# Influence of diesel fuel on seed germination

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**Abstract**

The use of plant-based systems to remediate contaminated soils has become an area of intense scientific study in recent years and it is apparent that plants which grow well in contaminated soils need to be identified and screened for use in phytoremediation technologies. This study investigated the effect of diesel fuel on germination of selected plant species. Germination response varied greatly with plant species and was species specific, as members of the same plant family showed differential sensitivity to diesel fuel contamination. Differences were also seen within plant subspecies. At relatively low levels of diesel fuel contamination, delayed seed emergence and reduced percentage germination was observed for the majority of plant species investigated. Results suggest the volatile fraction of diesel fuel played an influential role in delaying seed emergence and reducing percentage germination. In addition, the remaining diesel fuel in the soil added to this inhibitory effect on germination by physically impeding water and oxygen transfer between the seed and the surrounding soil environment, thus hindering the germination response.

*Keywords : germination, diesel fuel, volatile fraction, plant species, phytoremediation.*

*"Capsule" : The volatile fraction of diesel fuel played a major role in delaying seed emergence and reducing percentage germination.*

## 1. Introduction

Phytoremediation is the use of green plants and their associated microbiota to remediate contaminated soil, sediment and water. The use of plant-based systems for cleaning up wastes is not new. In fact, the use of plants to cleanse waters contaminated with organic and inorganic pollutants dates back hundreds of years (Cunningham *et al*., 1996) and forms the basis of reed bed, constructed wetland and municipal wastewater treatment technology that we use today. The concept of using plants to remediate contaminated soil is a more recent development and has become an area of intense scientific study.

Diesel fuel, on entering the terrestrial environment, will spread and seep into the soil. The downward migration of diesel fuel through the soil profile however is limited due to the physical properties of the fuel (Adam and Duncan, 2002). Under normal conditions, diesel fuel will be adsorbed in the organic rich surface soil, impeding downward migration. This makes diesel fuel contaminated soil a likely candidate for phytoremediation as the contaminant is held in the surface soil and within the rooting zone of most plant species. Plants have been shown to encourage organic contaminant degradation principally by providing an optimal environment for microbial proliferation in the rhizosphere (Kruger et al., 1997). These degradative processes are influenced not only by the rhizosphere microorganisms but also by unique properties of the host plant (Walton et al., 1994). If plants can be successfully established on polluted soils, then the plant-microbial interaction in the rhizosphere may provide enhanced breakdown of diesel fuel in vegetated soils as opposed to non vegetated soils. It is apparent that more plants that grow well in contaminated soils need to be identified and screened for use in phytoremediation technologies.

Grasses are used in remediation of contaminated soil due to their fibrous root systems with extensive surface area for microbial colonisation. The fibrous root system forms a continuous, dense rhizosphere, which provides ideal conditions for phytoremediation. Nitrogen fixing plants such as legumes have been used to remediate contaminated soil as nitrogen is a critical component in the mineralisation of organic contaminants in soil. When organic contaminants such as petroleum hydrocarbons contaminate soil, the added carbon stimulates microbial numbers but causes an imbalance in the soil C:N ratio which may result in immobilisation of soil nitrogen by the microbial biomass, leaving none available for plant growth. Legumes fix atmospheric nitrogen to produce their own source of nitrogen for growth therefore they may prove more successful at growing on petroleum hydrocarbon contaminated soil. In support of this statement, species of *Leguminosae* have been found to be the most abundant reinhabitors of petroleum hydrocarbon contaminated sites (Gudin and Syratt, 1975).

A selection of plant species including grasses, legumes, herbs and commercial crops were screened for their ability to germinate in diesel fuel contaminated soil. The most successful plant species would then be used in phytoremediation studies of diesel fuel contaminated soil. The work described here is a continuation of a study on the effect of diesel fuel on the growth of selected plant species (Adam and Duncan, 1999).

## 2. Materials and methods

### 2.1 Plant Screening

Twenty five plant species including grasses, legumes, herbs and commercial crops were screened for their ability to germinate in diesel fuel contaminated soil (Table 1). The soil used in this experiment was John Innes Seed Compost (textural class: Sand, 89.8% sand, 4.9% silt and 8.4% clay, organic matter content (LOI) 10.2% and total nitrogen content 0.19%). To obtain an even distribution of diesel fuel in the soil, diesel fuel was mixed with acetone before adding to the soil. The diesel:acetone was then mixed thoroughly through the soil and the acetone allowed to evaporate off in a fume cupboard. This procedure was used to prepare 25g and 50g diesel $kg^{-1}$ contaminated soil. Uncontaminated controls were prepared by adding acetone only to the soil. Ten grammes of uncontaminated, 25g and 50g diesel $kg^{-1}$ soil were weighed, in duplicate, into petri dishes. Seeds of each plant species investigated were planted in the appropriate petri dish and the soil moistened. The lids were replaced on the petri dishes and the petri dishes incubated at 22 °C ± 2 °C in the dark until the majority of seeds had germinated. The developing seedlings were then grown in light conditions at 22 °C ± 2 °C with a 16 hour light/8 hour dark cycle. The compost in the petri dishes was moistended when necessary and the percentage germination recorded after 7 and 14 days.

## 2.2 Germination of Plant Species at Lower Temperatures

Seeds of eight plant species were planted in 0g, 25g and 50g diesel $kg^{-1}$ soil as described above except the petri dishes were incubated at 8 °C ± 2 °C. Once germination was apparent in the majority of replicates the developing seedlings were subjected to light conditions at 22 °C ± 2 °C with a 16 hour light/8 hour dark cycle. The compost in the petri dishes was moistened when necessary and percentage germination recorded weekly for six weeks.

## 2.3 Germination of Plant Species in 'Aged' Soil

Seeds of five plant species were planted in 0g, 25g and 50g diesel $kg^{-1}$ soil that had previously been contaminated. The soil was contaminated as described above then stored in open polythene bags for three weeks to prepare contaminated soils with the more volatile diesel fuel components at a minimum. As before, the compost in the petri dishes was moistened then incubated at 22 °C ± 2 °C in the dark until germination was apparent. The developing seedlings were grown in light conditions at 22 °C ± 2 °C with a 16 hour light/8 hour dark cycle and percentage germination recorded after 7 and 14 days.

## 2.4 Viability of Diesel Fuel Soaked Seeds

The method for measuring germinating ability of Flax seeds was adapted from the method of Smith (1951).

Two hundred and forty Flax seeds were split into three groups (80 seeds in each). These seeds were subjected to the following treatments : 1) 80 seeds submerged and soaked in diesel fuel for 24 hours; 2) 80 seeds submerged and soaked in diesel fuel for 48 hours and 3) 80 seeds submerged and soaked in diesel fuel for 168 hours. Half the seeds from each treatment (40 seeds in total) were removed from the diesel fuel, lightly dried on a paper towel then placed in a 1.5% triphenyl tetrazolium chloride (TTC) solution. The seeds soaking in TTC solution were incubated at 22 °C ± 2 °C for 24 hours to determine the extent of diesel fuel penetration and damage to the viability of the seed. After 24 hours, seeds were removed from the TTC solution and dissected using a sharp blade to examine the extent of colour development at the embryo. Seeds were cut along the longitudinal axis to expose the embryo clearly. The colour developed was visually rated: red, pink and no colour which provided an estimate of viability: very viable, may be viable or non viable.

The remaining 40 seeds from each treatment were split into four groups of 10 seeds and planted in petri dishes containing John Innes Seed Compost. The compost in the petri dishes was moistened and incubated in the dark at 22 °C ± 2 °C until the first signs of germination, when they were allowed to grow in light conditions. The percentage germination of the pre soaked seeds was calculated. A control was set up for each test with uncontaminated seeds.

## 3. Results

### 3.1 Plant Screening Results

The ability of seeds to germinate in diesel fuel contaminated soil varied greatly depending on plant species (see Table 2). Some plant species were affected initially,

resulting in delayed seed emergence. Sheep's Fescue, Common Vetch, White Clover and Little Yellow Trefoil had low percentage germination rates in diesel fuel contaminated treatments at 7 days but germination increased significantly by 14 days. Inhibition of germination generally increased with increasing diesel fuel concentration. The ability to tolerate diesel fuel contamination whilst germinating was species specific as members of the same plant family showed differential sensitivity to diesel fuel contamination. This was clearly illustrated by the family *Gramineae* (Grasses) with some species germinating well (e.g. Westerwold's Ryegrass) whilst others had poor germination (e.g. Rough Meadow Grass). Differences were also seen within plant subspecies as illustrated by the differential sensitivity shown by the Bents. These results suggest whatever was affecting percentage germination initially was not persistent. Diesel fuel contains both volatile and non volatile components with the volatile fraction making up approximately 5-10% of the total product in most diesel fuels (unpublished results). The effect of the volatile fraction of diesel fuel was therefore the most likely explanation for this short lived, inhibitory effect on germination.

## 3.2 Effect of the Volatile Diesel Fuel Fraction on Germination

The inhibition of seed germination by the volatile fraction of diesel fuel was investigated in a series of germination experiments designed to minimise the concentration of volatile diesel fuel in contact with the germinating seeds.

Seeds were germinated at a lower temperature of 8°C to minimise the volatilisation of low molecular weight compounds but still allow germination to proceed. Measurements were made over the course of 6 weeks due to the slowed

germination response at the lower temperature. After 6 weeks, the percentage germination of most of the plants tested had improved significantly when compared with the 2 week results (see Table 3). Chewing's Fescue and Strong Creeping Red Fescue plants did particularly well under these conditions with percentage germination being very similar in the 0g, 25g and 50g diesel $kg^{-1}$ contaminated soils. There was no apparent effect of diesel fuel concentration on the percentage germination in any of the species examined except for Common Bent whose percentage germination remained significantly different from the control. Germinating seeds at lower temperatures appeared to slow volatilisation of diesel fuel components, which resulted in a less detrimental effect on germination. This observation has been noted by several authors for hydrocarbons at lower temperatures (Bossert and Bartha, 1984, Rogers *et al*., 1996).

Five of the plant species previously investigated were germinated in diesel fuel contaminated soil that had been 'aged' for three weeks before the seeds were sown to ensure the majority of volatile diesel fuel components were at a minimum. The results from this experiment were compared with the results from the original screening experiment as illustrated in Figure 1. The values show the ratio of percentage germination of each diesel fuel contaminated treatment as a fraction of the control, which is equal to 100% germination. Three of the plant species tested had higher percentage germination in the 'aged' soils compared to the freshly contaminated soil that was used in the initial screening experiment. The largest difference in germination was seen with Black Grass, which germinated poorly in freshly contaminated soil but improved greatly, particularly at the 25g diesel $kg^{-1}$ soil level, in 'aged' soil. At the lower level of contamination (25g diesel $kg^{-1}$ soil), most plants showed a slight increase in percentage germination in the 'aged' soil but a large improvement was seen at the

50g diesel $kg^{-1}$ soil level. The only exceptions were Strong Red Creeping Fescue and Chewing's Fescue who showed a similar response to both freshly contaminated and 'aged' diesel fuel contaminated soils at the 25g diesel $kg^{-1}$ treatment level but showed a larger improvement in the 'aged' soils at the 50g diesel $kg^{-1}$ treatment level.

These results suggest the volatile fraction of diesel fuel has an influential role in delaying seed emergence and reducing germination. However, results of the diesel fuel contaminated treatments with minimal volatile diesel fuel components were never as high as the control germination results. This suggests that the influence of the volatile fraction of diesel fuel is not the only factor inhibiting seed germination. The remaining diesel fuel in the soil still had some level of toxicity to the germinating seeds.

## 3.3 Effect of Diesel Fuel on Seed Viability

A method for determining the germinating ability of cereals using triphenyl tetrazolium chloride (TTC) was developed by Smith in 1951. In the presence of viable seed tissue, colourless solutions of TTC were reduced to insoluble, red triphenyl formazan (TPF). It was therefore possible to predict the germinating ability of seeds by observation of the embryo parts that were stained red by insoluble TPF deposits. A variation on this method was performed on a variety of seeds to determine whether this method of estimating seed viability could be used on seed species other than cereals. The results suggested that seeds with easily penetrable seeds coats were successfully classed whereas seeds with thick seed coats were difficult to assess (unpublished results). The difficulty in correctly assessing seeds with thick seed coats was probably due to the reduced penetrability of the TTC solution through their seed coats resulting in inconsistent penetration of the TTC solution. This lead to viable seeds being wrongly

classed as non viable. From this preliminary experiment, Flax seeds were found to be the most successful seed species for use in viability studies as they allowed easy penetration of the TTC solution through their seed coats facilitating rapid colour development (within 24 hours), the seeds were easy to dissect and the stained embryo was clearly visible.

Seeds of the Flax variety 'Viking', dissected after pre soaking in diesel fuel, showed that the majority of seeds had retained their viability even after soaking for 168 hours as illustrated in Table 4. Pre soaking for 48 and 168 hours showed only 2.5% of the seeds to be completely non viable suggesting that diesel fuel had penetrated into the seed, killing the embryo. Flax seeds planted in soil after pre soaking in diesel fuel differed from control seeds in their pattern of germination. Diesel fuel soaked seeds tended to have a longer lag phase preceding germination and the lag in germination seemed to increase with increasing soaking time as seen in Figure 2. The percentage germination after 5 days fell from 97.5% in the control to 87.5% after 24 hours soaking, to 67.5% and 42.5% after 48 and 168 hours respectively. Despite the longer lag period, the overall percentage germination of diesel fuel soaked seeds after 14 days was not badly affected except by the 168 hour pre soaking treatment, where the percentage germination fell from the 90% range for the other pre soaking times to 70% after 168 hours soaking. This is in agreement with the results of the TTC test where the majority of pre soaked seeds were showing signs of viability except at the 168 hour pre soaked treatment where fewer seeds were classed as viable. These results suggest the presence of diesel fuel was delaying seed emergence without adversely damaging the majority of seeds. This inhibitory effect on germination could be attributed more to physical constraints than biological damage on the seeds as the majority of seeds were showing tetrazolium chloride reducing properties which suggests respiring, healthy seeds

## 4. Discussion

The differential sensitivity of plants to hydrocarbon toxicity is well known (Crafts and Reiber, 1948, Currier, 1951, Baker, 1970, Warner *et al*., 1983, Gauvrit and Cabanne, 1993, Chaîneau *et al*., 1997) and exploited to man's benefit. For example, members of the family *Umbelliferae* (e.g. carrots) are notably tolerant to injury by lighter oils (low molecular weight, BP range 150-275°C) whereas grasses are intolerant. This specificity allowed oils to be successfully used as post emergence herbicides in vegetable crops (Gauvrit and Cabanne, 1993). Phytotoxicity was seen to increase with gravity through the series gasoline, kerosene, diesel fuel and heavy oil, indicating that the lighter fractions, either because they were more volatile or because the compounds present were less toxic, caused less long term damage to the plants than the heavier fraction (Crafts and Reiber, 1948). Highly volatile hydrocarbons, primarily those that are small and lightweight are able to move through cell membranes easily. Small hydrocarbon molecules, which penetrate into the plant, can cause toxic effects (van Overbeek and Blondeau, 1954), which although acute, are generally short lived. Acute toxicity caused by the lighter fraction of diesel fuel may explain the delayed seed emergence and reduction in percentage germination displayed by the plants in the initial screening experiment. When the concentration of volatile diesel fuel components was kept to a minimum, a significant increase in percentage germination was observed. This supports the theory that the volatile fraction of diesel fuel was affecting germination. Percentage germination in treatments where the volatile diesel fuel components were at a minimum were not however, as high as percentage germination in uncontaminated soil. This suggested some additional factor was influencing germination.

Seeds soaked in diesel fuel for varying lengths of time showed a lag period before germinating but the overall percentage germination rate after 14 days was not badly affected except by the 168 hour pre soaking treatment. This inhibitory effect on germination could be attributed more to physical constraints than biological damage to the seeds resulting from the physical and chemical characteristics of diesel fuel. Although the toxicity of diesel fuel has decreased in recent years due to the removal of many of the aromatic compounds present in diesel fuel, it is still toxic to plants at certain concentrations. The embryo of a seed could easily be injured or killed if it were to come in contact with diesel fuel. Seeds have a primary line of defence preventing diesel fuel penetration – their seed coat. The integrity and hardness of the seed coat affects the rate of oil penetration (Amakiri and Onofeghara, 1984). Only 2.5% of the 48 hour and 168 hour pre soaked seeds were classed as non viable. There was little indication that the embryo's were being killed in this investigation, as the majority of seeds were showing tetrazolium reducing properties, suggesting respiring, healthy seeds. Injury to the embryo may not have been fatal, but reduced the growth activity of the embryo which resulted in delayed seed emergence. These results indicate that seed coats resistant to oil penetration will be virtually unaffected and therefore, a prerequisite to embryo damage by oil is tissue penetration. Amakiri and Onofeghara (1984) showed that seeds of *Capsicum frutescens* retained almost 100% viability after 32 weeks pre soaking in crude oil but the lag phase preceding germination was increased threefold.

A more likely reason for the inhibitory effect of diesel fuel on germination is diesel fuels physical water repellent property. The film of diesel fuel around the seeds may act as a physical barrier, preventing or reducing both water and oxygen from entering the seeds. This would inhibit the germination response and explains why the

seeds still reduced TTC to TPF after being submerged in diesel fuel but had an extended lag phase before germinating.

Diesel fuel on entering the terrestrial environment will not migrate far into the soil profile due to the hydrophobic properties of the fuel. This means diesel fuel will be held in the surface soil and within the rooting zone of most seed species. Seeds planted into this contaminated soil will become coated with diesel fuel which is represented by soaking the seeds in diesel fuel, where the seeds are in contact with the diesel fuel at all times. Although soaking seeds in diesel fuel is not directly comparable to sowing seeds in diesel fuel contaminated soil, it allowed the seeds to be in contact with the contaminant whilst allowing the seeds to be studied for signs of germination. If the seeds were planted directly into diesel fuel contaminated soil this would not have been possible.

## 5. Conclusion

Germination of seeds in diesel fuel contaminated soil is highly dependent on plant species. Some species were notably tolerant whilst other species were completely intolerant of diesel fuel contamination. The ability to tolerate diesel fuel contamination was species specific, with members of the same plant family showing differential sensitivity to diesel fuel. Of the more tolerant plant species, a delay in seed emergence was generally observed. This delay in germination was shown to be caused, in part, to the volatile fraction of diesel fuel. When the concentration of volatile diesel fuel hydrocarbons surrounding the germinating seed was reduced, germination proceeded at a higher rate. However, the percentage germination of seeds in diesel fuel contaminated soil with volatile hydrocarbons at a minimum was never as high as in uncontaminated

soil suggesting some other factor was influencing germination. This inhibitory effect on germination may be attributed to the physical constraints induced by diesel fuel remaining in the soil on the seed. Diesel fuel would cause a film of oil to form around the seed which would act as a physical barrier, preventing or reducing both water and oxygen transfer to the seed. This physical impedance was shown to delay seed emergence and therefore could be an additional factor in the overall inhibitory effect of diesel fuel contamination on germination.

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Table 1. Common and scientific names of the 25 plant species used in the screening experiment.

| Common name | Scientific name |
|---|---|
| **Grasses** | |
| Cocksfoot | *Dactylis glomerata* L. |
| Creeping bent | *Agrostis stolonifera* L. |
| Highland bent | *Agrostis castellana* L. |
| Meadow bent | *Agrostis pratensis* L. |
| Common bent | *Agrostis capillaries* L. |
| Black grass | *Alopecurus myosuroides* Huds. |
| Couch grass | *Agropyron repens* L. |
| Sweet vernal grass | *Anthoxanthum odoratum* L. |
| Rough meadow grass | *Poa trivialis* L. |
| Westerwold's ryegrass | *Lolium multiflorum* L. |
| Sheep's fescue | *Festuca ovina* L. |
| Strong creeping red fescue | *Festuca rubra ssp. rubra* L. |
| Chewing's fescue | *Festuca rubra ssp. commutata* L. |
| Annual canary grass | *Phalaris canariensis* L. |
| **Herbs and Legumes** | |
| Black medick | *Medicago lupulina* L. |
| Fodder burnet | *Sanguisorba minor ssp. muricata* L. |
| Common vetch | *Vicia sativa* L. |
| Red clover | *Trifolium pratense L.* |
| White clover | *Trifolium album* L. |
| Little yellow trefoil | *Trifolium dubium L.* |
| Lucerne | *Medicago sativa* L. |
| **Commercial Crops** | |
| Oil seed rape cultivars Martina and Rocket | *Brassica napus var. olifera L.* |
| Flax cultivars Viking And Elise | *Linum usitatissumum* L. |

Plant species designated using a system of common names and scientific (binomial) names including authority (Gill and Vear, 1969).

Table 2 Average percentage germination[a], as fraction of control germination, of plant species exposed to varying concentrations of diesel fuel, measured 7 and 14 days after planting at 20 °C.

| Plant species | | % germination | | | |
|---|---|---|---|---|---|
| | | Diesel concentration g $kg^{-1}$ | | | |
| | | 25 | | 50 | |
| Latin Name | Common Name | 7 days | 14 days | 7 days | 14 days |
| **Grasses** | | | | | |
| Dactylis glomerata *L.* | Cocksfoot | 40 (a) | 38 (b) | 0 (c) | 0 (c) |
| *Agrostis stolonifera* L. | Creeping bent [b] | 33 (a) | 127 (b) | 6 (c) | 8 (d) |
| Agrostis castellana *L.* | Highland bent [b] | 53 (a) | 59 (b) | 60 (c) | 54 (d) |
| *Agrostis capillaris* L. | Common bent [b] | 28 (a) | 34 (b) | 10 (c) | 20 (d) |
| *Alopecurus myosuroides* Huds. | Black grass | 61 (*a) | 50 (a) | 9 (b) | 5 (b) |
| *Alopecurus pratensis* L. | Red grass | 65 (a) | 77 (b) | 0 (c) | 0 (c) |
| *Anthoxanthum odoratum* L. | Sweet vernal grass [b] | 56 (a) | 67 (b) | 7 (c) | 17 (d) |
| *Poa trivialis* L. | Rough meadow grass [b] | 5 (a) | 18 (b) | 0 (c) | 0 (c) |
| *Lolium multiflorum* L. | Westerwold's ryegrass | 86 (*a) | 82 (*a,b) | 55 (b) | 64 (a,b) |
| *Agropyron repens* L. | Couch grass | 0 (a) | 0 (a) | 0 (a) | 0 (a) |
| *Festuca ovina* L. | Sheep's fescue | 41(a) | 66 (*a) | 9 (a) | 41 (a) |
| *Festuca rubra ssp. rubra* L. | Strong creeping red fescue | 77 (a) | 107 (*b) | 23 (c) | 49 (d) |

| | | | | | |
|---|---|---|---|---|---|
| *Festuca rubra ssp. commutata* L. | Chewing's fescue | 68 (*a,b) | 119 (*a) | 32† (b,) | 42† (*b) |
| *Phalaris canariensis* L. | Annual canary grass | 83 (*a) | 83 (*a) | 17 (b) | 14 (b) |
| **Herbs and legumes** | | | | | |
| *Medicago lupulina* L. | Black medick | 80 (*a) | 100 (*a) | 113‡ (*a) | 120 (*a) |
| *Sanguisorba minor ssp. muricata* L. | Fodder burnet | 77 (a) | 107 (*b) | 23‡ (c) | 49 (d) |
| *Vicia sativa* L. | Common vetch | 41 (*a) | 94 (*b) | 19 (*a) | 66 (*a,b) |
| *Trifolium pratense* L. | Red clover | 92† (*a) | 100† (*a) | 69 (*a) | 71 (*a) |
| *Trifolium album* L. | White clover | 29 (a) | 53 (b) | 14 (c) | 18 (c) |
| *Trifolium dubium* L. | Little yellow trefoil | 0 (a) | 90 (*b) | 10 (a,c) | 45 (c) |
| *Medicago sativa*L. | Lucerne | 110 (*a) | 114 (*a) | 98 (*a) | 89 (*a) |
| **Commercial crops** | | | | | |
| *Brassica napus* var. *olifera* L. | Oil seed rape cv. Rocket | 111 (a) | 100 (*b) | 106 (c) | 95 (d) |
| *Brassica napus* var. *olifera* L. | Oil seed rape cv. Martina | 104 (a) | 100 (*b) | 99 (c) | 95 (d) |
| *Linum usitatissimum* L. | Flax cv. Viking | 82 (*a) | 89 (*a) | 73 (*a) | 51 (*a) |
| *Linum usitatissimum* L. | Flax cv. Elise | 100 (*a) | 102 (b) | 100 (*a) | 104 (c) |

[a] percentage germination equals every seed planted germinating and producing a sizeable shoot (> 2 mm), [b] these seed species were planted at a rate of 100 per replicate. The remaining seed species were planted 25 seeds per replicate.

Standard error (SE) < 5, n = 2 unless denoted: † SE < 10, ‡ SE <15.

Treatments labelled with the same letter (in parentheses) are not significantly different based on a Tukey LSD ($p<0.5$).

* indicates treatments that are not significantly different from the control in that set.

Table 3. Percentage germination, as a fraction of the control germination, of plant species exposed to varying concentrations of diesel fuel, measured 2, 4 and 6 weeks after planting at 8 °C.

| | % germination | | | | | |
|---|---|---|---|---|---|---|
| | Diesel concentration g $kg^{-1}$ | | | | | |
| Plant species | 25 | | | 50 | | |
| | 2 weeks | 4 weeks | 6 weeks | 2 weeks | 4 weeks | 6 weeks |
| Highland Bent | 0 (*a) | 32 (*b) | 68 (*c) | 0 (*a) | 27 (*b) | 71 (*c) |
| Common Bent | 0 (a) | 8 (a,c) | 12 (a,c) | 0 (a) | 12 (a,c) | 23 (b,c) |
| Sweet vernal grass | 56 (*a,b) | 67 (a,c) | 96 (*a,) | 11 (*b) | 48 (b,c) | 89 (*a,c) |
| Black grass | 50 (*a,b,c) | 95 (*b) | 75 (*a,b) | 8 (*c) | 60 (*b) | 58† (*b) |
| Rough meadow grass | 0 (*a) | 0 (*a) | 40 (*b) | 0 (*a) | 0 (*a) | 20 (*c) |
| Fodder burnet | 57 (*a) | 50 (*a) | 72 (*a) | 29 (*a) | 27 (a) | 53 (*a) |
| Chewing's fescue | 6 (a) | 65 (*b) | 94† (*b) | 6 (a) | 70† (*b) | 103 (*b) |
| Strong creeping red fescue | 20 (a) | 76 (*b) | 100 (*b) | 8 (a) | 78 (*b) | 91 (*b) |

Standard error (SE) < 5, n = 2 unless denoted:

† SE < 10,

Treatments labelled with the same letter (in parentheses) are not significantly different based on a Tukeys LSD ($p<0.5$).

* indicates treatments that are not significantly different from the control in that set.

Table 4. Development of TPF staining in viable seeds pre-soaked for varying lengths of time in diesel fuel.

| | Visual appearance of TTC reduction | | |
|---|---|---|---|
| Treatment | Development of stained colour | | |
| Soaked in diesel (hrs) | % Red | % Pink | % None (white) |
| 0 | 70.0 | 30.0 | 0.0 |
| 24 | 87.5 | 12.5 | 0.0 |
| 48 | 77.5 | 20.0 | 2.5 |
| 168 | 66.7 | 30.8 | 2.5 |

TPF, triphenyl formazan; TTC, triphenyl tetrazolium chloride

**Figure Legends**

Figure 1. Average percentage germination, as fraction of control germination, of varying plant species in ‘aged’ diesel fuel contaminated soil compared to freshly contaminated soil.

Average values given ± standard errors (SE), n=4.

Figure 2. Average percentage germination of Flax seeds pre soaked in diesel fuel.

Average values given ± standard errors (SE), n=4.

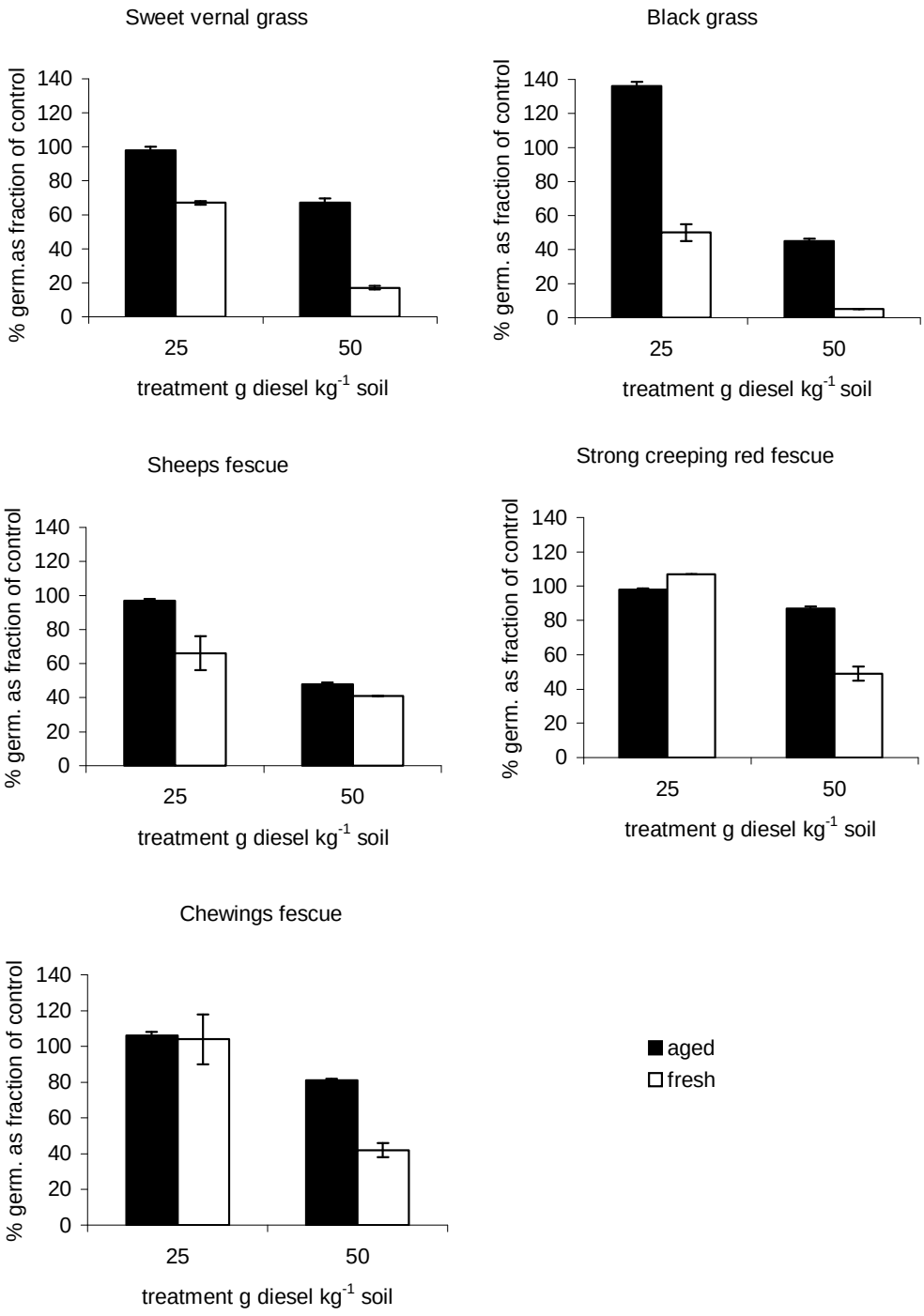
Sweet vernal grass
Black grass
Sheeps fescue
Strong creeping red fescue
Chewings fescue
% germ.as fraction of control
% germ. as fraction of control
0
20
40
60
80
100
120
140
25
50
treatment g diesel kg-1 soil
aged
fresh

Figure 1

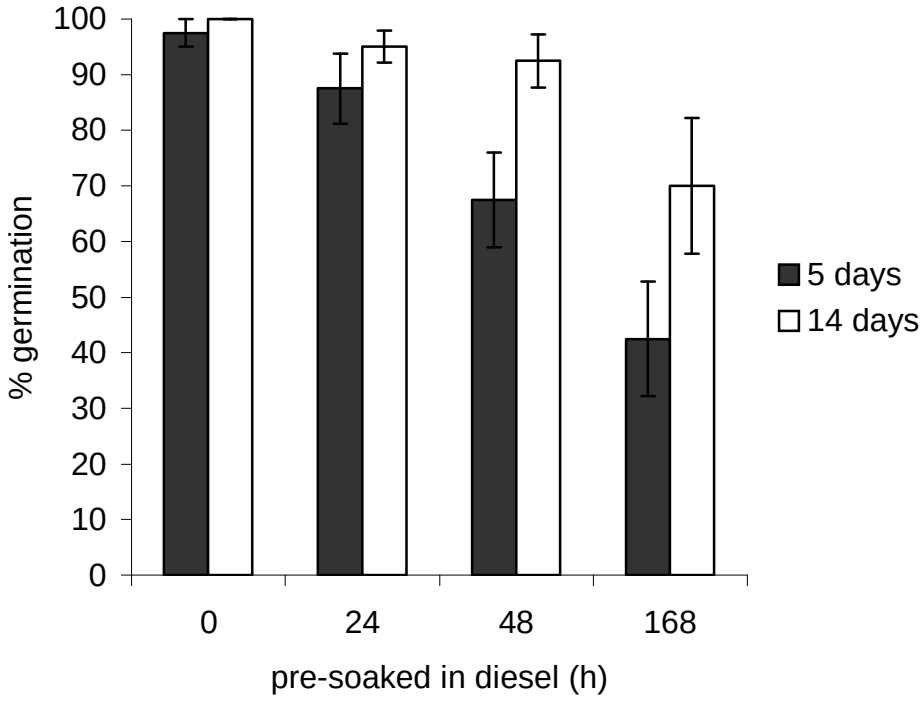
100
90
80
70
60
50
40
30
20
10
0
% germination
0
24
48
168
pre-soaked in diesel (h)
5 days
14 days

Figure 2