12
		Y × T	3	144	2.24	0.09
	0-1 cm	Y	1	48	6.38	0.01
		T	3	144	3.44	0.02
		Y × T	3	144	1.86	0.14
	1-5 cm	Y	1	24	3.31	0.08
		T	3	144	0.96	0.41
		Y × T	3	144	0.87	0.46
	0-5 cm	Y	1	48	7.83	<0.01
		T	3	144	2.67	0.06
		Y × T	3	144	1.85	0.14
	all	Y	1	48	11.89	<0.01
		T	3	144	3.09	0.03
		Y × T	3	144	2.11	0.10
Graminoids	litter	Y	1	24	11.59	<0.01
		T	3	144	2.88	0.08
		Y × T	3	144	1.63	0.19
	0-1 cm	Y	1	24	6.10	0.20
		T	3	144	0.57	0.63
		Y × T	3	144	1.17	0.32
	1-5 cm	Y	1	24	5.16	0.03
		T	3	144	0.86	0.46
		Y × T	3	144	0.63	0.59
	0-5 cm	Y	1	24	0.43	0.52
		T	3	144	0.73	0.53
		Y × T	3	144	1.24	0.30
	all	Y	1	24	3.42	0.08
		T	3	144	1.63	0.19
		Y × T	3	144	1.55	0.20

Table B2: ANOVA table for the effects of year (Y), treatment (T) (smoke, ash, or smoke plus ash), and year × treatment (Y × T) on species richness for seedlings within functional groups and total seedlings emerging from the soil seed bank

Seedlings emerging	Soil layer	Source of variation	numDF	denDF	F-value	P-value
Total seedling	litter	Y	1	24	33.58	<0.01
		T	3	144	6.74	<0.01
		Y × T	3	144	0.82	0.48
	0-1 cm	Y	1	24	12.41	<0.01
		T	3	144	9.83	<0.01
		Y × T	3	144	2.41	0.07
	1-5 cm	Y	1	48	0.07	0.79
		T	3	144	4.78	<0.01
		Y × T	3	144	0.13	0.94
	0-5 cm	Y	1	24	4.78	0.04
		T	3	144	6.65	<0.01
		Y × T	3	144	2.38	0.07
	all	Y	1	24	16.14	<0.01
		T	3	144	7.45	<0.01
		Y × T	3	144	1.98	0.12
Native	litter	Y	1	48	40.33	<0.01
		T	3	144	7.50	<0.01
		Y × T	3	144	0.34	0.80
	0-1 cm	Y	1	48	20.93	<0.01
		T	3	144	5.86	<0.01
		Y × T	3	144	2.08	0.11
	1-5 cm	Y	1	48	0.90	0.35
		T	3	144	2.77	0.04
		Y × T	3	144	0.11	0.95
	0-5 cm	Y	1	48	15.63	<0.01
		T	3	144	4.95	<0.01
		Y × T	3	144	1.08	0.36
	all	Y	1	48	43.66	<0.01
		T	3	144	5.48	<0.01
		Y × T	3	144	0.95	0.42
Nonnative	litter	Y	1	24	24.35	<0.01
		T	3	144	2.27	0.08
		Y × T	3	144	0.43	0.73
	0-1 cm	Y	1	24	2.75	0.11
		T	3	144	2.30	0.06
		Y × T	3	144	0.56	0.64
	1-5 cm	Y	1	24	0.01	0.92
		T	3	144	3.63	0.01
		Y × T	3	144	0.07	0.98
	0-5 cm	Y	1	24	1.51	0.23
		T	3	144	4.32	<0.01

Table B2 Continued

Seedlings emerging	Soil layer	Source of variation	numDF	denDF	F-value	P-value
		Y × T	3	144	0.45	0.72
	all	Y	1	24	4.12	0.05
		T	3	144	4.28	<0.01
		Y × T	3	144	0.64	0.59
Forbs	litter	Y	1	24	16.58	<0.01
		T	3	144	6.46	<0.01
		Y × T	3	144	0.76	0.52
	0-1 cm	Y	1	24	7.22	0.01
		T	3	144	8.57	<0.01
		Y × T	3	144	2.38	0.07
	1-5 cm	Y	1	48	0.45	0.50
		T	3	144	6.23	<0.01
		Y × T	3	144	0.07	0.97
	0-5 cm	Y	1	24	1.85	0.19
		T	3	144	5.50	<0.01
		Y × T	3	144	2.25	0.08
	all	Y	1	24	7.82	0.01
		T	3	144	6.71	<0.01
		Y × T	3	144	2.26	0.08
Graminoids	litter	Y	1	48	37.32	<0.01
		T	3	144	1.63	0.19
		Y × T	3	144	0.35	0.79
	0-1 cm	Y	1	48	16.65	<0.01
		T	3	144	1.72	0.18
		Y × T	3	144	0.66	0.58
	1-5 cm	Y	1	48	0.71	0.40
		T	3	144	1.02	0.39
		Y × T	3	144	0.19	0.91
	0-5 cm	Y	1	24	14.69	<0.01
		T	3	144	1.56	0.22
		Y × T	3	144	2.38	0.07
	all	Y	1	24	23.56	<0.01
		T	3	144	1.60	0.20
		Y × T	3	144	0.81	049

Table B3: ANOVA table for the effects of year (Y), treatment (T) (smoke, ash, or smoke plus ash), and year × treatment (Y × T) on the rate of seedling emergence rate of functional groups and total seedlings emerging from different soil layers in the soil seed bank

Affected species	Soil layer	Source of variation	numDF	denDF	F-value	P-value
Total	litter	Y	1	24	6.14	0.02
		T	3	144	3.42	0.02
		Y × T	3	144	7.65	<0.01
	0-1 cm	Y	1	48	3.78	0.06
		T	3	144	7.71	<0.01
		Y × T	3	144	7.65	<0.01
	1-5 cm	Y	1	24	2.42	0.13
		T	3	144	0.23	0.87
		Y × T	3	144	0.53	0.66
	0-5 cm	Y	1	24	2.07	0.06
		T	3	144	4.21	<0.01
		Y × T	3	144	3.99	<0.01
	all	Y	1	24	6.57	0.02
		T	3	144	7.55	<0.01
		Y × T	3	144	4.71	<0.01
Native	litter	Y	1	180	3.75	0.06
		T	3	180	4.07	<0.01
		Y × T	3	180	6.09	<0.01
	0-1 cm	Y	1	24	1.82	0.19
		T	3	144	5.18	<0.01
		Y × T	3	144	5.89	<0.01
	1-5 cm	Y	1	24	0.15	0.71
		T	3	144	1.88	0.14
		Y × T	3	144	1.32	0.27
	0-5 cm	Y	1	24	4.73	0.04
		T	3	144	2.93	0.04
		Y × T	3	144	2.46	0.05
	all	Y	1	24	5.91	0.02
		T	3	144	3.38	0.02
		Y × T	3	144	3.45	0.02
Non-native	litter	Y	1	47	1.77	0.19
		T	3	123	2.05	0.11
		Y × T	3	123	3.66	0.01
	0-1 cm	Y	1	22	0.03	0.96
		T	3	138	2.08	0.10
		Y × T	3	138	3.06	0.03
	1-5 cm	Y	1	47	0.04	0.85
		T	3	126	0.55	0.65
		Y × T	3	126	0.34	0.80
	0-5 cm	Y	1	24	0.70	0.41
		T	3	143	1.12	0.34

Table B3 Continued

Affected species	Soil layer	Source of variation	numDF	denDF	F-value	P-value
		Y × T	3	143	0.84	0.47
	all	Y	1	24	0.55	0.46
		T	3	144	0.52	0.67
		Y × T	3	144	1.93	0.13
Forbs	litter	Y	1	50	2.47	0.12
		T	3	137	5.51	<0.01
		Y × T	3	137	7.37	<0.01
	0-1 cm	Y	1	24	1.77	0.20
		T	3	144	6.71	<0.01
		Y × T	3	144	5.67	<0.01
	1-5 cm	Y	1	24	0.04	0.85
		T	3	144	1.60	0.19
		Y × T	3	144	1.53	0.21
	0-5 cm	Y	1	24	7.32	0.01
		T	3	144	3.60	0.02
		Y × T	3	144	3.02	0.03
	all	Y	1	24	9.19	<0.01
		T	3	144	4.30	<0.01
		Y × T	3	144	3.94	<0.01
Graminoids	litter	Y	1	22.5	1.22	0.28
		T	3	134	4.58	<0.01
		Y × T	3	134	6.07	<0.01
	0-1 cm	Y	1	48	0.44	0.51
		T	3	142	4.70	<0.01
		Y × T	3	142	3.27	0.02
	1-5 cm	Y	1	24	1.41	0.25
		T	3	138	0.36	0.78
		Y × T	3	138	0.63	0.60
	0-5 cm	Y	1	24	0.85	0.36
		T	3	144	2.22	0.09
		Y × T	3	144	1.61	0.19
	all	Y	1	24	0.02	0.89
		T	3	144	1.15	0.33
		Y × T	3	144	3.12	0.03

8.3 Appendix C: Analysis of variance (ANOVA) tables for the effects of smoke type and dilution on seed germination and seedling growth

Table C1: ANOVA tables for the effects of smoke type (T) and dilution (D) on total germination of tested species

Species	Germination conditions	Source of Variation	numDF	denDF	F-value	P-value
Artemisia frigida	10/0 °C Light/Darkness	T	2	119	12.10	<0.01
		D	5	119	15.51	<0.01
		T×D	10	119	6.62	<0.01
	10/0 °C Darkness	T	2	119	1.67	0.19
		D	5	119	36.43	<0.01
		T×D	10	119	5.54	<0.01
	25/15 °C Light/Darkness	T	2	119	2.74	0.07
		D	5	119	19.56	<0.01
		T×D	10	119	3.62	<0.01
	25/15 °C Darkness	T	2	119	4.61	0.01
		D	5	119	41.31	<0.01
		T×D	10	119	3.12	<0.01
Artemisia ludoviciana	10/0 °C Light/Darkness	T	2	119	0.34	0.71
		D	5	119	4.81	<0.01
		T×D	10	119	1.47	0.16
	10/0 °C Darkness	T	2	119	0.32	0.72
		D	5	119	12.99	<0.01
		T×D	10	119	0.77	0.65
	25/15 °C Light/Darkness	T	2	119	0.09	0.92
		D	5	119	2.32	0.05
		T×D	10	119	0.85	0.58
	25/15 °C Darkness	T	2	119	1.04	0.36
		D	5	119	15.78	<0.01
		T×D	10	119	0.97	0.48
Cirsium arvense	10/0 °C Light/Darkness	T	2	119	0.39	0.68
		D	5	119	1.67	0.19
		T×D	10	119	0.38	0.95
	10/0 °C Darkness	T	2	119	1.57	0.21
		D	5	119	2.00	0.08
		T×D	10	119	0.93	0.51
	25/15 °C Light/Darkness	T	2	119	2.09	0.13
		D	5	119	13.34	<0.01
		T×D	10	119	1.62	0.11
	25/15 °C Darkness	T	2	119	0.45	0.64
		D	5	119	18.90	<0.01
		T×D	10	119	1.42	0.18
Conyza canadensis	10/0 °C Light/Darkness	T	2	119	3.35	0.04
		D	5	119	5.47	<0.01
		T×D	10	119	1.92	0.05
	10/0 °C Darkness	T	2	119	1.39	0.25
		D	5	119	4.12	<0.01
		T×D	10	119	3.16	<0.01
	25/15 °C Light/Darkness	T	2	119	16.08	<0.01
		D	5	119	90.61	<0.01
		T×D	10	119	13.10	<0.01
	25/15 °C Darkness	T	2	119	15.44	<0.01
		D	5	119	13.45	<0.01
		T×D	10	119	12.84	<0.01

Table C2: ANOVA tables for the effects of smoke type (T) and dilution (D) on radical lengths of tested species

Species	Germination conditions	Source of Variation	numDF	denDF	F-value	P-value
Artemisia frigida	10/0 °C Light/Darkness	T	2	109	0.18	0.84
		D	5	109	1.89	0.10
		T×D	10	109	0.38	0.95
	10/0 °C Darkness	T	2	107	2.49	0.09
		D	5	107	2.04	0.13
		T×D	10	107	1.46	0.16
	25/15 °C Light/Darkness	T	2	110	1.86	0.16
		D	5	110	0.64	0.67
		T×D	10	110	1.17	0.32
	25/15 °C Darkness	T	2	108	2.68	0.07
		D	5	108	2.02	0.08
		T×D	10	108	0.77	0.66
Artemisia ludoviciana	10/0 °C Light/Darkness	T	2	115	1.64	0.20
		D	5	115	1.25	0.29
		T×D	10	115	0.80	0.63
	10/0 °C Darkness	T	2	116	0.21	0.81
		D	5	116	0.18	0.97
		T×D	10	116	0.18	1.00
	25/15 °C Light/Darkness	T	2	119	0.45	0.64
		D	5	119	1.80	0.12
		T×D	10	119	0.86	0.57
	25/15 °C Darkness	T	2	118	0.56	0.57
		D	5	118	2.88	0.02
		T×D	10	118	0.12	1.00
Cirsium arvense	25/15 °C Light/Darkness	T	2	115	2.06	0.13
		D	5	115	4.14	<0.01
		T×D	10	115	2.12	0.06
	25/15 °C Darkness	T	2	108	0.04	0.96
		D	5	108	9.57	<0.01
		T×D	10	108	0.68	0.74
Conyza canadensis	10/0 °C Light/Darkness	T	2	113	1.08	0.34
		D	5	113	2.90	0.02
		T×D	10	113	0.35	0.97
	10/0 °C Darkness	T	2	108	0.81	0.45
		D	5	109	2.18	0.06
		T×D	10	108	0.69	0.73
	25/15 °C Light/Darkness	T	2	123	8.52	<0.01
		D	5	123	23.41	<0.01
		T×D	10	123	3.01	<0.01
	25/15 °C Darkness	T	2	113	6.60	<0.01
		D	5	112	2.46	0.04
		T×D	10	112	5.34	<0.01

Table C3: ANOVA tables for the effects of smoke type (T) and dilution (D) on hypocotyl lengths of tested species

Species	Germination conditions	Source of Variation	numDF	denDF	F-value	P-value
Artemisia frigida	10/0 °C Light/Darkness	T	2	109	0.21	0.81
		D	5	109	0.87	0.50
		T×D	10	109	0.50	0.89
	10/0 °C Darkness	T	2	107	2.57	0.08
		D	5	107	2.23	0.06
		T×D	10	107	1.18	0.31
	25/15 °C Light/Darkness	T	2	110	0.68	0.51
		D	5	110	2.83	0.02
		T×D	10	110	0.71	0.72
	25/15 °C Darkness	T	2	108	0.67	0.51
		D	5	108	2.19	0.06
		T×D	10	108	0.36	0.96
Artemisia ludoviciana	10/0 °C Light/Darkness	T	2	115	1.49	0.23
		D	5	115	0.52	0.76
		T×D	10	115	0.91	0.52
	10/0 °C Darkness	T	2	116	0.34	0.71
		D	5	116	0.93	0.46
		T×D	10	116	0.40	0.94
	25/15 °C Light/Darkness	T	2	119	0.03	0.97
		D	5	119	0.85	0.51
		T×D	10	119	0.65	0.77
	25/15 °C Darkness	T	2	118	1.43	0.24
		D	5	118	2.57	0.03
		T×D	10	118	0.80	0.63
Cirsium arvense	25/15 °C Light/Darkness	T	2	115	1.28	0.28
		D	5	115	7.27	<0.01
		T×D	10	115	0.68	0.74
	25/15 °C Darkness	T	2	108	0.02	0.98
		D	5	108	5.96	<0.01
		T×D	10	108	0.31	0.98
Conyza canadensis	10/0 °C Light/Darkness	T	2	115	0.12	0.89
		D	5	115	1.43	0.22
		T×D	10	115	0.39	0.95
	10/0 °C Darkness	T	2	106	0.36	0.70
		D	5	107	1.56	0.18
		T×D	10	105	0.94	0.50
	25/15 °C Light/Darkness	T	2	120	1.98	0.14
		D	5	120	10.53	<0.01
		T×D	10	120	1.40	0.19
	25/15 °C Darkness	T	2	117	6.77	<0.01
		D	5	117	18.38	<0.01
		T×D	10	117	4.35	<0.01

Table C4: ANOVA tables for the effects of smoke type (T) and dilution (D) on total seedling lengths of tested species

Species	Germination conditions	Source of Variation	numDF	denDF	F-value	P-value
Artemisia frigida	10/0 °C Light/Darkness	T	2	109	0.06	0.95
		D	5	109	1.64	0.15
		T×D	10	109	0.38	0.95
	10/0 °C Darkness	T	2	107	2.93	0.06
		D	5	107	2.13	0.09
		T×D	10	107	1.66	0.10
	25/15 °C Light/Darkness	T	2	110	1.84	0.16
		D	5	110	0.38	0.86
		T×D	10	110	1.25	0.27
	25/15 °C Darkness	T	2	108	1.47	0.23
		D	5	108	2.02	0.08
		T×D	10	108	0.41	0.94
Artemisia ludoviciana	10/0 °C Light/Darkness	T	2	115	1.64	0.20
		D	5	115	1.16	0.33
		T×D	10	115	0.92	0.52
	10/0 °C Darkness	T	2	116	0.32	0.72
		D	5	116	0.45	0.81
		T×D	10	116	0.29	0.98
	25/15 °C Light/Darkness	T	2	119	0.31	0.73
		D	5	119	1.37	0.24
		T×D	10	119	0.79	0.63
	25/15 °C Darkness	T	2	118	0.90	0.41
		D	5	118	1.96	0.09
		T×D	10	118	0.44	0.92
Cirsium arvense	25/15 °C Light/Darkness	T	2	115	2.17	0.12
		D	5	115	6.72	<0.01
		T×D	10	115	1.53	0.14
	25/15 °C Darkness	T	2	108	0.03	0.97
		D	5	108	9.90	<0.01
		T×D	10	108	0.41	0.94
Conyza canadensis	10/0 °C Light/Darkness	T	2	119	0.90	0.41
		D	5	119	4.09	<0.01
		T×D	10	119	1.37	0.20
	10/0 °C Darkness	T	2	119	0.34	0.71
		D	5	119	5.23	<0.01
		T×D	10	119	0.84	0.59
	25/15 °C Light/Darkness	T	2	125	8.67	<0.01
		D	5	125	23.91	<0.01
		T×D	10	125	3.81	<0.01
	25/15 °C Darkness	T	2	118	9.97	<0.01
		D	5	118	14.41	<0.01
		T×D	10	118	7.27	<0.01

Table C5: ANOVA tables for the effects of smoke type (T) and dilution (D) on the days to 50% germination of tested species

Species	Germination conditions	Source of Variation	numDF	denDF	F-value	P-value
Artemisia frigida	10/0 °C Light/Darkness	T	2	114	0.21	0.81
		D	5	114	0.65	0.66
		T×D	10	114	0.71	0.71
	10/0 °C Darkness	T	2	114	2.07	0.13
		D	5	114	11.36	<0.01
		T×D	10	114	0.58	0.83
	25/15 °C Light/Darkness	T	2	113	0.16	0.85
		D	5	113	0.97	0.48
		T×D	10	113	0.77	0.65
	25/15 °C Darkness	T	2	112	0.51	0.60
		D	5	111	5.30	<0.01
		T×D	10	111	1.73	0.08
Artemisia ludoviciana	10/0 °C Light/Darkness	T	2	118	0.19	0.82
		D	5	118	0.71	0.62
		T×D	10	118	0.40	0.94
	10/0 °C Darkness	T	2	116	0.47	0.62
		D	5	116	2.23	0.06
		T×D	10	116	1.14	0.34
	25/15 °C Light/Darkness	T	2	119	1.08	0.34
		D	5	119	2.20	0.06
		T×D	10	119	0.74	0.69
	25/15 °C Darkness	T	2	118	1.75	0.18
		D	5	118	1.73	0.13
		T×D	10	118	0.98	0.46
Cirsium arvense	25/15 °C Light/Darkness	T	2	114	4.40	0.01
		D	5	114	2.58	0.03
		T×D	10	114	3.19	0.06
	25/15 °C Darkness	T	2	109	3.63	0.03
		D	5	109	6.49	<0.01
		T×D	10	109	1.88	0.06
Conyza canadensis	10/0 °C Light/Darkness	T	2	109	2.00	0.14
		D	5	109	16.19	<0.01
		T×D	10	109	1.68	0.09
	10/0 °C Darkness	T	2	91	3.18	0.06
		D	5	91	6.12	<0.01
		T×D	10	91	1.19	0.31
	25/15 °C Light/Darkness	T	2	120	0.06	0.95
		D	5	120	0.08	0.93
		T×D	10	120	0.12	0.89
	25/15 °C Darkness	T	2	120	0.07	0.94
		D	5	120	0.08	0.93
		T×D	10	120	0.15	0.87

8.4 Appendix D: Analysis of variance (ANOVA) tables for the effects of active fractions from alfalfa, prairie hay, and wheat straw, acetonitrile, and karrikinolide (KAR_1) on seed germination and seedling growth

Table D1: ANOVA table for the effects of active fractions from alfalfa, prairie hay, and wheat straw, acetonitrile, and karrikinolide (KAR_1) on total germination of tested species

Species	Germination conditions	Smoke type	numDF	denDF	F-value	P-value
Artemisia frigida	10/0 °C Light/Darkness	Acetonitrile	4	28	3.76	0.01
		Alfalfa	4	28	1.21	0.33
		Prairie hay	4	28	4.40	<0.01
		Wheat straw	4	28	2.22	0.09
		KAR_1	4	28	5.35	<0.01
	10/0 °C Darkness	Acetonitrile	4	28	0.85	0.51
		Alfalfa	4	28	1.22	0.33
		Prairie hay	4	28	1.35	0.28
		Wheat straw	4	28	0.54	0.70
		KAR_1	4	28	1.27	0.31
	25/15 °C Light/Darkness	Acetonitrile	4	28	1.04	0.41
		Alfalfa	4	28	0.89	0.48
		Prairie hay	4	28	1.50	0.23
		Wheat straw	4	28	0.55	0.70
		KAR_1	4	28	1.12	0.37
	25/15 °C Darkness	Acetonitrile	4	28	0.23	0.92
		Alfalfa	4	34	1.87	0.14
		Prairie hay	4	28	1.07	0.39
		Wheat straw	4	28	2.09	0.11
		KAR_1	4	28	1.81	0.15
Artemisia ludoviciana	10/0 °C Light/Darkness	Acetonitrile	4	28	1.02	0.42
		Alfalfa	4	28	2.34	0.08
		Prairie hay	4	28	2.12	0.11
		Wheat straw	4	28	2.22	0.09
		KAR_1	4	28	2.35	0.08
	10/0 °C Darkness	Acetonitrile	4	28	0.67	0.62
		Alfalfa	4	28	1.01	0.42
		Prairie hay	4	28	2.11	0.11
		Wheat straw	4	28	0.79	0.54
		KAR_1	4	28	1.74	0.17
	25/15 °C Light/Darkness	Acetonitrile	4	28	0.93	0.46
		Alfalfa	4	28	0.53	0.72
		Prairie hay	4	28	1.48	0.23
		Wheat straw	4	28	0.28	0.89
		KAR_1	4	28	0.87	0.50
	25/15 °C Darkness	Acetonitrile	4	28	1.19	0.34
		Alfalfa	4	28	1.44	0.25
		Prairie hay	4	28	2.84	0.04
		Wheat straw	4	28	2.84	0.04
		KAR_1	4	34	3.23	0.02
Cirsium arvense	10/0 °C Light/Darkness	Acetonitrile	4	34	0.38	0.82
		Alfalfa	4	28	0.10	0.98
		Prairie hay	4	35	0.90	0.47
		Wheat straw	4	28	1.46	0.24
		KAR_1	4	28	2.04	0.12
	10/0 °C Darkness	Acetonitrile	4	28	0.32	0.86
		Alfalfa	4	28	0.82	0.53
		Prairie hay	4	28	0.41	0.80
		Wheat straw	4	28	0.53	0.71
		KAR_1	4	28	0.84	0.51
	25/15 °C Light/Darkness	Acetonitrile	4	28	0.60	0.67
		Alfalfa	4	28	1.33	0.28

Table D1 Continued

Species	Germination conditions	Smoke type	numDF	denDF	F-value	P-value
		Prairie hay	4	28	0.90	0.48
		Wheat straw	4	28	0.56	0.69
		KAR_1	4	35	0.77	0.55
	25/15 °C Darkness	Acetonitrile	4	28	0.93	0.46
		Alfalfa	4	34	0.32	0.86
		Prairie hay	4	35	1.92	0.13
		Wheat straw	4	34	0.66	0.62
		KAR_1	4	35	0.36	0.84
Conyza canadensis	10/0 °C Light/Darkness	Acetonitrile	4	28	0.65	0.63
		Alfalfa	4	28	0.91	0.47
		Prairie hay	4	28	0.43	0.78
		Wheat straw	4	28	2.28	0.09
		KAR_1	4	28	1.27	0.30
	10/0 °C Darkness	Acetonitrile	4	34	1.45	0.24
		Alfalfa	4	28	1.13	0.36
		Prairie hay	4	28	0.59	0.67
		Wheat straw	4	28	0.69	0.61
		KAR_1	4	28	1.84	0.15
	25/15 °C Light/Darkness	Acetonitrile	4	28	0.53	0.72
		Alfalfa	4	35	0.98	0.43
		Prairie hay	4	28	3.49	0.02
		Wheat straw	4	28	3.12	0.03
		KAR_1	4	35	3.22	0.02
	25/15 °C Darkness	Acetonitrile	4	28	1.35	0.27
		Alfalfa	4	28	1.39	0.26
		Prairie hay	4	28	2.93	0.06
		Wheat straw	4	28	2.27	0.09
		KAR_1	4	28	1.31	0.29

Table D2: ANOVA table for the effects of active fractions from alfalfa, prairie hay, and wheat straw, acetonitrile, and karrikinolide (KAR_1) on radical lengths of tested species

Species	Germination conditions	Smoke type	numDF	denDF	F-value	P-value
Artemisia frigida	10/0 °C Light/Darkness	Acetonitrile	4	28	0.07	0.99
		Alfalfa	4	27	1.95	0.13
		Prairie hay	4	27	0.45	0.77
		Wheat straw	4	28	0.75	0.57
		KAR_1	4	28	1.00	0.42
	10/0 °C Darkness	Acetonitrile	4	28	1.91	0.14
		Alfalfa	4	27	0.87	0.49
		Prairie hay	4	27	1.01	0.42
		Wheat straw	4	27	0.69	0.60
		KAR_1	4	28	2.50	0.07
	25/15 °C Light/Darkness	Acetonitrile	4	28	1.29	0.30
		Alfalfa	4	28	1.24	0.32
		Prairie hay	4	28	2.79	0.06
		Wheat straw	4	28	2.29	0.08
		KAR_1	4	27	0.95	0.45
	25/15 °C Darkness	Acetonitrile	4	28	0.80	0.54
		Alfalfa	4	28	0.91	0.47
		Prairie hay	4	28	1.13	0.36
		Wheat straw	4	28	1.20	0.34
		KAR_1	4	28	1.53	0.22
Artemisia ludoviciana	10/0 °C Light/Darkness	Acetonitrile	4	28	0.79	0.54
		Alfalfa	4	27	1.84	0.15
		Prairie hay	4	26	0.87	0.49
		Wheat straw	4	28	0.23	0.92
		KAR_1	4	28	0.74	0.57
	10/0 °C Darkness	Acetonitrile	4	28	1.95	0.13
		Alfalfa	4	28	0.11	0.98
		Prairie hay	4	34	0.53	0.71
		Wheat straw	4	28	0.56	0.69
		KAR_1	4	28	0.66	0.63
	25/15 °C Light/Darkness	Acetonitrile	4	28	1.48	0.24
		Alfalfa	4	28	0.78	0.55
		Prairie hay	4	28	0.91	0.47
		Wheat straw	4	28	0.15	0.96
		KAR_1	4	28	1.38	0.27
	25/15 °C Darkness	Acetonitrile	4	28	1.36	0.27
		Alfalfa	4	28	0.89	0.48
		Prairie hay	4	28	0.44	0.78
		Wheat straw	4	28	0.36	0.83
		KAR_1	4	28	0.92	0.47
Cirsium arvense	25/15 °C Light/Darkness	Acetonitrile	4	35	0.50	0.74
		Alfalfa	4	28	1.35	0.28
		Prairie hay	4	34	2.46	0.07
		Wheat straw	4	28	0.93	0.46
		KAR_1	4	35	2.34	0.09
	25/15 °C Darkness	Acetonitrile	4	35	0.53	0.72
		Alfalfa	4	28	0.55	0.70
		Prairie hay	4	27	0.96	0.45
		Wheat straw	4	27	0.47	0.76
		KAR_1	4	35	0.22	0.92
Conyza canadensis	10/0 °C Light/Darkness	Acetonitrile	4	28	0.98	0.43
		Alfalfa	4	30	1.21	0.33
		Prairie hay	4	27	2.51	0.07
		Wheat straw	4	28	1.37	0.27
		KAR_1	4	28	0.86	0.50
	10/0 °C Darkness	Acetonitrile	4	25	2.16	0.10
		Alfalfa	4	32	1.74	0.17
		Prairie hay	4	32	0.47	0.76

Table D2 Continued

Species	Germination conditions	Smoke type	numDF	denDF	F-value	P-value
		Wheat straw	4	26	2.62	0.06
		KAR_1	4	23	0.94	0.46
	25/15 ℃ Light/Darkness	Acetonitrile	4	26	0.73	0.58
		Alfalfa	4	27	0.46	0.76
		Prairie hay	4	27	0.41	0.80
		Wheat straw	4	27	0.27	0.89
		KAR_1	4	28	0.45	0.78
	25/15 ℃ Darkness	Acetonitrile	4	24	1.76	0.17
		Alfalfa	4	24	2.24	0.09
		Prairie hay	4	24	2.29	0.09
		Wheat straw	4	24	1.66	0.19
		KAR_1	4	24	2.30	0.09

Table D3: ANOVA table for the effects of active fractions from alfalfa, prairie hay, and wheat straw, acetonitrile, and karrikinolide (KAR_1) on hypocotyl lengths of tested species

Species	Germination conditions	Smoke type	numDF	denDF	F-value	P-value
Artemisia frigida	10/0 °C Light/Darkness	Acetonitrile	4	28	1.23	0.32
		Alfalfa	4	27	0.56	0.69
		Prairie hay	4	28	0.23	0.92
		Wheat straw	4	28	0.98	0.44
		KAR_1	4	28	2.38	0.08
	10/0 °C Darkness	Acetonitrile	4	27	0.81	0.53
		Alfalfa	4	27	0.78	0.55
		Prairie hay	4	27	0.91	0.47
		Wheat straw	4	27	0.70	0.60
		KAR_1	4	27	1.97	0.13
	25/15 °C Light/Darkness	Acetonitrile	4	28	1.92	0.13
		Alfalfa	4	28	3.10	0.03
		Prairie hay	4	28	0.95	0.45
		Wheat straw	4	28	0.39	0.81
		KAR_1	4	27	0.46	0.77
	25/15 °C Darkness	Acetonitrile	4	28	0.44	0.78
		Alfalfa	4	34	0.58	0.68
		Prairie hay	4	28	1.92	0.13
		Wheat straw	4	28	0.39	0.81
		KAR_1	4	28	2.38	0.08
Artemisia ludoviciana	10/0 °C Light/Darkness	Acetonitrile	4	28	0.33	0.86
		Alfalfa	4	27	0.50	0.74
		Prairie hay	4	26	1.11	0.37
		Wheat straw	4	27	0.51	0.73
		KAR_1	4	28	2.20	0.10
	10/0 °C Darkness	Acetonitrile	4	28	0.63	0.65
		Alfalfa	4	27	0.60	0.67
		Prairie hay	4	28	0.99	0.43
		Wheat straw	4	28	0.65	0.63
		KAR_1	4	28	1.19	0.34
	25/15 °C Light/Darkness	Acetonitrile	4	28	0.38	0.82
		Alfalfa	4	34	1.29	0.30
		Prairie hay	4	35	0.09	0.98
		Wheat straw	4	28	0.22	0.93
		KAR_1	4	28	1.28	0.30
	25/15 °C Darkness	Acetonitrile	4	28	0.53	0.71
		Alfalfa	4	28	0.29	0.88
		Prairie hay	4	28	1.41	0.26
		Wheat straw	4	35	0.79	0.54
		KAR_1	4	28	0.51	0.73
Cirsium arvense	25/15 °C Light/Darkness	Acetonitrile	4	28	0.43	0.78
		Alfalfa	4	35	1.87	0.14
		Prairie hay	4	28	1.39	0.26
		Wheat straw	4	28	0.41	0.80
		KAR_1	4	28	0.46	0.77
	25/15 °C Darkness	Acetonitrile	4	28	2.50	0.07
		Alfalfa	4	28	1.47	0.24
		Prairie hay	4	28	2.30	0.08
		Wheat straw	4	27	1.44	0.25
		KAR_1	4	28	1.11	0.37
Conyza canadensis	10/0 °C Light/Darkness	Acetonitrile	4	28	0.11	0.98
		Alfalfa	4	33	0.32	0.86
		Prairie hay	4	27	2.63	0.06
		Wheat straw	4	28	0.21	0.93
		KAR_1	4	28	0.69	0.61
	10/0 °C Darkness	Acetonitrile	4	27	2.50	0.07
		Alfalfa	4	28	2.50	0.07
		Prairie hay	4	31	0.59	0.67

Table D3 Continued

Species	Germination conditions	Smoke type	numDF	denDF	F-value	P-value
		Wheat straw	4	25	0.42	0.79
		KAR_1	4	27	0.45	0.77
	25/15 °C Light/Darkness	Acetonitrile	4	32	2.54	0.06
		Alfalfa	4	27	0.80	0.54
		Prairie hay	4	27	1.63	0.20
		Wheat straw	4	27	0.89	0.48
		KAR_1	4	35	1.25	0.31
	25/15 °C Darkness	Acetonitrile	4	24	0.62	0.65
		Alfalfa	4	24	0.26	0.90
		Prairie hay	4	24	1.76	0.17
		Wheat straw	4	24	0.74	0.58
		KAR_1	4	24	0.04	1.00

Table D4: ANOVA table for the effects of active fractions from alfalfa, prairie hay, and wheat straw, acetonitrile, and karrikinolide (KAR_1) on total seedling lengths of tested species

Species	Germination conditions	Smoke type	numDF	denDF	F-value	P-value
Artemisia frigida	10/0 °C Light/Darkness	Acetonitrile	4	28	0.48	0.75
		Alfalfa	4	28	1.55	0.22
		Prairie hay	4	28	0.20	0.94
		Wheat straw	4	28	1.11	0.37
		KAR_1	4	28	1.69	0.18
	10/0 °C Darkness	Acetonitrile	4	28	1.73	0.17
		Alfalfa	4	28	1.31	0.29
		Prairie hay	4	28	0.65	0.63
		Wheat straw	4	28	1.32	0.29
		KAR_1	4	28	2.50	0.07
	25/15 °C Light/Darkness	Acetonitrile	4	28	1.98	0.12
		Alfalfa	4	28	1.92	0.14
		Prairie hay	4	28	1.49	0.23
		Wheat straw	4	28	1.07	0.39
		KAR_1	4	27	0.76	0.56
	25/15 °C Darkness	Acetonitrile	4	35	0.37	0.83
		Alfalfa	4	34	0.71	0.59
		Prairie hay	4	28	1.90	0.14
		Wheat straw	4	28	2.50	0.07
		KAR_1	4	28	0.64	0.64
Artemisia ludoviciana	10/0 °C Light/Darkness	Acetonitrile	4	28	1.01	0.42
		Alfalfa	4	28	0.74	0.58
		Prairie hay	4	28	0.96	0.44
		Wheat straw	4	28	0.52	0.72
		KAR_1	4	28	2.33	0.08
	10/0 °C Darkness	Acetonitrile	4	28	1.24	0.32
		Alfalfa	4	28	0.20	0.94
		Prairie hay	4	28	0.71	0.59
		Wheat straw	4	28	0.47	0.76
		KAR_1	4	28	0.44	0.78
	25/15 °C Light/Darkness	Acetonitrile	4	28	1.01	0.42
		Alfalfa	4	28	1.04	0.41
		Prairie hay	4	28	0.20	0.93
		Wheat straw	4	28	0.05	1.00
		KAR_1	4	28	1.55	0.21
	25/15 °C Darkness	Acetonitrile	4	27	1.80	0.16
		Alfalfa	4	28	0.51	0.73
		Prairie hay	4	28	0.64	0.64
		Wheat straw	4	28	0.25	0.90
		KAR_1	4	28	0.73	0.58
Cirsium arvense	25/15 °C Light/Darkness	Acetonitrile	4	28	0.30	0.88
		Alfalfa	4	28	2.54	0.06
		Prairie hay	4	28	2.54	0.06
		Wheat straw	4	28	0.75	0.56
		KAR_1	4	28	1.42	0.25
	25/15 °C Darkness	Acetonitrile	4	28	1.54	0.22
		Alfalfa	4	28	0.95	0.45
		Prairie hay	4	26	2.48	0.07
		Wheat straw	4	27	1.11	0.37
		KAR_1	4	28	0.53	0.72
Conyza canadensis	10/0 °C Light/Darkness	Acetonitrile	4	34	0.77	0.55
		Alfalfa	4	28	1.72	0.17
		Prairie hay	4	28	1.70	0.17
		Wheat straw	4	35	0.55	0.70
		KAR_1	4	28	0.28	0.89
	10/0 °C Darkness	Acetonitrile	4	28	1.41	0.26
		Alfalfa	4	34	2.33	0.08
		Prairie hay	4	34	1.60	0.20

Table D4 Continued

Species	Germination conditions	Smoke type	numDF	denDF	F-value	P-value
		Wheat straw	4	28	1.60	0.20
		KAR_1	4	28	1.35	0.28
	25/15 °C Light/Darkness	Acetonitrile	4	26	1.30	0.30
		Alfalfa	4	27	0.34	0.85
		Prairie hay	4	27	0.36	0.84
		Wheat straw	4	27	0.44	0.78
		KAR_1	4	28	0.49	0.74
	25/15 °C Darkness	Acetonitrile	4	24	0.35	0.84
		Alfalfa	4	24	0.49	0.74
		Prairie hay	4	24	0.83	0.52
		Wheat straw	4	24	0.08	0.99
		KAR_1	4	24	0.59	0.67

Table D5: ANOVA table for the effects of active fractions from alfalfa, prairie hay, and wheat straw, acetonitrile, and karrikinolide (KAR_1) on the days to 50% germination of tested species

Species	Germination conditions	Smoke type	numDF	denDF	F-value	P-value
Artemisia frigida	10/0 °C Light/Darkness	Acetonitrile	4	33	1.73	0.17
		Alfalfa	4	28	1.71	0.17
		Prairie hay	4	28	1.40	0.26
		Wheat straw	4	28	1.36	0.27
		KAR_1	4	28	0.95	0.45
	10/0 °C Darkness	Acetonitrile	4	28	0.44	0.78
		Alfalfa	4	28	0.83	0.52
		Prairie hay	4	26	0.91	0.47
		Wheat straw	4	28	1.42	0.25
		KAR_1	4	28	0.35	0.84
	25/15 °C Light/Darkness	Acetonitrile	4	28	1.17	0.35
		Alfalfa	4	34	2.43	0.07
		Prairie hay	4	35	0.21	0.93
		Wheat straw	4	28	0.42	0.79
		KAR_1	4	28	0.51	0.73
	25/15 °C Darkness	Acetonitrile	4	28	0.72	0.58
		Alfalfa	4	28	1.48	0.23
		Prairie hay	4	28	1.48	0.23
		Wheat straw	4	28	1.52	0.22
		KAR_1	4	28	1.20	0.33
Artemisia ludoviciana	10/0 °C Light/Darkness	Acetonitrile	4	28	1.22	0.32
		Alfalfa	4	23	0.09	0.98
		Prairie hay	4	28	1.31	0.29
		Wheat straw	4	28	0.42	0.79
		KAR_1	4	27	0.32	0.86
	10/0 °C Darkness	Acetonitrile	4	20	0.90	0.48
		Alfalfa	4	18	1.34	0.29
		Prairie hay	4	17	1.81	0.17
		Wheat straw	4	24	0.72	0.59
		KAR_1	4	19	1.26	0.32
	25/15 °C Light/Darkness	Acetonitrile	4	28	1.73	0.17
		Alfalfa	4	35	1.88	0.14
		Prairie hay	4	28	0.30	0.87
		Wheat straw	4	35	1.93	0.13
		KAR_1	4	28	0.94	0.46
	25/15 °C Darkness	Acetonitrile	4	28	0.38	0.82
		Alfalfa	4	28	1.54	0.22
		Prairie hay	4	28	1.05	0.40
		Wheat straw	4	28	1.83	0.15
		KAR_1	4	28	2.50	0.07
Cirsium arvense	25/15 °C Light/Darkness	Acetonitrile	4	28	0.52	0.72
		Alfalfa	4	28	1.84	0.15
		Prairie hay	4	34	1.00	0.42
		Wheat straw	4	35	1.17	0.34
		KAR_1	4	35	1.50	0.22
	25/15 °C Darkness	Acetonitrile	4	35	1.39	0.26
		Alfalfa	4	34	0.48	0.75
		Prairie hay	4	28	0.34	0.85
		Wheat straw	4	34	0.69	0.60
		KAR_1	4	28	1.26	0.31
Conyza canadensis	10/0 °C Light/Darkness	Acetonitrile	4	20	0.46	0.77
		Alfalfa	4	22	0.59	0.67
		Prairie hay	4	15	0.72	0.59
		Wheat straw	4	21	0.84	0.52
		KAR_1	4	21	1.24	0.32
	10/0 °C Darkness	Acetonitrile	4	10	0.38	0.82
		Alfalfa	4	4	0.80	0.58
		Prairie hay	4	14	1.08	0.40

Table D5 Continued

Species	Germination conditions	Smoke type	numDF	denDF	F-value	P-value
		Wheat straw	4	7	1.54	0.29
		KAR_1	4	7	0.40	0.80
	25/15 °C Light/Darkness	Acetonitrile	4	28	1.00	0.42
		Alfalfa	4	28	1.00	0.42
		Prairie hay	4	28	1.00	0.42
		Wheat straw	4	28	1.00	0.42
		KAR_1	4	35	2.33	0.07
	25/15 °C Darkness	Acetonitrile	4	28	1.75	0.17
		Alfalfa	4	28	1.00	0.42
		Prairie hay	4	35	1.47	0.23
		Wheat straw	4	28	1.00	0.42
		KAR_1	4	35	1.47	0.23