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Effects of Three Fire-Suppressant Foams on the Germination and Physiological Responses of Plants

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Abstract Suppressant foams used to fight forest fires may leave residual effects on surviving biota that managers need to consider prior to using them. We examined how three fire-suppressant foams (FSFs) (Forexpan S, Phos-Chek-WD881, and Silv-ex) affected seed germination and physiological responses of three plant species. Exposure to FSFs, whether in diluted concentrations or those typical in the field, reduced final germination percentages of seeds grown in petri dishes and within growth chambers. However, the FSFs did not cause total germination failure in any treatment. Inhibition of germination increased with longer exposure times, but only to diluted FSF solutions. Unlike in the laboratory experiments, none of the three FSFs affected seedling emergence when tested in field conditions. Further, we found no evidence of long-term phytotoxic effects on antioxidant enzyme activity nor chlorophyll content of the plant saplings. Therefore, although the three FSFs showed evidence of phytotoxicity to plants in laboratory tests, their actual impact on terrestrial ecosystems may be minimal. We suggest that the benefits of using these FSFs to protect plants in threatened forest ecosystems outweigh their minor risks.

Keywords Fire-suppressant foams · Seed · Germination · Phytotoxicity · Antioxidant enzyme · Wildfire

Introduction

The increasing frequency and severity of wildfires have generated global concern for the improvement of fire-fighting techniques (Couto-Vázquez et al. 2011). Various fire-fighting chemicals (FFCs) have been developed, including fire-suppressant foams (FSFs) and retardants to prevent the spread of fires (Larson et al. 1999). Residential areas typically are in close proximity to the lush mountainsides of South Korea, so FSFs frequently are needed to suppress forest fires (Kim et al. 2008). In Korea between the 1970s and 2000s, the number of large (over 100 ha) forest fires increased from 37 to 53 a year and the total burned area rose from 2,200 to 33,000 ha. Consequently, effects of forest fire-fighting techniques on the environment potentially have significant consequences that need to be examined (Lee 2010).

To date, the ecological impacts of FFCs have not been well studied so their effects on affected organisms and ecosystems remain poorly understood. Some research suggests, however, that the use of FFCs can significantly reduce germination rates of seeds (Angeler et al. 2004; Luna et al. 2007), lead to declines in species richness (Larson et al. 1999), and cause long-term changes in soil–plant ecosystems (Couto-Vázquez et al. 2011). Because of their stationary nature, plants are extremely susceptible to phytotoxic effects (Kalabokidis 2000), but these remain to

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be elucidated. If FFCs alter plant community structure, a cascade of ecological effects can be expected. For example, seed banks that survive and eventually regenerate (Meney et al. 1994) may cause shifts in species composition. In turn, such ecological changes might increase the potential for soil runoff and result in other physico-chemical changes (Marcos et al. 2000).

The effects of FSFs on the germination and physiological responses of plants are of special interest to ecologists as they potentially are of high significance to ecosystems. The Korean situation differs from that faced by fire fighters in many other parts of the world as fires typically occur from late winter to the middle of the spring, soon before seeds germinate and seedling growth periods. Moreover, fires can rapidly be fueled by the previous year's growth. In Korea, only one study has examined the ecological impact of FSFs, using simple germination and survival tests (Kim et al. 2008). Additional data thus are needed by managers so that they can knowledgeably assess whether to use FSFs when combating wildland fires. Fighting fires quickly and efficiently involves tradeoffs to the environments, such as potential residual damage FSFs impart to plant communities. Should germination or emergence rates change in post-fire fields, experimental studies are necessary to determine whether these changes come about due to the effects of the fire itself or the FSFs used to fight it.

We conducted this study to clearly delineate these tradeoffs. We examined germination rates of plant seeds, taking into account FSF concentration, developmental stage, and time of application. We conducted studies both in the laboratory and the field to understand whether FSFs would impart significant measurable effects in the field. We also measured the ecophysiological responses of treated plants to develop a better understanding of the mechanism of FSFs' effects on plants.

Materials and Methods

Description of Chemicals

We used the fire-suppressant foams Foxepan S (Angus Fire Ltd., Lancaster, England; mixture of hydrocarbon surfactants, glycol solvents and water; Angus Fire 2011), Phos-Chek-WD881 (ICL Performance Products LP, St. Louis, MO, USA; mixture of alpha-olefin sulfonate, 2,4-pentanediol, 2-methyl-, 1-dodecanol and d-limonene; ICL 2005) and Silv-ex (Ansul, Marinette, WI, USA; mixture of sodium and ammonium salts of fatty alcohol ether sulfates, higher alcohols and water; Ansul 2005). We selected these FSFs because their manufacturers provided detailed information on ingredients and all advised their use at the same maximum dilution rate of 1 %. This FSF concentration is typically used in helicopter-watering, the most common fire-fighting method in Korea (Kim et al. 2008). We tested both diluted FSFs and undiluted FSFs because evaporation and accumulation increase effective FSF concentrations to which plants are exposed in realistic field conditions (Adams and Simmons 1999). The same dilution rate has been used in other studies in Australia (Adams and Simmons 1999) and the USA (Buhl and Hamilton 1998; McDonald et al. 1997), but we are unaware of any previous studies that assessed the toxicity of the original FSFs before dilution.

Species Studied

For the germination test, we selected Korean red pine (*Pinus densiflora* S. et Z.). As one of the most abundant plant species in Korea (Lee et al. 2004), it benefits from the country's strongest fire management plan (Lee et al. 2005), but its responses to FSFs have not previously been studied. We conducted germination tests on pitch pine (*Pinus rigida* Miller) which, although not an indigenous species, thrives in Korea on a level comparable to that of *Pinus densiflora* (Hong et al. 1995). We also conducted studies on rape (*Brassica campestris* subsp. *napus* var. *nippo-oleifera* Makino) as a representative herbaceous plant species.

Sawtooth oak (*Quercus acutissima* Carr.), found throughout Korea (Kang et al. 2005), pin oak (*Quercus palustris* Muenchh), an introduced species, and *B. campestris* were selected to study FSF effects on antioxidant enzyme activity. Protein extraction from leaves is an essential step in measuring antioxidant enzyme activity (Song and Lee 2010), but we were unable to extract protein from the leaves of *P. densiflora* and *P. rigida* by the method of Bradford (1976). Therefore, we conducted these tests on the broadleaved species *Q. acutissima*, on which we had previously run assays (Song and Lee 2010), and *Q. palustris* as an introduced species like *P. rigida.* The seeds were vernalized for 4 weeks and sterilized for 10 min in 10 % sodium hypochlorite solution (USEPA 1996) before application.

Seed Germination Monitoring (Chamber Experiment)

Seeds were placed into 100 mL plastic bottles with either FSF solutions (experimental groups) or distilled water (DW) (controls). They were soaked for 1, 6, 12, or 24 h, in dark conditions at room temperature, while being gently shaken at 100 rpm. Owing to the high viscosity of the original FSF solutions, an orbital shaker was used to mix the seeds and FSFs thoroughly. After the soaking period, all samples were washed thoroughly with DW.

Half of the seeds were transferred into 100-mm petri dishes with one piece of filter paper (90 mm) and 5 mL of DW (Lin and Xing 2007) and the other half were transferred into 5 × 10 matrix plug trays (4.5 cm × 4.5 cm for each cell) filled with the growing soil (Sunshine Mix #5, Sun Gro Horticulture Canada, Alberta).Previous studies examining toxicological effects on germination usually have been conducted in petri dishes (Guenzi and McCalla 1962; Lin and Xing 2007). However, most seeds in forests germinate in litter soil, composed of decaying organic matter, rather than mineral soil (Tao et al. 1987). Thus, to simulate field conditions, we placed commercial soil composed mostly of organic matter into the matrix plug trays.

Each petri dish or pot cell contained ten seeds (ten replicates), and we recorded germination rates every third day (12 times total). *B. campestris* was measured only seven times due to mold and decomposition. Seeds were considered to have germinated when roots had grown 2 mm beyond the seed coat. All petri dishes and pot cells were kept in a growth chamber under a range of Organization for Economic Cooperation and Development guidelines (OECD 2003): temperature: 24 °C, humidity: 70 ± 25 %, photoperiod: 18 h light, and light intensity: 300 μE/m^2/s with protection from drying.

Seedling Emergence Monitoring (Field Experiment)

Field experiments were conducted on the southern slope of Gwanaksan mountain in Seoul, Korea. Vegetation at the field site is dominated by *P. densiflora* and *P. rigida,* and receives an average light intensity of 53.2 ± 1.7 % (mean ± SE of three replicates) as measured in open areas during the experimental period. We made plots and planted seeds (100 seeds per plot) in February, in advance of any FSF application. Seeds were planted at a depth of 0.5 cm in five replicates of 10 cm × 10 cm plots. For the first treatment, FSF solutions (1 % dilutions) were applied in late February at a concentration of 1 L/m^2, typical of local application standards (Kim et al. 2008) and similar to previous studies (Angeler et al. 2004). For the second treatment, FSFs were applied to some quadrats at this standard application rate (1 L/m^2) twice in early February and late February to simulate the effect of accumulated depositions due to repeated applications. For the third treatment, FSFs were applied to other quadrats, not previously treated, at the standard application rate (1 L/m^2) during the following month. To avoid edge effects, a 5 cm buffer zone was maintained on all sides of each quadrat in which no FSFs were applied. The emergence of seeds was checked every 15 days until late June.

Antioxidant Enzyme Activity and Chlorophyll Contents

In May of 2009, we transplanted 3-year-old *Q. acutissima* and *Q. palustris* into pots (16 cm diameter; 12 cm height) and placed them in a greenhouse. Seedlings of *B. campestris* were grown in plug trays in growth chamber before being transplanted into pots filled with growing soil which were also transported to the greenhouse. In early September, FSFs were applied to the pots at the dosages of either five (×5 treatment) or ten times (×10 treatment) the standard application rate. The higher rates were used because at the standard application rate (1 L/^{2}), the treatment did not penetrate through the topsoil to deeper layers. Thus, we reasoned that the FSFs were unlikely to make direct contact with plant roots. After the FSF application at these higher concentrations, water was supplied frequently but in small volumes to prevent leaching.

Total antioxidant capacity (TAC) and superoxide dismutase (SOD) activities were measured following protocols of Song and Lee (2010). The chlorophyll content of leaves was simultaneously measured by the dimethyl sulfoxide extraction method (Hiscox and Israelstam 1979). For the duration of the experiment, the average temperature of the greenhouse remained at 22.6 °C (measured by data logger HOBO H9; Onset Computer, Bourne, MA, USA).

Statistical Analyses

For the chamber germination experiment, we used multiway analysis of variance (ANOVA) to examine the effects of five fixed factors: species, chemical type, whether diluted or not, duration of soaking, petri dish or pot, as well as the interactions (up to secondary interactions) among them on germination rate. We considered duration of soaking as a categorical factor since it includes only two groups (1 and 24 h of soaking).

For the field experiment, we examined the effects of three fixed factors by ANOVA: species, chemical type, treatment, as well as the interactions (up to secondary interactions) among them on seedling emergence rate. For the antioxidant enzyme and chlorophyll content experiment, we also used ANOVA to examine the effects of three fixed factors: species, chemical type, dose (5 and 10 times), as well as the interactions (up to secondary interactions) on TAC, SOD, or chlorophyll.

We performed a backward model selection procedure based on the *P* value (criterion of 0.05) and the best model was presented in the results. Tukey's HSD test was conducted as a post hoc test for the significant terms. We adjusted p-values to correct family-wise error rates in a multiple comparison (i.e., to prevent accumulation of type 1 error). All statistical analyses were conducted in R 2.14.1 (http://www.r-project.org).

Results

Seed Germination Monitoring (Chamber Experiment)

Germination rates were lower in treatments of every species exposed to undiluted FSFs compared to controls (DW-1 h) in both petri dishes and pots (Table 1; Fig. 1). Diluted FSFs also inhibited germination rates for the seeds soaked in solutions for long time periods (12 and 24 h) (Table 1). Germination rates of petri dishes were significantly higher than those of pot treatments (Table 1; Fig. 1). Germination rates significantly decreased with increasing soaking time. Results of statistical analyses are shown in Table S1 in the Electronic Supplementary Material.

Germination rate also was affected by combinations of the treatments. There were significant interactions among five fixed factors: species, chemical type, whether diluted or not, petri dish–pot, and duration of soaking (Fig. 1; Table S1). Germination rates were consistently lower in treatments than controls (Table 1). We have highlighted the most important interactions (Figs. 1, S1).

Table 1 Germination rates (%) of *Pinus densiflora, Pinus rigida* and *Brassica campestris* in petri dish and pot experiments

Solution	Time (h)	FOREXPAN S		WD881		SILV-EX	
		Petri	Pot	Petri	Pot	Petri	Pot
Pinus densiflora							
Undiluted	1	6.0 ± 3.1	11.0 ± 2.8	9.0 ± 3.5	12.0 ± 5.5	11.0 ± 4.8	16.0 ± 3.7
	6	0.0 ± 0.0	0.0 ± 0.0	6.0 ± 3.4	1.0 ± 1.0	0.0 ± 0.0	5.0 ± 1.7
	12	1.0 ± 1.0	0.0 ± 0.0	1.0 ± 1.0	1.0 ± 1.0	1.0 ± 1.0	1.0 ± 1.0
	24	1.0 ± 1.0	8.0 ± 8.0	0.0 ± 0.0	0.0 ± 0.0	2.0 ± 1.3	0.0 ± 0.0
Diluted (1 %)	1	53.0 ± 3.7	49.0 ± 4.1	24.0 ± 5.0	51.0 ± 8.6	52.0 ± 3.6	25.0 ± 6.5
	6	11.0 ± 3.8	18.0 ± 3.3	9.0 ± 2.8	23.0 ± 4.0	14.0 ± 5.2	34.0 ± 6.4
	12	0.0 ± 0.0	6.0 ± 2.2	26.0 ± 4.5	4.0 ± 1.6	39.0 ± 5.3	5.0 ± 2.2
	24	0.0 ± 0.0	7.0 ± 2.1	6.0 ± 2.2	2.0 ± 2.0	12.0 ± 4.4	6.0 ± 2.2
DW	1	66.0 ± 3.1	37.0 ± 6.3	66.0 ± 3.1	37.0 ± 6.3	66.0 ± 3.13	37.0 ± 6.3
	24	36.0 ± 5.6	35.0 ± 4.8	36.0 ± 5.6	35.0 ± 4.8	36.0 ± 5.6	35.0 ± 4.8
Pinus rigida							
Undiluted	1	3.0 ± 2.1	14.0 ± 9.1	1.0 ± 1.5	3.0 ± 3.0	0.0 ± 0.0	3.0 ± 3.0
	6	6.0 ± 2.7	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0
	12	5.0 ± 4.0	1.0 ± 1.0	1.0 ± 1.0	1.0 ± 1.0	2.0 ± 2.0	0.0 ± 0.0
	24	1.0 ± 1.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0
Diluted (1 %)	1	72.0 ± 5.5	45.0 ± 7.8	71.0 ± 4.1	41.0 ± 4.1	54.0 ± 7.5	44.0 ± 8.6
	6	24.0 ± 5.8	12.0 ± 4.4	13.0 ± 7.0	15.0 ± 4.5	5.0 ± 2.2	22.0 ± 6.5
	12	51.0 ± 7.1	10.0 ± 3.7	28.0 ± 6.8	0.0 ± 0.0	15.0 ± 4.3	0.0 ± 0.0
	24	28.0 ± 7.1	12.0 ± 5.1	15.0 ± 9.9	3.0 ± 1.5	0.0 ± 0.0	0.0 ± 0.0
DW	1	86.0 ± 3.1	45.0 ± 4.5	86.0 ± 3.1	45.0 ± 4.5	86.0 ± 3.1	45.0 ± 4.5
	24	77.0 ± 5.2	64.0 ± 4.0	77.0 ± 5.2	64.0 ± 4.0	77.0 ± 5.2	64.0 ± 4.0
Brassica campestris							
Undiluted	1	8.0 ± 3.6	6.0 ± 1.6	12.0 ± 3.3	9.0 ± 1.0	3.0 ± 1.5	3.0 ± 1.5
	6	4.0 ± 1.6	9.0 ± 1.8	1.0 ± 1.0	2.0 ± 1.3	1.0 ± 1.0	1.0 ± 1.0
	12	4.0 ± 1.6	7.0 ± 2.6	9.0 ± 2.8	3.0 ± 1.5	1.0 ± 1.0	1.0 ± 1.0
	24	1.0 ± 1.0	2.0 ± 1.3	1.0 ± 1.0	0.0 ± 0.0	2.0 ± 1.3	2.0 ± 1.3
Diluted (1 %)	1	26.0 ± 1.6	8.0 ± 2.5	21.0 ± 3.5	7.0 ± 2.6	3.0 ± 1.5	4.0 ± 1.6
	6	21.0 ± 1.8	3.0 ± 1.5	14.0 ± 1.6	10.0 ± 2.6	4.0 ± 1.6	5.0 ± 1.7
	12	21.0 ± 1.8	7.0 ± 2.1	18.0 ± 8.9	2.0 ± 1.3	1.0 ± 1.0	1.0 ± 1.0
	24	4.0 ± 2.2	1.0 ± 1.0	8.0 ± 2.0	1.0 ± 1.0	3.0 ± 1.5	1.0 ± 1.0
DW	1	97.0 ± 1.5	86.0 ± 1.6	97.0 ± 1.5	86.0 ± 1.6	97.0 ± 1.5	86.0 ± 1.6
	24	98.0 ± 1.3	89.0 ± 1.8	98.0 ± 1.3	89.0 ± 1.8	98.0 ± 1.3	89.0 ± 1.8

The data are presented as the means and SE of ten replicates

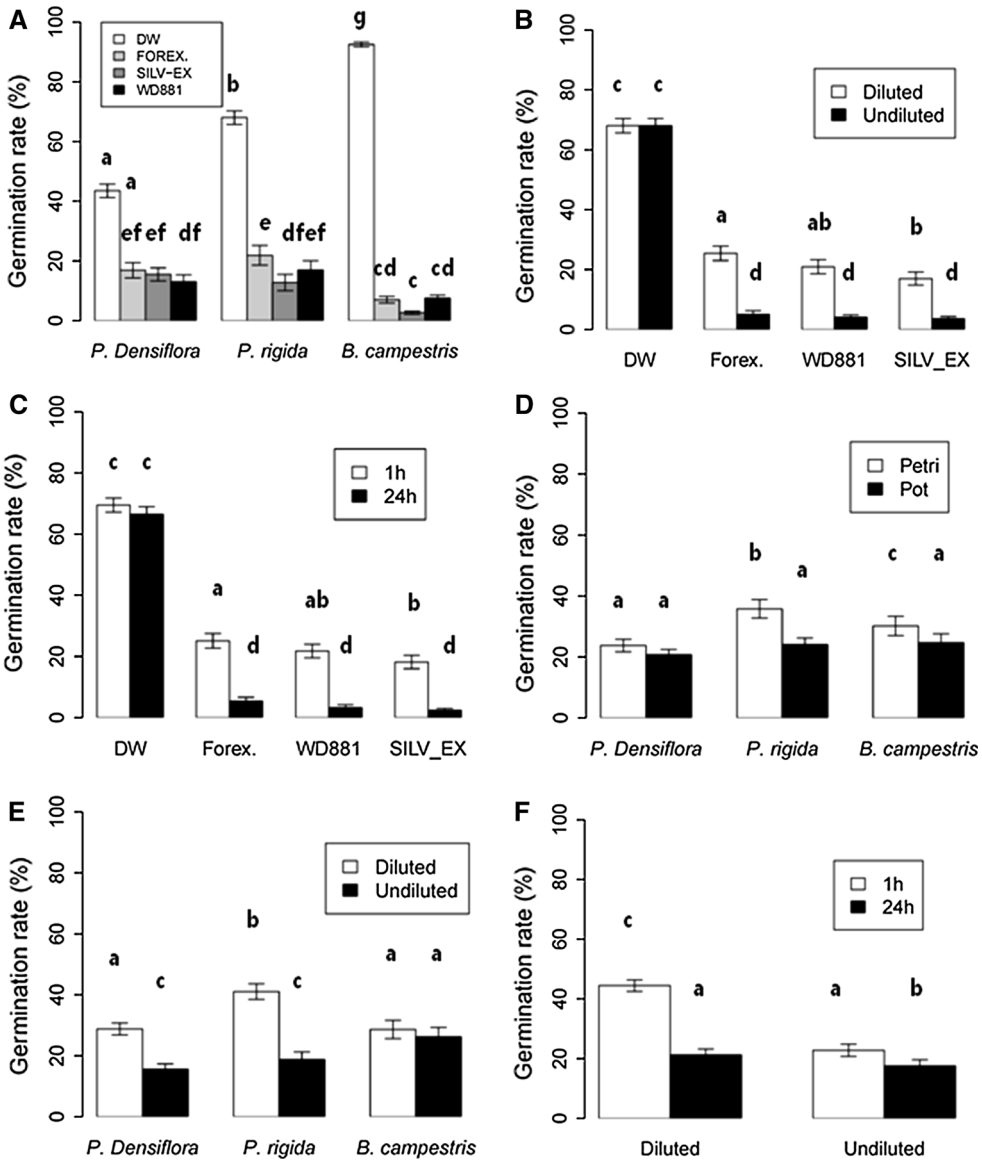

Fig. 1 Analysis of interactions among factors affecting seed germination experiments: **a** Solution and species, **b** Solution and dilution, **c** Solution and time, **d** Species and petri–pot, **e** Species and dilution, **f** Dilution and time. The *bars* and *error bars* represent the mean ± SE of; **a** 80 replicates, **b** and **c** 120 replicates, **d** and **e** 160 replicates, **f** 240 replicates. *Bars* having the same letter are not significantly different ($P > 0.05$)

Seedling Emergence Monitoring (Field Experiment)

No significant effects of FSFs on germination rates were found in the three treatments (FSFs applied at 1 L/m^2 in February and March; FSFs applied at 2 L/m^2 to certain quadrats in February; and the control group) (Table 2). However, species differed in their emergence rate ($F_{2.177} = 928.0$, $P < 0.001$). Figures of interactions with statistical significance are shown in Fig. S2 in the electronic supplementary material.

Antioxidant Enzyme Activity and Chlorophyll Contents

TAC did not significantly vary with concentration or among FSFs. For *Q. palustris* only, TAC readings were significantly higher than controls (Table 3; Fig. 2a). However, the increased values of TAC were small (Table 3). TAC results differed among species ($F_{2.107} = 47.09$, $P < 0.001$) and dose ($F_{1.107} = 7.67$, $P < 0.01$), but not among FSFs. The interaction between species and FSF ($F_{6.107} = 2.64$, $P < 0.05$), but none of the other interaction terms, was significant (Table 3; Figs. 2, S3).

Average values of SOD measures were about 15 % higher than controls (Fig. 2b), and Solution and Dose (5 and 10 times) showed significant differences between themselves, that "Solution" showed difference with $F_{3.115} = 5.48$ and $P < 0.01$ and "Dose" showed difference with $F_{1.115} = 9.94$ and $P < 0.01$. However, there were no significant interactions among factors (Table 3; Figs. 2, S3).

Total chlorophyll contents 1 week after the FSF treatments did significantly change for *Q. acutissima* and *B. campestris* (Table 3; Fig. 2c). The magnitude of these

Table 2 Germination rates of plants in the field after treatment with FSF

Species	Treatment	FOREXPAN S	WD881	SILV-EX
Pinus densiflora	February	5.6 ± 1.4	5.6 ± 1.2	6.0 ± 0.7
	February ×2	5.4 ± 0.9	6.0 ± 1.4	5.4 ± 0.9
	March	3.6 ± 0.7	6.0 ± 0.7	6.0 ± 0.7
	Control	4.2 ± 0.9	4.2 ± 0.9	4.2 ± 0.9
	February	9.0 ± 0.5	7.8 ± 0.9	8.8 ± 0.7
Pinus rigida	February ×2	7.4 ± 0.5	5.6 ± 1.2	6.8 ± 0.6
	March	6.6 ± 1.4	7.0 ± 0.7	8.0 ± 0.4
	Control	7.8 ± 0.9	7.8 ± 0.9	7.8 ± 0.9
	February	54.4 ± 6.1	53.8 ± 4.3	56.2 ± 5.4
Brassica campestris	February ×2	46.0 ± 3.0	50.6 ± 4.2	56.8 ± 9.6
	March	38.8 ± 4.7	45.0 ± 3.3	56.6 ± 3.1
	Control	49.4 ± 2.6	49.4 ± 2.6	49.4 ± 2.6

The data are presented as the means and SE of five replicates

February and March: Solutions are given at the rate of 1 L/m^2

February ×2: Solutions are given at the rate of 2 L/m^2 in February

FSFs fire-suppressant foams

Table 3 Antioxidant enzyme activities and total chlorophyll contents plants after treatment with FSFs

Antioxidant enzyme	*Quercus acutissima*		*Quercus palustris*		*Brassica campestris*	
	TAC	SOD	TAC	SOD	TAC	SOD
Control	0.18 ± 0.01	0.83 ± 0.13	0.42 ± 0.02	0.99 ± 0.00	0.14 ± 0.01	0.97 ± 0.01
FOREX. ×5	0.22 ± 0.02	0.88 ± 0.05	0.57 ± 0.04	1.05 ± 0.03	0.14 ± 0.01	1.02 ± 0.02
WD881 ×5	0.20 ± 0.01	0.97 ± 0.05	0.55 ± 0.06	1.02 ± 0.02	0.15 ± 0.01	0.98 ± 0.00
SILV-EX ×5	0.19 ± 0.02	0.95 ± 0.06	0.49 ± 0.08	1.02 ± 0.04	0.14 ± 0.01	1.01 ± 0.02
FOREX. ×10	0.27 ± 0.02	1.03 ± 0.02	0.64 ± 0.02	1.05 ± 0.04	0.16 ± 0.01	1.10 ± 0.04
WD881 ×10	0.23 ± 0.01	1.20 ± 0.14	0.55 ± 0.05	1.01 ± 0.01	0.16 ± 0.02	1.10 ± 0.05
SILV-EX ×10	0.26 ± 0.01	1.21 ± 0.13	0.64 ± 0.05	1.09 ± 0.05	0.17 ± 0.01	1.07 ± 0.05
Chlorophyll contents	*Quercus acutissima*		*Quercus palustris*		*Brassica campestris*	
	1 week	3 week	1 week	3 week	1 week	3 week
Control	31.3 ± 0.3	31.9 ± 0.5	36.3 ± 0.4	31.6 ± 0.9	28.2 ± 0.6	27.1 ± 0.4
FOREX. ×5	30.9 ± 0.3	31.4 ± 0.6	36.0 ± 0.3	33.2 ± 0.6	25.5 ± 0.5	26.8 ± 0.4
WD881 ×5	31.0 ± 0.4	32.1 ± 0.3	36.0 ± 0.4	32.1 ± 0.5	25.4 ± 0.1	26.2 ± 0.3
SILV-EX ×5	31.2 ± 0.3	31.2 ± 0.4	36.2 ± 0.3	32.8 ± 0.5	24.8 ± 0.3	26.6 ± 0.2
FOREX. ×10	28.6 ± 0.5	30.7 ± 0.4	35.9 ± 0.4	33.1 ± 0.4	24.1 ± 0.4	26.2 ± 0.4
WD881 ×10	30.4 ± 0.6	31.3 ± 0.5	36.0 ± 0.8	34.1 ± 0.9	25.1 ± 0.9	27.2 ± 0.2
SILV-EX ×10	28.3 ± 0.5	30.8 ± 0.4	35.6 ± 0.3	23.4 ± 0.9	24.3 ± 1.3	26.7 ± 0.5

The data are presented as the means and SE of five replicates

Numbers behind '×' indicates amount of solution applied (ex: 'x5' means solutions were given five times more than usual application rate of 1 L/m^2)

TAC: mM/mg protein, SOD: U/mg protein, Chlorophyll: mg/L

1 week 1 week after treatment, *3 week* 3 weeks after treatment

FSFs fire-suppressant foams, *FOREX*. FOREXPAN S

effects significantly differed among FSFs ($F_{3.105} = 20.56$, $P < 0.001$) and species ($F_{2.105} = 87.12$, $P < 0.001$). Species' responses to FSFs varied ($F_{6.105} = 4.61$, $P < 0.001$) as they did to different doses ($F_{2.105} = 3.12$, $P < 0.05$) at 3 weeks (Table 3; Figs. 2, S3). However, there were no significant interactions after 3 weeks.

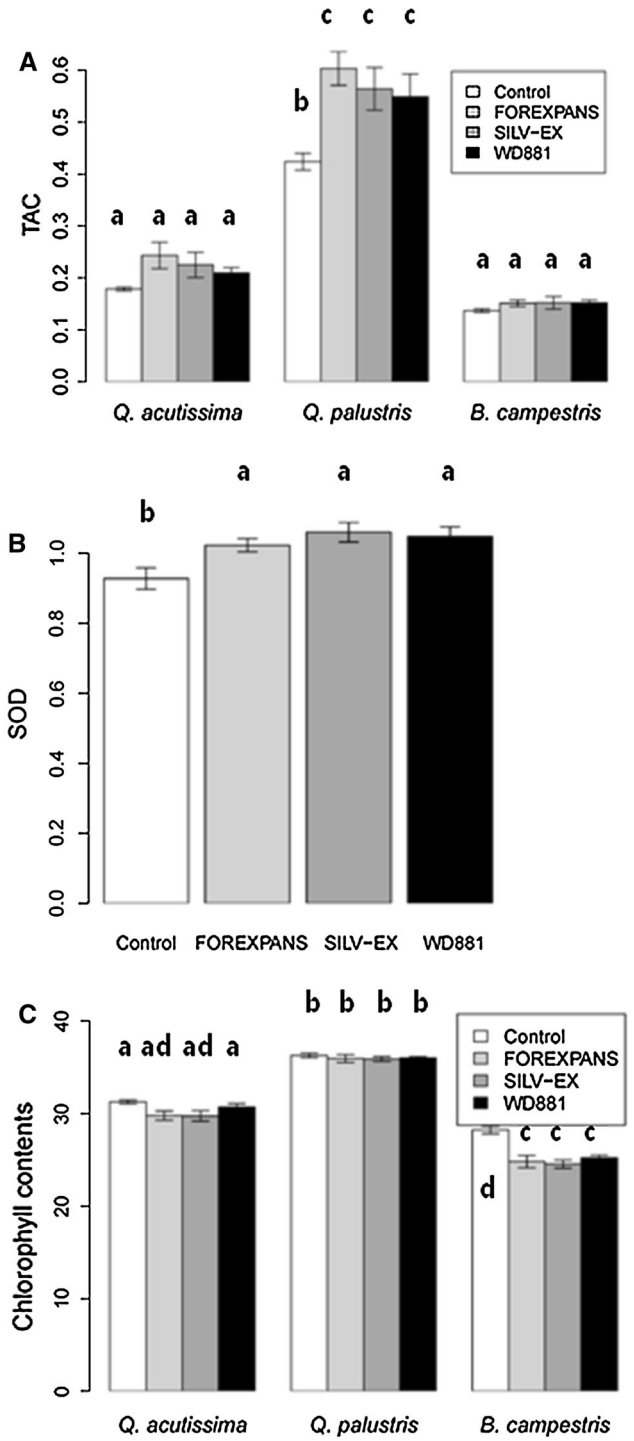

Fig. 2 Analysis of interactions among factors affecting physiological responses of plants after FSF application; **a** Species and solution interaction for TAC results, **b** Differences among solutions for SOD results, **c** Species and solution interaction for chlorophyll contents. The *bars* and *error bars* represent the mean ± SE of: **a** 5 replicates, **b** 15 replicates, **c** 5 replicates. *Bars* having the same letter are not significantly different ($P > 0.05$). TAC: mM/mg protein; SOD: U/mg protein, Chlorophyll: mg/L. *FSFs* Fire-suppressant foams, *TAC* Total antioxidant capacity, *SOD* Superoxide dismutase

Discussion

Seed Germination Monitoring (Chamber Experiment)

All FSF treatments resulted in germination rates significantly less than those of controls (DW) in every species tested, whether grown in petri dishes or pots (Table 1; Fig. 1a). *B campestris* was most sensitive to FSFs despite its controls (DW) showing higher germination rates. The toxicity of the FSFs decreased as their solutions were diluted (Fig. 1b), but germination was inhibited even in the most dilute treatments.

Increasing exposure time from 1 to 24 h dramatically decreased germination rates (Fig. 1c). Undiluted FSFs inhibited germination after every application, suggesting acute toxicity. Plant species with thick seed coats (*P. densiflora* and *P. rigida*) soaked in diluted FSF treatments briefly (1 h) showed much less inhibition than those soaked for longer times (24 h) (Table 1; Fig. 1f). Exposed to undiluted FSFs, species with thick seed coats showed similar or higher germination rates (e.g., pot experiments of *P. densiflora* treated with WD881) than controls (Table 1). This suggests that by weakening the seed coat, FSFs actually facilitate germination. Plants lacking a thick seed coat (i.e., *B. campestris*) suffered substantially reduced germination rates after exposure to all FSF treatments, compared to controls, but showed less effects of soaking time or dilution.

The germination rate of *B. campestris* under control conditions was higher than that of the other two species, but its seeds experienced lower germination rates than the other species even in the weakest FSF treatments (Table 1). Since its seed does not have a thick seed coat like the other two woody species, the FSFs can penetrate the seed easily. Therefore, *B. campestris* appears to be most at risk from these FSFs, even after brief exposure and in diluted concentrations. This result implies that small-seeded species without thick seed coats, particularly herbaceous plants, may be more vulnerable to FSFs. The phytotoxicity of FSFs on herbaceous plants has received little attention (Angeler et al. 2004; Luna et al. 2007), and as important ecological components of the forest understory, further research on them is crucial (Gilliam 2007).

Moreover, *P. densiflora* germinated at considerably reduced rates in the control group at long application times (DW-24 h treatment; Table 1), suggesting that some seed species are vulnerable to submersion stress (Table 1). Therefore, the effects of FSFs on seeds soaked with for long time periods (12 and 24 h) may be overestimated as a result of side effects attributable to their being submerged

in water. The inhibiting effects of submersion need to be considered in planning future experiments.

Overall, undiluted solutions of all three FSFs yielded very low germination rates for every species even when exposed for only 1 h, indicating the exceedingly phytotoxic nature of undiluted FSFs. As exposure time increased, germination rates of seeds exposed to diluted FSFs also decreased, highlighting the importance of exposure time. Therefore, further research about additional water supplies as soon as possible after extinguishing a fire to wash off FSFs and prevent prolonged exposure to the chemicals should be tested and inspected. Of course, immediate responses might be impracticable for a variety of reasons, as staff, water supplies, and funding may be exhausted, but managers cannot afford to lose sight of the important benefits of providing additional water supplies to ensure the recovery of plants in fire-damaged areas.

Conditions in the pot experiment more closely approximated those faced by plants in natural conditions than did those in petri dishes, but germination rates in these experiments were drastically reduced too (Figs. 1d, S1a,c,d in the Electronic Supplementary Material). These results suggest that seeds are exposed to FSFs that persist in soil, and thus soil seed banks remaining after a fire might be vulnerable to toxic effects over some time period following the fire. Importantly, while undiluted and diluted chemical treatments reduced final germination percentages, they did not prevent germination altogether. Hence, seeds that survive initial effects of FSF exposure on germination might replace the species' populations, but will subsequently be affected by exposure to contaminated soil. This effect might be ameliorated by efforts to wash off FSFs. However, the number of seeds that germinate still might be too low to effect restoration, dependent on likely requisite seed bank survival rates. We present in Table 1 germination rates for each FSF application that should be useful for assessing the likelihood of effective restoration in real scenarios.

Seedling Emergence Monitoring (Field Experiment)

Field experiments were conducted in the natural habitat of the selected species. Because local forest fires occur often during dry spring seasons (Kim et al. 2008), FSF treatments were applied in February and March. The emergence rates of the species in the field site initially were very low, even among controls (Table 2), but nonetheless did not substantially differ from those of plants in the FSF-treated plots. In the pot experiments, *Pinus* seeds tested in soil germinated at lower rates (Table 1), so we would expect to see this effect also in seed germination rates in the field. Emergence rates, always lower than germination rates, are more indicative of survival. Further, unlike the chamber experiments, continuous water was not supplied, so dry soil conditions in the field (Kim et al. 2008) would inhibit seedling emergence.

Neither the standard application rate of FSFs (1 L/m^2) nor even double this rate appeared to affect the seeds, indicating that FSFs do not damage seeds in the field. These results suggest that FSF applications may be safe for the maintenance of seed banks and should not hamper the eventual recovery of plant communities. The only significant differences that we found were among species, with *B. campestris* having a much higher emergence rate than the other two species (Fig. S2). Although germination rates were decreased by exposure to FSFs in our growth chamber experiments, we found no differences in emergence attributable to FSFs in the field. Therefore, other plant reactions such as antioxidant enzyme activity and chlorophyll contents were investigated to test potential toxicity of FSFs.

Antioxidant Enzyme Activity and Chlorophyll Contents

Understanding the dynamics of plant ecosystem recovery following the extinguishment of a fire requires tests to discriminate between effects attributable to fire damage and those resulting from FSF toxicity. In such tests, unlike in field experiments, germination rates can be determined under conditions that control precipitation and other factors that potentially could alter FSF toxicity. On the other hand, indoor experiments do not necessarily accurately reflect realistic effects of FSFs on plants. We thus conducted field experiments to investigate whether plants in natural conditions suffer acute toxicity on exposure to FSFs. Seedlings might be affected by FSFs even if they successfully germinate, so to obtain a more sensitive assay of potential FSF effects, we devised measures of physiological stresses experienced by seedlings in FSF-contaminated soil.

Since the plants were treated with FSF up to ten times the standard application rate, they might be expected to suffer more adverse effects owing to stress than those subject to the standard application. Also, this situation (expose to higher rate) can happen in actual fields when sprayed FSFs migrate to lower areas or gather at puddles of the mountain slope. However, our measures of TAC showed no evidence of increased stress. TAC did not significantly vary with concentration or among FSFs. For *Q. palustris* only, TAC readings were significantly higher than controls (Table 3; Fig. 2a). However, the increased values of TAC were small (Table 3), half as much as was previously measured just from transplanting the plants (Song and Lee 2010). These results suggest that plants are suffering less stress than expected.

Average values of SOD measures were about 15 % higher than controls (Fig. 2b), comparable to the 15 % increase caused by transplanting that we previously found (Song and Lee 2010). Therefore, the stress induced by the application of FSFs seems negligible and probably would not affect plant survival. Since environmental stress can influence plants' antioxidant activities (Song and Lee 2010) and SOD values are frequently used as an indicator of pollution stress (Koricheva et al. 1997), there would seem to be little reason to be concerned that FSFs damage plants by stressing them.

However, total chlorophyll contents 1 week after the FSF treatments did significantly change for *Q. acutissima* and *B. campestris* (Table 3; Fig. 2c), indicating that, at least by this measure, FSFs do affect plants' stress levels. Nevertheless, after 3 weeks the total chlorophyll contents of FSF-treated plants reverted to levels shown in the control group, indicating that plants have the ability to quickly recover from FSF stress.

For some species, application rate (5 and 10 times) significantly increased TAC and SOD and decreased chlorophyll contents (Fig. S3). Thus deposition in soil coupled with evaporation may have increased the effective concentration to which plants were exposed. This observation reinforces our suggestion that water supply should be provided after fire extinguishment. However, at realistic field application rates, as used in this research, FSFs should not be harmful to plants.

Although FSFs have some properties that are toxic to germination and emergence in experimental conditions, their overall effects on plants in natural ecosystems probably are minimal. Previous research and our ecophysiological results support the hypothesis that FFCs do not significantly affect plants in terrestrial ecosystems (Couto-Vázquez et al. 2011; Larson et al. 1999). How FSFs would impact aquatic ecosystems, however, is still unknown. Release of FSFs into aqueous systems is likely to harm some fishes, especially at larval stages (McDonald et al. 1997), as well as aquatic invertebrates (Buhl and Hamilton 1998). Therefore, where there is danger of migration of FSFs into aquatic environments, extra caution is warranted including post-application monitoring of FSF levels (Buhl and Hamilton 1998; McDonald et al. 1997).

Because of our inability to extract proteins from conifers, we substituted deciduous species in their place, so we could not estimate stress indicators such as antioxidant enzyme activity for *Pinus* species. Thus, we cannot be certain of the sensitivity of conifer species to FSFs. However, chlorophyll contents of experimental *P. densiflora* and *P. rigida* that we obtained prior to attempted protein extraction did not differ from those of controls (Table S2).

Conclusion

The FSFs Forexpan S, Phos-Chek-WD881, and Silv-ex reduced germination rates of seeds in laboratory experiments, regardless of whether they were applied in standard or diluted concentrations. More inhibition of germination occurred with longer exposure times, especially for diluted FSF concentrations. However, after exposure to standard concentrations, as typically used in FSF applications, at least some seeds always germinated, even in the longest exposure treatments.

By contrast, in field experiments, the three FSFs did not affect emergence rate in field experiments of any of the tested species. Neither woody species (*Pinus rigida* and *P. densiflora*) nor herbaceous species (*B. campestris)*, which showed the most vulnerability to the chemicals in laboratory experiments, were affected by FSFs in the field. Plant saplings showed only negligible stress responses, as measured by antioxidant enzyme activity and chlorophyll content, to FSFs even when the application rate was up to ten times higher than the standard application rate. Furthermore, physiological measurements on the plant saplings revealed no evidence of any long-term phytotoxic effects.

Therefore, although we have shown that the three fire-suppressant foams that we tested have some toxic properties, their effects on plants in terrestrial ecosystems probably are negligible. The efficacy of FSFs to protect plants in forest ecosystems against the threat of wildfires seems to outweigh any minor negative effects. We conclude that FSFs probably are safe for forest managers to use against fires.

Our conclusions must be tempered by the limitations of our experiment which only examined three Korean species. Although at typical application rates, even herbaceous plants appeared unaffected, further work might find that other species are more sensitive to FSFs. Because sprouting plays an important role in regeneration after fire (Bond and Midgley 2001) only minimal effects on trees would be expected. Where FSFs accumulate and become concentrated in soils after evaporation, the potential for damage might be higher but we suggest still not severe enough to outweigh potential fire damage of fire. Our conclusions agree with those of previous studies (Couto-Vázquez et al. 2011; Larson et al. 1999). Nonetheless, it would be prudent for forest managers to test germination and seedling sensitivities of the major species in their areas before applying FSFs to avoid unforeseen negative effects. Since effects on aquatic ecosystems still are uncertain, these habitats need to be monitored following the application of FSFs for additional negative effects.

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